Signs and symptoms of root rot in *Eucalyptus pellita* plantations in Indonesia

By

Luciasih Agustini
B.Sc. (Hons.)

School of Agricultural Science

UTAS

University of Tasmania

A research thesis submitted in fulfilment of the requirements for the Degree of Master Agricultural Science
August, 2009

Declarations of originality

This thesis contains no material which has been accepted for a degree or diploma in any university or other institutions, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person, except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Luciasih Agustini
(28 June 2010)

Authority of Access

This thesis may be made available for loan and limited copying in accordance with the Copyright Act of 1968.

Luciasih Agustini
(28 June 2010)

Abstract

This thesis investigates the identity of fungi causing root rot in *Eucalyptus pellita* plantations in Indonesia. It explores the question of how to detect root-rot infected *E. pellita* trees from their crown symptoms and whether root-rot disease can be detected at an early stage in the cycle of disease development.

This study described and identified the fungi associated with root-rot disease in *E. pellita*, putatively caused by a species of *Phellinus*. Macro- and microscopic observations and DNA analysis were used to describe sporocarp morphology and the fungal cultures growing from the symptomatic root samples. Results showed that at the 12 sites investigated, and contrary to expectations, species of *Phellinus* are less commonly associated with root-rot disease than are *Ganoderma philippii* and *G. mastosporum*. There were several potential fungal agents of root rot present at any one site; the sporocarp types observed and the external appearance of the roots were not consistently good indicators of the active pathogen as isolated from roots.

A visual assessment method to assess the crown condition of trees in plantations of *E. pellita* was developed. Eight aboveground variables were used as indicators to classify the *E. pellita* crowns into five different classes. Repeatability, reproducibility and reliability of this method were examined by conducting repeated surveys. Analysis of the data showed that the crown variables adequately discriminated between crown-condition classes when they were assessed by experienced assessors. However, in repeated surveys which were conducted by less experienced assessors, the crown variables did not sufficiently discriminate between crown-condition classes. Applicability of the method to indicate root-rot

incidence and severity at individual-tree level was tested during the first survey. An aboveground assessment of crown-condition using the methodology developed does indicate, at the plot level, the incidence and severity of root rot. At the tree level, the aboveground variables were not significantly correlated with root-rot incidence and severity, as indicated by Spearman correlation ($\alpha = 0.05$). There was an approximate probability of one out of two that poor crown health was associated with visible signs and symptoms on the roots exposed around the tree. Probability of these indicators for estimating root-rot incidence and severity in an individual tree is 61.4% and 41.6%, respectively.

A pathosystem model of *Eucalyptus nitens* trees artificially inoculated with *Armillaria luteobubalina* was set up to investigate early physiological responses associated with root-rot infection. Trees were inoculated with two different isolates of *A. luteobubalina*. Root systems were either wounded or left intact before inoculation. Three photosynthetic parameters, *i.e.* photosystem II yield (F_v/F_m), chlorophyll content and photosynthetic rate (A_{max}) were assessed during six-months of observation. Photosystem II yield was the most sensitive to root-rot infection. A significant difference in F_v/F_m between the unwounded control and other treatments was observed. Chlorophyll content and photosynthetic rate (A_{max}) decreased for all trees, including controls, during the period of the experiment. The decrease was more marked in treated than control trees. The root systems of inoculated trees were examined and reisolations of *A. luteobubalina* from symptomatic roots were carried out to confirm infection with *Armilllaria* of *E. nitens* trees. This preliminary trial of a model pathosystem was successful and did indicate that there were detectable physiological changes associated with early

infection by *Armillaria*. However the experiment required a longer duration for more widespread physiological changes to be detected.

The findings of this project reinforce the importance of comprehensive efforts to reduce the severity and incidence of root-rot disease, especially in terms of early detection. These efforts include the correct identification of fungal causal agent/s, the regular monitoring of crown condition, the application of physiological indicators such as photosystem II yield (F_v/F_m) to detect stress in plants, including that caused by root rot. Further studies are required to scale up these findings to an operational and cost effective level of plantation management. Pest management strategies, especially for root rot, such as site-hazard rating, species-site matching and mixed-planting systems are discussed in reference to plantation expansion in Indonesia.

Acknowledgements

I gratefully acknowledge the financial support of John Allwright Fellowship from ACIAR (Australian Centre for International Agricultural Research). Significant support was also provided by CSIRO (Commonwealth Scientific and Industrial Research Organisation) – Sustainable Ecosystems (Hobart, Australia); Centre for Biotechnology and Tree Improvement (CFBTI, Yogyakarta, Indonesia); PT. Arara Abadi (Sinar Mas Group, Riau, Indonesia).

I sincerely thank my supervisors Assoc. Prof. Caroline Mohammed, Dr. Chris Beadle, Dr. Karen Barry, and Dr. Anthony Francis for their patience, wisdom and encouragement during my study.

My deepest thanks go to Dr. Morag Glen and Dr. Vivi Yuskianti for their assistance in molecular analyses of my fungal collection and wonderful support during the writing process; Prof. David Ratkowsky for helping me with statistical analyses. I also wish to thanks Dr. Alieta Eyles, Dr. Sergey Shabala, Bryony Horton, David Page, Dale Worledge, Keith Churchill, Malcolm Hall, and Vin Patel for providing training and assistance with Chapter 4; Louise Oxley and Morag Porteus for English assistance.

My unlimited thanks to Dr. Anto Rimbawanto, Nur Hidayati, Desy Puspitasari, Mardai Unen, Heru Indrayadi, Fadjar Sagitarianto, Bayo Alhusaeri and all field crews at Arara Abadi for their great assistance with the fieldwork in Indonesia.

The completion of this thesis would not have been possible without support from my colleagues, especially Ragil Irianto and Sugeng Santosa, from Forest and Nature Conservation Research and Development Centre (FNCRDC, Bogor,

Indonesia), and sincere understanding and encouragement from my family – especially my mother who always supported me with her unconditional love; my best friends – Arief Pratisto, Devy Trusda, Dwi Kurnianto, Hanifah, Hary Sugiandi, Hartanti, Ira Fachira, Novy Marthiany, Popi Rismala, Ratih Lestarini, Safitri Wijanarti, Sofi Andriany, Puji Prihatinningsih, and Zulfiani who always accompanied me from a distance; and my very special friend who acted as a buffer when life got tough – Lilis Sadiyah who was always showing me the funny side of life; and Moch Ashari who always kept up my spirit to finish the revision process completely.

In particular, I would like to thank Joseph and Aranka Schmidt, Sári Karakoc, Najma Mahmud, Martha, Trirong Wan-ar-ree, Nghiem Quynh Chi, Tran Duc Vuong, Anung Riapanitra, Agni Lili Aryanti, Ifayanti Ridwan Saleh, Hamid Achmadi, Julia Roshalina, Rif'at Fadjrianto, Raul Simanjuntak, Istiana Prihatini, Purnamila Sulistyawati, Yantie, Azizah Vonna and others from the Indonesian Society in Hobart who made me feel 'at home' even though I was away from my family.

My greatest thanks to ALLAH who always provides everything I need in this 'journey'. He has been ever present in my life even when doubt and discouragement threatened. *Subhanallah walhamdulillah!*

Table of contents

Chapter 1 - General Introduction

1.1 Introduction

This thesis investigates the identity of fungi causing root rot in *Eucalyptus pellita* F. Muell. plantations in Indonesia. It explores the question of whether root-rot disease caused by the fungi identified can be detected at an early stage in the cycle of disease development.

1.2 Root rot in Indonesian plantations

Throughout the world, including Indonesia, plantation forestry is becoming the primary supply of wood as that from natural forests declines and the demand for wood both from domestic and international markets continues to increase (Rimbawanto 2006). Plantation forests not only provide a wood supply for industry and other uses, but contribute to reduced logging of natural forest, as well as providing an alternative livelihood for local communities (FAO 1999; Parrotta 1992; Tiarks, Nambiar *et al.* 1998; UNEP 2007).

In regard to these issues, since the mid-1980s, the Government of Indonesia has launched a program for rehabilitation of unproductive *Imperata* sp. grassland and secondary scrubland into industrial forest in islands other than Java (Rimbawanto 2006). The area of plantations, which is particularly planted with fast-growing species, in Indonesia has dramatically expanded since that time. Acacias, such as *Acacia mangium* Willd, *A. auriculiformis* A. Cunn. Ex Benth, *A. crassicarpa* A.Cunn. Ex Benth, *A. aulacocarpa* A. Cunn Ex Benth, and

eucalypts, such as *Eucalyptus pellita* F. Muell, *E. urophyla* ST. Blake, *E. deglupta* Blume and *E. alba* Reinw Ex Blume are the species most planted by the plantation industry in Indonesia (Leksono 2004).

Besides providing new timber resources, monocultures of fast-growing trees may be at greater risk of pest and disease outbreaks. Root-rot disease in forestry reduces productivity by slowing tree growth, predisposing trees to wind-throw and insect attack, and causing mortality (Morrison, Williams *et al.* 1991). Root rot is considered one of the most damaging diseases to Indonesia's acacia plantation resources; in the *A. mangium.* estate, high levels of tree mortality due to root rot are now being observed during the second and third-rotations and therefore the impact is very real (Eyles, Beadle *et al.* 2008; Glen, Bougher *et al.* 2009; Irianto, Barry *et al.* 2006; Irianto, Barry *et al.* 2003).

Root-rot fungi are normal components of forest ecosystems. In un-disturbed forest ecosystems, root fungi and their hosts are in a dynamic equilibrium. The establishment of plantations and associated management practices (such as introducing exotic species that may be susceptible to local diseases, and regular harvesting that provides many stumps as new food sources for the fungi) breaks this balance (Morrison, Merler *et al.* 1991). This can lead to situations like that seen in *A. mangium* plantations in Indonesia where root-rot incidence increases from one rotation to the next. The disease incidence of second rotation stands of *A. mangium* aged 3-5 yrs old has been recorded as between 3 and 28% (Irianto, Barry *et al.* 2006). *Ganoderma philippii* (Bres. & Henn. ex Sacc) Bress. has been reported as the causal agent for the majority of root-rot disease in *A. mangium* plantation (Glen, Bougher *et al.* 2009; Irianto, Barry *et al.* 2006).

Eucalyptus pellita has been identified as a promising species for industrial plantations and a potentially viable alternative to *A. mangium* as a source of pulp wood. This eucalypt species had better and healthier growth compared to other eucalypts tested, i.e. *E. urophylla* S.T. Blake. and *E. urophylla* × *E. grandis* Hill ex. Maiden, on the same site in South Sumatera (Hardiyanto 2003). Harwood, Alloysious *et al.* (1997) also concluded that *E. pellita* appears more resistant to foliar diseases than other eucalypt species planted in humid tropical environments. Some plantation companies, such as PT. Arara Abadi and PT. Success Perawang Industry in Riau, PT. Wira Karya Sakti in Jambi, PT. Inhutani III in South Kalimantan, PT. Korintiga Hutani in Central Kalimantan, and PT. Emma Sawa in Papua have planted *E. pellita* on an operational scale, with the idea that it could be less susceptible to root disease caused by *Ganoderma* sp (Leksono 2004).

However, in recent surveys of plantations owned by Sinar Mas, *E. pellita* has been observed to be susceptible to several diseases, *viz* root rot caused by a *Phellinus* species, dieback caused by *Botrydiplodia* sp., and wilt disease caused by the bacteria *Ralstonia solanacearum* Smith. Of these diseases bacterial wilt and fungal root rot caused are the potentially the most damaging pathogens (Mardai Unen 2007, pers.comm.).

In order to manage fungal root-rot outbreaks in *E. pellita* plantations, the causal agents must be established and described so that they can be recognised in the field. Methodology for the detection of this disease, preferably at an early stage of development, is required. Thus, this research project investigates the causal agents, their recognition, and develops detection methods that can be applied in *E. pellita* plantations in Indonesia in particular.

1.3 The identification and detection of fungal root rot disease in trees

Manion (1991) defines *signs* as a structure of the biotic causal agents of disease and *symptoms* as a phenomenon of plant reaction in response to disease invasion. Most root diseases do not always show specific or characteristic above-ground symptoms of crown ill-health until a tree is near death. In this case, diagnosing the causal agent/s of the disease (usually in advanced stages of the disease) relies on the presence of identifiable sporocarps, other fungal structures (e.g. mycelial cords or rhizomorphs) or the isolation and identification of the organism in question from infected roots which are excavated (Manion 1991).

In a survey held in February 2007 at a trial plot in an *E. pellita* plantation in Riau province (Central Sumatra) of Indonesia, basidiomes identified as belonging to a species of the genus *Phellinus* and rotted wood with a honeycomb-like pattern also characteristic of infection by a species of *Phellinus* were observed. These observations were associated with a large number of dead trees in this plot. However, it has never been proven that the root-rot disease observed in commercial stands of *E. pellita* is caused by a species of *Phellinus* or whether other fungi are involved.

Tree health can be considered in a pathological or broader physiological sense (Stone 1998) and is a term which encompasses how damaging factors, both biotic and abiotic, affect tree growth, crown condition and survival (Stone and Haywood 2006). The development of an effective system of health management and intervention strategies for forest trees is dependent upon regular forest health surveillance i.e. the recognition and quantification of the visual symptoms of abiotic and biotic damage. For root diseases, crown condition (i.e. crown

appearance and size) should be an important parameter in the description of symptom development. Morrison, Williams *et al.* (1991) and Guthardt-Göerg and Vollenweider (2007) have reported that damage to the root vascular system caused by fungal invasion is manifest in observable leaf symptoms. In young stands of *E. pellita* in Indonesia surveillance of crown condition is ad hoc, there has been no attempt to describe a healthy crown and whether symptoms if seen in the crown relate to an early stage of root rot infection or whether they indicate that the tree will die, as is often the case with fungal root-rot disease. The detection of root rot can never be entirely based on foliar symptoms e.g. in rubber, abiotic stress factors interfering with normal physiological processes result in similar visible crown symptoms to those of root-rot infection (Peries 1965). As similar symptoms are induced by a number of biotic and abiotic factors, the presence of signs that are specific to particular causal agent/s will contribute to a correct diagnosis of the type of root-rot disease. It is important to combine crown symptoms with other signs of root rot in order to develop a reliable detection method for this disease.

Symptoms present in the crown are a manifestation of physiological and morphological changes in response to the disease and develop because of functional disruption to cells or tissues within individual leaves. There is subsequently visible damage to leaf clusters, canopy contraction, and the development of stand gaps caused by tree death (Stone, Coops *et al.* 2000). Stone, Coops *et al.* (2000) also proposed a relationship between the different stages of symptom development associated with eucalypt canopy decline and certain measurable physiological or morphological attributes (Fig. 1.1). An increased understanding of the pre-visual (Fig 1.1) but physiologically measurable changes in a tree as it becomes unhealthy and the relative timing of these events is an

important first step to developing systems for early disease detection, especially for root-rot diseases which rarely show any observable symptoms until near death.

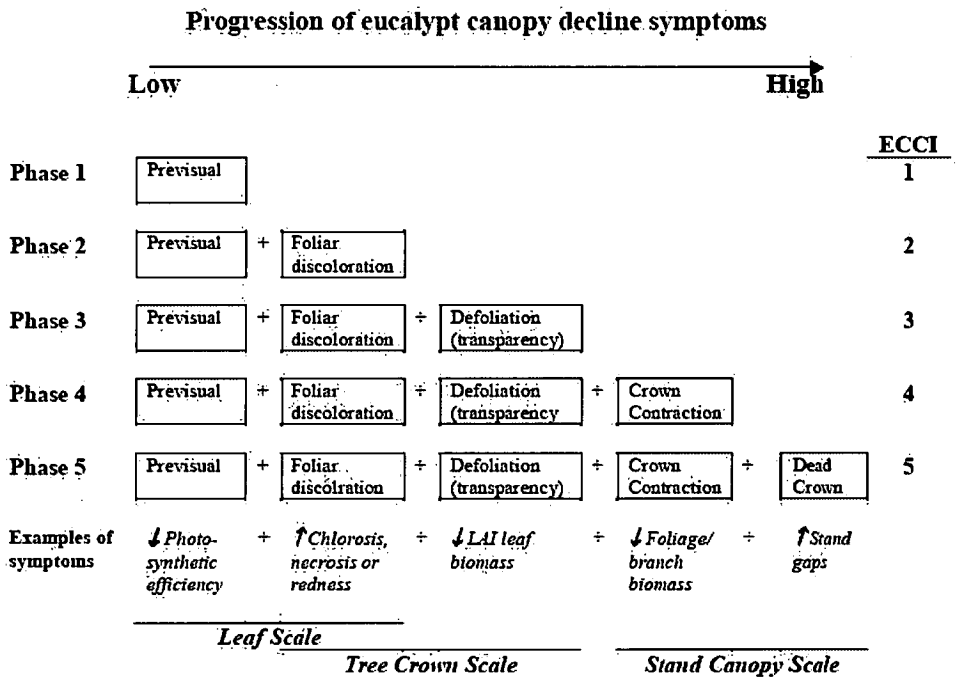


Figure 1.1 A conceptual model illustrating the structural hierarchy of the Eucalypt Canopy Condition Index (ECCI) (Stone, Coops *et al.* 2000)

1.4 Thesis aims

The specific aims of this project were:

1. To accurately characterise root disease in *E. pellita* by examining and describing in detail the fungal signs collected from the suspected root-rot area. Several sites in *E. pellita* plantations in Indonesia that have been reported with root-rot disease were surveyed. Basidiomes and infected root samples were collected for further investigation (Chapter 2).
2. To develop an operational system of crown-condition assessment for *E. pellita* stands and to test if this system is useful for early detection of root-rot disease. Several indicators of crown condition in plantation *E. pellita* were quantified

and linked to visual assessments of the incidence, severity and type of infection by carrying out root excavation and observation, sporocarp collection and fungal isolation (Chapter 3).

3. To provide physiological evidence that can be used to assist in the recognition of root rot in its early stages of infection in eucalypts. *E. nitens* Deane & Maiden saplings grown in Hobart were artificially inoculated with the root-rot fungus *Armillaria luteobubalina* Watling & Kile, and monitored at regular intervals after inoculation for physiological changes, namely photosynthetic capacity (A_{max}), photosystem II yield (F_v/F_m via chlorophyll fluorescence) and chlorophyll content (Chapter 4).

Chapter 2 – Recognizing root rot in *Eucalyptus pellita* plantations

2.1 Introduction

The pulpwood plantation estate in Indonesia is largely based on species from two genera, *Acacia* and *Eucalyptus*. The *Acacia* estate is the more mature estate and some parts are now in third rotation with the total area planted exceeding 1M hectares. *Eucalyptus pellita* is increasingly planted in Indonesia instead of *A. mangium* because of its high productivity and perceived lower susceptibility to root-rot diseases. Sinar Mas, one of the largest forestry plantation companies in Indonesia has planted *E. pellita* in major areas of their concession (Mardai Unen, pers.comm. 2007).

2.1.1 The causal agents of root rot disease in Indonesia

Basal stem rot and root rot in trees caused by basidiomycetes are naturally and widely occurring diseases on a wide range of hosts. Indonesia supports substantial areas of plantation estates; palm oil production from oil palm (*Elaeis guineensis* Jacq.), latex production from rubber (*Hevea brasiliensis* Müll.Arg), and forest species from the genera *Acacia* and *Eucalyptus* (mainly pulpwood production from *Acacia mangium* Willd. and *Eucalyptus pellita* F.Muell.). These are all susceptible to the root-rot group of basidiomycete pathogens such as *Rigidoporus microporus* (Fr.) Overeem syn. *Rigidoporus lignosus* (Klotzsch) on rubber (*Hevea brasiliensis*) and sentang trees (*Azadirachta excelsa* (Jack) Jacobs); *Phellinus noxius* (Corner) Cunningham on teak (*Tectona grandis* L.f.), sentang and

A. mangium; *Ganoderma* spp. on *A. mangium*; *Ganoderma orbiforme* (Fr.) Ryvarden [as 'orbiformum'] syn. *Ganoderma boninense* Pat., on oil palm (Farid and Lee 2006; Farid, Lee *et al.* 2005; Guyot and Flori 2002; Irianto, Barry *et al.* 2006). *Ganoderma* basal rot appears to be the single major disease constraint to sustainable production of oil palm throughout Asia (Ariffin, Idris *et al.* 2000; Durand-Gasselin, Asmady *et al.* 2005; Flood, Hasan *et al.* 2000; Sigh 1991; Turner 1981).

More than one root-rot fungal species may be pathogenic on a single host species; for example in Indonesia and Malaysia, red-root disease, *Ganoderma philippii* (Bres. & Henn.ex Sacc.) Bres. is the second most significant root disease of rubber after *R. lignosus* (Chee 1990; Lim 1977; Rubber Research Institute of Malaysia - RRIM 1961). *Phellinus noxius* is also pathogenic on rubber, but less aggressive than *R. lignosus* or *G. philippii* (Rubber Research Institute of Malaysia - RRIM 1974). There are at least three root-rot pathogens associated with root-rot in *A. mangium*. In Malaysia and Indonesia, two *Ganoderma* species and *P. noxius* have also been isolated from *Acacia* plantations affected by root rot (Glen, Bougher *et al.* 2009).

These basidiomycete root-rot fungi are facultative saprophytes as well as pathogens and can survive for long periods on woody debris (Morrison, Merler *et al.* 1991; Turner 1965); see the life cycle of *P. noxius* illustrated in Fig. 2.1. Sources of inoculum therefore include debris from the previous rotation e.g. slash or colonised debris, litter, coarse roots and stumps. Spread of the pathogen is commonly through contact with an existing source of dead wood supporting the pathogen saprophytically or a living infected root. The pathogens also appear to spread by spores because trees in areas previously with no history of root rot

become infected. However in a diseased area the predominant means of pathogen spread is by root contact with infected material. The only strategy available for disease management in plantation monocultures is one that contains the presence of the pathogen at acceptable levels.

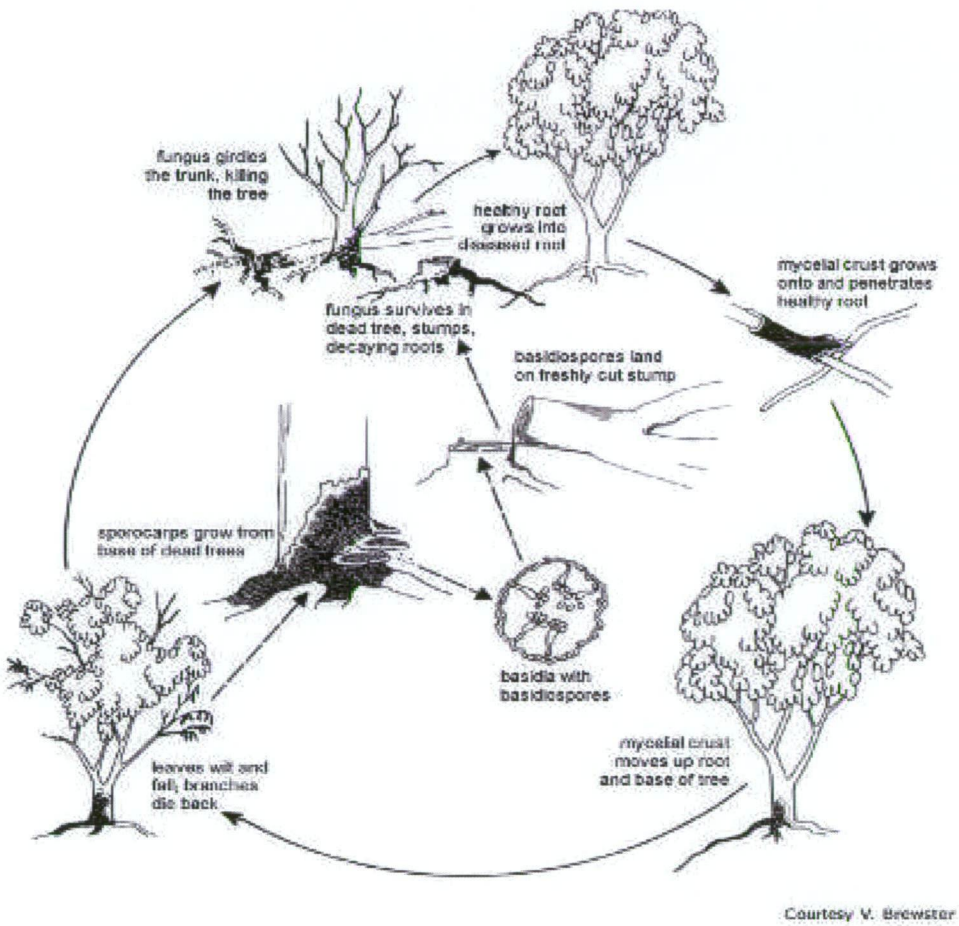


Figure 2.1: Brown root rot (*Phellinus noxius*) disease cycle and epidemiology (reproduced from Brooks, F. E. 2002. Brown root rot. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2002-0923-01).

Pot inoculation experiments to test host susceptibility have indicated that *E. pellita* is less susceptible than *A. mangium* to *Ganoderma* root disease (H. Indrayadi, pers.com. 2007). A large number of dead trees associated with many

Phellinus sporocarps at one site led the company to hypothesise that a *Phellinus* species, possibly *P. noxius*, could be a major pathogen in *E. pellita.* However, recognising the causal agent(s) primarily responsible for root-rot disease is not necessarily straightforward since several pathogens may be present at a site. Root diseases may not show a specific aboveground symptom; general symptoms include an overall decline in crown condition, poor growth rate, and poor foliage condition. Fungal signs present belowground on the root will vary depending on the fungal species present (Blanchard and Tattar 1981) and it is unlikely that all fungi in an area or even on an affected host will be responsible for a particular disease.

2.1.2 Recognition of root rot by fungal signs

Signs of a disease are defined as observable evidence of the disease causal agent (Manion 1991), e.g. for root-rot fungi this may include the presence of mycelia, rhizomorphs, and specific fruiting structures. Because signs are a direct product of the pathogen, they are more useful in the diagnosis and identification of the disease than symptoms which are the plant's response to the effects of the pathogen. Different pathogens can cause similar symptoms (Kavanagh 2005). Signs are characteristic of the fungus when it interacts with a particular host. However, not all characters produced by all fungi present on the host are indicative of a particular disease. For example fungal structures found on a diseased host may belong to saprobic fungi or secondarily invading pathogens. Some pathogens can also penetrate plant surfaces without causing disease due to the resistance of the hosts (Lucas, Campbell *et al.* 1992).

A number of root-rot pathogens produce characteristic signs on infected roots that can be used to reliably identify the disease organism. Those signs directly related to the process by which a pathogen causes disease are the most useful for identifying particular disease causal agents. For instance, in mixed coniferous stands in British Columbia, basal resinosis coupled with the presence of mycelial fans in the bark or cambium were accepted as evidence of *Armillaria ostoyae* (Romagn.) Herink infection (Bloomberg and Morrison 1989; Morrison, Pellow *et al.* 2000). In SE Asia *G. philippii* affected roots are covered by a reddish-brown rhizomorphic skin which ranges from a sparse network to a continuous fungal skin covering the infected root; a white mottling pattern is evident on the underside of the infected bark and there is a distinct fungal odour (Fig.2.2.A). *Rigidoporus lignosus* is a rhizomorphic root-infecting fungus the rhizomorphs of which often cover infected roots extensively. The rhizomorphs growing on the surface of the root are white (especially at the growing ends), possess many branches and are firmly attached to the surface of the roots. The epiphytic growth of rhizomorphs (Fig.2.2.B) may extend 1- to - 5 m ahead of actual penetration of live rubber roots and descend the full length of the tap root to a depth of 50 m. A thick, dark brown to black crust forming around infected roots and lower stems is characteristic of brown root-rot disease caused by *P. noxius* in Queensland (Bolland 1984). Mycelium is present between the bark and sapwood. Decayed wood is white, soft and crumbly, laced with brown pseudosclerotial plates that may darken with age giving the rotted wood a honeycomb-like appearance (Ivory 1996); see Fig.2.2.C. In the acacia and eucalypt plantations in Indonesia root-rot infections show *Phellinus*-like honeycomb rot but do not usually exhibit the typical brown crust on roots or on the base of the stem.

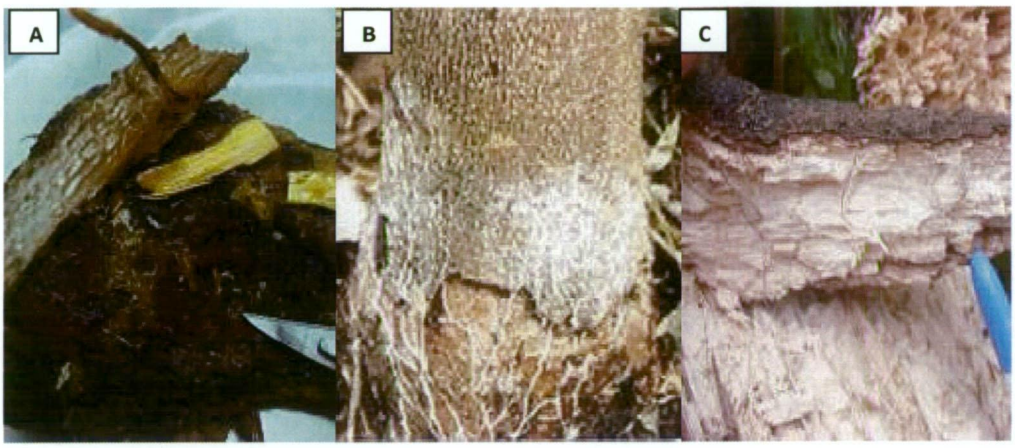


Fig. 2.2 Characteristic of signs of root-rot fungi: (A) *G. philippii* ; (B) *R. lignolosus*, source: http://www.bayer.co.id/ina/cs_cp_problems.php?_rd=37 ; (C) *P. noxius*.

It is conceivable that related species may produce similar signs on the roots even though they have substantially different biological characteristics, such as their level of pathogenicity and dispersal mechanisms. Glen, Bougher *et al.* (2009) concluded that the signs of red root-rot diseases present in *A. mangium* plantation in Indonesia and Malaysia were associated with more than one fungal species. This suggests that *G. philippii* and *G. mastoporum* produce similar signs. Thus, examination of the signs associated with diseased roots followed by isolation and identification of the fungal cultures are a necessary first step in investigating a new host/pathosystem (Kavanagh 2005), such as root rot in *E. pellita*.

The presence of particular basidiomycete root-rot pathogens in the field is also indicated when sporocarps are produced. The morphology of cultures isolated from the reproductive tissue of sporocarps can also be diagnostic although basidiomycete cultures associated with wood decay have received little recent attention and guidelines for their identification rely exclusively on northern hemisphere authors (Nobles 1948; Nobles 1958; Nobles 1965; Stalpers 1978).

The identification of sporocarps as a tool in the field depends on whether and how well they have been previously described and if the taxonomy is stable. Unfortunately both the mycological and pathological understanding of basidiomycete genera and species associated with root-rot disease is often very poor especially in tropical regions. The taxonomy of *Phellinus* and allied genera is in a state of flux and is an area of active but limited research (Corner 1991; Dai 1999; Loguercio-Leite and Wright 1995; Nunez and Ryvarden 2000; Wagner and Fischer 2002). More than 300 *Ganoderma* species have been named, many on only one collection with narrow or poorly defined species concepts based on the highly variable morphology of the fruiting bodies (Seo and Kirk 2000). Ryvarden (1994) proposed a ten-year moratorium on the description of new *Ganoderma* species because such a large number of synonyms exist. However some species, such as *G. lucidum* P.Karst., may represent a complex of six or more, as yet unnamed, species (Hseu, Wang *et al.* 1996).

Even though signs have greater diagnostic value for root-rot disease than crown dieback symptoms, there are several caveats to their application. They can be overlooked or misinterpreted and so careful observation and interpretation is necessary. A sporocarp may have been previously described taxonomically but its role as a causal agent of root-rot disease may not have been established or even recognised. Many fungi are present in the environment and a number are associated with dead trees, including those that have died of root rot. These fungi may produce structures such as sporocarps on the host dead tissue, or cause an identifiable rot pattern but the fungus was not the primary organism responsible for killing the host. For example, the presence of rhizomorphs did not specifically indicate the incidence of *Armillaria* root rot since they may be present without

causing infection especially if they belong to a less pathogenic *Armillaria* species (Greig and Strouts 1977; Redfern and Filip 1991).

2.1.3 Molecular Identification of Root-rot Fungi

Molecular techniques have been proved a valuable tool for fungal identification. In some respects, these techniques have superceded traditional morphological identification as they allow rapid and unambiguous identification of organisms, including fungal pathogens (Njambere *et al.* 2010). The basic idea of the technique is to compare the DNA (deoxyribonucleic acid) properties of organisms, including fungi, in order to identify and determine their phylogenetic relationships. Effectively DNA provides an abundance of taxonomic characters for the identification of organisms that have inadequate morphological characters to create the same certainty of identification (Glen 2006).

There are several molecular techniques that can be used for fungal identification. Basically the techniques consist of four steps, *i.e.* (a) DNA extraction and purification; (b) DNA amplification, (c) DNA sequencing; and (d) genera and/or species determination through comparison with a database of sequences from identified fungi (Glen 2006). Many protocols and manufactured kits are available for extracting and purifying DNA. PCR (polymerase chain reaction) is used to amplify a small portion of genomic DNA. For this amplification, primers (short DNA fragments), oligonucleotides, polymerase enzymes and desired temperature (which is a series of repeated heating and cooling), and buffers solutions are required. The commonly used primer is ITS (internal transcribed spacer). The ITS is a region of the nuclear ribosomal repeated unit that is a very useful locus for species identification and subgeneric

phylogenetic inference in sequence-based mycological research (Olson and Stenlid 2000, Nilsson *et al.* 2008).

Molecular techniques have been applied for identification of root-rot fungi. Park *et al.* (1994) determined phylogenetic relationships of some Ganoderma species through analysing their intraspecific allozyme variation. Moncalvo *et al.* (1995) and Hseu *et al.* (1996) adopted DNA sequences and ramdomly amplified polymorphic DNA (RAPD) as the tool for analysing relationships of the *Ganoderma lucidum* complex. ITS and IGS-1 (the first intergenic spacer) sequences have successfully identified *Armillaria* species from Indo-Malaysia and Chile as species that had the highest similarity to *Armillaria novae-zelandiae* and *A. limonea*, respectively (Coetzee *et al.* 2003). Glen *et al.* (2009) who used fungal-specific primer combination (ITS1-F/ITS-4) identified that *Ganoderma philippii*, *G. mastosporum*, *G.* aff.*steyaertanum*, G. *australe* and *Amauroderma rugosum* are species associated with root-rot disease of *Acacia mangium* plantation in Indonesia and Malaysia.

In this study, molecular techniques were used tools to identify putative causal agents of root rot as only a few sporocarps were found in the area surveyed.

2.1.4 Research objectives

This study uses two hypotheses, namely: ***(A)*** that *Phellinus* sp. is the most common fungal agent associated with root disease in the examined *E. pellita* plantations (this was expected to be reflected in the fungal signs present in the infected areas); ***(B)*** Sporocarps and fungal signs observed on the roots suggest the same root-rot causal agents that present in a particular area. In order to test these hypotheses, the objectives of Chapter 2 were:

1. To examine several compartments of *E. pellita* plantations affected by root rot and make detailed records of the fungal signs found at these sites.
2. To identify sporocarps and cultures obtained from these, using DNA analysis and morphological characteristics.
3. To determine the identity of fungi associated with signs or structures on the roots by the molecular identification of cultures isolated from the structures.
4. To compare the suite of fungi identified from sporocarps with that from fungal signs such as crusts and infected root tissue. This will indicate if the different methods provide the same indication of the possible pathogens present in the area.

The following questions could then be answered:

a. Are species of *Phellinus* the most common pathogenic fungi recovered from isolates of infected *E. pellita* examined? If so, this would support hypothesis ***A***.
b. Do sequences from sporocarps and root isolates match? If so, this would support hypothesis ***B***.

2.2 Materials and Methods

The research strategy was based on a series of field and laboratory studies which were conducted in several research institutes. Field surveys were carried out in *E. pellita* plantations belonging to P.T. Arara Abadi (Sinar Mas Group) in Perawang (Riau Province, Indonesia). Fungal isolations, both from root samples and sporocarps, were conducted at the Pest and Disease Laboratory of P.T. Arara Abadi and at the Centre for Biotechnology and Tree Improvement (CFBTI) in

Yogyakarta, Indonesia. DNA extraction and PCR analysis of fungal cultures was conducted at the Genetic & Molecular Laboratory of CFBTI. Macroscopic features of the fresh sporocarps were described in the field and sporocarp herbarium specimens were air-dried at P.T. Arara Abadi's laboratory then brought to CFBTI for microscopic examination. Due to the insufficient magnification of the microscope at the CFBTI, the herbarium samples were brought to Forest Health Laboratory of CSIRO Sustainable Ecosystem (Hobart, Australia). All herbarium specimens were gamma-irradiated upon importation, in accordance with the terms of import permit IP07020082. DNA extraction and PCR analysis of sporocarps were conducted at the Forest Health Laboratory of CSIRO Sustainable Ecosystems, Hobart. DNA sequencing of the PCR products, both from fungal cultures and sporocarps, were carried out by Macrogen Inc. (Seoul, Korea).

2.2.1 Study sites and samples collection

Twelve sites suspected of experiencing root -ot problems were surveyed. The land use history of the areas surveyed were recorded including information on planting techniques and any silvicultural treatments applied. Root-rot incidence at each site was examined by a walk-through disease assessment method. Exploration of root-rot incidence commenced at 100 m from the road. Two teams explored the area in two different directions for 30 min, finding and recording any signs and symptoms of root-rot incidence. Sporocarps of putatively pathogenic species and root samples from symptomatic trees were collected (Table. 2.1).

Table 2.1. Description of the sites visited during field survey

No.	Compt.	Site history	Sporocarp code	Notes
1	246	First rotation *E. pellita*, clone EP05 (4th rotation after *A. mangium*); age 1.5 yr.	---	
2	250	First rotation of *E. pellita* clone EP05 (4th rotation after *A. mangium*); age 1.5 yr.	---	The trees had bacterial wilt disease when young.
3	223	First rotation *E. pellita* clone EP05 (4th rotation after *A. mangium*); age 1.5 yr.	E 8549 E 8550 E 8552	This plot had previously experienced at least 40-50% tree mortalities during the 3rd rotation of *A. mangium*.
4	173	First rotation *E. pellita* (4th rotation after *A. mangium*); age 1.5 yr.	---	Crown condition was very variable.
5	175	First rotation *E. pellita* (4th rotation after *A. mangium*); age 1 yr.	---	The crowns looked were fairly uniform though some trees were flowering and had smaller and sparser crowns.
6	236	First rotation *E. pellita* clone EP05 (the 4th rotation after *A. mangium*); age 1.5 yr.	E 8538 E 8539 E 8543	This site is a trial to test different types of planting holes.
7	071	First rotation *E. pellita* clone EP05 (4th rotation after *A. mangium*); age 5 yr.	---	This is a sloping site which was used for fertilisation trials. Water availability at this site was not uniform and possibly water was deficient in places.
8	063-A	First rotation *E. pellita* clone EP05 (4th rotation after *A. mangium*) growing adjacent to an infected 3rd rotation planting of *A. mangium*.	---	The site had been de-stumped prior to planting to test if this reduced root-rot incidence.
9	063-B	First rotation *E. pellita* clone EP05 (4th rotation after *A. mangium*) growing adjacent to an *A. crassicarpa* planting with high root rot incidence.	---	This site was not a part the de-stumping trial. Bamboo was growing throughout this site.
10	1A	Demonstration plot of 5 yr - *E. pellita* clone. A rubber plantation had originally been located at this site	E 8540 E 8544 E 8546 E 8547	High levels of tree mortality causing big gaps were obvious on this site.
11	5A	Nine months-coppice of *E. pellita* clone EP05 planted on ex rubber plantation that had replaced cleared native forest	E 8548	Trees showed evidence of herbicide damage.
12	2C	Clonal eucalypt resistance trial to root rot, age 2 yr., planted on ex rubber plantation that had replaced cleared native forest.	E 8541	Wildling rubber trees had grown on the limed windrow of rubber tree debris.

2.2.2 Description of fungal signs

The root collars and primary lateral roots of trees with crowns showing symptoms of ill health and their neighbouring trees were excavated to examine the root systems for the presence of fungal signs. The investigators were looking for

structures such as brown crusts on the root surface, red rhizomorphic crusts or skins, a brown stocking at the collar such as typically found with *P. noxius*, mycelial fans, particular types of rotten wood such as the honeycomb rot typical of *P. noxius*, psedosclerotial plates ("blacklines") and sporocarps. Excavation was carried out to about a distance of 50 cm around the base of a tree. The number of trees excavated at each plot varied. Three compartments, i.e. compt. 223, 236 and 1A, had more excavated trees than the others because these sites were used as monitored plots for the study presented in chapter 3. The observed signs on each root system were recorded, photographs were taken. Samples of roots and sporocarps were also taken. Based on these observations, the signs were classified into five major groups as described in Table 2.2.

2.2.3 Fungal isolations

Fungal isolations were carried out with the root samples and sporocarps by placing small pieces that had been surface-sterilised into tubes containing slopes of selective medium (MAT). This medium was prepared by autoclaving 1% malt extract agar (MEA) for 30 min at 120°C. Fifty ppm penicillin, 50 ppm streptomycin, 25 ppm polymixin and 230 ppm thiabendazole were added into the autoclaved MEA while it was cooling (at < 60°C). Surface sterilisation was carried out through a series of washing steps, namely: 2 min in tap water, 2-3 min in 20% Chlorox™ (Hypochlorite solution), and three times washed in sterile distilled-water. Root and sporocarp segments were put on paper tissue and left until they dried, then one piece of each sample was placed into each tube. Five replications were made for both root samples and sporocarps. Sporulating and fast-growing hyphae that grew within one day to one week were considered as contaminants,

and only non-sporulating and relatively slow-growing (grew within 2-4 weeks) hyphae that grew from the root samples or the sporocarp segments were subcultured onto MAT plates and incubated for 4-8 weeks at 25-26°C. Macroscopic morphology of the cultures was recorded and classified into three major groups, namely putative *Phellinus*, putative *Ganoderma* and non target fungi. Putative *Phellinus* was indicated by white, fast-growing mycelium that gradually turned brown over time, especially near the inoculum source; a brown crust was formed on the old cultures (Farid, Lee *et al.* 2005). Putative *Ganoderma* was indicated by white, slow-growing mycelium with scattered powder-like mycelium over the surface and the underside of the culture cream with irregular brown areas (Anonymous 2008). Other cultures with a morphology that did not fit with either the putative *Phellinus* or putative *Ganoderma* isolates were put in the non target group. Due to culture morphological variation, the putative *Phellinus* and *Ganoderma* were separated into several groups as listed in Table 2.3. Appendix 2.6 lists sporocarps, root-sign samples and cultures and the relevant compartment from which they were taken.

2.2.4 Description of sporocarps

Twelve putative pathogenic sporocarps were collected. All sporocarps were examined and their macroscopic features recorded when they were still fresh. Each collection was given a unique herbarium accession number ("E" numbers). The characteristics noted for fresh fruit bodies in the field were: size, shape, colour, surface texture of the fruit body, pore surface colour and the number of pores per millimetre. Photos were taken and sketches (with a scale) were made in the field to facilitate the description of sporocarps. Colours for macroscopic

features were recorded by the collector referring to the Methuen handbook of colour (Kornerup and Wanscher 1961). Specimens were preserved as air-dried herbarium collections using a desiccator.

For microscopic examination in the laboratory, each specimen to be examined was cut transversely and several thin sections taken from the cut surface, context and pores. The microscopic features of the sections (mounted in 3% KOH) were observed under the microscope (Axioscope-ZEISS). Average spore dimensions were based on measurements of 10 spores (if any spores were observed). Average dimensions of basidia (if observed), hymenial elements or other hyphae were obtained from measurements of five of each of these elements. Lactophenol cotton blue was applied to visualise hyaline structures. Tissue and spore colours were determined in water, KOH and Melzer's solution for sections mounted directly in these media. Spore length includes the hilar appendix but neither length nor width includes the ornamentation or perisporium. Photographs of spores were taken at 1000x and annotated with a scale bar, other elements have separate bar indicators with the relevant scales. Fungal identity was determined based on Ryvarden and Johansen (1980) of '*A preliminary polypore flora of East Africa*' and Glen, Bougher *et al.* (2009)

2.2.5 Molecular Analysis

Molecular work was carried out in collaboration with Dr Vivi Yuskianti (Centre for Biotechnology and Tree Improvement, Yogyakarta) and Dr Morag Glen (CSIRO Sustainable Ecosystems, Hobart). DNA was extracted according to Glen, Bougher *et al.* (2009). Twenty (20) mg or less of dried herbarium material was frozen with liquid nitrogen (poured into the 1.5 ml microcentrifuge tube),

ground with a motorised micro-pestle with the addition of a few drops of extraction buffer (Reader and Broda 1985) during grinding. The micro-pestle was rinsed with the remainder of the 250 µl aliquot of extraction buffer to wash off any adhering fungal material. For fungal cultures, a mycelial plug approx. 0.25cm^2 was ground with a motorised micro-pestle and 250 µl of extraction buffer added. The ground samples were incubated for 30-90 minutes at 65°C then centrifuged at maximum speed 14000 rpm (Eppendorf 5415 D) for 15 minutes.

For each sample, 200µl of supernatant was transferred to a new 1.5ml micro-centrifuge tube to which 10µl of glass-milk (Sigma silica, 100 mg/ml in phosphate buffered saline) and 800 µl of NaI (1mg/ml) had been added. The resulting mixtures were shaken briefly (using a vortex mixer) then incubated for 15 minutes on ice with occasional manual shaking. Two or three washing steps were then carried out, where the samples were centrifuged for 10 seconds at maximum speed to pellet the glass-milk and DNA, the supernatant discarded and then the pellet re-suspended (shaken on the vortex mixer) and centrifuged ready for the next wash step. Wash buffer (50 mM NaCl, 10mM TrisHCl pH 7.5, 2.5 mM EDTA, 50) v/v ethanol) was used for the first washing step and 100 % ethanol for the one or two subsequent wash steps. After discarding the supernatant from the last ethanol wash, the tubes were inverted and left to dry (either on the bench-top over-night or in the laminar-flow hood) for approximately 1 hour (or until the tubes appeared dry). The DNA was re-suspended in 25µl of TE buffer and incubated at 45°C for 10 minutes. The samples were centrifuged at maximum speed for one minute and then 20 µl supernatant was removed into a fresh microtube.

PCR amplification of the ribosomal DNA internal transcribed spacers (rDNA ITS) was carried out using either an Applied Biosystems 2720 Thermal

Cycler or a MJ Research Inc. PTC-100™ Thermal Controller with the following program: 2 min at 95°C, followed by 35 cycles of 95°C for 30 s, 56°C for 1 min, 72°C for 2 min and final extension of 72°C of 8 min. PCR reactions contained reaction buffer [67 mM TRIS_HCl pH 8.8m 16.6 mM (NH4)2SO4, 0.45% Triton X-100, 0.2 mg/mL gelatine] (Fisher Biotech, West Perth, Western Australia), 2 mM $MgCl_2$, 0.2 µg/µL bovine serum albumin (Fisher Biotech), 0.2 mM dNTPs (Promega Corp., Madison, WI, USA), 0.25 µM primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White, Bruns *et al.* 1990), 1.1 units *Tth*+ DNA polymerase (Fisher Biotech), with 5 µL DNA template. For all reactions, 1/10 dilutions of the PCR product were made (in case a nested PCR was required) before 5µl of the remaining PCR product was loaded onto 1% agarose gels to visualise the product using Blue Loading Dye. PCR products were sent to Macrogen Inc. (Seoul, Korea) for DNA sequencing.

DNA sequences were edited in the Seqman module of the Lasergene package (DNAStar, Madison, WI, USA). Public and private databases were searched for matching sequences and identifications were based on sequence similarity to morphologically identified sporocarps or isolates from these. Where sequences did not provide a link to morphologically identified collections, isolates with over 98% sequence similarity were grouped and taxonomic information was derived from similarity to sequences in public DNA databases.

2.3 Results

2.3.1 Site inspections

Sporocarps, other signs or both (Fig. 2.3; Fig 2.4 and Appendix 2.1), indicating the presence of *Phellinus* root-rot were observed in compartments 1A, 2C, 5A and 236 (four of twelve sites observed, three of which were planting trials). Compartment (Compt.) 1A is a demonstration plot of *E. pellita* clone EP05, Compt. 2C is a trial investigating clonal resistance to root rot, and Compt. 5A is a coppicing trial. Indicators of *Phellinus* root rot on operational plantation sites were only found in Compt. 236, however, these were less prominent than *Ganoderma* red root-rot on this site. *Ganoderma* root-rot signs were observed in compartments. 071, 173, 223, 236 and 246 (five of twelve sites observed). In compartments 175 and 250, even though the trees were stressed (indicated by sparse crown and fruit production), no obvious root-rot signs were found. No evidence of infection in living trees was observed in Compt. 063(A) (close to infected *A. mangium* stands) or Compt. 063(B) (next to an infected *A. crassicarpa* stand) even though some of the dead trees, stumps and the roots of acacia trees were covered with red rhizomorphic skins characteristic of the presence of *Ganoderma.*

2.3.2 Fungal structures on roots

140 root samples collected in diseased areas were classified into five groups as summarised in Table 2.2. Photographs of these signs are presented in Appendix 2.1.

Table 2.2. The most common types of fungal structures and associated rot types found on root s in the study

Group code	Fungal sign	Number of samples
RS-1	Black crust on the root's bark and a network of black or brown lines underneath the bark and in the wood. The wood becoming white spongy with a honeycomb-like pattern of black or brown lines at an advanced stage of decomposition	22
RS-2	Black crust on the root's bark and irregular pattern of black lines dividing different rot types.	44
RS-3	Red rhizomorphs on the root surface, white-yellowish mycelia mat under the root's bark; small "pockets" of white-yellowish mycelia growing through the root woody tissue underneath the bark.	50
RS-4	White mycelia growing on the root's surface, but the root tissue underneath the bark looks healthy.	7
RS-5	The root has a yellowish-brown to dark brown crust and/or resupinate fungal material on its surface; underneath the bark the woody tissue has a brown stringy texture.	17

2.3.3 Morphology of fungal isolates

157 isolates were morphologically grouped as putative *Phellinus*, putative *Ganoderma*, or as 'non-target' fungi. Fungal cultures assigned to the putative *Phellinus* category were further discriminated by their differing morphology into seven sub-groups. The fungal cultures in the putative *Ganoderma* category were also further divided into six sub-groups. The categories and groups are described in Table 2.3. Photographs of the macroscopic morphology of *Phellinus* and *Ganoderma* cultures are illustrated in Appendix 2.2.

Table 2.3. The primary distinguishing features of cultural morphological groups found in the study

Morphological group code	Morphology of fungal culture	Number of isolates
Putative Phellinus		
Ph. 1	Mat is cream-white mat, reverse light brown. May have patchy white mycelial clumps, and brown crust.	7
Ph. 2	Mat is cream-white, with dense white to very light brown aerial mycelia, "brown lines" (pseudo-sclerotial plates) present, reverse yellowish to brown.	7

Ph. 3	Mat is cream-white to brownish, may have mycelial clumps, brown to dark-brown feathery, submerged mycelia were observed in the underside of the cultures.	20
Ph. 4	Mat is cream-white to cinnamon-brown, may have patchy mycelial clumps.	14
Ph. 5	Mat is brownish-white with dark-brown, submerged mycelia.	8
Ph. 6	Mat is cinnamon-brown with brown circular zones, a thin layer of white aerial hyphae is present.	6
Ph. 7	Mat is brownish white with irregular light-brown, submerged mycelia.	4
Putative Ganoderma		
Gd. 1	Mat is white powdery at the centre with patchy-cottony mycelia clumps, margins feathery.	11
Gd. 2	Mat is white powdery at the centre, patchy-cottony mycelial clumps around margins, cinnamon-brown to dark-brown submerged mycelium.	7
Gd. 3	Mat is white powdery at the centre, margins feathery, cinnamon-brown to dark-brown where submerged.	11
Gd. 4	Mat is white powdery and layered feathery, may be yellowish-brown crustose around the centre and/or patchy at margins.	13
Gd. 5	Mat is white to very pale brown amd velvety.	3
Gd. 6	Mat is white dense woolly or fluffy to powdery around the centre and fine and feathery at the margin.	7
Non Target isolates	Various	39

Note: Isolates were grown from both symptomatic root samples and sporocarps

2.3.4 Description of sporocarps

Phellinus noxius (Corner) G.Cunn. *Bull. N.Z. Dept. Sci. Industr. Res., Pl. Dis. Div.* 164:221, 1965.

Synonymy
Fomes noxius Corner, Gardeners' Bull. Straits Settlements 5(12):324, 1932.
Phellinidium noxium (Corner) Bondartseva & S. Herrera, Mikol. Fitopatol. 26(1): 13 (1992)

Macroscopic
Basidiocarp perennial, solitary to imbricate, pileate broadly attached, effuso-reflexed to resupinate, consistency hard. *Pileus* dimidate, flat, petaloid to spathulate, 5-15 cm wide, up to 15 cm length, 0.6-6.1cm thick, glabrous when mature but irregularly zoned and bumpy, dark brown (5-F4 – 7-F2) to black, white basal mycelium near the point of attachment to the wood. *Crust* hard, 0.2-0.8 mm thick, thinner toward the margin, brown (5-F4) to black. *Pileus context* essentially a single layer, blackening in KOH, often with white mycelia strands oriented radially in the direction of growth in cross-section, 8-30 mm thick, yellow-brown (5-D8) to brown (6-E7). *Pileus margin* rounded-obtuse, paler than the rest of the pileus or pore surface. *Pore surface* greyish brown (5-E1) to dark-browny grey (7-

F8), pores small and round, 6-11 per mm. *Tubes* where multiple tube layers are present are distinctly stratified, yellowish brown (5-E8) to dark brown (6-F2), up to 14 mm length.

Microscopic

Hyphal system dimitic, generative hyphae difficult to see and possibly collapsed, in tubes and the context, thin-walled, hyaline to very pale yellow, 2.6-5.1 µm in diameter, skeletal hyphae yellowish brown, 3.8-6.3 µm in diameter. *Pileus crust* palisade cells, yellowish brown, 2.5-6.5 µm wide. *Pileus context* predominantly thick-walled, yellowish brown skeletal hyphae 3-6µm wide. *Dissepiments* interwoven, darker than the pileus context, predominantly brown skeletal hyphae (2.6-7.8 µm), 59.3-84.6 µm wide, 134.4-185.3 µm axes. *Setal hyphae* abundant in tubes, thick walled, usually projecting into the hymenium, obtuse to acute, yellowish brown, 6-9 µm wide and 48.3-71.6µm length. *Setae* none. *Basidia* not clearly seen. *Basidiospores* elliptical to ovoid, smooth and thin-walled, hyaline, 4.5-4.78 x 3.18-3.5 µm.

Material examined:

INDONESIA: Sumatra, Riau: Compt. 1A-Bunut, on *E. pellita* stump, L. Agustini *et al.*, 31 May 2008 (E8540); Sumatra, Riau: Compt. 2C-Rasau Kuning, on *E. pellita* stump, L. Agustini *et al.*, 31 May 2008 (E8541); Sumatra, Riau: Compt. 236-Rasau Kuning, on *E. pellita* stump, L. Agustini *et. al*, 31 May 2008 (E8543); Sumatra, Riau: Compt. 1A-Bunut, on *E. pellita* stump, L. Agustini *et al.*, 31 May 2008 (E8544); Sumatra, Riau: Compt. 1A-Bunut, on *E. pellita* stump, L. Agustini *et al.*, 31 May 2008 (E8546); Sumatra, Riau: Compt. 1A-Bunut, on *E. pellita* stump, L. Agustini, *et al.*, 31 May 2008 (E8547); Sumatra, Riau: Compt. 5A-Kampung Nias, on un known stump, L. Agustini *et al.*, 31 May 2008 (E8548).

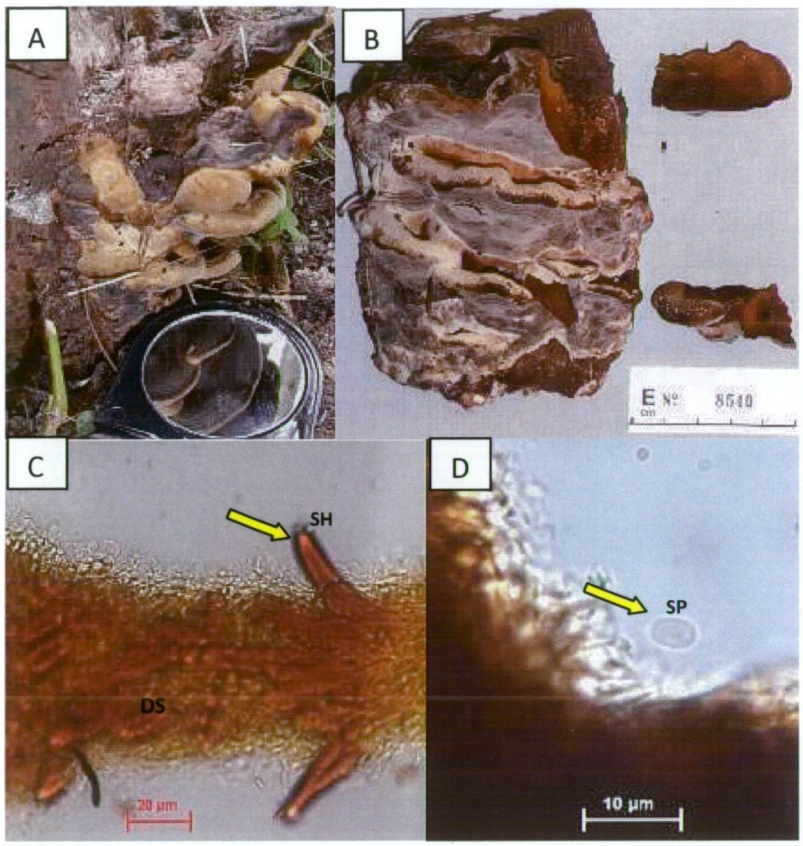


Figure 2.3. Morphology of *Phellinus noxius*. (A). attached on an *E. pellita* stump; (B) preserved as a herbarium specimen; (C) and (D) microscopic features, DS= Dissepiment, SH = Setal hyphae, SP = spore.

Ganoderma mastoporum (Lév.) Pat., Bull. Soc. Mycol. Fr. 5(2,3): 71, 1889

Synonymy:
Elfvingia mastopora (Lév.) Imazeki, Bull. Gov. Forest Exp. St. Tokyo 57: 104 (1952)
Fomes mastoporus (Lév.) Cooke, Grevillea 13(no. 68): 119 (1885)
Polyporus mastoporus Lév., Annls Sci. Nat., Bot., sér. 3 2: 182 (1844)
Scindalma mastoporum (Lév.) Kuntze, Revis. Gen. pl. (Leipzig) 3(2): 519 (1898)

Macroscopic
Basidiocarp annual, growing singly or in a small group, shape variable, stipitate, relatively light, will bend slightly but with a brittle crust. *Stipe* hard, up to 3 cm long, 2 cm wide and 1-2 cm thick, cylindric to slightly vertically flattered; matte, dull dark-brown (5-F2), no obvious base mycelium. *Stipe crust* thin, hard, brittle, dark brown (darker than 6-F2). *Stipe context* single layer with dark inclusions, fibrous and concentrically zoned like the pileus context and continuous with it. *Pileus* applannate broadly attached to dimidate or stipitate spathulate with multiple lobes, from 5 to 12.3 cm wide, 3.5 to 8.9 cm long from substrate including the lateral stipe, 0.8 to 1.2 cm thick, surface laccate red-brown to dark red-brown (10-F5 to 11-F5) becoming dull dark-brown (7-F2) with aging and weathering; pileus with concentric zones, but also with bumps and irregularities. *Crust* very thin,

brittle, dark red-brown (11-F5). *Pileus context* more or less single layer with hard, brown to dark-brown inclusions, concentrically zoned in direction of pileus expansion in cross-section, silky fibrous and compressible, yellowish-brown to dark-brown (6-E8 to 7-F7), thickness ranges from 2-7 mm. *Pileus margin* acute to squarely obtuse and vertically ridged, extending under the pileus about 2-3 mm to border the pore surface. *Pore surface* more or less same shape and area as the underside of the pileus, when young off-white to yellowish gray (5-A2 to 5-C2) and bruising pinkish brown, dull dark to grayish-brown (8-F4 to 5-E2) with age and no longer bruising, 4-8 pores/mm. *Tubes* 5-7 mm long and dark brown (8-F4 to 8-E3).

Microscopic

Hyphal system trimitic. *Pileus crust,* palisade cells squat with flattish tops, yellowish brown, 9.5-14.1 µm long and 3.63 – 6.19 µm wide. *Pileus context,* predominantly yellow-brown skeletal hyphae (3-5 µm broad) with some paler, coiling binding hyphae (0.6-0.9 µm). *Dissepiments,* interwoven, concolorous with the pileus context, predominantly brown skeletal hyphae (2-5 µm) and binding hyphae (0.7-0.8µm) with some hyaline generative hyphae (1.2-2.4µm) nearby the hymenium, 86.3 – 96.5 µm width and axes 174 – 221µm. *Hymenium* difficult to distinguish. *Basidia* four-spored, hyaline. *Basidiospores Ganoderma*-type, pale yellow-brown, echinulate, truncate ellipsoid, 6.0-9.7 x 3.6-5.4 µm. *Stipe crust and context* as for pileus crust and context.

Material examined:

INDONESIA: Sumatra, Riau: Compt.236-Rasau Kuning, on *A. mangium* stump, L. Agustini *et.al*, 31 May 2008 (E8538); Sumatra, Riau: Compt.236-Rasau Kuning, on *A. mangium* stump, L. Agustini *et.al,* 31 May 2008 (E8539); Sumatra, Riau: Compt.223-Rasau Kuning, on a living *E. pellita* tree, L. Agustini *et.al*, 2 June 2008 (E8549); Sumatra, Riau: Compt.223-Rasau Kuning, on a dead standing *E. pellita* tree, L. Agustini *et.al*, 2 June 2008 (E8552).

Comments

This species appears to show considerable variation in pore surface colour with age, maturity and weathering initially pale but darkening dramatically. The large range of spore sizes may be an artefact of the reliance on spores trapped in the basidiocarp in this study. Ideally, had more time been available, spore prints from fresh basidiocarps would have been made to provide only mature spores.

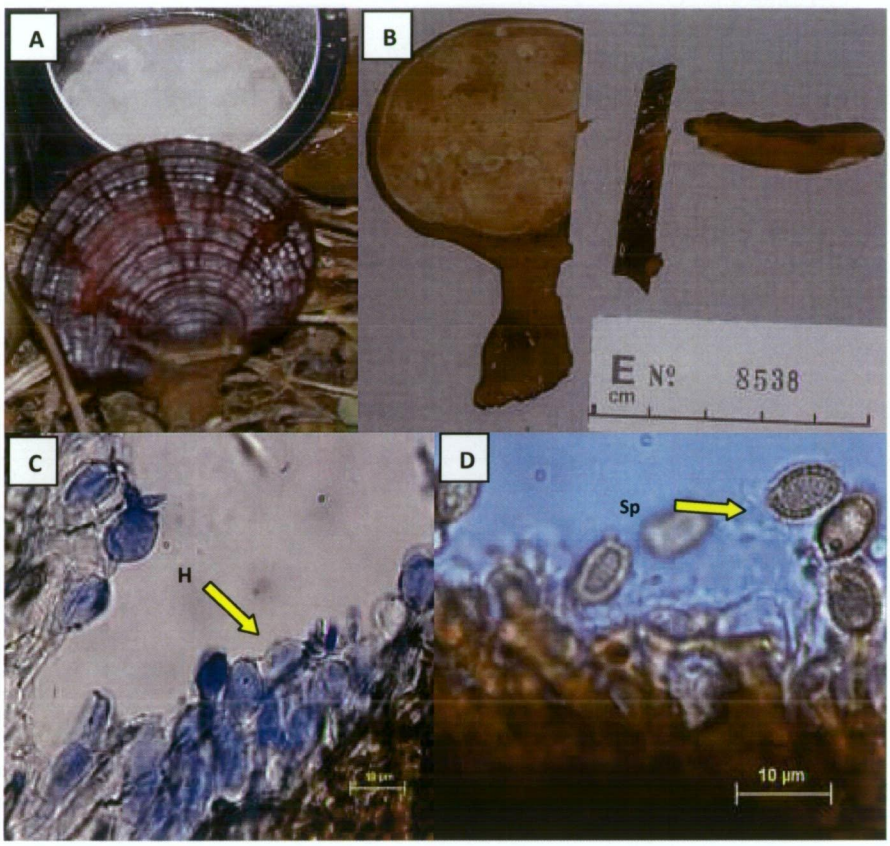


Figure 2.4. Morphology of *Ganoderma mastoporum*. (A). grown on forest debris; (B) preserved as a herbarium specimen; (C) and (D) microscopic features, H= hymenium, Sp = basidiospores

2.3.5 Molecular Analysis

Of the 56 isolates considered likely to be *Phellinus* sp., based on morphological criteria, 36 gave no PCR product using the primers ITS1-F/ITS4. The rDNA ITS sequences of root isolates E10W-33, E10W-34, 10A-27(b),11B-29 and 11C-27 had greater than 99% similarity to GenBank accessions EF065630 – EF065634 and EF079827 from *Phellinus noxius*, and to sporocarp collections E8544 (Appendix 2.3.A). The next closest match was AY558635, *Inonotus pachyphloeus.* Additional isolates and collections also had sequences with high similarity to this group, though sequencing results were often noisy or incomplete. Problems in amplifying and sequencing the ITS of this species are often

encountered and noisy sequences are probably caused by polymorphisms including indels in the rDNA repeats (Glen and Yuskianti, unpublished). Clean sequences for such isolates/collections can only be obtained by cloning, which is beyond the scope of this project. Despite the poor sequence quality, BLAST searches of GenBank retrieved *P. noxius* as the closest matches and it was possible to align the noisy or partial sequences with the other *P. noxius* sequences (Appendix 2.3.B). Those cultures from root samples (11C-39, 11B-5, 11B-11, 11C-36, E10F-17, 11C-12, 10A-30, 11B-13, E9W-27A, E2W-5, E6W-11 and E10W-34) and sporocarps (E8541, E8543, E8546 and E8548) were also considered to be *P. noxius* on the basis of sequence similarity and cultural morphology. PCR amplification of many other potential *P. noxius* isolates was unsuccessful, preventing their identification by DNA sequences.

Root isolate 11C-6 had approximately 200 bp of clean sequence and 400 bp of slightly noisy sequence. Nonetheless, it had 98% similarity to GenBank accession AY558635 (*Inonotus pachyphloeus*) and sporocarp T61, also morphologically consistent with *I. pachyphloeus*. A sequence alignment of those three is included (Appendix 2.3.C). The next closest sequences were the *P. noxius* sequences, GenBank accessions EF065630 – EF065634 and EF079827.

All other rDNA ITS sequences are included in Appendices 2.4 and 2.5. For identification as *Ganoderma philippii*, rDNA ITS sequences were 98-100% similar to sequences from morphologically identified specimens or cultures from morphologically identified specimens, and no other species had greater than 96% similarity. For *Ganoderma mastoporum/cupreum*, rDNA ITS sequences were 98-100% similar to sequences from morphologically identified specimens or cultures from morphologically identified specimens of *G. mastoporum*, and no other

species had greater than 94% similarity apart from *G. cupreum*, which is indistinguishable from *G. mastoporum* based on ITS sequences and may be synonymous (Glen, Bougher *et al.* 2009). In addition, some cultures were identified as *G. philippii* or *G. mastoporum/cupreum* based on species-specific amplification using primers targeting highly variable regions of the ITS (Glen *et al.* in preparation). These primers have been tested on a broad range of basidiomycete fungi and are specific for the target species against all other species tested, including 10 species of *Ganoderma* (Glen and Yuskianti 2009, pers.comm).

Isolates were placed in the *Ganoderma australe* group, based on 97-100% sequence similarity to morphologically identified collections of *G. australe* and less than 94% similarity to any other species. Isolates were identified as *Ganoderma subresinosum* based on 99.5% sequence similarity to morphologically identified collections of *G. subresinosum* (Murrill) Humphrey and less than 93% similarity to any other species. The ITS sequence of *Amauroderma/Ganoderma* sp.A was 99% similar to GenBank accession AY605709 (an unidentified basidiomycete), 99-100% similar to other isolates collected in the ACIAR root-rot project (Glen 2009, pers. comm.) and 93% similar to *G. subresinosum.*

Isolates designated *Lenzites aff. elegans* (Spreng.) Pat. were 98% similar to GenBank accessions EU661879 (*Trametes elegans* [Spreng.:Fr.] Fr.) and AY684178 (*T. Palisotii* [Fr.] Imazeki), both synonyms of *Lenzites elegans.* The next closest species were several *Pycnoporus* spp., with 95% similarity. *Phlebiopsis* sp. 1 were all 95% similar to EU118662 (*Phlebiopsis flavidoalba* [Cooke] Hjortstam.) and EF174437 (*P. gigantea* [Fr.] Jülich) plus about 16 other *P. gigantea* sequences and less than 91% similar to any other genus. *Gymnopilus* sp.1. had 98.5% similarity to AY280979, *Gymnopilus purpureosquamulosus* Høil.,

AY280992 *G. luteofolius* (Peck) Singer and AY280974 *G. aeruginosus* (Peck) Singer and less than 90% similarity to any other genus. *Gymnopilus* sp. 2 had 99% similarity to AY280980 *Gymnopilus dilepis* (Berk. & Broome) Singer., AY280991 *G. lepidotus* Hesler. and EU401709 *G. ochraceus* Høil. and less than 91% similarity to any other genus.

Several isolates had highly similar ITS sequences, with up to 1% sequence variation. These were 99% similar to AY216475 (*Marasmius cladophyllus* Berk.), FJ711050 and FJ711051 (both *Tinctoporellus epimiltinus* [Berk. & Broome] Ryvarden.). None of these accessions are linked to published papers, though FJ711051 was derived from a CBS (Centraal Bureau voor Schimmelculturs) isolate 389.61, for which isolation and identification details are available online (http://www.cbs.knaw.nl/). It is therefore considered that AY216475 may be derived from a misidentified isolate and these isolates are considered to be closely related to *Tinctoporellus epimiltinus.*

One isolate had 99% similarity to GenBank accession AY939879, a *Cerrena* sp (D'Souza, Tiwari *et al.* 2006) with 91% similarity to several sequences from *Cerrena unicolor* (Bulliard. Fries) Murrill. and up to 90% similarity to other basidiomycete species. It is considered likely to be a *Cerrena* sp. Another isolate had 90% similarity to AY593868 (*Rigidoporus ulmarius* [Sowerby] Imazeki) and a range of other basidiomycete species and is designated Basiodiomycete sp. A. It is considered likely to be a species of *Rigidoporus*, but a lack of available sequences precludes a sufficient degree of confidence in that identification.

Sequencing of some isolates was interrupted, possibly by sequence variation among repeats of the rDNA (Glen, Bougher *et al.* 2009), so a full length sequence was not obtained. Partial sequences from some isolates were identical to

several GenBank entries (AF525074, AF525075, DQ44306, DQ444307) from *Neonothopanus nambi* (Speg.) RH Petersen & Krisai. and had less than 96% similarity to any other species, these isolates were designated *Aff. Neonothopanus nambi.* All sequences are included in Appendices 2.3, 2.4 and 2.5.

2.3.6 DNA analysis of fungal isolates obtained from different types of fungal signs/structures and rot types

In order to develop reliable signs and symptoms that will lead us to particular root-rot causal agents, the fungal structures or rot types from which isolates of a sub-group were obtained (Table 2.2), the sub-group morphology of a culture (Table 2.3), and associated molecular identifications are summarised in Table 2.4.

RS-1 morphologically resulted in 95% *Phellinus* isolates, however molecular identification shows that only about 17% (4 of 23) of the isolates have been identified either as *Phellinus noxius* or other *Phellinus* groups. While RS-2, morphologically resulted in 56% Phellinus isolates, 5% Ganoderma isolates and 39% non target isolates. Molecular identification of the isolates grown from the root samples of RS-2 shows that only about 16% (7 of 43) of the isolates have been identified as either confirmed or affiliated to *Phellinus noxius*. From these types of samples, we isolated 40% (17 of 43) non target fungi of which is six isolates were identified as *Phlebiopsis* sp.1.

Table 2.4. Associations between a) the root structure from which the culture was derived in the field, b) macroscopic morphology group of fungal culture and c) fungal identity (based on molecular identification). Numbers in the bracket indicate the number of examined-isolates

Root signs [a]	Cultural morphology [b]		Molecular ID [c]
RS-1 (20)	Gd. 6	(1)	unidentified (1)
	Ph.1	(5)	Unidentified (5)
	Ph. 2	(3)	Unidentified (3)
	Ph. 3	(8)	*Phellinus* group (1); unidentified (7)
	Ph. 4	(3)	*P. noxius* (1); unidentified (2)
	Ph. 5	(2)	*P. noxius* (1); *Phellinus* group (1)
	Ph. 6	(1)	unidentified (1)
RS-2 (42)	Gd. 4	(2)	*G. philippii* (1); unidentified (1)
	Ph. 1	(1)	Unidentified (1)
	Ph. 2	(2)	*Inonotus* aff. *Pachyphloeus* (1); *P. noxius* (1)
	Ph. 3	(8)	*Phellinus* group (3); Unidentified (5)
	Ph. 4	(3)	Unidentified (3)
	Ph. 5	(4)	*Phellinus* group (1); unidentified (3)
	Ph. 6	(4)	*P. noxius* (1); unidentified (3)
	Ph.7	(2)	*Phellinus* group (1); unidentified (3)
	Non Target	(17)	Aff. *Neonothopanus nambi* (2); Aff. *Tinctoporellus epimiltinus* (1); Basidiomycete sp.A (1); *Cerena* sp. (1); *Gymnopilus* sp. 1 (1); *Phlebiopsis* sp.1 (6); Hypocreales (1); unidentified (4).
RS-3 (45)	Gd. 1	(11)	*G. australe* group (1); *G. philippii* (8); unidentified (2)
	Gd. 2	(6)	*G. philippii* (5); unidentified (1)
	Gd. 3	(11)	*G. australe* group (1); *G. mastoporum* (2); *G. philippii* (8)
	Gd. 4	(10)	*G. australe* group (2); *G. mastoporum* (1); *G. philippii* (6); unidentified (1) .
	Gd. 5	(3)	*Amauroderma/ Ganoderma* sp. A (1); *G. philippii* (1); unidentified(1)
	Gd. 6	(1)	*G. mastosporum* (1)
	Ph. 4	(2)	*P. noxius* (1); unidentified (1)
	Non Target	(3)	Aff. *Neonothopanus nambi* (1) ; *Gymnopilus* sp.1 (1); unidentified (1)
RS-4 (6)	Ph. 4	(1)	unidentified (1)
	Non Target	(5)	Aff. *Tinctoporellus epimiltinus* (1); *Phlebiopsis* sp.1 (3); unidentified (1)
RS-5 (17)	Ph. 4	(3)	*Phellinus* group (1); unidentified (2)
	Ph. 5	(2)	Unidentified (2)
	Ph. 6	(1)	unidentified (1)
	Non Target	(14)	Aff. *Lenzites elegans* (2); Aff. *Tinctoporellus epimiltinus* (6); *Phlebiopsis* sp.1 (2); Zygomycetes (1); unidentified (3)

Notes:

- *If there is any mismatch between the sum of numbers in cultural morphology column (b) and the total number of root signs (a), it because some root samples yielded more than one type of culture morphology.*
- *The unidentified samples were caused by the lack of PCR amplification.*
- *Total numbers of root signs (a) are different with what is stated in Table 2.2.because some root samples failed to be isolated (RS-1 was 2 samples; RS-2 was 2 samples; RS-3 was 5 samples; and RS-4 was 1 sample that did not result in any cultures due to contamination or not growing).*

A larger number of isolates, *viz* 83% and 47% from RS-1 and RS-2 respectively, cannot be identified molecularly due to negative results of PCR of the isolates. Ninety per cent (42 of 47) of the isolates grown from RS-3 which showed typical red root-rot signs were morphologically identified as *Ganoderma* isolates. Molecularly, the isolates were identified as *G. australe* group (4 of 47), *G. mastoporum* (4 of 47), *G. philippii* (28 of 47) and *Amauroderma/ Ganoderma* sp.A (1 of 47). Even though the sporocarps of *G. philippii* were not found, the isolation from the RS-3 yielded 62% of *G. philippii*. *Phlebiopsis* sp.1 was isolated as well from RS-4 and RS-5. Photographs, the description, detailed information of isolate code and molecular identification is presented on the Appendix 2.2.

2.4 Discussion

Incidence of red root rot caused by *G. philippii* in the *E. pellita* stands appears to be associated with the planting history of the area. Some eucalypt sites with root-rot disease were previously planted to *A. mangium* which is susceptible to *G. philippii*. This finding shows that a current assumption that *E. pellita* is less susceptible to *G. philippii* and other *Ganoderma* species should be treated with caution.

In surveyed *E. pellita* plantations, brown root rot caused by *Phellinus* sp. seemed less prominent than red root rot caused by *Ganoderma* spp. This means that hypothesis ***A*** was not supported, and we conclude that although *Phellinus* species are present in operational and experimental *E. pellita* plantations, they are not the most common fungal agent associated with root disease across all compartments examined.Sporocarps that were present at a site do not automatically indicate that active pathogens are in the area. Even though no sporocarps of

G. philippii were observed during the survey, fungal cultures isolated from infected roots with 'small pockets'of white-yellowish mycelia were more frequently identified as *G. philippii.* It appears from molecular analysis of isolates obtained from infected root material that *G. philippii* is the predominant pathogen in *E. pellita* plantation even though no fruiting bodies of this species were found in the current study. However sporocarps of *Phellinus noxius* or *Ganoderma mastoporum* were recovered from some of the areas that also yielded these species from root isolates. It was found that sporocarp diversity does not indicate the same suite of possible root-rot fungi as isolations from roots. Thus hypothesis ***B*** which states that sporocarps and fungal signs observed on the roots suggest the same root-rot causal agents is not supported.

It is useful to note that three of four sites with *Phellinus* were ex rubber (the one site that was ex- *A. mangium* had a .mixture of *Phellinus* and *Ganoderma* sporocarps) and all five sites with *Ganoderma* were ex-*A. mangium.* This indicates that previous vegetation may play an important role in determining the resident pathogen population in a given area. The pathogenicity of *Ganoderma philippii* and the relative susceptibility of *E. pellita* and other tree species to this fungus is currently under investigation in a related Australian Centre for International Agricultural Research project (FST 2003/048). Similar experiments for other species of fungi are planned for future projects. If sufficient data on previous vegetation type, root-rot type and root-rot incidence were available it might be possible to assess the relative susceptibility of different hosts to different fungi by changes in the levels of disease between different rotations. However, the lack of accurate characterisation of root symptoms and sporocarps addressed by this thesis means such data is, to the best of my knowledge, unavailable; and the natural

pathogen expansion from one rotation to another is not well characterised and would complicate and confuse the interpretation of changes in disease levels.

Root signs and rot types are useful indicators of particular genera of pathogens. For instance, rot type RS1 (blacklines with honeycomb-like pattern) was fairly consistently associated with *Phellinus noxius* or allied *Phellinus* species. However black lines by themselves are not always indicative of *Phellinus* and can be associated with other fungi especially if these black lines are associated with rot types that are not characteristically honeycomb in pattern. Different individuals of the same fungal species occupying the same substrate have also been reported to induce formation of "blacklines" as well e.g. (Darus, Seman *et al.* 1989). Rot type RS3 (red rhizomorphs on the root surface, with mat or small 'pockets' of white-yellowish mycelia growing underneath the bark through the woody tissue) is the typical of *Ganoderma* root rot. As for *Phellinus* these fungal signs were associated with one of several *Ganoderma* species.

There are some differences in morphological features between sporocarps collected in this study and those described by Ryvarden and Johansen (1980) and Glen, Bougher (2009) for *P. noxius* and *G. mastoporum*, repectivelly. The generative hyphae of *P. noxius* specimens found in this study are thinner, and the basidiospores are slightly larger than those described by Ryvarden and Johansen (1980). *Ganoderma mastosporum* specimens collected during the study are stipitate while those described by Glen, Bougher, *et al.* (2009) are sessile; and the number of pores/mm is much smaller than the description stated on Glen, Bougher *et al.* (2009). However, molecular identification suggests that these sporocarps are in the same species.

The low number of *Phellinus* spp. isolates for which we obtained PCR products is likely to be a result of PCR inhibition. Many fungal species contain substances that inhibit the polymerase enzyme and this is a common problem with species of *Phellinus* (Glen 2009, pers.com). The problem can be overcome by further purification of the DNA or by attempting PCR with several dilutions of the DNA extract. There was insufficient time to complete this in the current project.

This study also discovered another fungal species that warrants investigation as a potential root-rot biocontrol. Some cultures which were grouped as "non target" fungi were identified as belonging to the genus *Phlebiopsis*. *Phlebiopsis gigantea* has been demonstrated to be an effective prophylactic biocontrol for root rot caused by *Heterobasidion annosum* (Annesi, Curcio *et al.* 2005; Berglund, Rönnberg *et al.* 2005; Grieg 1976; Kallio and Hallaksela 1979; Nicolotti and Gonthier 2005; Pratt 2000; Sierota 2003). The ability of *Phlebiopsis gigantea* to prevent germination of *H. annosum s.l.* spores was noticed during the 1950's by John Rishbeth. During the following decades, *P. gigantea* has repeatedly been confirmed to be an effective preventative control agent against *Heterobasidion* infections. However over the past 50 years in plantation forestry worldwide *P. gigantea* has been the only commercial biological control agent developed and successful at reducing the incidence of root rot (*Heterobasidion annosum*) in plantation conifers.

The application of mechanical management strategies such as debris removal and trenching are not environmentally or economically feasible on a large scale. There have been relatively few successful cases of intraspecific variability in tree resistance against different root-rot pathogens and both eucalypts and acacia are susceptible to root-rot fungal species. Finding a species of *Phlebiopsis* isolated

from the roots of trees in the area which is infected with root-rot fungi (*Phellinus noxius* was isolated from this area) and which offers a possible biocontrol for root-rot disease is an extremely significant result and must be further investigated.

2.5 Conclusions

Phellinus species are not the most common pathogenic fungi in the *E. pellita* plantations surveyed. *Ganoderma philippii* appears to be the most prominent root-rot causing fungus in *E. pellita* stands.

Sporocarps cannot be relied upon to indicate all potentially pathogenic species or even the most common species recovered from infected root material. Fungal isolation from infected roots seems to be a more reliable indicator of the active pathogens responsible for the disease incidence.

In order to obtain a more comprehensive understanding of root-rot fungi in *E. pellita* plantations, this study suggests further research on:

- Systematic and wider field surveys that quantify the estate level problem of root rot, its causal agent(s) and effect on the productivity of *E. pellita* plantations.
- Pathogenicity testing of fungal root-rot pathogens and different genotypes of the same species in order to obtain more information about pathogenicity and host resistance.
- Testing of the antagonism of the *Phleobiopsis* sp. isolated in this study against both *Phellinus* spp. and *Ganoderma* spp. in order to explore the possibility of using *Phlebiopsis* isolates as a biocontrol for root-rot disease in *E. pellita* stands and other tropical forestry.

Appendix 2.1 – *Illustrations of fungal signs present on the roots and associated rot types*

Code: RS-1

Description of root: Black crust (BC) on bark. A network of black (BL) or brown lines (BrL) in the rotten wood which is white and spongy with a honeycomb (HC) appearance when the stage of rot is advanced.

Samples collected: E10W-33, E10W-34, E10W-35, E10W-36, 10A-0, 10A-1, 10A-6, 10A-7, 10A-8, 10A-9, 10A-10, 10A-11, 10A-14, 10A-15, 10A-16, 10A-17, 10A-21, 10A-24, 10A-27, 10A-28, 10A-30, 10A-37.

Code: RS-2

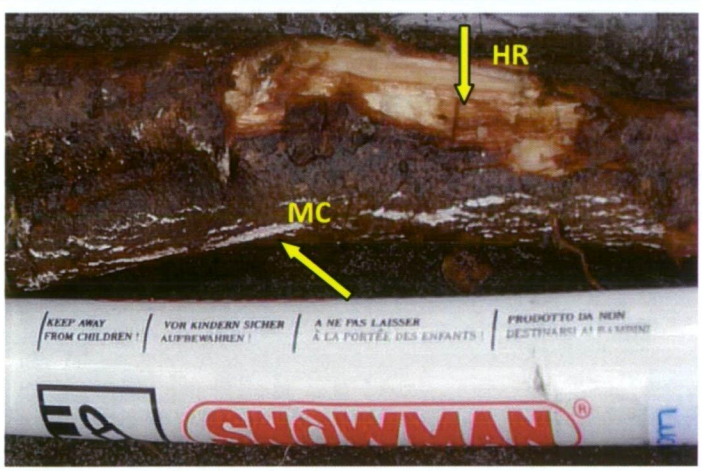
Description of root section: Black crust on the root's bark and irregular pattern of black lines associated with different rot types

Samples collected: E7W-25, E7W-26, E11W-29, E11W-30, 3A-1, 3A-22, 3A-23, 3A-25, 3A-28, 3C-0, 3C-3, 3C-11, 3C-19, 3C-21, 11A-0, 11A-37, 11A-40, 11B-0, 11B-2, 11B-3, 11B-4, 11B-5, 11B-6, 11B-9, 11B-11, 11B-12, 11B-13, 11B-16, 11B-18, 11B-20, 11B-21, 11B-22, 11C-0, 11C-1, 11C-2, 11C-6, 11C-12, 11C-14, 11C-18, 11C-27, 11C-29, 11C-32, 11C-35, 11C-36, 11C-39.

Code: RS-3

Description of root: Red rhizomorphs (RR) on the root surface, white-yellowish mycelia mat (MM) under the root's bark; small "pockets" of white-yellowish mycelia grow through the root woody tissue from underneath the bark.

Samples collected: E1W-1, E1W-2, E1W-3, E3W-7, E4W-8, E5W-9, E5W-10, E6W-11, E6W-12, E6W-13, E9W-27, E9W-28, E11W-31, Am8W-32, 3A-0, 3A-7, 3A-11, 3A-29, 3A-36, 3B-0, 3B-14, 3B-21, 3B-28, 3C-5, 3C-8, 3C-10, 3C-29, 3C-40, 6A-0, 6A-2, 6A-11, 6A-20, 6A-22, 6A-23, 6A-24, 6A-27, 6B-0, 6B-1, 6B-6, 6B-8, 6B-11, 6B-24, 6B-39, 6C-0, 6C-10, 6C-23, 6C-25, 6C-30, 6C-31, 6C-38



Code: RS-4

Description of root: White mycelia(MC) grow on the root's surface, but the root tissue underneath the bark looks healthy (HR = healthy root).

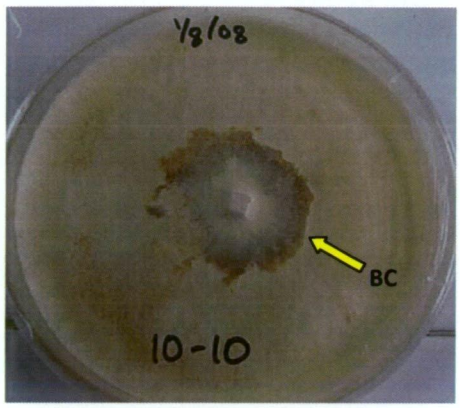
Samples collected: 10A-38, 11A-1, 11A-4, 11A-5, 11A-29, 11A-30, 11A-32.

Code: RS-5

Description of root: The root has a yellowish-brown to dark brown or black crust (BC) and/or resupinate fungi on its surface; underneath the bark the woody tissue has a brown stringy (BrS) texture.

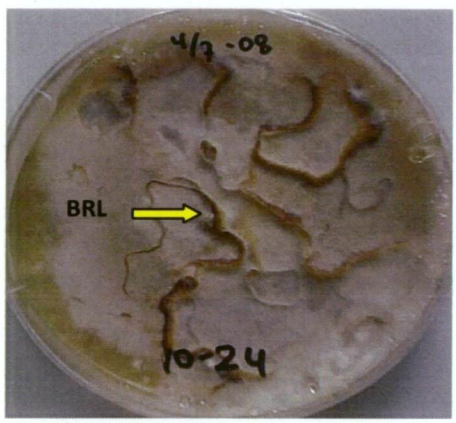
Samples collected: E1W-4, E2W-5, E11W-29, E11W-38, 11A-6, 11A-19, 11B-24, 11B-25, 11B-26, 11B-29, 11B-30, 11B-33, 11B-35, 11B-38, 11C-5, 11C-10, 11C-34.

Appendix 2.2 – *Illustration of macroscopic morphology of the cultures*



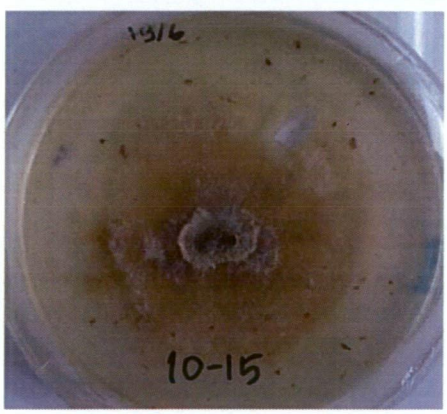
Code: Ph.1

Description: Mat is cream-white mat, reverse light brown. May have patchy white mycelial clumps, and brown crust (BC).
Cultures examined: E8548, 10A-0, 10A-9, 10A-10, 10A-11, 10A-21, 11B-20.
Molecular identification: PCR negative (all specimens)



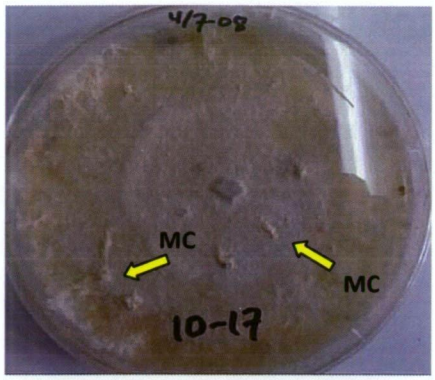
Code: Ph.2

Description: Mat is cream-white, with dense white to very light brown aerial mycelia, "brown lines (BRL)"or pseudo-sclerotia present.
Cultures examined: E8546, E8548, 10A-1, 10A-15, 10A-24, 11C-6, 11C-27.
Molecular identification: *P. noxius* (1), *Inonotus aff pachyphloeus* (1), PCR negative (5).



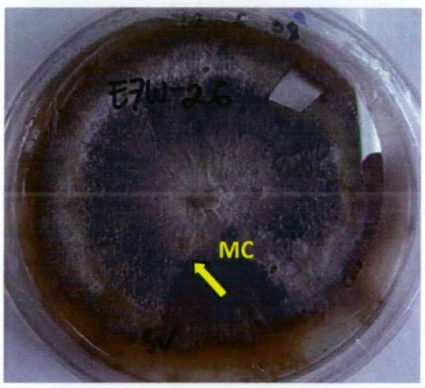
Code: Ph.3

Description: Mat is cream-white to brownish, may have mycelial clumps, brown to dark-brown feathery, submerged mycelia were observed in the underside of the cultures.
Cultures examined: E8543, E8540, E8544, E10W-34, E11W-29, E11W-30, E8541, 10A-6, 10A-8, 10A-14, 10A-15, 10A-27A, 10A-28, 10A-30, 11B-0, 11B-2, 11B-11, 11C-32, 11C-36, 11C-39.
Molecular identification: *Phellinus* group (5), PCR negative (15).



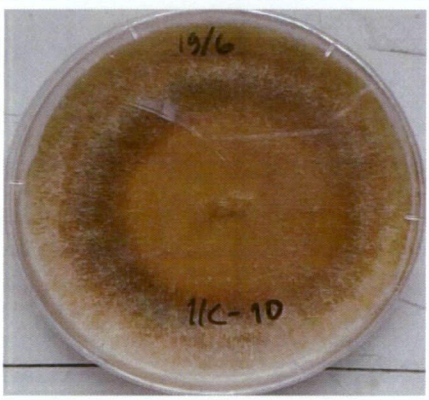
Code: Ph.4

Description: Mats cream-white to cinnamon-brown, may have patchy aerial mycelia clumps (MC).
Samples examined: E6W-11, E8543, E9W-27A, E10W-36, E8548, E11W-29, E11W-30, 3C-0, 10A-17, 10A-27B, 10A-38, 11B-29, 11C-0, 11C-1.
Molecular identification: *P. noxius* (3), PCR negative (11).



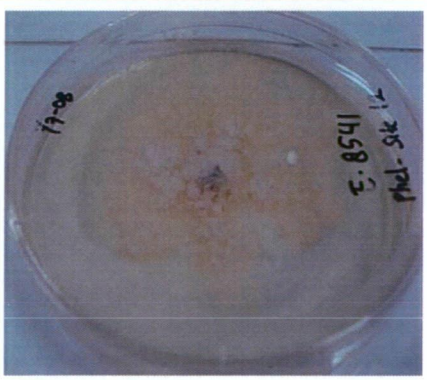
Code: Ph.5

Description: Mat is brownish-white with dark-brown, submerged mycelia; may have patchy mycelia clumps.
Samples examined: E2W-5, E7W-26, E10W-33, E11W-29, 10A-30, 11B-5, 11B-6, 11C-2.
Molecular identification: *P. noxius* (1), *Phellinus* group (1), PCR negative (5).



Code: Ph.6

Description: Mats cinnamon-brown with brown circular zones, thin layers of white aerial mycelia present.
Samples examined: 10A-15, 11B-12, 11B-22, 11C-10, 11C-12, 11C-14.
Molecular identification: *P. noxius* (1), PCR negative (5)



Code: Ph.7

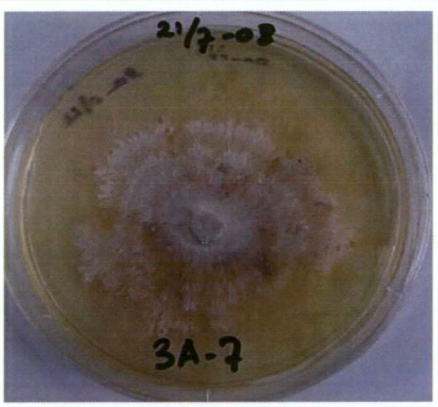
Description: Mat is brownish white with irregular light-brown, submerged mycelia.
Cultures examined: E8543, E8541, 3C-3, 11B-13.
Molecular identification: *Phellinus* group (1), PCR negative (3)

Code: Gd.1

Description: Mats white powdery at the centre, patchy-cottony mycelia clumps (MC), margins light-feathery.
Cultures examined: Am8W-32, E1W-1, E9W-28, 3C-8, 3C-11, 6A-20, 6A-22, 6A-24, 6B-24B, 6C-23, 6C-31, 6C-38.
Molecular identification: *G. Philippii* (9) , *G. australe* group (1), PCR negative (2)

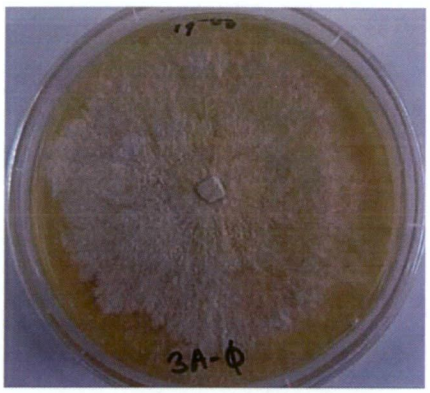
Code: Gd.2

Description: Mats white powdery at the centre, patchy-cottony mycelia clumps around margins, cinnamon-brown to dark-brown submerged.
Cultures examined: E8538, 3B-0, 3C-29, 3C-40, 6C-10, 6C-25, 6C-30.
Molecular identification: *G. philippii* (5), *G. mastosporum* (1); PCR negative (1).



Code: Gd.3

Description: Mats white powdery at the centre, margins feathery, cinnamon-brown to dark-brown submerged.
Cultures examined: E6W-12, E6W-13A/B, 3A-7, 3A-11, 3B-28, 6A-2, 6A-23, 6B-0, 6B-1, 6B-8, 6B-24A.
Molecular identification: *G. australe* group (1), *G. philippii* (8), *G. mastosporum* (2).

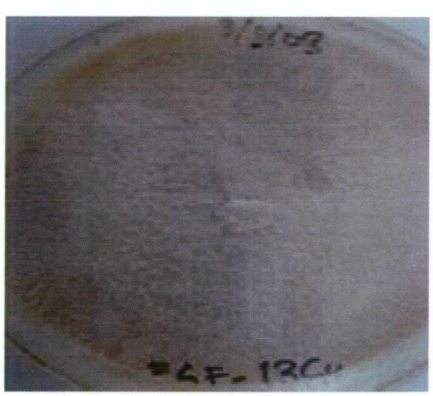


Code: Gd.4

Description: Mats white powdery and layered feathery, may have yellowish-brown crustose around the centre and/or patchy at margins.
Cultures examined: E1W-3, E8539, E5W-10, E7W-25, 3A-0, 3A-22, 3A-36, 3B-14, 3B-21, 6A-0, 6A-11, 6B-11, 6B-39.
Molecular identification: *G. mastosporum* (2), *G. australe* group (2), *G. philippii* (7), PCR negative (2).

Code: Gd.5

Description: Mats white to very pale brown velvety, may have patchy mycelial clumps.
Cultures examined: E9W-27, 3C-10, 6A-27.
Molecular identification: *G. philippii* (1), *Amauroderma/ Ganoderma sp. A* (1), PCR negative (1).



Code: Gd.6
Description: Mats white dense woolly or fluffy powdery around the centre, fine feathery at margins.
Cultures examined: E8542, E8551, E8539(a,b,c) , 3C-5, 10A-37.
Molecular identification: *G. mastosporum* (2), *G. subresinosum* (1), PCR negative (4).

Appendix 2.3 – *Alignment of rDNA ITS sequences from isolates in Phellinus group and sporocarps collected during this project*

A. Alignment of rDNA ITS sequences from *Phellinus noxius* isolates and sporocarps collected during this project or the broader ACIAR root-rot project, including six DNA sequences of *Phellinus noxius* isolates from GenBank for comparison. The sequence for T157 is incomplete. All other sequences are highly similar, with up to 1% sequence variation including an 8-base-pair indel near nucleotide 200.

```
                61         71         81         91         101        111
E8544           AAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCTGGTCGGTCTCTGGACTTGCAT
EF065632        -----------ATGAGTTTTTTAAAGTAAACTTGATGCTGGTCGGTCTCTGGACTTGCAT
EF065633        -----------ATGAGTTTTTTAAAGTAAGCTTGATGCTGGTGGGTCTCTGGACTTGCAT
EF079827        -----------ATGAGTTTTTTAAAGTAAGCTTGATGCTGGTGGGTCTCTGGACTTGCAT
EF065630        -----------ATGAGTTTTTTAAAGTAAGCTTGATGCTGGTGGGTCTCTGGACTTGCAT
EF065634        -----------ATGAGTTTTTTAAAGTAAGCTTGATGCTGGTGGGTCTCTGGACTTGCAT
EF065631        -----------ATGAGTTTTTTAAAGTAAGCTTGATGCTGGTGGGTCTCTGGACTTGCAT
E10W-33         AAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCTGGTGGGTCTCTGGACTTGCAT
11B-29          AAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCTGGTGGGTCTCTGGACCTGCAT
10A-27(b)       AAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCTGGCCGGTCTCTGGACTTGCAT
11C-27          AAGGATCATTAATGAGTTTTTTAAAGTAAGCTTGATGCTGGTGGGTCTCTGGACTTGCAT

                121        131        141        151        161        171
E8544           GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
EF065632        GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
EF065633        GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
EF079827        GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
EF065630        GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
EF065634        GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
EF065631        GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
E10W-33         GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
11B-29          GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
10A-27(b)       GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA
11C-27          GTGCTCAGTTTGCGCTCATCCATCTCACACCTGTGCACTTACTGAAGAGAGAGAGGGAGA

                181        191        201        211        221        231
E8544           GGGAGAGTGGTTTATTCGTTTATTCATTTATTCGTGTATTCAACTCANAAGTCTTCAATC
EF065632        GGGAGAGTGGTTTATTCGTTTATTCATTTATTCGTGTATTCAACTCAAA-GTCTTCAATC
EF065633        GGGAGAGTGGTTTATTCGTTTATTCATTTATTCGTGTATTCAACTCAAA-GTCTTCAATC
EF079827        GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATC
EF065630        GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATC
EF065634        GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATC
EF065631        GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATT
E10W-33         GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATC
11B-29          GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATC
10A-27(b)       GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-GTCTTCAATC
11C-27          GGGAGAGTGGTTTATTCGT--------TTATTCGTGTATTCAACTCAAA-TTCTTCAATC

                241        251        261        271        281        291
E8544           TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
EF065632        TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
EF065633        TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
EF079827        TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
EF065630        TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
EF065634        TCCCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
EF065631        TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
E10W-33         TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
11B-29          TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
10A-27(b)       TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
11C-27          TCTCTTTTGACTTTATAATAAACAACTATATTGTTTGTGTAGAATGCATTAGCCTCATTG
```

```
                301         311         321         331         341         351
E8544           TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
EF065632        TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
EF065633        TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
EF079827        TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCCCTCGCATCGATGAAG
EF065630        TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
EF065634        TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
EF065631        TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
E10W-33         TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
11B-29          TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
10A-27(b)       TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
11C-27          TAGGTGAAATA-ACTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG

                361         371         381         391         401         411
E8544           AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
EF065632        AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
EF065633        AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
EF079827        AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
EF065630        AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
EF065634        AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
EF065631        AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
E10W-33         AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
11B-29          AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
10A-27(b)       AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
11C-27          AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT

                421         431         441         451         461         471
E8544           GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
EF065632        GAACGCACCTTGCACTCCTTGGTATTCCGGGGAGTATGCCTGTTTGAGTGTCATGTTAAT
EF065633        GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
EF079827        GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
EF065630        GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
EF065634        GGACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
EF065631        GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
E10W-33         GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
11B-29          GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
10A-27(b)       GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT
11C-27          GAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGTTAAT

                481         491         501         511         521         531
E8544           CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTRATATTGGACTTGGGGACTGCTGGC
EF065632        CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
EF065633        CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
EF079827        CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
EF065630        CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
EF065634        CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
EF065631        CTCAATACAACATTTTTTTGTAACTAAGAA-GTGTTAATATTGGACTTGGGGACTGCTGGC
E10W-33         CTCAATACAACATTTTTTTGTAACTAAAAAAGTGTTGATATTGGACTTGGGGACTGCTGGC
11B-29          CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTAATATTGGACTTGGGGACTGCTGGC
10A-27(b)       CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
11C-27          CTCAATACAACATTTTTTTGTAACTAAAAA-GTGTTGATATTGGACTTGGGGACTGCTGGC
```

```
                541         551         561         571         581         591
E8544           GT--RAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
EF065632        GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
EF065633        GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
EF079827        GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
EF065630        GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
EF065634        GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
EF065631        GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
E10W-33         GT--GAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
11B-29          GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
10A-27(b)       GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT
11C-27          GT--AAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTTGCTCGAGTAATTGGTGTAAT

                601         611         621         631         641         651
E8544           AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGYCTGCTTCTAATCGTCTTGTAAT
EF065632        AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
EF065633        AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
EF079827        AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
EF065630        AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
EF065634        AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
EF065631        AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
E10W-33         AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
11B-29          AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
10A-27(b)       AGTTTCTAACATTCACCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT
11C-27          AGTTTCTAACATTCGCCGTTTACACTTGCTAATAGAGTCTGCTTCTAATCGTCTTGTAAT

                661         671         681         691         701         711
E8544           GAGACAAACGACTTAACTTTGACCTTTGGCCTCAAATCAGG------------------
EF065632        GAGACAAAC-ACTTAACTTTGACCTTTGGCC----------------------------
EF065633        GAGACAAAG-ACTTAACTTTGACCTTTGGCC----------------------------
EF079827        GAGACAAAC-ACTTAACTTTGACCTTTGGCC----------------------------
EF065630        GAGACAAAC-ACTTAACTTTGACCTTTGGCC----------------------------
EF065634        GAGACAAAC-ACTTAACTTTGACCTTTGGCC----------------------------
EF065631        GAGACAAAC-ACTTAACTTTGACCTTTGGCC----------------------------
E10W-33         GAGACAAAC-ACTTAACTTTGACCTT-GGCCTCAAANTCAGGTAG---------------
-
11B-29          GAGACAAAC-ACTTAACTTTGACCTT-GGCCTCAAATCAGNTAN----------------
10A-27(b)       GAGACAAAC---TTAACTTTGACC-----------------------------------
11C-27          GAGACAAAC---TTAACTTTGACCTTTGGCCTCAAATCAGGTAG----------------
```

B. Alignment of partial and noisy sequences of putative *Phellinus noxius* cultures and sporocarps.

```
                61        71        81        91        101       111
11C-39          ------------------------------------------A-GTA-GCTTGATGCT
11B-5           ------------------------GGGGNTTTNGAGTTTTTTAN-GTAAGCTTGATGCT
11B-11          ------------------------------------------TA-GTAAGCTTGATGCT
11C-36          ------------------------------------------TA-GTAAGCTTGATGCT
E10F-17         ----------------------GGATCATTAATGAGTTTTTTAAAGTAAGCTTGATGCT
11C-12          TTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCT
E8544           TTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCT
10A-30          --TCCGTAGGGGAACCTGGGGAAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCG
11B-13          --GCCGTAGGTGAACGTGCGGAAGGATCATTAATGAGTTTTATAAAGTAAGCTTGATGCT
E8543           ------------------------------------------TAAAAACGATGCT
E9W-27A         ------------------------------------------------------------
E8546           TTCGTAGGAGGACATGCGGGAGGAT--CATCATTGAGTTTTTTTAAATAAAAATGATGCT
E8541           ------------------------------------------------------------
E8548           CCCAGGTGGGCACCTGCGGCAAGTATCCTTAATTCATTTTTTAAAATCTACAATGATGCT
E2W-5           ------------ACGTGCGGAAGGATCATTAAGGAGTTTTTGAGGGGGAACTTGAGACT
10A-27(b)       --TCCGTAGGTGAACCTGCGGAAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCT
E10W-33         --TCCGTAGGTGAACCTGCGGAAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCT
11B-29          --TCCGTAGGTGAACCTGCGGAAGGATCATTAATGAGTTTTTTAAAGTAAACTTGATGCT
11C-27          --TCCGTAGGTGAACCTGCGGAAGGATCATTAATGAGTTTTTTAAAGTAAGCTTGATGCT
E6W-11          ------------------------------------------------------------
T61             --TCCGTAGGTGAACCTGCGGAAGGATCATTATTGAGTTTAACAAAGTGGACTTGATGCT
E10W-34         --------------------------CATTAATGAGTTTTTTAAAGTAAACTTGATGCT


                121       131       141       151       161       171
11C-39          GGTCGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
11B-5           GGTGGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
11B-11          GGTCGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
11C-36          GGTCGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
E10F-17         GGTGGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
11C-12          GGTCGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
E8544           GGTCGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
10A-30          GGTCGGTTTTTGGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCCCCC-CTGGG
11B-13          GGGGGGTCTCAGGACTTCCATGTGCTCAGTTTGCTCT---CACCCATCTCACCC-CTGTG
E8543           GGGGGGTGTCTGGAGACGCACATGCGCAGTGTGTGCT---CACACATATCTCAC-CTGTG
E9W-27A         ------------------------------------------------------------
E8546           GGTCGGGCTTTTGAGTTGCATCTGGTCCGCATTTGGT---CCTCCTTCTTCCAC-CTCTG
E8541           --------------------------CAGTTTTCGCTT--CATCCATCTCACAC-CCGTG
E8548           GGTAGGTTTCTCGGATTTTCATGATGTCAGTTGCGCT---CATCCATTTCTCAATCTGTC
E2W-5           GATCAGTCTCGAAACTTGCAAGGGGTCAGTTTGGGGGT--CATCCATCTCACAC-ATATG
10A-27(b)       GGCCGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
E10W-33         GGTGGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
11B-29          GGTGGGTCTCTGGACCTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
11C-27          GGTGGGTCTCTGGACTTGCATGTGCTCAGTTTGCGCT---CATCCATCTCACAC-CTGTG
E6W-11          ------------------------------------------------------------
T61             GGCATGTCTCTGGACTTGCATGTGCTCAGTCTGCGCT---CATCCAYTTCACAC-CTGTG
E10W-34         GGTCGGTCTCTGGACTTGCATGTGCTCAGTTTGGGCT---CATCCATCTCACAC-CGGTG
```

```
                181       191       201       211       221       231
11C-39          CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGKGR------------------------
11B-5           CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATGCGTTTATTCATTTATTCGT
11B-11          CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGGGR------------------------
11C-36          CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGK--------------------------
E10F-17         CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTAGTTTATTCGTTTATTY--------GT
11C-12          CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATTCGTTTATTCATTTATTCGT
E8544           CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATTCGTTTATTCATTTATTCGT
10A-30          CCCTTACTGAAGAGAGAGGGGGAGGGGGAGAGGGGTTTTTTTGTTTTTTCATTTTTTTGG
11B-13          CACTTAGAGAAGAGAGAGGGGGAGAGGGAGAGTGGTTTTTTCGT--------TTTTTTGT
E8543           CGCTTTTTGAGAAGAGAGAGAGAGGGGGAGAGGTGTATTCGCGTATTCACATATACGT
E9W-27A         ------------------------------------------------------------
E8546           CACCTTTTGAAGAAGGGGGGGGGGGGGGGGGGGGGTATTCGTTTATTCATCTATTCGT
E8541           CACTTTTTGAAGAGAGAGAGGGAGAGGGAGAGAGGTTTATTTGTGTATTCATTTATTCGT
E8548           CACTTGTTGAATAGAGATAGGGCGAGGGAGAGTGCT-TAGTGGTCTATTCATTTATTCGT
E2W-5           CGC-TTAAGAAGAGGGAGAGGGGGGGGGAGAGGGGGGTATACGTTTGTTCATTTATTCGT
10A-27(b)       CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATTCGT--------TTATTCGT
E10W-33         CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATTCGT--------TTATTCGT
11B-29          CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATTCGT--------TTATTCGT
11C-27          CACTTACTGAAGAGAGAGAGGGAGAGGGAGAGTGGTTTATTCGT--------TTATTCGT
E6W-11          ------------------------------------------------------------
T61             CACTTTCAAAGGGGGATTGGATCTTATTAGATAGATTT----------------------
E10W-34         CACTTACTGAAGAGAGAGAAGG-GAGGAAGAGGGGTTTATTCGTTTATTCATTTATTCGT


                241       251       261       271       281       291
11C-39          ------------------------------------------------------------
11B-5           GTATACAACTCAAA-GTCTTCAATCTCTCTTTTGACTTTATAATAAACAAC---------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         GTATTCAACTCAAA-GTYTTCAATCTYTYTTTTGACTTTATAATAAACAACTATATTGTT
11C-12          GTGTTCAACTCAAA-GTTTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
E8544           GTATTCAACTCANAAGTCTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
10A-30          GTTTTCAACTCAAA-TTTTTCAATTTTTTTTTTGACTTTATAAAAAACAAATATATTGTT
11B-13          GTTTTCAACTCAAA-TTTTTCAATTTTTTTTTTGACTTTATAATAAACAAATATATTGTT
E8543           GTGTTCAAATCAAA-ATGTTCAAAATCTCTTTTGACTTTATAAAAAACACATATATATTT
E9W-27A         ------------------------------------------------------------
E8546           GTATTCAACACAAA-ATCTTCAAAATCTTTTTTTACTTTTTAATAAAAAACTATATTGTT
E8541           GTATTCAACTCTAA-GTCTTCACTCTCTCTTTTGACTTTATAATAAACACCTATATTGTT
E8548           GTATTCCACTCAAA-GTCTTCGATCTCTCTTTCGACTTTATAATAAACAACTATATTGTT
E2W-5           GTATTCAACTGGGA-GTCTTCAATCTGTGGTTTGACTTTATAATAAACAACGATATGGTT
10A-27(b)       GTATTCAACTCAAA-GTCTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
E10W-33         GTATTCAACTCAAA-GTCTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
11B-29          GTATTCAACTCAAA-GTCTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
11C-27          GTATTCAACTCAAA-TTCTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
E6W-11          ----------------------------------------AAACA-CTATATTGTT
T61             GYAAAGATCTTCGAACAGTTCAGTTCTTCTTTACATAT---A-TAAACA-CTATATTGTT
E10W-34         GTATTCAACTCAAA-GTCTTCAATCTCTCTTTTGACTTTATAATAAACAACTATATTGTT
```

```
                301       311       321       331       341       351
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         TGTGTAGAATGCMTTARCCTCMTTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
11C-12          TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
E8544           TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
10A-30          TGTGTAGAACGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
11B-13          TGTGTAGAACGCATTAGCCTCATTGTAGGTGAAATA-AATATACAACTTTCAACAACGGA
E8543           TGTGTGGAAAACGCTAGCCTCACATTAGGGGAAAAA-AATATACACAATTCAACACAGGA
E9W-27A         --------------------CTTATTATATGCAAAA-TCTATACACCATTTAAAAACAGA
E8546           TTTGTAGAAAGCATTTGCCCCATTGTAGAGGAAAAA-AATATACAAATTTCAACAAAGGA
E8541           TGTGTAGAAAGCATTAGCCTCACTGTAGAGGAAATA-ACTATACAACTTTCAACAAAGGA
E8548           TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATTCAACTTTCAACAACGGA
E2W-5           TGTGTAGAATGAATAAGGCTCAG-GAAGGTAAAATA-ACTATACAACTGTCAACAACGGA
10A-27(b)       TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
E10W-33         TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
11B-29          TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
11C-27          TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
E6W-11          TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA
T61             TGTGTAGAATGTACTTGCCTCTT-GTAGGTGAATAATACTATACAACTTTCAACAACGGA
E10W-34         TGTGTAGAATGCATTAGCCTCATTGTAGGTGAAATA-ACTATACAACTTTCAACAACGGA

                361       371       381       391       401       411
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         TYTYTTGGCTCTYGCATYGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
11C-12          TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
E8544           TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
10A-30          TCTTTTGGCTCTCGCATAGAAGAAGAACACAGAGAAATGCGATAAGTAATGAGAATCGCA
11B-13          TCTTTTGGCTCTCGCATAGAAGAAGAACGCAGAGAAATGCGATAAATAATGAGAATTGCA
E8543           TCTCTCTGCTCTCGCGTCGAGGAAAAGAGCACCGAAAAGCGATAAATAAAATGAAATGCC
E9W-27A         TATCTTTTCTCTCGCATCTATATGAAGAGCAGCGAAAAATGATAAATAAAATGAGAATCG
E8546           GATCTTGGGTCTCGCATCGAGAAAAAAAGCAGAGAAAAATGATAAGTAAAATGAATTGCA
E8541           TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAAAGCGATAAATAAAGTGAAATGCA
E8548           TCTCTTGGCTCTCGCATCGCTGAAGAACCCAGCGAAATGCGATAAGTAATGCGAATTGCA
E2W-5           TCTGGTGGCTCTGGCAGCAATGAAGAGCGCAG-GAAATGGGGTGGGGAATGGAAATTGCA
10A-27(b)       TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
E10W-33         TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
11B-29          TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
11C-27          TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
E6W-11          TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
T61             TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
E10W-34         TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
```

```
                421       431       441       451       461       471
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         GAATTCAGTGAATCATYGAATYTTTGAACSCMCCYTGCMCTCCTTGGTATTYCGAGGAGT
11C-12          GAATTCAGTGAATCATCGAATCTTTGAACGCCCCTTGCACTCCTTGGTATTCCGAGGAGT
E8544           GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
10A-30          GAACTCAGAGAATCATAGAATCTTAGAACGCACCTCGCACTCCTTGGTATTCCGAGGAGT
11B-13          GAACTCAGAGAATCATAGAATCTTTGAACGCACCTCGCACTCCTGGGTATTCCGAGGAGT
E8543           CAGATCACTGAATCTTCGAGTATTTGAGAGCGCCCCGCACTCCCCGGTATTTTGAGGAGT
E9W-27A         CAATTCACAGAATCTCCTAATATCTTAACACACCCTTCACTCCCTTTTATTTTGAGGAGA
E8546           GAATTCAGTGAATCTTAGAATCTTTGAACGCACCCTGCACTCCTTGGGGTTTCGAGGAGT
E8541           GAATTCTCTGAATCATAGAATCTTTGAAAGCACCTCGCACTCCCTGGTATTCCGAGGAGA
E8548           GAATTCTGTGAATCATCGAATCTTTGAACGCACCTTGCACTCC-TGGTATTCCGAGGAGT
E2W-5           GGGGG-AGTGAATCAT-GAATCTTGGAACGCGCCGGAGAATCCTTGGTGGTCGGGGAAAA
10A-27(b)       GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
E10W-33         GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
11B-29          GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
11C-27          GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
E6W-11          GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
T61             GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT
E10W-34         GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGT


                481       491       501       511       521       531
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         ATGCCTGTTTGAGTGTCATGTTAATYTCAATACAAC-MCTTTTTGTAACTAAAAAG-TGT
11C-12          ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
E8544           ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
10A-30          ACGCCTGTTTGAGTCTCATGTTAATCTCAATACAAC-ATTTTTTGTAAAAAAAAAG-TTT
11B-13          ACGCCTGTTTGAGTCTCATGTTAATCTCAATACAAC-ATTTTTTGTAAATAAAAAG-TGT
E8543           ATACCCGTTTGAGAGTCACATTAAAATCACAACAAC-ACTTTTTGTAAATAAAAAA-AGT
E9W-27A         ATACCCCTTTTAGAGTCACATTAATCTCACAACAAA-ACATTTTTTAAAAAAAAAA-AGT
E8546           ATGCCCGTGTGAGAGTCACGTTAAAATCAAAACAAC-ACTTTTTGTAACTAAAAAA-TGT
E8541           ATGCCTGTTTGAGAGTCATGTTTATCTCAAAACAAC-ATTTTTTTTAACTAAAAAA-TGT
E8548           -TCCCTGTTCGAGAGTCACGTTAATCTCAATACAAC-ATTTTTTGTAACCAAAAAG-TGT
E2W-5           AGGCCTGTTTGAGTGTGATGTTAAGCTCAATACAAC-ATTTGTGGTAAGTAAAAAG-TAT
10A-27(b)       ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
E10W-33         ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAAGTGT
11B-29          ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
11C-27          ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
E6W-11          ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
T61             ATGCCTGTTTGAGTGTCATGTTAATCTCAATTCAAC-ATGTTTTTG----------TGT
E10W-34         ATGCCTGTTTGAGTGTCATGTTAATCTCAATACAAC-ATTTTTTGTAACTAAAAAG-TGT
```

```
                541       551       561       571       581       591
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         TAATATTGGACTT-GGGGRCTGCTGGSGT--AAGT---YGGCTTYTYTTGAATGCMTTAG
11C-12          TAATATTGGACTT-GGGGACTGCTGGCGT--AAGT---CGGCTTCTCTTGAATGCATTAG
E8544           TRATATTGGACTT-GGGGACTGCTGGCGT--RAGT---CGGCTTCTCTTGAATGCATTAG
10A-30          TAATATTGGACGG-GGGGACTGGGGGCGA--AAGT---CGGCTTCTCTAGAACGCATTAG
11B-13          TGATATTGGACGG-GGGGACTGGGGGCGA--AAGT---CGGCTTCTCGAGAACGCATTAG
E8543           TAATATTTGGGAT-GGGGGGTGCGGGGGT--GAGT---GTGGTTTTCTTGAAAACACAAT
E9W-27A         TAAAATATTACAT-GGGGAGAGCTCGGGC--GAGT---CTCCTCTTCTTTTAAACACTTT
E8546           TTTTATTGGACTT-GGGGGGTGCTGGGGT--AAAT---CGGCTTTTCTTGAAAACACAAG
E8541           GAATATTTGAGTT-GGGGACAGCTGGCGC--GAGT---CTGGGTCTCTTGAATACATTAG
E8548           TGAAATTGGTCTT-GGGGACCGTTCGCGT--GACT---CGGCTTCTCTTGAATGCCTCAG
E2W-5           TAATATGGGGGTT-GGGGAG-GCTGACGT--AAGT---CGGATTAACTAGAAGAAATTAG
10A-27(b)       TGATATTGGACTT-GGGGACTGCTGGCGT--AAGT---CGGCTTCTCTTGAATGCATTAG
E10W-33         TGATATTGGACTT-GGGGACTGCTGGCGT--GAGT---CGGCTTCTCTTGAATGCATTAG
11B-29          TAATATTGGACTT-GGGGACTGCTGGCGT--AAGT---CGGCTTCTCTTGAATGCATTAG
11C-27          TGATATTGGACTT-GGGGACTGCTGGCGT--AAGT---CGGCTTCTCTTGAATGCATTAG
E6W-11          TAATATTGGACTT-GGGGACTGCTGGCGT--GAGT---CGGCTTCTCTTGAATGCATTAG
T61             TTGAATTGGACTT-GGAGTCTGCTGGCGTCAAAGT---CGGCTTCTCTTGAATGCATTAG
E10W-34         TGATATTGGACTT-GGGGACTGCTGGCGT--AAGT---CGGCTTCTCTTGAATGCATTAG
```

```
                601       611       621       631       641       651
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTYTAACMTTCMCCG-TTTACMC
11C-12          CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTTTAACATTCACCG-TTTACAC
E8544           CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCACCG-TTTACAC
10A-30          GTGGGCTTTTGCT----AGAGTAATTGGTGTAAGAGTTTATCACATTCCCCG-TTCACAC
11B-13          GGGGGCTTTTGCT----AGAGTAATTGGTGTAATAGTTTATCACATTCCCCG-TTCACAC
E8543           ATGGGGGTTTTCT----CTAGTAATAGGGGTAATAATTTT-CACACTCTCCC-CTTTCAC
E9W-27A         CGGTGCTTTTTCT----CGGGTAAAAGGGGTAATAGTTTTTCTCACTCTCCG-CTTTCAC
E8546           ATGGGGGTTTTTT----CGAGAAAAAGGGGTAATATTTCT-AACACTCTCC--CGGGCAC
E8541           CTGGGCTTTTTCG----CGAGTAATAGGTGTAATAGAGTTTAACACTCTCCC-CGTACAC
E8548           CGGGGTTTTTTCT----TGAGTAATTGGTGTAATAGTTTGTAACTTTCACCG-GGTACAC
E2W-5           CGGGGC-TAAACT----GGAGTAATAG-TGTAATAATTTG-TACATGCACCG-TTTACAC
10A-27(b)       CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCACCG-TTTACAC
E10W-33         CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCACCG-TTTACAC
11B-29          CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCACCG-TTTACAC
11C-27          CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCGCCG-TTTACAC
E6W-11          CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCACCG-TTTACAC
T61             CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTCTCTACATTCGCCG--TTACAC
E10W-34         CTGGGCTTTTGCT----CGAGTAATTGGTGTAATAGTTTCTAACATTCACCG-TTTACAC
```

```
                661       671       681       691       701       711
11C-39          ------------------------------------------------------------
11B-5           ------------------------------------------------------------
11B-11          ------------------------------------------------------------
11C-36          ------------------------------------------------------------
E10F-17         TTGCTAATA-GAG---TYTGCTTYTAATCGTCTTGTAATGAGACM--AACTTAACCTTTG
11C-12          TTGCTAATA-GAG---TCTGCTTCTAATCGTCTTGTAATGAGACAAACACTTAACTT-TG
E8544           TTGCTAATA-GAG---YCTGCTTCTAATCGTCTTGTAATGAGACAAACACTTAACTT-TG
10A-30          GTGATAAGA-GAG---TCTGCTTCTAATCCTCGTGTAATGACACAAAC--TTAACTT-TG
11B-13          TTGATAATA-GAG---TGTGCTTATAATTGTCGTGTAATGAGACAAAC--TTAACTT-TG
E8543           TCGCTAATA-GAG---TGTGCTTCTATTAGTCTCGTAATGAGACACACACTTAACTC-TG
E9W-27A         TGGCTAATA-AAG---TCTGCGACTGGAGGTCTTGTGAATAGACACACACTTAACTC-TA
E8546           TTGCGAAAA-GAG---CCTGCTCCTCGGCGTAGTGTGAAGAGACACAG-CTTAACTC-TG
E8541           A-GCTCATA-GAG---ACTGCTTCTAATCGTCTTGTAAAGAGACACACACTTAACTT-GA
E8548           TTGCTAATA-GAG---TCC-CTTCTAATCGTCTTGTAATGAGACACACAGTTACTTT-GC
E2W-5           T-TCTGATG-GA----AGGGGTTGTAATCGTCGT-TAAGAAAACAAAG-----------
10A-27(b)       TTGCTAATA-GAG---TCTGCTTCTAATCGTCTTGTAATGAGACAAAC--TTAACTT-TG
E10W-33         TTGCTAATA-GAG---TCTGCTTCTAATCGTCTTGTAATGAGACAAACACTTAACTT-TG
11B-29          TTGCTAATA-GAG---TCTGCTTCTAATCGTCTTGTAATGAGACAAACACTTAACTT-TG
11C-27          TTGCTAATA-GAG---TCTGCTTCTAATCGTCTTGTAATGAGACAAAC--TTAACTT-TG
E6W-11          TTGCTAATA-GAG---TCTGCTTCTAATCGTCTTGTAATGAGACAAACACTTAACTT-TG
T61             TTGCTTAGA-AAG---TCTGCTTCTAACCGTCTTGTAATGAGACAATA---TAACTT-TG
E10W-34         TTGCTAATA-GAG---TATGGTTCTAATCGTCTGGTAAGGAGACAAA-GACTAACTT-TG
```

```
                721       731       741       751       761       771
11C-39          ------------------------------------------------------
11B-5           ------------------------------------------------------
11B-11          ------------------------------------------------------
11C-36          ------------------------------------------------------
E10F-17         ACCTT-GGCCTCAAATCAGGTAGG---------------------------------
11C-12          ACCTT-GGCCTCAAGTTCAG-------------------------------------
E8544           ACCTTTGGCCTCAAATCAGG-------------------------------------
10A-30          ACCTT-GGCCTCAAAT-CAGGT-----------------------------------
11B-13          ACCTT-GGCCTCAAATTCAGGT-----------------------------------
E8543           AGC------------------------------------------------------
E9W-27A         ------------------------------------------------------
E8546           ACCG-----------------------------------------------------
E8541           CCCTTGCCTCAAAACCAAGGGAGAATCAGGG--------------------------
E8548           CNCGGGCCYCAAATTCT----------------------------------------
E2W-5           ------------------------------------------------------
10A-27(b)       ACC------------------------------------------------------
E10W-33         ACCTT-GGCCTCAAANTCAGGTAG---------------------------------
11B-29          ACCTT-GGCCTCAAATCAGNTAN----------------------------------
11C-27          ACCTTTGGCCTCAAATCAGGTAG----------------------------------
E6W-11          ACCTTTGGCCTCAAATCAGGTAG----------------------------------
T61             ACTTTTGGCCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAA
E10W-34         ACC------------------------------------------------------
```

C. Alignment of rDNA ITS sequence from isolate 11C-6 with those from T61 and GenBank accession AY558635 (CBS isolate 193.37, *Inonotus pachyphloeus*). Despite a stretch of noisy sequence between nucleotides 100 and 500 for isolate 11C-6, there are few mismatches with T61 and the GenBank sequence.

```
                    1          11         21         31         41         51
T61                 TTCTTGGTCCATTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11C-6               TTCTTGGTC-ATTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGACCCTGCGGA
AY558635            ------------------------------------------------------------

                    61         71         81         91         101        111
T61                 AGGATCATTATTGAGTTTAACAAAGTGGACTTGATGCTGGCATGTCTCTGGACTTGCATG
11C-6               AGGATCATTATTGAGTTTAACAAAGTGGACTTGATGCTGGCATGTCTCTGGACTTGCATG
AY558635            ----------TTGAGTTTAACAAAGCGGACTTGATGCTGGCATGTCTCTGGACTTGCATG

                    121        131        141        151        161        171
T61                 TGCTCAGTCTGCGCTCATCCAYTTCACACCTGTGCACTTTCAAAGGGGGATTGGATCTTA
11C-6               TGCTCAGTCTGCGTTCATCCACTTCACCCCTGTGCACTTTCAAAGGGGGATTGGATCTTA
AY558635            TGCTCAGTCTGCGCTCATCCACTTCACACCTGTGCACTTTCAAAGGGGGATTGGATCTTA

                    181        191        201        211        221        231
T61                 TTAGATAGATTTGYAAAGATCTTCGAACAGTTCAGTTCTTCTTTACATATATAAACACTA
11C-6               TTAGATAGATTTGCAAAGTTCTTCGACCAGTTCAGTTTTTCTTTACATATATAACCACTA
AY558635            TTAGATAGATTTGCAAAGATCTTCGAACAGTTCAGTTCTTCTTTACATATATAAACACTA

                    241        251        261        271        281        291
T61                 TATTGTTTGTGTAGAATGTACTTGCCTCTTGTAGGTGAATAATACTATACAACTTTCAAC
11C-6               TATTGTTTGTGTAGAATGTACTTSCYTCTTGTAGGTGAATAATACTATMCAACTTTCAAC
AY558635            TATTGTTTGTGTAGAATGTACTTGCCTCTTGTAGGTGAATAATACTATACAACTTTCAAC

                    301        311        321        331        341        351
T61                 AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA
11C-6               AACGGATTTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA
AY558635            AACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA

                    361        371        381        391        401        411
T61                 ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCG
11C-6               ATTGCAGAATTCAGTGAATCATGGAATCTTTGAACGCCCCTTGCACTCCTTGGTATTCCG
AY558635            ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCG

                    421        431        441        451        461        471
T61                 AGGAGTATGCCTGTTTGAGTGTCATGTTAATCTCAATTCAACATGTTTTTGTGTTTGAAT
11C-6               AGGAGTATCCCTGTTTGAGTGTCATGTTAATCTCAATTCAACATGTTTTTGTGTTTGAAT
AY558635            AGGAGTATGCCTGTTTGAGTGTCATGTTAATCTCAATTCAACATGTTTTTGTGTTTGAAT

                    481        491        501        511        521        531
T61                 TGGACTTGGAGTCTGCTGGCGTCAAAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTT
11C-6               TGGACTTGGAGTCTGCGGGCGTCAAAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTT
AY558635            TGGACTTGGAGTCTGCTGGCGTCAAAGTCGGCTTCTCTTGAATGCATTAGCTGGGCTTTT

                    541        551        561        571        581        591
T61                 GCTCGAGTAATTGGTGTAATAGTTCTCTACATTCGCCGTTACACTTGCTTAGAAAGTCTG
11C-6               GCTCGAGTAATTGGTGTAATAGTTCTCTACATTCGCCGTTACACTTGCTTAGAAAGTCTG
AY558635            GCTCGAGTAATTGGTGTAATAGTTCTCTACATTCGCCGTTACACTTGCTTAGAAAGTCTG

                    601        611        621        631        641        651
T61                 CTTCTAACCGTCTTGTAATGAGACAATATAACTTTGACTTTTGGCCTC-AAATCAGGTAG
11C-6               CTTCTAACCGTCTTGTAATGAGACAATATAACTTTGACTTTT-GGCCT------------
AY558635            CTTCTAACCGTCTTGTAATGAGACAATATAACTTTGACTTTTGGCCTCCAAATCAGGTAG
```

Appendix 2.4 – *Alignment of rDNA ITS sequences from isolates in Ganoderma group and sporocarps collected during this project.*

A. Alignment of ITS sequences from Am8W-32, E1W-1, E5W-10, E7W-25, and E9W-28 with GenBank accession AJ608713 (*Ganoderma philippii*). All other isolates of *Ganoderma philippii* were identified by species-specific PCR.

```
                1          11         21         31         41         51
E7W-25          GGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
AM8W-32         GGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
E1W-1           GGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
AJ608713        GGTCCATTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
E5W-10          GGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT
E9W-28          GGTCATTTAGAGGAAGTAAAAGTCGTAGCAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT

                61         71         81         91         101        111
E7W-25          CATTACCGAGTCTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCCGCTCA
AM8W_32         CATTACCGAGTCTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCCGCTCA
E1W-1           CATTACCGAGTCTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCCGCTCA
AJ608713        CATTACCGAGTCTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCCGCTCA
E5W-10          CATTACCGAGTCTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCCGCTCA
E9W-28          CATTACCGAGTCTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCCGCTCA

                121        131        141        151        161        171
E7W-25          TCCACTCTACACCTGTGCACTCACTGTGGGCTTCAGATCGTGAAGCGGG-CTCTTTGCTG
AM8W-32         TCCACTCTACACCTGTGCACTCACTGTGGGCTTCAGATCGTGAAGCGGG-CTCTTTGCTG
E1W-1           TCCACTCTACACCTGTGCACTCACTGTGGGCTTCAGATCGTGAAGCGGG-CTCTTTGCTG
AJ608713        TCCACTCTACACCTGTGCACTCACTGTGGGCTTCAGATCGTGAAGCGGG-CTCTTTGCTG
E5W-10          TCCACTCTACACCTGTGCACTCACTGTGGGCTTCAGATCGTGAAGCGGG-CTCTTTGCTG
E9W-28          TCCACTCTACACCTGTGCACTCGCGGTGGGCTTCAGATCGTGAAGCGGGGCTCTTTGCTG

                181        191        201        211        221        231
E7W-25          GGCTTGCGAAGCGTGTCTGTGCCTGCGTTTATTACAAACTCTATAAAGTATCAGAATGTG
AM8W-32         GGCTTGCGAAGCGTGTCTGTGCCTGCGTTTATTACAAACTCTATAAAGTATCAGAATGCG
E1W-1           GGCTTGCGAAGCGTGTCTGTGCCTGCGTTTATTACAAACTCTATAAAGTATCAGAATGTG
AJ608713        GGCTTGCGAAGCGTGTCTGTGCCTGCGTTTATTACAAACTCTATAAAGTATCAGAATGTG
E5W-10          GGCTTGCGAAGCGTGTCTGTGCCTGCGTTTATTACAAACTCTATAAAGTATCAGAATGTG
E9W-28          GGCTTGCAAAGCGTGTCTGTGCCTGCGTTTATTACAAACTCTATAAAGTATCAGAATGTG

                241        251        261        271        281        291
E7W-25          TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC
AM8W-32         TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC
E1W-1           TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC
AJ608713        TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC
E5W-10          TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC
E9W-28          TAGTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC

                301        311        321        331        341        351
E7W-25          GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AM8W-32         GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
E1W-1           GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AJ608713        GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
E5W-10          GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
E9W-28          GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG

                361        371        381        391        401        411
E7W-25          AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
AM8W-32         AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
E1W-1           AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
AJ608713        AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
E5W-10          AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
E9W-28          AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA
```

```
           421        431        441        451        461        471
E7W-25      TGAAATCTTCAACCTACAAGCTTTTGTGGTTTTGTAGGCTTGGACTTGGAGGCTTGTCGG
AM8W-32     TGAAATCTTCAACCTACAAGCTTTTGTGGTTTTGTAGGCTTGGACTTGGAGGCTTGTCGG
E1W-1       TGAAATCTTCAACCTACAAGCTTTTGTGGTTTTGTAGGCTTGGACTTGGAGGCTTGTCGG
AJ608713    TGAAATCTTCAACCTACAAGCTTTTGTGGTTTTGTAGGCTTGGACTTGGAGGCTTGTCGG
E5W-10      TGAAATCTTCAACCTACAAGCTTTTGTGGTTTTGTAGGCTTGGACTTGGAGGCTTGTCGG
E9W-28      TGAAATCTTCAACCTACAAGCTTTTGTGGTTTTGTAGGCTTGGACTTGGAGGCTTGTCGG

           481        491        501        511        521        531
E7W-25      CCGTTCTCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGG
AM8W-32     CCGTTCTCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGG
E1W-1       CCGTTCTCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGG
AJ608713    CCGTTCTCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCRGATCGGCTCTCGG
E5W-10      CCGTTCTCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGG
E9W-28      CCGTTCTCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTGGCAGATCGGCTCTCGG

           541        551        561        571        581        591
E7W-25      TGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAATCGTCTCAGTTG
AM8W-32     TGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAATCGTCTCAGTTG
E1W-1       TGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAATCGTCTCAGTTG
AJ608713    TGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAATCGTCTCAGTTG
E5W_10      TGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAATCGTCTCAGTTG
E9W-28      TGTGATAATGTGTACGCCGCAACCGTGAAGCGTT-GACGAGCTTCTAATCGTCTCAGT-G

           601        611        621        631        641        651
E7W-25      AAGACAGCTTTATGACCTCTGACCTCAA--------------------------------
AM8W-32     AAGACAGCTTTATGACCTCTGACCTCAA--------------------------------
E1W-1       AAGACAGCTTTATGACCTCGG-CCTCAA--------------------------------
AJ608713    AAGACAGCTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCAT
E5W-10      AAGACAGCTTTATGACCTCTGACCTCAATTCAGGTAGG----------------------
E9W-28      AAGACAGCTTTATGACCTGT-ACCTCAAGT-------------------------------
```

B. Alignment of ITS sequence from E8538 with GenBank accession AJ627585 (*Ganoderma mastoporum).* All other isolates of *Ganoderma mastoporum* were identified by species-specific PCR.

```
                1          11         21         31         41         51
E8538           CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG
AJ627585        ----------------------------AACAAGGTTTCCGTAGGTGAACCTGCGGAAG

               61         71         81         91        101        111
E8538           GATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTACGAGGCATTGTGCACGCCCTG
AJ627585        GATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTACGAGGCATYGTGCACGCCCTG

              121        131        141        151        161        171
E8538           CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTACGGATTGTGGAGCGGGCTCTTCA
AJ627585        CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTACGGATCGTGGAGCGGGCTCTTCG

              181        191        201        211        221        231
E8538           CGGAGCTTGTGAAGCGCTTCTGTGCCTGCGTTTTACAACAAACACTTTAAAAGTATTAGA
AJ627585        CGGAGCTTGTGAAGCGCTTCTGTGCCTGCGTTTTATAACAAACACTTTAAAAGTATTAGA

              241        251        261        271        281        291
E8538           ATGTGTATTGCGATGTAGCGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTC
AJ627585        ATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTC

              301        311        321        331        341        351
E8538           GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
AJ627585        GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT

              361        371        381        391        401        411
E8538           CATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
AJ627585        CATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG

              421        431        441        451        461        471
E8538           TGTCATGAAATCTTCAACCTACAAGCTTTTTAATGGGTTTGTAGGCTTGGACTTGGAGGC
AJ627585        TGTCATGAAATCTTCAACCTACAAGCTTTTTAATGGGTTTGTAGGCTTGGACTTGGAGGC

              481        491        501        511        521        531
E8538           TTGTCGGTCTTTATTGGTCGGCTCCTCTCAAATGCATTAGCTTGGTTCCT-GCGGATCGG
AJ627585        TTGTCGGTCTTTATTGGTCGGCTCCTYTCAAATGCATTAGCTTGGTTCCTTGCGGATCGG

              541        551        561        571        581        591
E8538           CTTGTCGGTGTGATAATGTTTACGCCGCGACCGAGAA----------------------
AJ627585        CTTGTCGGTGTGATAATGTCTACGCCGCGACCGAGAAGCGTTTTTGGGCGAGCTTCTAGT

              601        611        621        631        641        651
E8538           ------------------------------------------------------------
AJ627585        CGTCTCTGTATAGAGACAATCTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCT
```

C. Alignment of ITS sequences from isolates 3A-7, 3B-14, 3A-36, 3C-8 and 10A-37 with GenBank accessions EU239383, EU239389 and EU239389 (*Ganoderma australe*).

```
                1         11        21        31        41        51
3A-7            ----------------------------------------TGCGGAAGGATCATTATC
3B-14           ----GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATC
3A-36           TTGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATC
3C-8            --GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATC
10A-37          ---GAGAAGTAAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATC
EU239389        ----------------------------------------------------GATCATTATC
EU239390        ----------------------------------------------------GATCATTATC
EU239383        ----------------------------------------------------GATCATTATC

                61        71        81        91        101       111
3A-7            GAGTTAATTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCGGGTCATCCAC
3B-14           GAGTTAATTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCGGCTCATCCAC
3A-36           GAGTTAATTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCGGCTCATCCAC
3C-8            GAGTTAATTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCGGCTCATCCAC
10A-37          GAGTCAATTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCGGCTCATCCAC
EU239389        GAGTTAATTGACGGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCGGCTCATCCAC
EU239390        GAGTT--CTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCGTCCAC
EU239383        GAGTT--CTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCGTCCAC

                121       131       141       151       161       171
3A-7            GCTCTTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTTAAACGGGCTCGTTTATTCG
3B-14           GCTCTTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTTAAACGGGCTCGTTTATTCG
3A-36           GCTCTTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTTAAACGGGCTCGTTTATTCG
3C-8            GCTCTTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTTAAACGGGCTCGTTTATTCG
10A-37          GCTCTTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTTAAACGGGCTCGTTTATTCG
EU239389        GCTCTTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTTAAACGGGCTCGTTTATTCG
EU239390        TC---TACACCTGTGCACTTACTGTGGGTTTACGGGTCGTGAAGCGGGCTCGTTTGTTCG
EU239383        TC---TACACCTGTGCACTTACTGTGGGTTTACGGGTCGTGAAACGGGCTCGTTTGTTCG

                181       191       201       211       221       231
3A-7            GGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACACACAAACACTATAAAGTATTAGA
3B-14           GGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACACACAAACACTATAAAGTATTAGA
3A-36           GGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACACACAAACACTATAAAGTATTAGA
3C-8            GGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACACACAAACACTATAAAGTATTAGA
10A-37          GGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACACACAAACACTACAAAGTATTAGA
EU239389        GGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACACACAAACACTATAAAGTATTAGA
EU239390        GGCTTGTCGAGCGCACTTGTTGCCTGCGTTTATC----ACAAACTCTATAAAGTATCAGA
EU239383        GGCTTGTCGAGCGCACTTGTTGCCTGCGTTTATC----ACAAACTCTATAAAGTATCAGA

                241       251       261       271       281       291
3A-7            ATGAATTGGGTAAATCGGGATATACAATATC---ATACAACTTTCAGCAACGGATCTCTT
3B-14           ATGAATTGGGTAAATCGGGATATACAATATC---ATACAACTTTCAGCAACGGATCTCTT
3A-36           ATGAATTGGGTAAATCGGGATATACAATATC---ATACAACTTTCAGCAACGGATCTCTT
3C-8            ATGAATTGGGTAAATCGGGATATACAATATC---ATACAACTTTCAGCAACGGATCTCTT
10A-37          ATGAATTGGGTAAATCGGGATAGACAATATC---ATACAACTTTCAGCAACGGATCTTTT
EU239389        ATGAATTGGGTAAATCGGGATATACA-TATC---ATACAACTTTCAGCAACGGATCTCTT
EU239390        ATG-------TGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTT
EU239383        ATG-------TGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTT

                301       311       321       331       341       351
3A-7            GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
3B-14           GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
3A-36           GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
3C-8            GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
10A-37          GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
EU239389        GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTT
EU239390        GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
EU239383        GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
```

```
                361         371         381         391         401         411
3A-7            AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
3B-14           AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
3A-36           AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
3C-8            AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
10A-37          AGTGAATCATTGAATCTTTGAACGCACCCTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
EU239389        AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
EU239390        AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
EU239383        AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT

                421         431         441         451         461         471
3A-7            GTTTGAGTGTCATTGAATCTTCAACTTACAAGCTTTTTTTATTGAAGGCTT-GTAGGCTT
3B-14           GTTTGAGTGTCATTGAATCTTCAACTTACAAGCTTTTCTTATTGAAGGCTT-GTAGGCTT
3A-36           GTTTGAGTGTCATTGAATCTTCAACTTACAAGCTTTTCTTATTGAAGGCTT-GTAGGCTT
3C-8            GTTTGAGTGTCATTGAATCTTCAACTTACAAGCTTTTCTTATTGAAGGCTT-GTAGGCTT
10A-37          GTTTGAGTGTCATTGAATTTTCAACTTACAAGCTTTTTTTATTGAAGGCTT-GTAGGCTT
EU239389        GTTTGAGTGTCATTGAATCTTCAACTTACAAGCTTTTCTTATTGAAGGCTTTGTAGGCTT
EU239390        GTTTGAGTGTCATTGAATCTTCAACTTACAAGCTTTTCTTATTGAAGGCTTTGTAGGCTT
EU239383        GTTTGAGTGTCATGAAATCTTCAACCTACAAACTT--CTTATGG--GGCTT-GTAGGCTT

                481         491         501         511         521         531
3A-7            GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAAAGCATTAGCTT
3B-14           GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAAAGCATTAGCTT
3A-36           GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAATGCATTAGCTT
3C-8            GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAATGCATTAGCTT
10A-37          GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAATGCATTAGCTT
EU239389        GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAATGCATTAGCTT
EU239390        GGATTTGGAGGCTTGTCGGACTTTATTATACGGGTCGGCTCCTCTTAAATGCATTAGCTT
EU239383        GGACTTGGAGGCTTGTCGGACT--ACTATATAGGTCGGCTCCTCTTAAATGCATTAGCTT

                541         551         561         571         581         591
3A-7            GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
3B-14           GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
3A-36           GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
3C-8            GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
10A-37          GGTTCCTTTGCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
EU239389        GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
EU239390        GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG
EU239383        GGTTCCTT-GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTG

                601         611         621         631         641         651
3A-7            TTTGGAACGAGCTTCTAATGGTCTCGTTAGAGAGAC-AAGTCTTAATGAGCTA-GACCTC
3B-14           TTTGGAACGAGCTTCTAATGGTCTCGTTAGAGAGACCAAGTTTTAATGAGCTCTGACCTC
3A-36           TTTGGAACGAGCTTCTAATGGTCTCGTTAGAGAGAC-AAGTTTTAATGAGCTCTGACCTC
3C-8            TTTGGAACGAGCTTCTAATGGTCTCGTTAGAGAGAC-AAGTTTTAATGAGCTCTGACCTC
10A-37          TTTGGAACGAGCTTCTAATGGTCTCGTTAGAGAGAC-AAGTTTTA-TGACCTCTGACCTC
EU239389        TTCGGAACGAGCTTCTAATGGTCTCGTTAGAGAGAC-AACCTTTA-TGACCTCTGACCTC
EU239390        TTTGGG-CGAGCTTCTAATCGTCTCGTTAGAGAGAC-AACCTTTA-TGACCTCTGACCTC
EU239383        TTTGGG-CGAGCTTCTAATCGTCTCGTTAGAGAGAC-AACCTTTA-TGACCTCTGACCTC

                661         671
3A-7            A----------
3B-14           AAATCAGGTAG-
3A-36           AAATCAGGTAG-
3C-8            AAATCAGGTAG-
10A-37          AAATCAGGTAG-
EU239389        AAATCAGG----
EU239390        AAATCAGGTAGG
EU239383        AAATCAGGTAGG
```

D. **Alignment of ITS sequences from E3F-41 with GenBank accession AJ627583 (*Ganoderma subresinosum*).**

```
                1         11        21        31        41        51
E3F-41          TCTTGGTCAATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
AJ627583        ----------GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA

               61        71        81        91        101       111
E3F-41          AGG-ATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCC
AJ627583        AGGGATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCC

               121       131       141       151       161       171
E3F-41          TGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAATGCGTGGAATGAGGCCTT
AJ627583        TGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAATGCGTGGAATGAGGCCTT

               181       191       201       211       221       231
E3F-41          TACGGGCTCGTGAAGCGGGTTGTGCCTGCGTTTATTACAAACACTATAAAGTATAAGAAC
AJ627583        TACGGGCTCGTGAAGCGGGTTGTGCCTGCGTTTATTACAAACACTATAAAGTATAAGAAC

               241       251       261       271       281       291
E3F-41          GTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGC
AJ627583        GTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGC

               301       311       321       331       341       351
E3F-41          ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA
AJ627583        ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA

               361       371       381       391       401       411
E3F-41          TCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTG
AJ627583        TCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTG

               421       431       441       451       461       471
E3F-41          TCATGAAATCTTCAATCTACAAACCTTTGCGGGTTTTGCAGATTTGGACTTGGAGGCTTG
AJ627583        TCATGAAATCTTCAATCTACAAACCTTTGCGGGTTTTGCAGATTTGGATTTGGAGGCTTG

               481       491       501       511       521       531
E3F-41          TCGGCCTGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTTACGGT
AJ627583        TCGGCCTGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTTACGGT

               541       551       561       571       581       591
E3F-41          GTGATAATTGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTC-TAATCGTCTCGTTG
AJ627583        GTGATAATTGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCCTAATCGTCTCGTTG

               601       611       621       631       641       651
E3F-41          GAGACATCTTATCGACCTCTGACCTCAAATCAGGTA----------------------
AJ627583        GAGACATCTTATTGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCAT
```

Appendix 2.5 – *Alignment of rDNA ITS sequences from fungal isolates in non-target group collected during this project.*

A. Alignment of ITS sequences from 11A-4, 11A-6, 11A-30, 11A-32, 11A-37, 11B-3, 11B-9, 11B-16, 11C-18, 11C-34, and 11C-35 with GenBank accessions EU118662 (*Phlebiopsis flavidoalba*) and DQ320133 (*Phlebiopsis gigantea*).

```
                1          11         21         31         41          51
11A-4           ----------------GGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11B-3           ----------------GAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11A-32          TCTTGGTC-ATTTAGAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11B-9           TCTTGGTCCATTTAGAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11C-18          TCTTGGTC-ATTTAGAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11C-34          TCTTGGTCCATTTAGAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11C-35          TCTTGGTCCATTTAGAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11A-30          --------------GAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11A-37          -----------TTAGAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11A-6           --------------GAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
11B-16          --------------GAGGAAGTAAAAGTCGTAACAAGGTT-TCCGTAGGTGAACCTGCGG
EU118662        ----------ATTAGAGGAAGTANA-GTCGTAACTACGTTCTCCGTAGGTGAACCTGCGG
DQ320133        ------------------------------------------------------------

                61         71         81         91         101         111
11A-4           AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11B-3           AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11A-32          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11B-9           AAGGATCATTATYGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11C-18          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11C-34          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11C-35          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11A-30          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11A-37          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11A-6           AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
11B-16          AAGGATCATTATCGAGTTTTGAACGGGTTGTTGCTGGCCTCA-TAC-GGGGCATGTGCAC
EU118662        AAGGATCATTATCGAGTTTTGAACGGGTTGTAGCTGGCTTCAGTAC-GAGGCATGTGCAC
DQ320133        ------------CCGGTTTTGAACGGGTTGTAGCTGGCCTCA-TACTGTGGCATGTGCAC

                121        131        141        151        161         171
11A-4           GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11B-3           GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11A-32          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11B-9           GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11C-18          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11C-34          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11C-35          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11A-30          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11A-37          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11A-6           GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
11B-16          GCCTGACTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTAGTGTGAAAGGTTG
EU118662        GCTCGACTTCATCCACTCCTTAACCTCTGTGCACTTATTGTAGAYTAGT--GAAAGGCTG
DQ320133        GCCTGTCTTCATCCACTCTTCAACCTCTGTGCACTTATTGTAGGCTGGT--GAAGGGTCG

                181        191        201        211        221         231
11A-4           CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11B-3           CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11A-32          CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11B-9           CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11C-18          CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11C-34          CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11C-35          CAT--TTATTTGCGAYTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11A_30          CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11A-37          CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11A-6           CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
11B-16          CAT--TTATTTGCGACTGGAAGCCTG-TCTACGTTTTACTACAAACGCTTCAGTTATAGA
EU118662        CAT--TCATTTGTGGCTGGAAGCCTG-TCTACGTTTTACCACAAACGCTTCAGTTATAGA
DQ320133        CATAGTAATGTGCGGCTTGAAGCCTGGTCTACGTTTTACTACAAACGCTTCAGTTATAGA
```

```
                241         251         261         271         281         291
11A-4           ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11B-3           ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11A-32          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11B-9           ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11C-18          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11C-34          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11C-35          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11A-30          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11A-37          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11A-6           ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
11B-16          ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
EU118662        ATGTTTATCTGCGTATAACGCATTTA-TATACAACTTTCAGCAACGGATCTCTTGGCTCT
DQ320133        ATGTCTATCTGCGTATAACGCATTTAATATACAACTTTCAGCAACGGATCTCTTGGCTCT

                301         311         321         331         341         351
11A-4           CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11B-3           CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11A-32          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11B-9           CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11C-18          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11C-34          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11C-35          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11A-30          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11A-37          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11A-6           CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
11B-16          CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
EU118662        CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
DQ320133        CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA

                361         371         381         391         401         411
11A-4           TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11B-3           TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11A-32          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11B-9           TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11C-18          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11C-34          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11C-35          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11A-30          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11A-37          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11A-6           TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
11B-16          TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
EU118662        TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA
DQ320133        TCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGA

                421         431         441         451         461         471
11A-4           GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11B-3           GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11A-32          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11B-9           GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11C-18          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11C-34          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11C-35          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11A-30          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11A-37          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11A-6           GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
11B-16          GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
EU118662        GTGTCATGGAATTCTCAACTTCTAATCCTTTTTTGTATTGGAAGCTTGGATTTGGAGGCT
DQ320133        GTGTCATGGAATTCTCAACTTCTAATACTTTTTTGTATCAGAAGCTTGGATTTGGAGGCT
```

```
                481        491        501        511        521        531
11A-4           TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11B-3           TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11A-32          TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11B-9           TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11C-18          TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11C-34          TGTGCTGGCTC---TAAC-GAGTYCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11C-35          TGTGCTGGCTC---TAAC-GAGTYCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11A-30          TGTGCTGGCTC---TAAC-GAGTYCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11A-37          TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11A-6           TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
11B-16          TGTGCTGGCTC---TAAC-GAGTCCGGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
EU118662        TGTGCTGGCTCCTTTGTT-GAGTC-GGCTCCTCTTAAATGAATTAGCGTGAATCACTATG
DQ320133        CGTGCTGGCTCTCTCGTTAGAGTC-GGCTCCTCTTAAATGAATTAGCGTGAATCACTATG

                541        551        561        571        581        591
11A-4           GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11B-3           GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11A-32          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11B-9           GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11C-18          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11C-34          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11C-35          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11A-30          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11A-37          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11A-6           GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
11B-16          GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAATC-AAGTTCTCG
EU118662        GATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTATTAAAATAAGTTCTCG
DQ320133        GATCGCTTCGGTGTGATAATTATCTGCGCCGTAGTCGTGAAGTATTAATAAAAGTTCTCG

                601        611        621        631        641        651
11A-4           CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTGACCTCAAATCAGGTAG---
11B-3           CTTCTAATCGTCCTTCACCGGACAATTAACCCTGACTTTTGACCTCAAATCAGGTAG---
11A-32          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACT----------------------
11B-9           CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTGACCTCAAAT-----------
11C-18          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTGACCTCAAATCA--------
11C-34          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTGACCTCAAATCAGGTA----
11C-35          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTCGACCTCAAATCAGGTAG----
11A-30          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTGACCTCAAATCAGGTAGG---
11A-37          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTGACCTCAAATCAGGTAG----
11A-6           CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTGACCTCAAATCAGGT-----
11B-16          CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTGACCTCAAATCAGGTAG---
EU118662        CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTTGACCTCAAATCAGGTAGGA
DQ320133        CTTCTAATCGTCCTTCACGGGACAATTAACCCTGACTTTTTGACCTCAAATCAGGTAGGA
```

B. Alignment of ITS sequences from 11A-1, 11A-19, 11A-40, 11B-24, 11B-30, 11B-33, 11B-35 and 11B-38a with GenBank accession FJ711051 (*Tinctoporellus epimiltinus*).

```
                1          11         21         31         41         51
11B-33          ----------TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11B-38a         ----------TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11A-40          TCTTGGTCCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11A-1           TCTTGGTCCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11B-35          ----------GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11A-19          ----------TTGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11B-30          ----------CATTGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
11B-24          ----------AGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGA
FJ711051        ------------------------------------------------------------

                61         71         81         91         101        111
11B-33          AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACAGGCATGTGCACACCTCA
11B-38a         AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACTGGCATGTGCACACCTCA
11A-40          AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACRGGCATGTGCACACCTCA
11A-1           AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACRGGCATGTGCACACCTCA
11B-35          AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACAGGCATGTGCACACCTCA
11A-19          AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACRGGCATGTGCACACCTCA
11B-30          AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACYGGCATGTGCACACCTCA
11B-24          AGGATCATTAACGAGTTGAACGGGGTTGTAGCTGGCCTTCACWGGCATGTGCACACCTCA
FJ711051        ----------------------------TAGCTGGCCTTCACGGGCATGTGCACACCTCA

                121        131        141        151        161        171
11B-33          CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11B-38a         CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11A-40          CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11A-1           CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11B-35          CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11A-19          CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11B-30          CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
11B-24          CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT
FJ711051        CTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCGAGAGGCCGCGCTTGCGTGGTT

                181        191        201        211        221        231
11B-33          GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11B-38a         GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11A-40          GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11A-1           GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11B-35          GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11A-19          GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11B-30          GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
11B-24          GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC
FJ711051        GATCGGGCTCACGTCTATTACAAACTCTTCAGTATCAGAATGTGTATCGCGATGTAACGC

                241        251        261        271        281        291
11B-33          ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11B-38a         ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11A-40          ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11A-1           ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11B-35          ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11A-19          ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11B-30          ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
11B-24          ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
FJ711051        ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
```

```
                301        311        321        331        341        351
11B-33          AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11B-38a         AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11A-40          AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11A-1           AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11B-35          AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11A-19          AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11B-30          AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
11B-24          AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
FJ711051        AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC

                361        371        381        391        401        411
11B-33          TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11B-38a         TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11A-40          TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11A-1           TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11B-35          TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11A-19          TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11B-30          TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
11B-24          TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA
FJ711051        TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAACCTA

                421        431        441        451        461        471
11B-33          TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCTTTACSGTCGGCTC
11B-38a         TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCCTTACCGTCAGCTC
11A-40          TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCTTTACCGTCGGCTC
11A-1           TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCTTTACCGTCGGCTC
11B-35          TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCTTTACSGTCGGCTC
11A-19          TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCTTTACCGTCGGCTC
11B-30          TAAATCCTTGYGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCYTTACCGTCAGCTC
11B-24          TAAATCCTTGYGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCYTTACCGTCRGCTC
FJ711051        TAAATCCTTGTGGTTTTTAGGCTTGGACTTGGAGGCTTTTGCTGGCTTTACCGTCGGCTC

                481        491        501        511        521        531
11B-33          CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11B-38a         CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11A-40          CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11A-1           CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11B-35          CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11A-19          CTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11B-30          CTCTTAAAYGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
11B-24          CTCTTAAAYGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG
FJ711051        CTCTTAAACGCATTAGCTTGATTCCTTGCGGATCGGCTCTCAGTGTGATAATTATCTGCG

                541        551        561        571        581        591
11B-33          CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTTGAGACAAACACTTTGACA
11B-38a         CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCCTTGAGACAAACACT------
11A-40          CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTTGAGACAAACACTTTGACA
11A-1           CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTTGAGACAAACACTTTGACA
11B-35          CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTTGAGACAAACACTTTGACA
11A-19          CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTTGAGACAAACACTTTGACA
11B-30          CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCCTTGAGACAAACACTTTGACA
11B-24          CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCCTTGAGACAAACACTTTGACA
FJ711051        CTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCTTCGAGACAAACACTTTGACA

                601        611        621        631        641
11B-33          T----------------------------------------------
11B-38a         ----------------------------------------------
11A-40          TCTGACCTCAAATCAGGTA-----------------------------
11A-1           TCTGACCTCAAATCAGGTAGG---------------------------
11B-35          TCTGACCTCAAATCAGG-------------------------------
11A-19          TCTGACCTCAAATCAGGTA-----------------------------
11B-30          TCTGACCTCAAATCAGGTAG----------------------------
11B-24          TCTGACCTCAAATCAGGTAG----------------------------
FJ711051        TCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATAT
```

C. Alignment of partial ITS sequences from 3A-23, 3A-29 and 3C-21(b) with GenBank accession DQ444306 (*Neonothopanus nambi*).

```
                1         11         21         31         41         51
DQ444306        GAAATGTTTTGAAGGGGATTGTTGCTGGCCTATAACAAGGCATGTGCACATCTTCTTTCA
3A-29           ------------------------------------------------------------
3A-23           ----------------------------------AAGGCATGTGCACATCTTCTTTCA
3C-21(b)        ------------------------------------------------------------

                61         71         81         91         101        111
DQ444306        ATCTATTCATCCACCTGTGCATCTTTTTTGTAGGAACCCTATAT-AGGATGGTTGAACCGG
3A-29           ------------------------------------------------------------
3A-23           ATCTATTCATCCACCTGTGCATCTTTTTTGTAGGAACCCTATAT-AGGATGGTTGAACCGG
3C-21(b)        ------------------------------------------------------------

                121        131        141        151        161        171
DQ444306        GGGTCTATTACTTCTGTTGT-----AGGCCTTGTTTGAC-AGTCCTGG-GGTTTCTATGT
3A-29           ------------------------------------------------TTTCTATGT
3A-23           GGGTCTATTACTTCTGTTGT-----AGGCCTTGTTTGAC-AGTCCTGG-GGTTTCTATGT
3C-21(b)        ------------------------------------------------------------

                181        191        201        211        221        231
DQ444306        CTTACAAACTCTAATGAAATGTATCTGAATGTCATTTATTGGGACTTAACTGGCCCTCTA
3A-29           CTTACAAACTTTAATGAATGTATTCTGAATGTCATTTATTGGGACTTAACTGGCCCTCTA
3A-23           ------------------------------------------------------------
3C-21(b)        CTTACAAACTCTAATGAAA-GTATTTGAA--------------------------------

                241        251        261        271        281        291
DQ444306        AACTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA
3A-29           AACTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA
3A-23           ------------------------------------------------------------
3C-21(b)        ------------------------------------------------------------

                301        311        321        331        341        351
DQ444306        AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT
3A-29           AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT
3A-23           ------------------------------------------------------------
3C-21(b)        ------------------------------------------------------------

                361        371        381        391        401        411
DQ444306        TGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTCA
3A-29           TGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTCA
3A-23           ------------------------------------------------------------
3C-21(b)        ------------------------------------------------------------

                421        431        441        451        461        471
DQ444306        CAAGTTTTGTAGCTTCTGAGGCTTGGATTGTGGAGGCTTGCTGGCATCTAAGATGCATTT
3A-29           CAAGTTT-GTAGCTTTTGAGGCTTGGATTGTGGAGGCTTGCTGGCATTTAAGATGCATT-
3A-23           ------------------------------------------------------------
3C-21(b)        ------------------------------------------------------------

                481        491        501        511        521        531
DQ444306        GGCTCCTCTTAAAAGCATTAGTAGAAACCAATTGTTGGACTACCTTTGGTGTGATAATTA
3A-29           GGCTCCTYTTAAAAGCATTAGTAGAAACCAATTGTTGGACTACCTTTGGTGTGATAATTA
3A-23           ------------------------------------------------------------
3C-21(b)        ------------------------------------------------------------

                541        551        561        571        581        591
DQ444306        TCTACGCCTTGGTGTTCTATCTGACAAAAGGGTCTCTTTGGTTGGGATAGTTGCAAACGA
3A-29           TTTACGCCTTGGTGTTCTATCTGAC------------------------------------
3A-23           ------------------------------------------------------------
3C-21(b)        ------------------------------------------------------------

                601        611        621        631        641        651
DQ444306        GAGTTTGCTCTGTCTGTTTT-AACTGTCAAAGAGGCTTTGGGGTGTCTGCTCTCTAACTG
3A-29           ------------------------------------------------------------
3A-23           ---------------GTTTTTAACTGTCAAAGAGGCTTTGGGGTGTCTGCTCTCTAACTG
3C-21(b)        -----------------TTTTAACTGTCAAAGAGGCTTTGGGGTGTCTGCTCTCTAACTG
```

```
                661         671         681         691         701
DQ444306        TCTGTTTGACGGACAACTAATTGATTTGTTTGACC-----------
3A-29           ----------------------------------------------
3A-23           TCTGTTTGACGGACAACTAATTGATTTGTCGACCTCAAATCA-----
3C-21(b)        TCTGTTTGACGGACAACTAATTGATTTGTTGACCTCAAATCAGGTAG
```

D. Alignment of ITS sequences from 11B-25 and 11B-26 with GenBank accession EU661879 (*Trametes elegans = Lenzites elegans*).

```
                1         11        21        31        41        51
11B-25          GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAACGA
11B-26          ------------------CAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAACGA
EU661879        -------------------------TCCGTAGGTGAACCTGCGGAAGGATCATTAACGA

                61        71        81        91        101       111
11B-25          GTTCTGACATGGGTTGTAGCTGGCCTCACGAGGCATGTGCACGCCCTGCTCATCCACTCT
11B-26          GT-CTGACATGGGTTGTAGCTGGCCTCACGAGGCATGTGCACGCCCTGCTCATCCACTCT
EU661879        GTTTTGACATGGGTTGTAGCTGGCCTCACGAGGCATGTGCACGCCCTGCTCATCCACTCT

                121       131       141       151       161       171
11B-25          ACACCTGTGCACTTACTGTAGGTTTGGCGTGGGCTTCGAGGGCCTTCACGGGCTTTTGAG
11B-26          ACACCTGTGCACTTACTGTAGGTTTGGCGTGGGCTTCGAGGGCCTTCACGGGCTTTTGAG
EU661879        ACACCTGTGCACTTACTGTAGGTTTGGCGTGGGCTTTCGGGGCCTTCACGGGCTTTGAGA

                181       191       201       211       221       231
11B-25          GCATTCTGCCTGCCTATGTATCACTACAAACACTATAAAGTAACAGAATGTAATCGCGTC
11B-26          GCATTCTGCCTGCCTATGTATCACTACAAACACTATAAAGTAACAGAATGTAATCGCGTC
EU661879        GCATTCTGCCTGCCTATGTATCACTATAAACACTACGAAGTAACAGAATGTAATCGCGTC

                241       251       261       271       281       291
11B-25          TAACGCATCTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC
11B-26          TAACGCATCTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC
EU661879        TAACGCATCTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC

                301       311       321       331       341       351
11B-25          GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
11B-26          GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
EU661879        GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA

                361       371       381       391       401       411
11B-25          CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGTATTCTC
11B-26          CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGTATTCTC
EU661879        CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGTATTCTC

                421       431       441       451       461       471
11B-25          AACCCACACATCCTTGTGATGCTTGTGAGGCTTGGACTTGGAGGCTTGCTGGCCCGTCGC
11B-26          AACCCACACATCCTTGTGATGCTTGTGAGGCTTGGACTTGGAGGCTTGCTGGCCCGTCGC
EU661879        AACCCACACATCCTTGTGATGCTTGTGAGGCTTGGACTTGGAGGCTTGCTGGCCCATCGC

                481       491       501       511       521       531
11B-25          GGTCGGCTCCTCTTGAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCAGTGTGATAA
11B-26          GGTCGGCTCCTCTTGAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCAGTGTGATAA
EU661879        GGTCGGCTCCTCTTGAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCAGTGTGATAA

                541       551       561       571       581       591
11B-25          TTGTCTACGCTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCCTGCTAGGGACAAC
11B-26          TTGTCTACGCTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCCTGCTAGGGACAAC
EU661879        TTGTCTACGCTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCCTGCTAGGGACAAT

                601       611       621       631       641       651
11B-25          TTACTTGACATCTGACCTCAAATCAGGTA----------------------------
11B-26          TTACTTGACATCTGACCTCA-------------------------------------
EU661879        TTACTTGACATCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATA

                661
11B-25          ---------
11B-26          ---------
EU661879        AGCGGAGGA
```

E. Alignment of ITS sequences from E9W-27B and E8818C (isolated from a sporocarp of a *Ganoderma* sp. in the ACIAR root-rot project) with GenBank accession AY605709 (unidentified basidiomycete).

```
                1         11        21        31        41        51
E9W-27B         ------------------------------------------------------------
AY605709        GTCGTACTACCGATTGAATGGCTTAGTGAGGTCTTGGGATTGGCTTCGGGGAGCCGGCAA
E8818C          ------------------------------------------------------------

               61        71        81        91        101       111
E9W-27B         ----------------------------------------TTAGAGGAAGTAAAAGTCGT
AY605709        CGGCACCCTGTCGCTGAGAACTTGATCAAACTTGGTCA-TTTAGAGGAAGTAAAAGTCGT
E8818C          ----------------------------TCTTGGTCAATTTAGAGGAAGTAAAAGTCGT

              121       131       141       151       161       171
E9W-27B         AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTA
AY605709        AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTA
E8818C          AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTA

              181       191       201       211       221       231
E9W-27B         GCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTG
AY605709        GCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACATGTGCACTTACTG
E8818C          GCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTG

              241       251       261       271       281       291
E9W-27B         TGGGTTTCAGACGGCGTAGCGAGCCTTTACGGGTTCGTGAAAGCGTCTGTGCCTGCGTTT
AY605709        TGGGTTTCAGACGGNGTAGCGAGCCTTTACGGGTTCGTGAAAGCGTCTGTGCCTGCGTTT
E8818C          TGGGTTTCAGACGGTGTAGCGAGCCTTTACGGGTTCGTGAAAGCGTCTGTGCCTGCGTTT

              301       311       321       331       341       351
E9W-27B         ATTACAAACTCTTACAAGTAAATGAATGTGTATTGCGATATAACGCATCTATATACAACT
AY605709        ATTACAAACTCTTACAAGTAAATGAATGTGTATTGCGATATAACGCATCTATATACAACT
E8818C          ATTACAAACTCTTACAAGTAAATGAATGTGTATTGCGATATAACGCATCTATATACAACT

              361       371       381       391       401       411
E9W-27B         TTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
AY605709        TTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
E8818C          TTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA

              421       431       441       451       461       471
E9W-27B         ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGT
AY605709        ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGT
E8818C          ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGT

              481       491       501       511       521       531
E9W-27B         ATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTATAAACCTTTGCGGG
AY605709        ATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTATAAACCTTTGCGGG
E8818C          ATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTATAAACCTTTGCGGG

              541       551       561       571       581       591
E9W-27B         TTTGTAGGCTTGGATTTGGAGGCTTGTCGGCCTAACGGTCGGCTCCTCTTAAATGCATTA
AY605709        TTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCTAANGGTCGGCTCCTCTTAAATGCATTA
E8818C          TTTGTAGGCTTGGATTTGGAGGCTTGTCGGCCTAACGGTCGGCTCCTCTTAAATGCATTA

              601       611       621       631       641       651
E9W-27B         GCTTGATTCCTTGCGGATCGGCTCTCGGTGTGATAATTGTCTACGCCGCGACCGTGAAGC
AY605709        GCTTGATTCCTTGCGGATCGGCTCTCGGTGTGATAATTGTCTACGCCGCGACCGTGAAGC
E8818C          GCTTGATTCCTTGCGGATCGGCTCTCGGTGTGATAATTGTCTACGCCGCGACCGTGAAGC

              661       671       681       691       701       711
E9W-27B         GTTTGGCGAGCTTCTAATCGTCTCTTATGAGACAACACATTGACCTCTGACCTCAAATCA
AY605709        GTTTGGCGAGCTTCTAATCGTCTCTTATGAGACAACACATTGACCTCTGACCTCAAATCA
E8818C          GTTTGGCGAGCTTCTAATCGTCTCTTATGAGACAACACATTGACCTCTGACCTCA-----
```

F. Alignment of ITS sequences from 11B-18 with GenBank accession AY593868 (*Rigidoporus ulmarius*).

```
                1         11        21        31        41        51
AY593868        --------ATAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAAC
11B-18          TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAAC

                61        71        81        91        101       111
AY593868        GAATTGCGTTCGGGGTTGTTGCTGGTTT-CTTTTTGA--------AGCATGTGCACACCT
11B-18          GAATTGCGTTCGGGGTTGTTGCTGGTTTTCTTTTTAACAGGAGAGAACATGTGCACGCCT

                121       131       141       151       161       171
AY593868        CGCAATCCATTTTCAAACCACACATGTGCACTTCAGAGGGAGA-CCTCTTCTGGTCTCTC
11B-18          CGCAATCCATTT-CAAACCACACTTGTGCACTTCAGAGGGGGAGCCTCTCTTGGCCTCTC

                181       191       201       211       221       231
AY593868        CTCTTTTCAT---TACAAACCACAATAAAGTCTTTTGTATTATTGATCGTATGATAAACT
11B-18          CTTCTTTCATCACTACAAACCACTTTAAAGTCTTTTGTATTTGTTGGTTAACTATAATGT

                241       251       261       271       281       291
AY593868        AAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA
11B-18          TAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA

                301       311       321       331       341       351
AY593868        TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTG
11B-18          TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTG

                361       371       381       391       401       411
AY593868        CGCTCCTTGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAATCTCAAC
11B-18          CGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGTAATTCTCAATCTCAAC

                421       431       441       451       461       471
AY593868        TTGTTTGTTGTAGATTGGATTTGGGAGCTTGTCGTGCCTCTT----------AGAGGTT
11B-18          TTYTTTGTTGTGGATTGGATTTGGGAGCTTGTYGTGTCTCTTTCTATWATGAAAGAGGTT

                481       491       501       511       521       531
AY593868        AGACTCTCCTTGAATGCATTAGCTCGGTCACGTAGTTTGCCCGACGGTTCACGGTGTGAT
11B-18          AGACTCTCCTTGAATGCATTAGCTCGGTCACGTAGTTTGCCYGACGGTTCACGGTGTGAT

                541       551       561       571       581       591
AY593868        AGTTTCACTTCATCGCCGTTCTAACCTTTGGTGCCTGTGTTTTTACCGGCTTCTAATCTC
11B-18          AGTCTCACTTCATCGCCGTTCTAACTGTTGGTGCCTGTGTTTTTGCCGGCTTCTAATCTC

                601       611       621       631       641       651
AY593868        TGGCAATCAGTGTCTTTCATTAGATATTGACACGCCTATA-ACTTTAACGCTTGACCTCA
11B-18          TGGCC-------TCTTT---TTCAAAGTG----GCCTTTACACTTTTGATACTGACCTCA

                661       671       681       691       701
AY593868        AATCAGGTAGGATTACCCGCTGAACTTAAGCATATCAATAAGCCGGG
11B-18          AATCAGGT-----------------------------------
```

G. Alignment of ITS sequence from 11B-4 with GenBank accession FJ010208 (*Cerrena* sp.).

```
                1          11         21         31         41         51
11B-4           GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGA
FJ010208        -------------------------------------------GGAAGGATCATTAATGA

               61         71         81         91        101        111
11B-4           ATTTTATGGCGGAATTGTAGCTGGCCCCAACCGGGCATGTGCACATTCTGTTCATTCCAT
FJ010208        ATTTTATGGCGGAATTGTAGCTGGCCCCAACCGGGCATGTGCACATTCTGTTCATTCCAT

              121        131        141        151        161        171
11B-4           TCTCATACACCTCTGTGCACTTTACATAGGTTTGGTATAGAAAAGGTCTTTATTGACTTT
FJ010208        TCTCATACACCTCTGTGCACTTTACATAGGTTTGGTATAGAAAAGGTCTTTATTGACTTT

              181        191        201        211        221        231
11B-4           GGAAATACTGACCTATGCTTTTATAAACGCTTCAGTTTTAGAATGTCATCCGCGTATAAC
FJ010208        GGAAATACTGACCTATGCTTTTACAAACGCTTCAGTTTTAGAATGTCATCCGCGTATAAC

              241        251        261        271        281        291
11B-4           GCAATAAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC
FJ010208        GCAATAAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC

              301        311        321        331        341        351
11B-4           GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAT
FJ010208        GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAT

              361        371        381        391        401        411
11B-4           CTTGCGCCCTTTGGTATTCYGAAGGGCATGCCTGTTTGAGTGTCATGGTATTCTCAATAC
FJ010208        CTTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGTGTCATGGTATTCTCAATAC

              421        431        441        451        461        471
11B-4           CCCAAATCTTTGCGGATAAGGGTGTGTTGGACTTGGAGGTTTTTGCAGGTAATGATTGWR
FJ010208        CCCAAATCTTTGCGGATAAGGGTGTGTTGGACTTGGAGGTTTTTGCAGGTAATGATTGTA

              481        491        501        511        521        531
11B-4           TTACCAGCTCCTCTTAAATGCATTAGCAGAGATAATACTGCTACTCTCCAGTGTGATAAT
FJ010208        TTACCAGCTCCTCTTAAATGCATTAGCAGAGATAATACTGCTACTCTCCAGTGTGATAAT

              541        551        561        571        581        591
11B-4           TGTCTACACTGTTAGTAGTGCGGTATAACAAAATGTCTATGCTTCTAATCGTCTTCGGAC
FJ010208        TGTCTACACTGTTAGTAGTGCGGTATAACAAAATGTCTATGCTTCTAATCGTCTTCGGAC

              601        611        621        631        641        651
11B-4           AACTTTTGACAATCTGACCTCAAATCAGGTA--------------------------
FJ010208        AACTTTTGACAATCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATC
```

H. Alignment of ITS sequences from 11C-29, E10F-20 and E3W-7 with GenBank accessions AY280979 (*Gymnopilus purpureosquamulosus)* and AY280980 (*Gymnopilus dilepus*).

```
                1         11        21        31        41        51
11C-29          -TCTTGGTCCAATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG
E3W-7           ------------------------------------------------------------
AY280979        TTCTTGGTC--ATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG
E10F-20         ---------------GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG
AY280980        ----------------AGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG

                61        71        81        91        101       111
11C-29          GAAGGATCATTATTGAATAAACTTGATGTAGTTGAGCTGACTCTCTCGGGAGTATGTGCT
E3W-7           ------------------------------------------------------------
AY280979        GAAGGATCATTATTGAATAAACTTGATGTAGTTGAGCTGACTCTCTCGGGAGTATGTGCT
E10F-20         GAAGGATCATTATTGAATAAACTTGGCGTGGTTGAGCTGACTCTCTCGGGAGTATGTGCT
AY280980        GAAGGATCATTATTGAATAAACTTGGCGTGGTTGAGCTGACTCTCTCGAGAGTATGTGCT

                121       131       141       151       161       171
11C-29          CGCTCGTCATCTTTATCTTTCCACCTGTGCACTTTTTGTAGATTTGGATGTAACTGTCCG
E3W-7           -------------------------TGCACTTTTTGTAGATTTGGATGTAACTGTCCG
AY280979        CGCTCGTCATCTTTATCTTTCCACCTGTGCACTTTTTGTAGATTTGGATGTAACTGTCCG
E10F-20         CGCTCGTCATCTTTATCTTTCCACCTGTGCACTTTTTGTAGATTTGGATGTAACTTTCTG
AY280980        CGCTCGTCATCTTTATCTTTCCACCTGTGCACTTTTTGTAGATTTGGATGTAACTTTCTG

                181       191       201       211       221       231
11C-29          AGGTAACTCGGTTGGGAGGAATGCTATCTCTGATGGCTTTCCTTGTATGTCCAAGTCTAT
E3W-7           AGGTAACTCGGTTGGGAGGAATGCTATCTCTGATGGCTTTCCTTGTATGTCCAAGTCTAT
AY280979        AGGCAACTCGGTTGGGAGGAATGCTGTCTCTGATGGCTTTCCTTGTATGTCCAAGTCTAT
E10F-20         AGGCAACTCAGTTGGGAGGAATGCTATTTC-GATGGCTTTCCTTGTATGTCCAAGTCTAT
AY280980        AGGCAACTCAGTTGGGAGGAATGCTATTTC-GATGGCTTTCCTTGTATGTCCAAGTCTAT

                241       251       261       271       281       291
11C-29          GTTTTCATATACTCCAAGTATGTAACAGAATGTATCATTGGGCCTTGTGCCTATAAACTA
E3W-7           GTTTTCATATACTCCAAGTATGTAACAGAATGTATCATTGGGCCTTGTGCCTATAAACTA
AY280979        GTTTTCATATACTCCAAGTATGTAACAGAATGTATCACTGGGCCTTGTGCCTATAAACTA
E10F-20         GTTTTCATATACTCCAAGTATGTAACAGAATGTATCACTGGGCCTTGTGCCTATAAACCT
AY280980        GTTTTCATATACTCCAAGTATGTAACAGAATGTATCACTGGGCCTTGTGCCTATAAACCT

                301       311       321       331       341       351
11C-29          TATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG
E3W-7           TATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG
AY280979        TATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG
E10F-20         TATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG
AY280980        TATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG

                361       371       381       391       401       411
11C-29          CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCG
E3W-7           CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCG
AY280979        CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCG
E10F-20         CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCG
AY280980        CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCG

                421       431       441       451       461       471
11C-29          CCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTTACTAG
E3W-7           CCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTTACTAG
AY280979        CCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTTACTAG
E10F-20         CCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTTACTAG
AY280980        CCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTTACTAG

                481       491       501       511       521       531
11C-29          CTTTTGCGAAGTAATGGCTTGGACTTGGGGGTCTTTTT-GCTGGTTTCGAAAGAGATCTGC
E3W-7           CTTTTGCGAAGTAATGGCTTGGACTTGGGGGTCTTTTT-GCTGGTTTCGAAAGAGATCTGC
AY280979        CTTTTGCGAAGTAATGGCTTGGACTTGGGGGTCTTTTTTGCTGGTTTCGAAAGAGATCTGC
E10F-20         CTTTTGCGAAGTAATGGCTTGGATGTGGGGGTCTTTTT-GCTGGTTTCGAAAGAAATCTGC
AY280980        CTTTTGCGAAGTAATGGCTTGGATGTGGGGGTCTTTTT-GCTGGTTTCGAAAGAAATCTGC
```

```
                541         551         561         571         581         591
11C-29          TCCCCTTAAATGCATTAGCCGGTGCCCCGCGTGGACCGTCTATTGGTGTGATAATTATCT
E3W-7           TCCCCTTAAATGCATTAGCCGGTGCCCCGCGTGGACCGTCTATTGGTGTGATAATTATGT
AY280979        TCCCCTTAAATGCATTAGCCGGTGCCCCGCGTGGACCGTCTATTGGTGTGATAATTATCT
E10F-20         TCCCCTTAAATGYATTAGCCGGTGCCCCGCGTGGACCGTCTATTGGTGTGATAATTATCT
AY280980        TCCCCTTAAATGCATTAGCCGGTGCCCCGCGTGGACCGTCTATTGGTGTGATAATTATCT

                601         611         621         631         641         651
11C-29          ACGCCGTTAGATGTCTGCTATTAAATGGGAT-GCGCTGCTTCTAATCGTCCTCT-AGGAC
E3W-7           ACGCCGTTAGATGTCTGGTATTAAATGGGAG-GCGCTGCTTCTAATCGTCCTCT-AGGAC
AY280979        ACGCCGTTAGATGTCTGCTATTAAATGGGAT-GTGCTGCTTCTAATCGTCCTTC-AGGAC
E10F-20         ACGCCGTTAGACGTCTGCTATTAAATGGGWTTGCGCTGCTTCTAATCGTCCTCTTAGGAC
AY280980        ACGCCGTTAGACGTCTGCTATTAAATGGGATTGCGCTGCTTCTAATCGTCCTCTTAGGAC

                661         671         681         691         701         711
11C-29          AAT-TATTGACCATT-GACCTCAAATCAGG----------------------------
E3W-7           AAT-TATGG-CCAGT-GACCTCA---------------------------------
AY280979        AAT-TATTGACCATTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA--------
E10F-20         AATCTATTGACCATT-GACCTCAAATCAGGTAGG------------------------
AY280980        AAT-TATTGACCATTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA
```

Appendix 2.6 – Compartment of origin for basidiocarps, root-sign samples and cultures

Material	Type of material	Material code	Compartment	Notes
Sporocarp	*Phellinus noxius*	E8540	Compt. 1A - Bunut	
Sporocarp	*Phellinus noxius*	E8541	Compt. 2C - Rasau Kuning	
Sporocarp	*Phellinus noxius*	E8543	Compt.236 - Rasau Kuning	
Sporocarp	*Phellinus noxius*	E8544	Compt. 1A - Bunut	
Sporocarp	*Phellinus noxius*	E8546	Compt. 1A - Bunut	
Sporocarp	*Phellinus noxius*	E8547	Compt. 1A - Bunut	
Sporocarp	*Phellinus noxius*	E8548	Compt. 5A - Kampung Nias	
Sporocarp	*Ganoderma mastoporum*	E8538	Compt.236 - Rasau Kuning	
Sporocarp	*Ganoderma mastoporum*	E8539	Compt.236 - Rasau Kuning	
Sporocarp	*Ganoderma mastoporum*	E8549	Compt.223 - Rasau Kuning	
Sporocarp	*Ganoderma mastoporum*	E8552	Compt.223 - Rasau Kuning	
Sporocarp	Unidentified	E8550	Compt.223 – Rasau Kuning	Rotten internally
Root signs	RS-1	E10W-33	Compt 1A – Bunut	
Root signs	RS-1	E10W-34	Compt 1A – Bunut	
Root signs	RS-1	E10W-35	Compt 1A – Bunut	Isolation was failed
Root signs	RS-1	E10W-36	Compt 1A – Bunut	
Root signs	RS-1	10A-0	Compt 1A – Bunut	
Root signs	RS-1	10A-1	Compt 1A – Bunut	
Root signs	RS-1	10A-6	Compt 1A – Bunut	
Root signs	RS-1	10A-7	Compt 1A – Bunut	Isolation was failed
Root signs	RS-1	10A-8	Compt 1A – Bunut	
Root signs	RS-1	10A-9	Compt 1A - Bunut	
Root signs	RS-1	10A-10	Compt 1A – Bunut	
Root signs	RS-1	10A-11	Compt 1A – Bunut	
Root signs	RS-1	10A-14	Compt 1A – Bunut	
Root signs	RS-1	10A-15	Compt 1A – Bunut	
Root signs	RS-1	10A-16	Compt 1A – Bunut	Isolation was failed
Root signs	RS-1	10A-17	Compt 1A – Bunut	
Root signs	RS-1	10A-21	Compt 1A – Bunut	
Root signs	RS-1	10A-24	Compt 1A – Bunut	
Root signs	RS-1	10A-27	Compt 1A – Bunut	
Root signs	RS-1	10A-28	Compt 1A – Bunut	
Root signs	RS-1	10A-30	Compt 1A – Bunut	
Root signs	RS-1	10A-37	Compt 1A – Bunut	
Root signs	RS-2	E7W-25	Compt 071 – Rasau Kuning	
Root signs	RS-2	E7W-26	Compt 071 – Rasu Kuning	
Root signs	RS-2	E11W-29	Block 5A – Kampung Nias	
Root signs	RS-2	E11W-30	Block 5A – Kampung Nias	
Root signs	RS-2	3A-1	Compt 223 – Rasau Kuning	
Root signs	RS-2	3A-22	Compt 223 – Rasau Kuning	
Root signs	RS-2	3A-23	Compt 223 – Rasau Kuning	
Root signs	RS-2	3A-25	Compt 223 – Rasau Kuning	
Root signs	RS-2	3A-28	Compt 223 – Rasau Kuning	
Root signs	RS-2	3C-0	Compt 223 – Rasau Kuning	
Root signs	RS-2	3C-3	Compt 223 – Rasau Kuning	

Root signs	RS-2	3C-11	Compt 223 – Rasau Kuning	
Root signs	RS-2	3C-19	Compt 223 – Rasau Kuning	
Root signs	RS-2	3C-21	Compt 223 – Rasau Kuning	Isolation was failed
Root signs	RS-2	11A-37	Block 5A – Kampung Nias	
Root signs	RS-2	11A-40	Block 5A – Kampung Nias	
Root signs	RS-2	11B-0	Block 5A – Kampung Nias	
Root signs	RS-2	11B-2	Block 5A – Kampung Nias	
Root signs	RS-2	11B-3	Block 5A – Kampung Nias	
Root signs	RS-2	11B-4	Block 5A – Kampung Nias	
Root signs	RS-2	11B-5	Block 5A – Kampung Nias	
Root signs	RS-2	11B-6	Block 5A – Kampung Nias	
Root signs	RS-2	11B-9	Block 5A – Kampung Nias	
Root signs	RS-2	11B-11	Block 5A – Kampung Nias	
Root signs	RS-2	11B-12	Block 5A – Kampung Nias	
Root signs	RS-2	11B-13	Block 5A – Kampung Nias	
Root signs	RS-2	11B-16	Block 5A – Kampung Nias	
Root signs	RS-2	11B-18	Block 5A – Kampung Nias	
Root signs	RS-2	11B-20	Block 5A – Kampung Nias	
Root signs	RS-2	11B-21	Block 5A – Kampung Nias	Isolation was failed
Root signs	RS-2	11B-22	Block 5A – Kampung Nias	
Root signs	RS-2	11C-0	Block 5A – Kampung Nias	
Root signs	RS-2	11C-1	Block 5A – Kampung Nias	
Root signs	RS-2	11C-2	Block 5A – Kampung Nias	
Root signs	RS-2	11C-6	Block 5A – Kampung Nias	
Root signs	RS-2	11C-12	Block 5A – Kampung Nias	
Root signs	RS-2	11C-14	Block 5A – Kampung Nias	
Root signs	RS-2	11C-18	Block 5A – Kampung Nias	
Root signs	RS-2	11C-27	Block 5A – Kampung Nias	
Root signs	RS-2	11C-29	Block 5A – Kampung Nias	
Root signs	RS-2	11C-32	Block 5A – Kampung Nias	
Root signs	RS-2	11C-35	Block 5A – Kampung Nias	
Root signs	RS-2	11C-36	Block 5A – Kampung Nias	
Root signs	RS-2	11C-40	Block 5A – Kampung Nias	
Root signs	RS-3	E1W-1	Compt 246 – Rasau Kuning	
Root signs	RS-3	E1W-2	Compt 246 – Rasau Kuning	Isolation was failed
Root signs	RS-3	E1W-3	Compt 246 – Rasau Kuning	
Root signs	RS-3	E3W-7	Compt 223 – Rasau Kuning	
Root signs	RS-3	E4W-8	Compt 173 – Rasau Kuning	
Root signs	RS-3	E5W-9	Compt 175 – Rasau Kuning	Isolation was failed
Root signs	RS-3	E5W-10	Compt 175 – Rasau Kuning	
Root signs	RS-3	E6W-11	Compt 236 – Rasau Kuning	
Root signs	RS-3	E6W-12	Compt 236 – Rasau Kuning	
Root signs	RS-3	E6W-13	Compt 236 – Rasau Kuning	
Root signs	RS-3	E9W-27	Compt 063 B – Rasau Kuning	
Root signs	RS-3	E9W-28	Compt 063 B – Rasau Kuning	
Root signs	RS-3	E11W-31	Block 5A – Kampung Nias	Isolation was failed
Root signs	RS-3	Am8W-32	Compt 063 A – Rasau Kuning	
Root signs	RS-3	3A-0	Compt 223 – Rasau Kuning	
Root signs	RS-3	3A-7	Compt 223 – Rasau Kuning	
Root signs	RS-3	3A-11	Compt 223 – Rasau Kuning	

Root signs	RS-3	3A-29	Compt 223 – Rasau Kuning	
Root signs	RS-3	3A-36	Compt 223 – Rasau Kuning	
Root signs	RS-3	3B-0	Compt 223 – Rasau Kuning	
Root signs	RS-3	3B-14	Compt 223 – Rasau Kuning	
Root signs	RS-3	3B-21	Compt 223 – Rasau Kuning	
Root signs	RS-3	3B-28	Compt 223 – Rasau Kuning	
Root signs	RS-3	3C-5	Compt 223 – Rasau Kuning	
Root signs	RS-3	3C-8	Compt 223 – Rasau Kuning	
Root signs	RS-3	3C-10	Compt 223 – Rasau Kuning	
Root signs	RS-3	3C-29	Compt 223 – Rasau Kuning	
Root signs	RS-3	3C-40	Compt 223 – Rasau Kuning	
Root signs	RS-3	6A-0	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-2	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-11	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-20	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-22	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-23	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-24	Compt 236 – Rasau Kuning	
Root signs	RS-3	6A-27	Compt 236 – Rasau Kuning	
Root signs	RS-3	6B-0	Compt 236 – Rasau Kuning	
Root signs	RS-3	6B-1	Compt 236 – Rasau Kuning	
Root signs	RS-3	6B-6	Compt 236 – Rasau Kuning	Isolation was failed
Root signs	RS-3	6B-8	Compt 236 – Rasau Kuning	
Root signs	RS-3	6B-11	Compt 236 – Rasau Kuning	
Root signs	RS-3	6B-24	Compt 236 – Rasau Kuning	
Root signs	RS-3	6B-39	Compt 236 – Rasau Kuning	
Root signs	RS-3	6C-0	Compt 236 – Rasau Kuning	Isolation was failed
Root signs	RS-3	6C-10	Compt 236 – Rasau Kuning	
Root signs	RS-3	6C-23	Compt 236 – Rasau Kuning	
Root signs	RS-3	6C-25	Compt 236 – Rasau Kuning	
Root signs	RS-3	6C-30	Compt 236 – Rasau Kuning	
Root signs	RS-3	6C-31	Compt 236 – Rasau Kuning	
Root signs	RS-3	6C-38	Compt 236 – Rasau Kuning	
Root signs	RS-4	10A-38	Block 1A – Bunut	
Root signs	RS-4	11A-1	Block 5A – Kampung Nias	
Root signs	RS-4	11A-4	Block 5A – Kampung Nias	
Root signs	RS-4	11A-5	Block 5A – Kampung Nias	Isolation was failed
Root signs	RS-4	11A-29	Block 5A – Kampung Nias	
Root signs	RS-4	11A-30	Block 5A – Kampung Nias	
Root signs	RS-4	11A-32	Block 5A – Kampung Nias	
Root signs	RS-5	E1W-4	Compt 246 – Rasau Kuning	
Root signs	RS-5	E2W-5	Compt 250 – Rasau Kuning	
Root signs	RS-5	E11W-29	Block 5A – Kampung Nias	
Root signs	RS-5	E11W-38	Block 5A – Kampung Nlas	
Root signs	RS-5	11A-6	Block 5A – Kampung Nias	
Root signs	RS-5	11A-19	Block 5A – Kampung Nias	
Root signs	RS-5	11B-24	Block 5A – Kampung Nias	
Root signs	RS-5	11B-25	Block 5A – Kampung Nias	
Root signs	RS-5	11B-26	Block 5A – Kampung Nias	
Root signs	RS-5	11B-29	Block 5A – Kampung Nlas	

Root signs	RS-5	11B-30	Block 5A – Kampung Nias	
Root signs	RS-5	11B-33	Block 5A – Kampung Nias	
Root signs	RS-5	11B-35	Block 5A – Kampung Nias	
Root signs	RS-5	11B-38	Block 5A – Kampung Nias	
Root signs	RS-5	11C-5	Block 5A – Kampung Nias	
Root signs	RS-5	11C-10	Block 5A – Kampung Nias	
Root signs	RS-5	11C-34	Block 5A – Kampung Nias	

Material	Type of material	Root Signs	Material code	Compartment	Molecular ID
Cultures	Ph.1	-	**E8548**	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.1	RS-1	10A-0	Block 1A – Bunut	Unidentified
Cultures	Ph.1	RS-1	10A-9	Block 1A – Bunut	Unidentified
Cultures	Ph.1	RS-1	10A-10	Block 1A – Bunut	Unidentified
Cultures	Ph.1	RS-1	10A-11	Block 1A – Bunut	Unidentified
Cultures	Ph.1	RS-1	10A-21	Block 1A – Bunut	Unidentified
Cultures	Ph.1	RS-2	11B-20	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.2	-	**E8546**	Block 1A – Kampung Nias	Unidentified
Cultures	Ph.2	-	**E8548**	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.2	RS-1	10A-1	Block 1A – Bunut	Unidentified
Cultures	Ph.2	RS-1	10A-15	Block 1A – Bunut	Unidentified
Cultures	Ph.2	RS-1	10A-24	Block 1A – Bunut	Unidentified
Cultures	Ph.2	RS-2	11C-6	Block 5A – Rasau Kuning	*Inonotus* aff.pachyphloeus
Cultures	Ph.2	RS-2	11C-27	Block 5A – Rasau Kuning	*Phellinus noxius*
Cultures	Ph.3	-	**E8543**	Compt 236 – Rasau Kuning	Unidentified
Cultures	Ph.3	-	**E8540**	Block 1A – Bunut	*Phellinus* group
Cultures	Ph.3	-	**E8544**	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	E10W-34	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-2	E11W-29	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.3	RS-2	E11W-30	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.3	-	**E8541**	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.3	RS-1	10A-6	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	10A-8	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	10A-14	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	10A-15	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	10A-27A	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	10A-28	Block 1A – Bunut	Unidentified
Cultures	Ph.3	RS-1	10A-30	Block 1A – Bunut	*Phellinus* group
Cultures	Ph.3	RS-2	11B-0	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.3	RS-2	11B-2	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.3	RS-2	11B-11	Block 5A – Kampung Nias	*Phellinus* group
Cultures	Ph.3	RS-2	11C-32	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.3	RS-2	11C-36	Block 5A – Kampung Nias	*Phellinus* group
Cultures	Ph.3	RS-2	11C-39	Block 5A – Kampung Nias	*Phellinus* group
Cultures	Ph.4	RS-3	E6W-11	Compt 236 – Rasau Kuning	*Phellinus noxius*
Cultures	Ph.4	-	**E8543**	Compt 236 – Rasau Kuning	Unidentified
Cultures	Ph.4	RS-3	E9W-27A	Compt 063B – Rasau Kuning	Unidentified
Cultures	Ph.4	RS-1	E10W-36	Block 1A – Bunut	Unidentified
Cultures	Ph.4	-	**E8548**	Block 5A – Kampung Nias	Unidentified

Cultures	Ph.4	RS-5	E11W-29	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.4	RS-5	E11W-30	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.4	RS-2	3C-0	Compt 223 – Rasau Kuning	Unidentified
Cultures	Ph.4	RS-1	10A-17	Block 1A – Bunut	Unidentified
Cultures	Ph.4	RS-1	10A-27B	Block 1A – Bunut	*Phellinus noxius*
Cultures	Ph.4	RS-4	10A-38	Block 1A – Bunut	Unidentified
Cultures	Ph.4	RS-5	11B-29	Block 1A – Kampung Nias	*Phellinus noxius*
Cultures	Ph.4	RS-2	11C-0	Block 1A – Kampung Nias	Unidentified
Cultures	Ph.4	RS-2	11C-1	Block 1A – Kampung Nias	Unidentified
Cultures	Ph.5	RS-5	E2W-5	Compt 250 – Rasau Kuning	Unidentified
Cultures	Ph.5	RS-2	E7W-26	Compt 071 – Rasau Kuning	Unidentified
Cultures	Ph.5	RS-1	E10W-33	Block 1A – Bunut	*Phellinus noxius*
Cultures	Ph.5	RS-5	E11W-29	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.5	RS-1	10A-30	Block 1A – Kampung Nias	Phellinus group
Cultures	Ph.5	RS-2	11B-5	Block 5A – Kampung NIas	Phellinus group
Cultures	Ph.5	RS-2	11B-6	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.5	RS-2	11C-2	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.6	RS-1	10A-15	Block 1A – Bunut	Unidentified
Cultures	Ph.6	RS-2	11B-12	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.6	RS-2	11B-22	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.6	RS-5	11C-10	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.6	RS-2	11C-12	Block 5A – Kampung Nias	*Phellinus noxius*
Cultures	Ph.6	RS-2	11C-14	Block 5A – Kampung Nias	Unidentified
Cultures	Ph.7	-	**E8543**	Compt 236 – Rasau Kuning	Unidentified
Cultures	Ph.7	-	**E8541**	Block 2C – Rasau Kuning	Unidentified
Cultures	Ph.7	RS-2	3C-3	Compt 223 – Rasau Kuning	Unidentified
Cultures	Ph.7	RS-2	11B-13	Block 5A – Kampung Nias	*Phellinus* group
Cultures	Gd.1	RS-3	Am8W-32	Compt 063A – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	E1W-1	Compt 246 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	E9W-28	Compt 063B – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	3C-8	Compt 223 – Rasau Kuning	*G. australe* group
Cultures	Gd.1	RS-2	3C-11	Compt 223 – Rasau Kuning	Unidentified
Cultures	Gd.1	RS-3	6A-20	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	6A-22	Compt 236 – Rasau Kuning	Unidentified
Cultures	Gd.1	RS-3	6A-24	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	6B-24B	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	6C-23	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	6C-31	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.1	RS-3	6C-38	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.2	-	**E8538**	Compt 236 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.2	RS-3	3B-0	Compt 223 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.2	RS-3	3C-29	Compt 223 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.2	RS-3	3C-40	Compt 223 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.2	RS-3	6C-10	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.2	RS-3	6C-25	Compt 236 – Rasau Kuning	Unidentified
Cultures	Gd.2	RS-3	6C-30	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	E6W-12	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	E6W-13A/B	Compt 236 – Rasau Kuning	*Ganoderma philippii*

Cultures	Gd.3	RS-3	3A-7	Compt 223 – Rasau Kuning	*G. australe* group
Cultures	Gd.3	RS-3	3A-11	Compt 223 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.3	RS-3	3B-28	Compt 223 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.3	RS-3	6A-2	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	6A-23	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	6B-0	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	6B-1	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	6B-8	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.3	RS-3	6B-24A	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-3	E1W-3	Compt 246 – Rasau Kuning	Unidentified
Cultures	Gd.4	-	**E8539**	Compt 223 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.4	RS-3	E5W-10	Compt 175 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-2	E7W-25	Compt 071 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-3	3A-0	Compt 223 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.4	RS-2	3A-22	Compt 223 – Rasau Kuning	Unidentified
Cultures	Gd.4	RS-3	3A-36	Compt 223 – Rasau Kuning	G. australe group
Cultures	Gd.4	RS-3	3B-14	Compt 223 – Rasau Kuning	G. australe group
Cultures	Gd.4	RS-3	3B-21	Compt 223 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-3	6A-0	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-3	6A-11	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-3	6B-11	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.4	RS-3	6B-39	Compt 236 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.5	RS-3	E9W-27	Compt 063B – Rasau Kuning	*Amauroderma/Ganoderma* sp
Cultures	Gd.5	RS-3	3C-10	Compt 223 – Rasau Kuning	*Ganoderma philippii*
Cultures	Gd.5	RS-3	6A-27	Compt 236 – Rasau Kuning	Unidentified
Cultures	Gd.6	-	**E8542**	Compt 063A – Rasau Kuning	Unidentified
Cultures	Gd.6	-	**E8551**	Compt 223 – Rasau Kuning	*G. subresinosum*
Cultures	Gd.6	-	**E8539**	Compt 236 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.6	RS-3	3C-5	Compt 223 – Rasau Kuning	*G. mastoporum*
Cultures	Gd.6	RS-1	10A-37	Block 1A – Bunut	Unidentified
Cultures	Non Target	RS-2	11C-29	Block 5A – Kampung Nias	Gymnopilus sp 1
Cultures	Non Target	RS-2	3A-25	Compt 223 – Rasau Kuning	*Hypocreales* (contaminant?)
Cultures	Non Target	RS-5	11B-38 (b)	Block 5A – Kampung Nias	Unidentified
Cultures	Non Target	RS-2	3A-1	Compt 223 – Rasau Kuning	Unidentified
Cultures	Non Target	RS-2	3C-19	Compt 223 – Rasau Kuning	Unidentified
Cultures	Non Target	RS-2	11A-37	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-2	11B-16	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-2	11B-3	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-2	11B-9	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-2	11C-18	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-2	11C-35	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-2	3A-23	Compt 223 – Rasau Kuning	Aff. *Neonothopanus nambi*
Cultures	Non Target	RS-2	3C-21 (b)	Compt 223 – Rasau Kuning	Aff. *Neonothopanus nambi*
Cultures	Non Target	RS-5	11B-38 (a)	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-2	11B-18	Block 5A – Kampung Nias	*Basidiomycete* sp. 3
Cultures	Non Target	RS-2	11B-4	Block 5A – Kampung Nias	*Cerrena* sp
Cultures	Non Target	RS-2	3A-28	Compt 223 – Rasau Kuning	Unidentified

Cultures	Non Target	RS-3	E3W-7	Compt 223 – Rasau Kuning	*Gymnopilus* sp. 1
Cultures	Non Target	RS-3	E4W-8	Compt 173 – Rasau Kuning	Unidentified
Cultures	Non Target	RS-3	3A-29	Compt 223 – Rasau Kuning	Aff. *Neonothopanus nambi*
Cultures	Non Target	RS-4	11A-30	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-4	11A-32	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-4	11A-4	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-4	11A-1	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-4	11A-29	Block 5A – Kampung Nias	Unidentified
Cultures	Non Target	RS-5	11C-5	Block 5A – Kampung Nias	Unidentified
Cultures	Non Target	RS-5	E11W-38	Block 5A – Kampung Nias	Unidentified
Cultures	Non Target	RS-5	E1W-4	Block 5A – Kampung Nias	Zygomycete
Cultures	Non Target	RS-2	11A-0	Block 5A – Kampung Nias	Unidentified
Cultures	Non Target	RS-5	11A-19	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-2	11A-40	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-5	11A-6	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-5	11C-34	Block 5A – Kampung Nias	*Phlebiopsis* sp. 1
Cultures	Non Target	RS-5	11B-25	Block 5A – Kampung Nias	Aff. *Lenzites elegans*
Cultures	Non Target	RS-5	11B-26	Block 5A – Kampung Nias	Aff. *Lenzites elegans*
Cultures	Non Target	RS-5	11B-24	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-5	11B-30	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-5	11B-33	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*
Cultures	Non Target	RS-5	11B-35	Block 5A – Kampung Nias	Aff. *Tinctoporellus epimiltinus*

Chapter 3 - Assessing crown condition in *E. pellita*: Applicability for root-rot detection

3.1 Introduction

3.1.1 Crown condition, tree health, forest health and Forest Health Surveillance

The condition of tree crowns relates directly to the productivity of those trees as it is the crown that converts solar radiation into the photosynthate required by the trees for growth and proper functioning (Schomaker, Zarnoch *et al.* 2007). If one defines health as "The general condition of the body with respect to the efficient or inefficient discharge of functions" (Anonymous 2009) then for a tree planted to produce as much pulp as possible, it may be considered healthiest in that state which best facilitates this. Thus a dead or dying tree is definitely considered unhealthy. Health has also been defined as the absence of diseases (Haskell, Norton *et al.* 1992). Disease can be defined as a "deviation in the normal functioning of a plant caused by some type of persistent agent" (Manion 1991). In this context, the health of a tree can be evaluated by several indicators such as crown condition, growth rate, and any visual signs of disease causal agents (Kolb, Wagner *et al.* 1994). These definitions allow the idea of health to be applied to forest ecosystems at several scales ranging from individual trees to the landscape level, based either on the productivity of the forest or the absence of disease.

Judgment of the health of a stand does not, however, necessarily require that *all* trees in the stand be healthy (Kolb, Wagner *et al.* 1994). From a stand perspective, tree mortality does not automatically indicate that the *stand* is unhealthy, provided that the rate of mortality is not greater than the capacity of the

stand for replacement, either by new trees or compensatory growth by surviving trees.

The health of a forest ecosystem is a complex idea to define and is determined by both societal objectives and the interaction of biotic and abiotic processes (Kolb, Wagner *et al.* 1994). Forest health is defined subjectively depending on a range of uses from purely commercial plantations to undisturbed native forests (Old, Coops *et al.* 1999).

The different interpretations of what is meant by forest health have influenced the approaches taken to monitoring forests (Stone and Haywood 2006). Commercial forest owners tend to associate forest health with those agents or processes that potentially reduce tree productivity – this is the definition with most applicability to the current study; in non-commercial forest sectors, forest health surveillance is viewed as a means of tracking the protection of biodiversity and conservation of ecosystem processes (Stone, Old *et al.* 2001).

Even though the term forest health is being increasingly used in forestry and natural resource management, in many cases, "forest health" is used without a clear definition (Kolb, Wagner *et al.* 1994) and often synonymously with the term "forest condition" (Percy and Ferretti 2004). Forest condition however has a broader meaning than forest health. Forest health has commonly been used to describe the degree to which pests and diseases potentially disrupt the normal processes of the trees; while forest condition is usually applied in relation to the descriptive indicators used in routine forest assessments (Percy 2002). Thus, forest condition might be described as poor if silvicultural management has had a negative impact on biodiversity even though the health of the trees remains excellent.

3.1.2 Forest-health surveillance in Indonesia

In Australia as well as in New Zealand, USA and some European countries, systematic surveys to detect and map biotic damage are referred to as forest health surveillance (Carnegie 2008). The term monitoring is reserved for the regular inspection of a particular health problem and a detailed assessment of the development of this problem so that intervention can prevent further damage.

In Indonesia, forest-health surveillance is not yet routinely applied in either native forests or plantations. During 1996 – 2001, an ITTO (*International Timber Trade Organisation*) project (PD 16/95 Rev.2 [F]) in collaboration with the USDA (*United States Department of Agriculture*) Forest Service attempted to develop a forest health surveillance system linked to the sustainability of Indonesian tropical rain forest. This project was mainly focused on tree productivity, biodiversity, and site quality (Putra, Sutisna *et al.* 2001; Soekotjo and Sutisna 1997; Supriyanto, Soekotjo *et al.* 2001; Sutisna, Putra *et al.* 2001). However, the outcomes of this project have never been applied in Indonesian forestry. Forest-health surveillance is still considered a minor issue by the Indonesian Government (M.F. Fahada 2009, pers.comm). Despite the rapid expansion of Indonesia's plantation forests to meet increasing demand for timber, pulp and paper, there is no published information that describes any programs for the assessment of forest health, or reports the long-term trends in their health status.

3.1.3 Crown condition as an indicator of forest health

The most common indicator for forest health is tree health (Innes 1993) which is usually indicated by crown condition (Stone and Haywood 2006; Zarnoch, Bechtold *et al.* 2004). Large dense crowns are associated with vigorous growth rates, while trees with sparsely foliated crowns and/or showing little or no growth are probably in a state of decline (Zarnoch, Bechtold *et al.* 2004). Several methods have been used to assess crown condition. Visual estimation is one widely used approach where the surveys are carried out by air, roadside drive-bys or ground inspections (Innes 1993; Stone, Coops *et al.* 2000). More objective and repeatable methods that employ reference photographs, digital image-analysis (Mizoue and Masutani 2003; Redfern and Boswell 2004), and systematic procedures to assess and monitor crown damage (Stone, Matsuki *et al.* 2003) reduce subjectivity and improve data quality.

Redfern and Boswell (2004) used two standard reference photographs for each tree species. One was the absolute standard that represented the ideal tree for a species. The second represented a reference tree that carried the maximum amount of foliage under growing conditions in a specific locality. These reference photographs were used to standardise the assessment of crown density or transparency when viewed against the sky (Redfern and Boswell 2004). A semi-automatic image-analysis system – CROCO (i.e. CROwn Condition) assesses crown transparency from photographs (Mizoue 2002; Mizoue and Dobbertin 2003). Hemispherical digital images that use a fish-eye lens attached to a digital camera have been used to quantify changes to the crown associated with *Phythopthora cinnamomi* infestation in *Banksia* shrubland, *Banksia* woodland and *Eucalyptus marginata* Donn Ex Sm. forest biomes in Western Australia (Crane and

Shearer 2007). The Crown Damage Index was developed in Australia (Stone, Matsuki *et al.* 2003) for young eucalypts to provide a standardised, repeatable and statistically valid measure of pest and disease damage so that quantitative comparisons can be made irrespective of the cause of the damage or site.

Improvements in sensor capabilities on both airborne and satellite platforms have made these remote-sensing techniques an attractive option for forest monitoring and for the acquisition of spatially explicit data that permit the integration of forest inventory and health assessments (Carnegie 2008; Goodwin, Coops *et al.* 2005; Johnson and Wittwer 2008; Stone, Turner *et al.* 2008). Although remote sensing can provide frequent temporal and spatial monitoring across large areas (Stone *et al.* 2008), ground-based assessments at the individual tree level are still required to validate and interpret data.

3.1.4 Crown condition as an indicator for root rot

Much research has been directed towards understanding the effect of environmental stresses, pests and diseases on the tree crown (Edgar, Kile *et al.* 1976; Fox and Curry 1980; Podger 1972; Vollenweider and Gunthardt-Goerg 2006; Zarnoch, Bechtold *et al.* 2004). Only a few studies have sought to examine the relationship between crown symptoms and root condition, and these have yielded variable results (Omdal, Shaw *et al.* 2004). Several above-ground indicators, i.e. standing dead trees, dead and downed trees, shortened internodes and discoloured foliage, were statistically reliable for estimating the total number of infected Douglas-fir trees growing in an area occupied by *Phellinus weirii* on southern Vancouver Island, British Columbia (Wallis and Bloomberg 1981). Foliage chlorosis, dieback and wilting have been observed in crowns of

Eucalyptus grandis Hill Ex Maiden trees that were infected with *Ganoderma sculpturatum* (Lloyd) Ryvarden. (Kile 2000). Filip (1986), who surveyed crown and root-collar symptoms of three species of conifer (Douglas fir, Grand fir, and Ponderosa pine) that were infected by *Armillaria ostoyae* (Romagn.) Herink., *Heterobasidion annosum* (Fr.) Bref. or *Phellinus weirii* (Murr.) Gilbertson in central Washington State, reported that root disease was very difficult to detect above ground in trees with less than 30% of the root system decayed. This relationship between severity of root-rot disease and crown symptoms is rarely investigated or quantified.

Plantations of *Eucalyptus pellita* F. Muell. are now being developed as a major wood source for the pulp and paper industry in Indonesia. A surveillance system could provide data to help develop strategies to better manage pest and diseases and prevent their build up, such as has been the experience with root rot in *Acacia mangium* Willd plantations. This study took the first step towards health surveillance by developing a system to visually estimate the crown condition of *E. pellita*. The method was developed on sites with a history of root-rot disease in the previous rotations of *A. mangium* and where *E. pellita* also appeared to be attacked by root-rot fungi. The desired outcome was to link crown condition to a known disease affecting tree and stand health.

3.1.5 Research objectives

The research was conducted with following objectives:

1) To develop a method for assessing the crown condition of *E. pellita* based on crown characteristics potentially indicative of tree health. The repeatability, reproducibility and reliability of the method were examined. **Repeatability** is defined as "within observer" error; that is, the variation that occurs among

measurements made by the same observer. **Reproducibility** is "between observer" error, and it is usually traced to differences among observers who obtain different measurements while using the same gauge (Smith, Mc Crary *et al.* 2007). **Reliability** is the capacity of product, system or method to perform their required function (relatively) without failure, in specified environments and with a desired confidence, over a period of observation (Kececioglu 1991);

2) To determine if any correlation can be found between crown condition as assessed by the crown indicators and root-rot severity.

3.2 Materials & Methods

3.2.1 Study area

The crown condition of trees was surveyed in the first week of June 2008 (survey I), the fourth week of October 2008 (survey II) and the third week of February 2009 (survey III) in three stands of *E. pellita* located at three different sites (Table 3.1). All stands were affected by root-rot diseases (see Chapter 2). Three plots were established in Compartments 223 and 236 at Rasau Kuning and a single plot in Block 1A at Bunut. The plots are on the same soil type, that is well-drained, red-yellow podsolic soil with a loamy-sand to sandy texture. Detailed information about these three sites is provided in Table 3.1.

Table 3.1. Description of the monitored sites

Site	Plot	GPS point	Planting History	Age
Compt.223-Rasau Kuning	A	E: 101 36.666 N: 0 44.567	The 1st rotation of EP05 (4th rotation after *A. mangium*). This site was badly affected by root rot when it was planted to *A. mangium*.	1.5 years old. (planted in Jan 2007)
	B	E: 101 36.661 N: 0 44.615		
	C	E: 101 36.660 N: 0 44.665		
Compt.236-Rasau Kuning	A	E: 101 34.140 N: 0 44.985	The1st rotation of EP05 (5th rotation after *A. mangium*)	1 year 9 months old (planted in Oct 2006).
	B	E: 101 34.117 N: 0 44.982		
	C	E: 101 34.100 N: 0 44 985		
Block 1A-Bunut	A	E: 101 36.642 N: 0 41.549	Demonstration plot of EP05. Planted on ex-contractor site with some *A. mangium*, before ex-rubber.	5.5 years old. (planted in Jan 2003).

Note: EP05 is a clone of E. pellita

3.2.2 Sampling strategy

In survey I, trees to be assessed for both crown condition and root-rot severity were sampled across a series of transects positioned to intersect at the first tree killed in a disease centre (Fig. 3.1). Disease centres are gaps in the plantation formed by tree death. If the gap originates from a point source of infection and spreads outwards from there, as is common in root rot (Irianto, Barry *et al.* 2006; Shaw, Stage *et al.* 1991) the dead tree with the smallest diameter should be, everything else being equal, the one killed first and the tree closest to the original inoculum. By finding this tree and centering the system of transects on this tree, the design aimed to minimise the number of trees to be sampled yet include as many trees as possible with different stages of disease development. For this design to be the most effective, the pathogen would need to spread outwards uniformly from the initial inoculum. The total number of sampled trees in this survey was 287 trees (i.e. 41 trees on each of seven plots). Eighty of them were missing or dead due to root-rot infection as confirmed through root-system

excavation. It was envisaged that this sampling strategy would provide data appropriate for developing a crown scoring methodology and also testing for correlation between crown condition and root-rot incidence and severity.

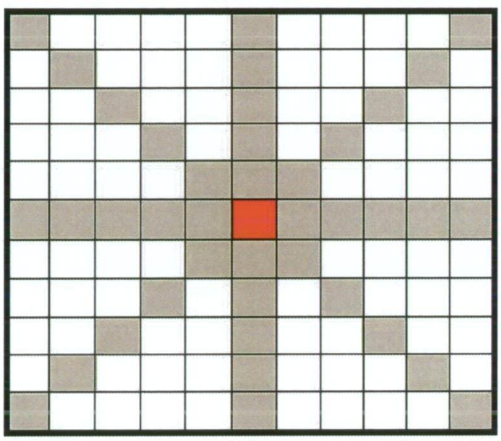


Figure 3.1. Survey I sampling design. The tree represented by the red square is the "disease centre". Olive squares represent trees in one of the four intersecting transects.

Surveys II and III, which were aimed to test the reliability of the crown scoring system that had been developed during survey I, were expanded to assess all trees on the monitored plots (121 trees on each of the 7 plots i.e. 890 trees in total). The proportion of dead or missing trees observed during these repeated surveys, 37% (327 trees) and 40% (354 trees) in Survey II and Survey III respectively, were higher than that observed in Survey I (28%).

3.2.3 Discriminant analysis

Discriminant analysis (DA) was used to address the specific aims of the following sections 3.2.4.2 (method reliability); 3.2.5 (refinement and reanalysis of the crown scoring system); 3.2.6 (aboveground indicators as indicators of root-rot).

Discriminant analysis (DA) is a statistical technique for assessing the variables distinguishing groups in an *a priori* classification and classifying observations into the groups of this classification based on a series of predictors or variables through a three-step process:

- forming and testing the significance of a set of non-correlated discriminant functions or linear combinations of the variables where values are as similar as possible within the *a priori* groups and as dissimilar as possible between them,
- indicating to which of the *a priori* groups each observation would be expected to belong based on the observed predictor variables and these discriminant functions. The functions can also be used to predict which class / category new individuals most likely belong based on their values for the observed variables (Klecka 1980),
- comparison of the match between the *a priori* group membership and group membership expected by application of the discriminant functions. Observations where the expected group matches the *a priori* group may be considered 'correctly classified'. This provides an indication of how well the measured variables can be used to determine the *a priori* group.

The relative importance of a particular variable in each discriminant function is interpreted by means of standardized coefficients (β) which are given for each variable in each discriminant or canonical function. The larger the standardized coefficient the greater the contribution of the respective variable to the discrimination between groups (Poulsen and French 2009). In addition, the between-group discrimination by each function can be identified by looking at the mean for the function across the group as represented by the centroid (Lachenbruch 1975). The capacity of DA and the data to classify the observations

into their *a priori* groups, is gauged by the number of observations that have been correctly classified over the total number of observations.

The DAs were run at significance level $\alpha = 0.05$ and equality of within-class covariance matrices was assumed. All the analyses used XLSTAT2009®.

3.2.4 Assessment of crown condition - development of scoring methodology

Prior to the assessment exercises, different crown conditions were observed in various stands, reference photos were taken (Fig. 3.2) and then sorted into classes that represented steps in the progression of trees from healthy to dead. These classes were then described (Table 3.2). This description became a reference for judging overall crown condition and assigning a crown condition class during Surveys I, II and III.

Apart from assessing crown-condition class based on the overall impression of crown condition (Table 3.2 and Fig. 3.2), the trees were assessed using several separate measured/estimated above-ground indicators, namely:

1. Crown dominance (dominant, co-dominant/sub-dominant, or suppressed converted into numerical categories – 3 was dominant, 2 co-dominant/subdominant, and 1 was suppressed).
2. Tree height (measured using a hypsometer – Suunto PM5/1520P™).
3. Diameter at breast height (DBH).
4. Live crown ratio (the ratio of the live crown [measured from the top of the tree to the lowest green branch] to the total tree height. This variable was expressed as a percentage of the tree height).
5. Crown density (visually estimated and expressed as a percentage of that on the reference tree for the site). Percentage of crown density was defined by

comparing each respective tree to the tree that appeared to be the most healthy and vigorous with good crown condition at the site (the reference tree).

6. Percentage of new and old foliage observed in the crown
7. Crown colour (estimated as three percentages % green, % yellow-green and % yellow foliage)

Table 3.2. Tree crown condition classes as illustrated by reference photographs in Figure 3.2.

Crown condition class	Description
0 (Dead)	Tree recently dead (Fig.3.2.A). Crown dead or missing
1 (Severely stressed)	Tree height mostly suppressed; sparse crown dominated by yellowing foliage and/or some epicormic growth (Fig.3.2.B).
2 (Moderately stressed)	Tree height partially suppressed with sparse crown. Epicormic shoots and flowers and/or fruit may be observed in response to stress (Fig.3.2.C).
3 (Lightly stressed)	Subdominant or co-dominant tree with moderate crown density and some yellow-green foliage (Fig.3.2.D).
4 (Healthy)	Subdominant or co-dominant tree with a dense crown (Fig.3.2.E).
5 (Perfectly healthy)	Dominant tree with very dense crown and green foliage (Fig.3.2.F).

Figure 3.2. Reference pictures of crown condition in *E. pellita*. (A) The crown was scored as "0"; (B) the crown was scored as "1"; (C) the crown was scored as "2"; (D) the crown was scored as "3"; (E) the crown was scored as "4"; and (F) the crown was scored as "5".

3.2.4.1 User repeatability and reproducibility tests

Repeatability and reproducibility tests were carried out in order to gauge the subjectivity of visual classification and estimations. Three assessors, who had different levels of experience in crown-condition scoring, independently assessed the crown condition of 16 trees at the Compt. 236 – Rasau Kuning district in the morning and in the afternoon of one day, a time interval considered short enough to minimise the chance that the condition of the tree might have changed. Assessor 1 who had been trained and had some previous experience in crown assessment of eucalypts, both in native and plantation forests was the standard-assessor in this

study against which the performance of the others was compared. Assessor 2 and Assessor 3 were trained during the study. Assessor 2, had had a short period of training prior to the initial survey, had more experience than Assessor 3 who only obtained the method description immediately before the repeatability and reproducibility tests were conducted. Record sheets were collected after each assessment and assessors were asked to work independently and without any exchange of information. In this study, repeatability was determined by comparing the data from the same assessor working at different times of observation; reproducibility was a comparison of data from different assessors for the same trees.

The consistency of each assessor on scoring the crown indicators during the morning and the afternoon assessments (repeatability) and the variation among assessors (reproducibility) were quantified by comparing the mean values (± SE) for all trees for each of the estimated variables with their standard errors and range of data. Differences between the mean results for condition class, % crown density, % new foliage, % yellow-green foliage and % yellow foliage estimated by different assessors and by individual assessors at different times were analysed statistically using one-way ANOVA at significance level $\alpha = 0.05$.

Data obtained by Assessor 1 were used as a standard for the crown scoring data. To gauge the effect of assessors, the mean of the differences between variable values estimated by the two other assessors were compared graphically to those estimated by the standard assessor for the two observation times.

3.2.4.2 Method reliability

Discriminant analysis was applied to the tree data sets of surveys to examine the reliability of the crown condition assessment method. Our method can be considered reliable if the same above-ground indicators consistently differentiate crown condition classes over successive assessments. The importance of each above-ground indicator in determining the observed trees into a particular crown-condition class is indicated by the standardized canonical discriminant function coefficients and the degree of visual separation of the class centroids (multi-dimensional means) on factor axes produced from the discriminant analyses. In these analyses, crown-condition class is set as the dependent variable, the seven continuous above-ground indicators, tree height, DBH, crown density, live crown ratio, and percentages of new, yellow and yellow-green foliage as quantitative explanatory variables, and three levels of tree dominance as a qualitative explanatory variable.

3.2.5 Refinement and reanalysis of the crown scoring system

Following preliminary statistical analyses, it appeared that the less trained assessors had difficulty distinguishing some of the crown-condition classes. To attempt to account for this the data from some of the classes were merged into new classes. Trees in crown-condition classes 'five' (perfectly healthy, dominant trees with very dense crowns and green foliage) and 'four' (healthy, subdominant or co-dominant trees with a dense green crown) were merged into category III (healthy). Trees in classes 'two' (moderately stressed, partially suppressed trees with sparse crowns and epicormic shoots and flowers and/or fruit present) and 'three' (lightly stressed, subdominant or co-dominant trees with moderate crown density and some

yellow-green foliage) were merged into category II (stressed). Trees in class 'one' (severely stressed, suppressed trees with sparse crowns dominated by yellowing foliage and/or some epicormic growth) were left as category I (severely stressed). The percentages of new, yellow-green and yellow foliage were excluded because these variables were considered to be the most difficult characteristics to score and thus the most prone to poor estimation. The merged and reduced data were analysed using discriminant analysis as for the unmerged data. The above-ground variables DBH, tree height, crown density and live crown ratio were used as quantitative explanatory variables and dominance was again made a qualitative explanatory variable with three levels. Class centroids (multi-dimensional means) on factor axes were produced from discriminant analyses. Confusion matrices for the estimation samples of the two discriminant analyses on the original and merged data were compared.

3.2.6 Aboveground indicators as indicators of root-rot

Root assessments were only carried out during survey I. Root-rot incidence and its severity were assessed by excavation of the root collar and primary lateral roots to a distance of 0.5 m around the tree and about 0.3 m depth. The number of lateral roots showing any sign of root rot and the total number of exposed lateral roots were recorded and the percentage of infected lateral roots determined.

The correlation between above-ground symptoms and root-rot severity was investigated by comparing the above-ground data to both the percentage of lateral roots infected and root-rot severity classes based on these percentages. There were four classes of root-rot severity, namely: (1) *totally infected* (100% of excavated roots infected) (2) *highly infected* (50 – 99% of excavated roots infected); (3)

partly infected (<50% of excavated roots infected); and (4) *healthy* (no infection visible on any exposed roots). Correlation between root-rot severity, both as percentage of lateral roots infected and severity classes based on this, and above-ground indicators was assessed by Spearman Correlation at significance level $\alpha = 0.05$. Analyses were carried out both with dead trees included and with them excluded.

Discriminant analysis was used to test the usefulness of above-ground crown characteristics as indicators for root-rot incidence and severity. Two analyses were carried out, one using root-rot incidence, either infected or healthy, as the dependant variable and the other using the root-rot severity classes defined above. Crown dominance was a qualitative explanatory variable with three levels and tree height, DBH, crown density, live crown ratio, and percentages of new, yellow-green and yellow foliage were qualitative explanatory variables.

3.3 Results

3.3.1 Method development for assessing crown condition

3.3.1.1 Repeatability and reproducibility of the method

For the repeatability ("within" assessor) test, there were no significant differences in values for the same trees assessed twice for all the crown indicators, except for the estimates of crown condition class and percentage of yellow foliage by Assessor-3 (Table 3.3). The reproducibility ("between" assessors) test showed that Assessor-3 generally gave a higher crown-condition class score than the other two assessors for the same tree. Assessor-3 also tended to give lower scores for new foliage and yellow foliage. Except for the percentage of yellow foliage of the

Assessor-3, mean differences among assessors for the reproducibility test were <10% (Fig. 3.3).

Table 3.3 Means (±SE) of the visual estimation for three assessors in the morning and afternoon.

Assessors	Time	Crown indicators				
		Condition class	% Crown density	% New foliage	% Yellow-green foliage	% Yellow foliage
1	AM	3.8 ± 0.14 ^{c}	71.9 ± 2.08 ^{a}	21.3 ± 2.02 ^{ab}	15.6 ± 2.58 ^{a}	11.3 ± 1.25 ^{a}
	PM	3.7 ± 0.14 ^{c}	71.3 ± 2.56 ^{a}	21.3 ± 2.21 ^{ab}	15.6 ± 2.73 ^{a}	11.9 ± 1.64 ^{a}
2	AM	3.9 ± 0.10 ^{bc}	70.6 ± 2.32 ^{a}	20.6 ± 2.13 ^{ab}	15.6 ± 2.03 ^{a}	11.9 ± 1.01 ^{a}
	PM	4.0 ± 0.11 ^{bc}	70.0 ± 2.42 ^{a}	23.8 ± 2.21 ^{a}	13.8 ± 2.21 ^{a}	10.6 ± 0.63 ^{a}
3	AM	4.2 ± 0.08 ^{b}	71.3 ± 2.39 ^{a}	17.5 ± 1.44 ^{b}	15.0 ± 2.24 ^{a}	6.9 ± 1.50 ^{b}
	PM	4..9 ± 0.09 ^{a}	71.9 ± 3.19 ^{a}	16.9 ± 1.51 ^{b}	19.4 ± 2.95 ^{a}	1.3 ± 1.25 ^{c}

Note:
The values followed with different letters within the same column are significantly different at significance level α = 0.05, as determined by ANOVA for each indicator separately.

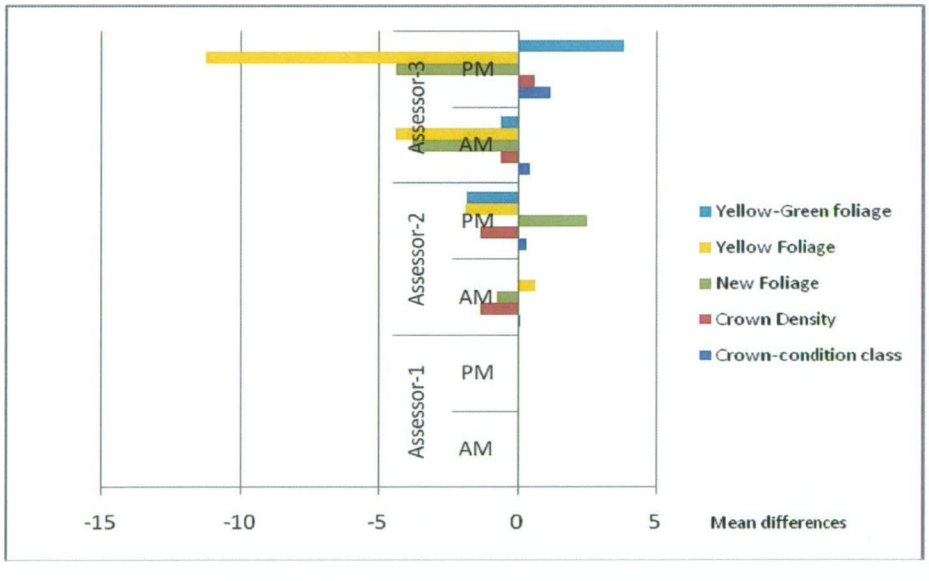


Figure 3.3. Mean differences between visual estimation for three assessors in the morning (AM) and afternoon (PM) assessments (the most experienced assessor, Assessor-1, is used as a standard)

3.3.1.2 Reliability of the method

The distribution of the class centroids (multi-dimensional means) on factor axes produced from the discriminant analyses is shown in Fig. 3.4. Five crown-condition classes were observed in Surveys I and II (Figs. 3.4.A, B), while Survey III only gives four crown-condition classes (Fig. 3.4.C). This is because the observers who conducted the last survey did not find any trees that were considered to be in class 5 (perfectly healthy crown). In Survey-I, crown condition classes 1, 2 and 3 were well-discriminated as indicated by the distances between the centroids relative to the spread of points, but that classes 4 and 5 were similar and overlapped to a much greater degree (Fig. 3.4.A). The second and the third surveys showed that the distance between the groups was less than in survey I and all classes overlapped to a much greater degree (Fig. 3.4.B, C).

A comparison of the influence of the above-ground variables on the first discriminant functions of each data set is presented in Table 3.4. These data can be used to indicate the consistency of different users in understanding and applying the scoring system. Survey-I and -II data showed that crown density ($\beta = 0.706$ and 0.903 in surveys I and II, respectively) and dominance-1 ($\beta =$ -0.634 and -0.502 in surveys I and II, respectively) had the greatest influence on crown-condition classification. In Survey-III, tree height and live crown ratio had the greatest influence on crown-condition classification ($\beta = 0.777$ and 0.501, respectively). Interestingly in survey III, the β value for crown density, which had most influenced crown-condition classification in surveys I and II, was only 0.393.

Table 3.4. The standardised canonical discriminant function coefficient (β) of above-ground indicators for the three surveys

Above-ground indicators	Standardised canonical (β) coefficient*		
	Survey I	Survey II	Survey III
Crown Dominance : 1	**-0.634**	**-0.502**	-0.095
2	0.000	0.000	0.000
3	-0.185	0.104	0.191
Height	-0.168	0.031	**0.777**
DBH	0.130	0.269	0.060
Crown density	**0.706**	**0.903**	0.393
Live crown ratio	0.260	-0.042	**0.501**
New foliage	0.194	-0.088	-0.001
Yellow-green foliage	-0.082	-0.163	0.020
Yellow foliage	0.176	0.315	0.175

Note:

* *The β values were taken from the first factor (F1) that represents 86.42%, 87.74% and 68.72% of the variances of the data sets in surveys I, II and III, respectively.*

** *The two variables having the most influence on the discriminant function are in bold.*

3.3.1.3 Refinement and reanalysis of the crown scoring system

Condensing the crown-condition classification from five into three classes and reducing the explanatory variables from eight to five resulted in better discrimination between classes (Fig. 3.5). This was confirmed by the higher percentage of trees that were correctly classified in the confusion matrix (Table 3.5).

Table 3.5. A summary of the confusion matrix for the original and merged scoring system.

Survey	% observed trees that have been correctly classified	
	The original system	*The merged system*
I	72.96	84.80
II	60.69	71.74
III	73.86	75.97

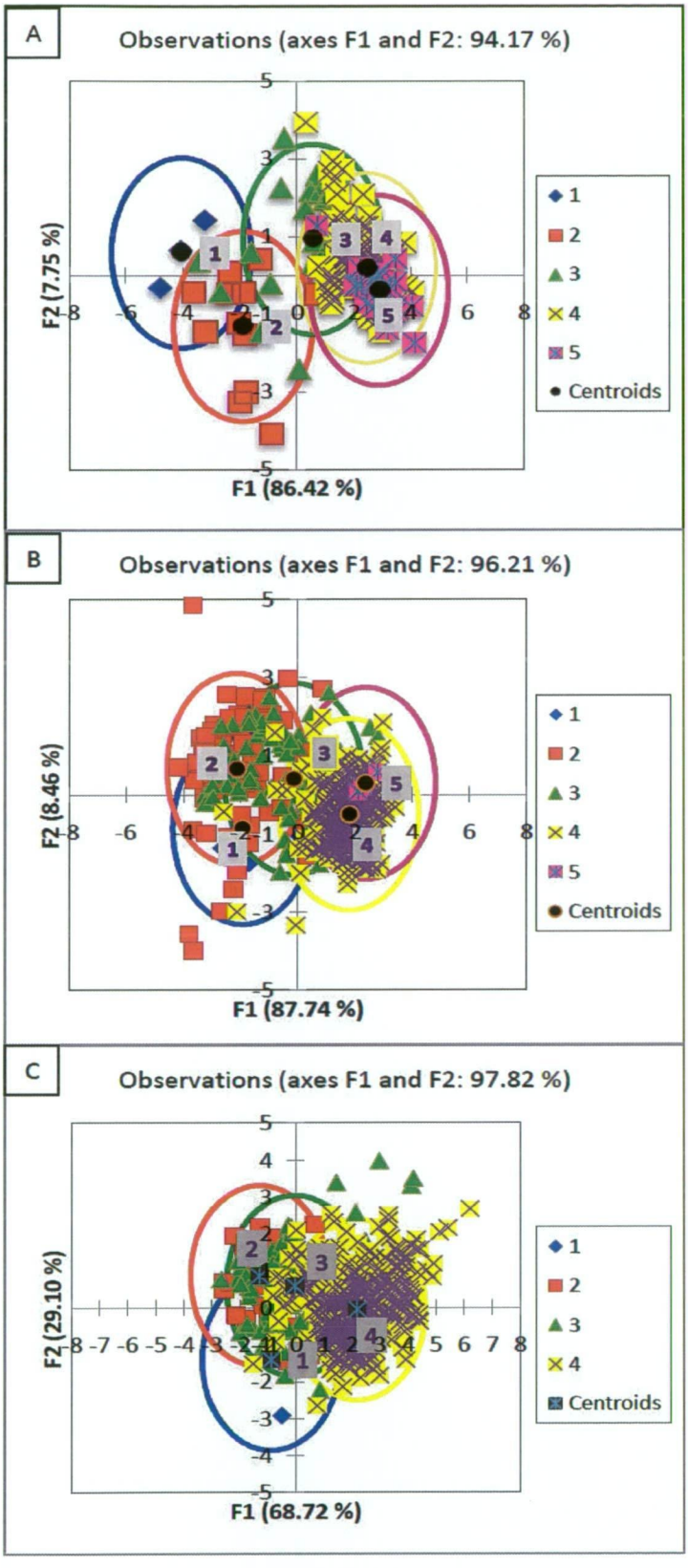


Figure 3.4 Discrimination between observed crown-condition classes when eight above-ground indicators are used as explanatory variables. (A) Survey I; (B) Survey II; and (C) Survey III.

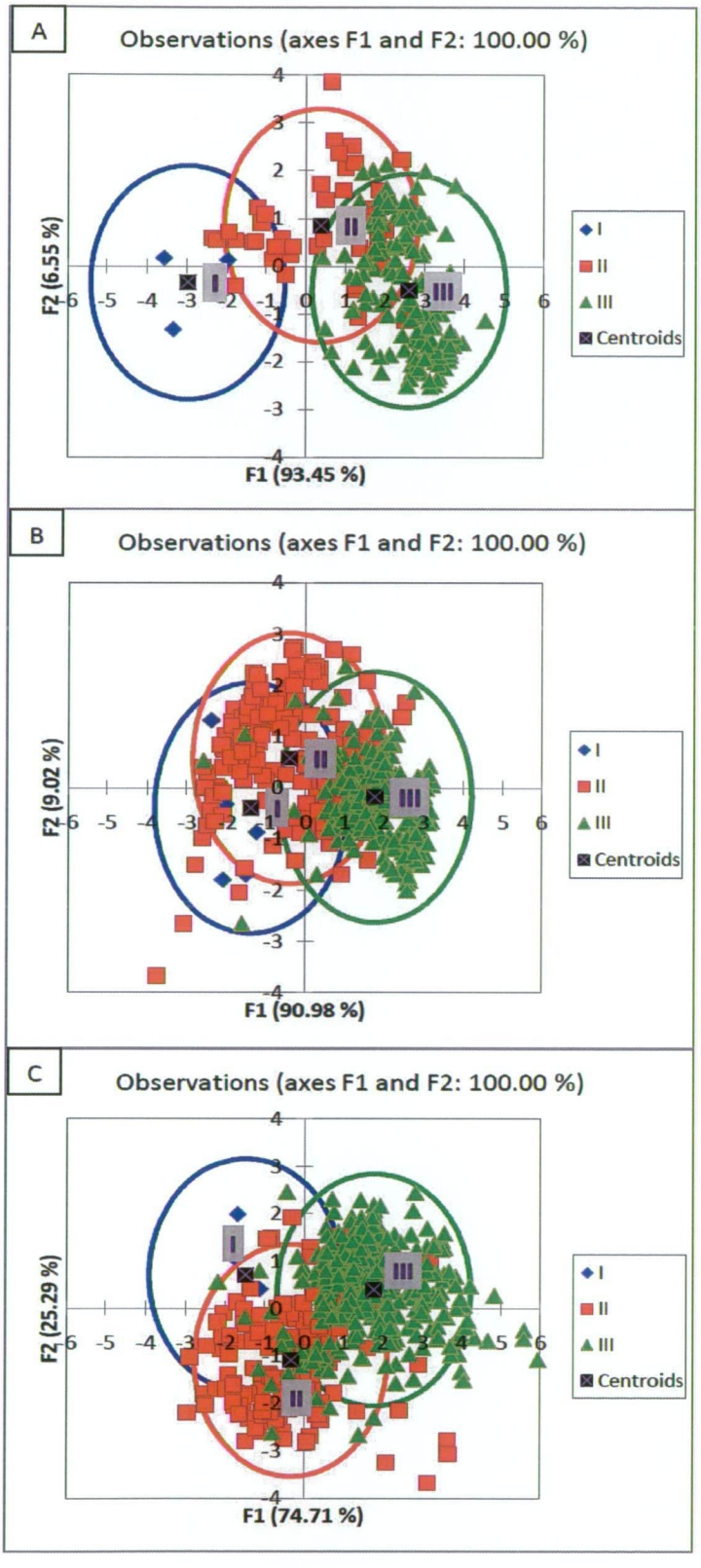


Figure 3.5. Discrimination between merged crown-condition classes when five above-ground indicators are used as explanatory variables. (A) Survey I; (B) Survey II; and (C) Survey III.

3.3.2 Crown condition as an indicator of root-rot incidence and severity

When dead trees were included, above-ground indicators of crown condition were strongly correlated with root-rot incidence and severity (p-values <0.0001); when dead trees were excluded, no significant correlations were observed (Table 3.6).

Table 3.6 Spearman's correlation (S) of the above-ground indicators with percentage root infection and root-rot severity classes.

Above-ground indicators	% root infection				Root-rot severity classes			
	Dead tree included		Dead tree excluded		Dead tree included		Dead tree excluded	
	S	p-value	S	p-value	S	p-value	S	p-value
Dominance	**-0.711**	<0.0001	-0.123	0.078	**0.711**	<0.0001	0.120	0.086
Height	**-0.650**	<0.0001	-0.013	0.853	**0.650**	<0.0001	0.012	0.864
DBH	**-0.655**	<0.0001	-0.020	0.773	**0.656**	<0.0001	0.020	0.775
Crown density	**-0.682**	<0.0001	-0.104	0.137	**0.683**	<0.0001	0.102	0.143
Live crown ratio	**-0.674**	<0.0001	-0.068	0.328	**0.675**	<0.0001	0.069	0.322
New foliage	**-0.661**	<0.0001	0.014	0.842	**0.661**	<0.0001	-0.016	0.814
Yellow-green foliage	**-0.650**	<0.0001	0.054	0.442	**0.652**	<0.0001	-0.051	0.468
Yellow foliage	**-0.416**	<0.0001	-0.056	0.425	**0.488**	<0.0001	0.057	0.416

Note: Values in bold are significantly different from 0 with significance level α=0.05.

Discriminant analysis (DA) showed that, using this method, the probability of the above-ground crown condition indicating root-rot incidence and severity was 61.4% (calculated: {30+97}/207) and 41.6% (calculated: {58+11+9+8}/207), respectively (Tables 3.7 and 3.8). This poor capacity of the DA to correctly predict root-rot status is shown by the high degree of overlap between groups of infected- and healthy-roots (Fig.3.6.A), and between highly infected, partly infected and healthy roots (Fig.3.6.B).

Table 3.7 The reliability of above-ground crown condition to indicate root-rot incidence (**N** is number of trees).

Actual observation		Predicted group memberships		% correct
Groups	**N**	***Infected***	***Healthy***	
Infected	48	**30**	18	62.5
Healthy	159	62	**97**	61.0
Total	**207**	92	115	**61.4**

Table 3.8 The reliability of the above-ground crown condition to indicate root-rot severity (**N** is number of trees).

Actual observation		Predicted group memberships				% correct
Groups	**N**	***Healthy***	***Partly infected***	***Highly infected***	***Totally infected***	
Healthy	159	**58**	16	44	41	36.5
Partly infected	24	7	**11**	1	5	45.8
Highly infected	12	0	2	**9**	1	75.0
Totally infected	12	2	2	0	**8**	66.7
Total	**207**	67	31	54	55	**41.6**

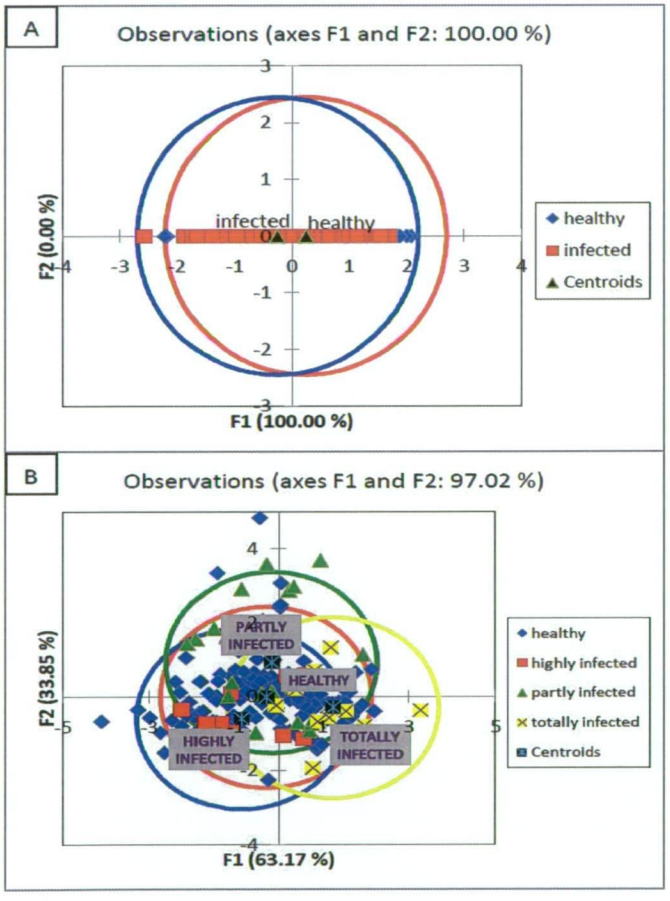


Figure 3.6. Discrimination between infection severity categories when above-ground crown condition was used to indicate root-rot incidence (A) and severity (B).

3.4 Discussion

This study investigated whether a repeatable, reproducible and reliable method for crown-condition measurement could be developed to meet a primary need for a forest-health surveillance program in Indonesia. Based on the analysis of data in Survey 1 the assessment method appears to offer sufficient repeatability for a single assessor, and reproducibility between assessors, to allow its use by foresters after they have received a minimum amount of training. However, experience and personal style of the assessors influenced the repeatability of particular indicators; in the worst case the least experienced assessor gave significantly different results for two variables between morning- and afternoon-assessments on the same day. The examination of reproducibility showed that mean differences among assessors were within what Smith, Pinkard *et al.* (2005) considered an acceptable range of error (±10%). The main issues with the least experienced assessor involved the scoring of overall crown condition class and percentage yellow foliage, suggesting that these indicators might require the most training and calibration between assessors. Scoring foliage colour was particularly susceptible to observer error because the *E. pellita* clones in this study (EP05) are quite tall and the crown is clumped in the high canopy (Fig.3.7). Good reference photographs and assessment of crowns from different directions/angles might help to reduce observer bias.

Figure 3.7 Feature of 5.5 years-old *E. pellita* (clone EP05)

There was some evidence of a lack of consistent scoring between surveys. The β values for discriminant analyses of surveys I and II were consistent suggesting that the crown-condition scoring system was reliable. The description of each crown-condition class in Table 3.2 explicitly stated that crown density and tree dominance were taken into consideration to classify the crown condition, and the assessors who conducted surveys I and II seemed to obtain a correct understanding in applying the method to assess the crown condition; while survey III gave a different and inconsistent result. This may have been due to incorrect scoring and indicated an apparent difficulty for assessors in Survey III in making reliable estimates of crown density.

Crown density estimates the proportion of crown volume that contains green foliage and reproductive structures on a network of live branches (Wulff 2004) and is widely used to characterise tree condition as well as tree health (Schomaker, Zarnoch *et al.* 2007). Dense crowns are often indicative of healthy

trees, conversely sparse crowns, unless a characteristic of the species, are often associated with trees in a state of decline (Solberg and Strand 1999). Zarnoch, Bechtold *et al.* (2004) developed a crown rating system for *Pinus ponderosa* by examining crown density, terminal branch growth, dead branches and needle colour and found that crown density was the most important discriminant of crown condition. Innes (2004) however stated that a tree with good crown condition cannot be classified as healthy simply because it has dense foliage. Foliage appearance and loss in trees, especially those with root-rot disease, is often the end result of a series of changes in tree condition and leaf fall may only occur at a late stage of a biotically-driven damaging event (Farid, Lee *et al.* 2005; Morrison, Merler *et al.* 1991; Wallis and Bloomberg 1981). Since crown condition is such an important explanatory variable, inconsistent and/or incorrect scoring may explain, in part, why the crown condition classes overlapped in the discriminant analyses in this study.

Surveys II and III were conducted by two different teams with different levels of training; one person was common to Surveys I and II; there was no assessor calibration in surveys II and III. This study has already shown that the visual estimation required by the method is subject to variation among observers. However the results from this study can assist the development of standard procedures, rank those indicators e.g. crown density and colour that most strongly influence the assessment process, and describe the requirements for assessor training. The consistent use of trained teams for crown-condition assessment is a fundamental message being delivered from this study.

The merging of crown-condition classes from five to three, and the reduction of above-ground indicators from eight to five increased the percentage of

trees that were correctly classified. To reduce the possibility of misinterpretation of crown-condition during assessment, minimising the number of crown classes used and eliminating explanatory variables that potentially bias the data because of the difficulties of field observation of these variables is recommended.

Early and intermediate symptoms of root rot are hard to recognise from the appearance of the crown because changes to above-ground explanatory variables in trees infected with root rot only occur when the root system has reached a fairly advanced stage of infection (Wallis and Bloomberg 1981). In this study correlations between deteriorating crown symptoms and the severity of root-disease infection were significant when the dead trees were included. This observation reflects the fact that most of the trees with root rot encountered in this survey were already dead, and so the above-ground characteristics of dead trees were a good indicator that such trees would have root rot. However, when correlations were examined for living trees only, they were inadequate for a root-rot rating system based on above-ground crown condition. This was due, in part, to the relatively small number of trees with root rot that were still alive. This finding reinforced the.conclusion that root rot is a 'sudden death disease' and because of this it will always be challenging to design a forest-health surveillance system using above-ground indicators of crown condition to estimate root-rot incidence and severity in living trees.

It is generally accepted that root-to-root contact is the primary means of root-rot spread (Ariffin, Idris *et al.* 2000; Wallis and Bloomberg 1981), after infection by primary inoculum. Trees are therefore likely to get root rot from their infected neighbours (Irianto, Barry *et al.* 2006). If above-ground indicators cannot be used to accurately indicate the actual incidence and severity of root rot then the

number of dead trees may be a better surrogate for these variables and for predicting rotation-length losses.

It should be noted that to the best of my knowledge, only one clone of *Eucalyptus pellita*, EP05 is planted industrially by P.T. Arara Abadi. This severely limited my ability to sample root-rot incidence over a wide genetic range of the host and means that other clones or varieties of *E. pellita* may behave in quite a different manner. Nevertheless, and especially given the apparently widespread planting of this single clone, the results presented here have significance for a large part of P.T. Arara Abadi's plantation estate.

This study used first-rotation *E. pellita* planted in an area with a history of root-rot infection of *A. mangium*. In Compt. 223 and Compt. 1A levels of tree mortality were around 40% on the monitored plots. In concert with the results from Chapter 2, there are strong indications that *E. pellita* may be more susceptible to root rot than previously thought.

3.5 Conclusion

Crown-condition scoring methodology is potentially applicable for monitoring changes in the crown-condition of *E. pellita* trees with a prerequisite of a well-trained assessment team. Minimising the crown-condition classes required and selecting indicator variables that strongly influence the classification are suggested.

It was not possible to reliably indicate the actual trees infected with root rot based on above-ground symptoms or individual crown condition assessments. However the impact of the disease was plainly observed at site level by crown

condition assessments and has already caused unacceptable levels of tree death. Levels of tree death in the experimental plots should not be generalised to all *E. pellita* compartments or plantations. Extensive and appropriately sampled observations of root-rot status at different sites are needed. Nevertheless, as a preliminary investigation into root rot in *E. pellita*, this study found that severe damage similar to that caused in *A. mangium* plantations can also occur in *E. pellita* plantations. It is an important and timely warning to seek and apply appropriate management strategies that can protect *E. pellita* plantations from more severe damage.

Chapter 4 – *Eucalyptus nitens* and *Armillaria luteobubalina* as a pathosystem model to investigate physiological responses at initial stages of the root-rot infection

4.1 Introduction

4.1.1 Contribution of plant physiological studies to the development of a root-rot early detection method

A major issue for the pulpwood, oil palm and rubber industries is early root-rot disease detection at a stage which might allow the implementation of effective remedial measures. Because root-rot disease is hidden below ground, early detection is difficult because individual trees can remain apparently healthy above ground until damage to the root system is severe (Newsam 1964; Sariah 2000). Even if remedial measures have not been established or are not feasible, the delineation of areas affected by root rot could permit a more accurate estimation of current and future losses to this disease.

Symptoms such as thinning crowns, growth reduction and/or foliage chlorosis have been proven to be useful in detecting trees with root infection (Morrison, Williams *et al.* 1991; Omdal, Shaw *et al.* 2004). A study of symptoms of root rot present in the crowns of *Eucalyptus pellita* was presented in Chapter 3. Because symptoms that present in the crown are a manifestation of physiological and morphological changes occurring in individual leaves, quantitative measures that are indicative of functional disruption at leaf level can potentially be used for monitoring forest condition (Gunthardt-Goerg and Vollenweider 2007; Luyssaert, Raitio *et al.* 2002; Stone, Coops *et al.* 2000). The determination of the early

physiological responses of eucalypts to a root-rot pathogen could be valuable in developing technology to detect root rot in the tree at an early stage.

Remote sensing technology is a potentially powerful tool to greatly enhance our capability for mapping forest condition as it allows identification of even subtle changes in the biochemical composition of leaves in the canopy that could indicate early stages of disease infection (Hall, Hilker *et al.* 2008). High spectral resolution remote sensing has been used for accurate mapping of vegetation condition, although the extraction of physiologically relevant information is not a trivial exercise (Hilker, Coops *et al.* 2008). A study in Malaysia using imagery captured by an AISA airborne hyperspectral imaging spectrometer showed the potential of such technology to detect and map oil palms affected by Basal Stem Rot (*Ganoderma boninense*) (Shafri and Hamdan 2009), discriminating the physiological changes occurring with diseased and stressed trees from those of healthy trees (Haniff, Ismail *et al.* 2005). This chapter contributes to the development of an early detection method for root rot in eucalypts.

4.1.2 Photosynthetic responses to pathogen invasion

While symptoms such as thinning crowns, growth reduction and foliage chlorosis have been studied extensively and have proven to be useful in detecting trees with root infection (Morrison, Williams *et al.* 1991; Omdal, Shaw *et al.* 2004), there has been little research on the effect of root disease on the host's physiology before the visual symptoms appear. Understanding the physiological outcome of phytopathogen infection is the key to understanding the plant's reaction to disease (Shaw and Kile 1991).

A plant pathosystem, which is defined in terms of the phenomenon of parasitism, is concerned with the interaction between plants and parasites. A parasite can be regarded as a pathogen when it causes disease in the plant host. The complete process of disease development, which is known as pathogenesis, is determined by interactions between a host, a pathogen, and the environmental conditions (Agrios 2005; Lucas 1998)

As with other stressful environmental conditions, pathogenesis leads to changes to several physiological processes in the host plant (Beadle 2000; Guest and Brown 1997) . Photosynthetic capacity is a useful parameter for monitoring these physiological changes. Various stressful agents reduce the photosynthetic capacity of growing plants due to their influence on one or more of the partial processes associated with photosynthesis (Dubey 1997). This influence may include decreased light-energy utilization, chlorophyll content, destruction of the chloroplasts' fine structures, degradation of photosystem (PS) II, alteration of biochemical processes, etc. (Berger, Papadopoulos *et al.* 2004; Berger, Sinha *et al.* 2007; Chou, Bundock *et al.* 2000; Dubey 1997; Lopes and Berger 2001; Meyer, Saccardt *et al.* 2001; Robert, Bancal *et al.* 2004; Sharma and Hall 1992; Sigh and Dubey 1995).

Chlorotic symptoms and necrotic areas on the foliage may be indicative of pathogen invasion and photosynthetic disruption (Issac 1992). The degree of inhibition of photosynthesis may be indicative of the aggressiveness of the pathogen (Guest and Brown 1997). Root pathogens, such as *Armillaria* sp., which occupy and alter the host's vascular tissue (Morrison, Williams *et al.* 1991) may influence photosynthetic activity indirectly by affecting the pathways of water flow in the xylem. The impact of root rot on photosynthetic activities will then be

similar to the disruptions caused by water stress that is associated with decreased stomatal conductance, a lowering of intercellular CO_2, decreased chlorophyll level, changes in ultrastructure of chloroplasts, alteration in electron transport and decreased activity of Rubisco (Dubey 1997).

4.1.3 Physiological basis for Armillaria root diseases

Armillaria is a genus of fungi with a worldwide distribution. Many of its species are capable of causing root- and butt-rot diseases and the eventual death of susceptible host species (Dunne, Glen *et al.* 2002; Kile 2000). In the northern hemisphere two of the most pathogenic species in native and planted forests are *Armillaria mellea* (Vahl) P. Kumm. and *A. ostoyae* (Romagn.) Herink. (Hood, Redfern *et al.* 1991). *Armillaria luteobubalina* Watling & Kile in the southern hemisphere is native to Australia and is a significant pathogen in natural ecosystems including a wide range of native eucalypt forests, especially dry sclerophyll eucalypt forest (Kile, Watling *et al.* 1983), forest plantations, fruit crops and ornamental plants (Coetzee, Wingfield *et al.* 2001; Menge and Ploetz 2003; Morrison, Pellow *et al.* 2000; Onsando 1997; Wago and Shaw 1985). Edgar, Kile *et al.* (1976) reported that death caused by *A. luteobubalina* in eucalypts as old as 25 years could appear suddenly, with trees showing no significant symptoms of decline before death.

More than 50 families and over of 200 plant species have been recorded as susceptible hosts of *A. luteobubalina,* including *Eucalyptus nitens* (Deane & Maiden) (Shearer, Crane *et al.* 1998). In Tasmania only two cases of *A. luteobubalina* have been reported in *E. nitens* plantations; in 3-year- and 6-year-old plantations in Kamena (near Burnie) and on the Woolnorth property in the far NW Tasmania, respectively (Wardlaw 2000). Although the incidence of

root rot in plantations is relatively low in these cases, it illustrates that *E. nitens* is not resistant to *A. luteobubalina*. If suitable inoculum is present and the environmental conditions are conducive to disease development, *A. luteobubalina* can potentially kill young trees of *E. nitens*.

Although physiological aspects of phytopathogenesis have been studied for decades, those for root-rot diseases, especially in relation to hardwood trees, have received little attention. Root-rot diseases are usually first recognised after the expression of visual symptoms, such as reduction of shoot growth, changes in foliage characteristics and stress-induced reproduction (Morrison, Merler *et al.* 1991). Changes in physiological processes that occur before the expression of visual symptoms are not well understood. Morrison, Williams *et al.* (1991) proposed two theories that might explain the physiological basis of symptom development of *Armillaria* root rot: (i) that physiological changes were directly affected by disruption of the host's vascular system and (ii) that metabolic toxins produced by the *Armillaria* species induced changes in the physiological behaviour of the host, particularly the foliage.

The pathogenic effect on photosynthetic capacity can be examined in part by measuring photosystem II performance via chlorophyll fluorescence. The intensity of fluorescence emitted from dark-adapted leaves is sensitive to any changes in the photosynthetic apparatus caused by both biotic and abiotic stress (Berger, Sinha *et al.* 2007; Bonfig, Schreiber *et al.* 2006; Rolando and Little 2003). Logan, Adams *et al.* (2007) recommended that chlorophyll fluorescence be applied along with other methods that characterise photosynthesis, such as gas exchange and foliar pigment composition. Ploetz and Schaffer (1987) have reported that Phytophthora root rot caused by *Phytophthora cinnamomi* Rands.

reduced photosynthesis, transpiration and stomatal conductance of avocado (*Persea Americana* Miller) seedlings. This same disease also reduced the water status of chestnuts saplings (Maurel, Robin *et al.* 2001). Thus it is apparent that plant pathogens can alter several physiological processes of their hosts, although the mechanisms by which fungal invaders affect photosynthetic and related processes still remain unclear. It is also very probable that different pathogens affect their host's photosynthetic pathways in more than one way. Specific studies of particular pathosystems still need to be explored.

4.1.4 Research objective

This study sought to quantify physiological changes of the host plant in response to root disease through examining a root-rot pathosystem model of *A. luteobubalina* and *E. nitens*. In this experiment, the hypothesis that root infection will alter processes associated with photosynthesis before the visual expression of disease symptoms is tested. To better address the mechanistic background to changes in physiological behaviour, measures of photosynthetic capacity (A_{max}), photosystem (PS) II yield (F_v/F_m via chlorophyll fluorescence) and chlorophyll content in *E. nitens* saplings that had been artificially inoculated with *A. luteobubalina* were related to progressive root damage during disease development.

4.2 Material and methods

4.2.1 Plants and isolates

Forty-two two-year old *E. nitens* saplings were re-potted into 30-cm diameter plastic pots containing a potting-mix medium which contained soil, sand,

and pine-bark compost. The plants had been previously planted in perlite-vermiculite (1:1) media (Appendix 4.1). The mixed-soil medium was chosen because it appeared more suitable for the inoculum to be maintained in a viable state as tested in a preliminary trial (Appendix 4.2). The plants were fertilised and well-watered before inoculation.

The fungal cultures were obtained by isolating from mycelial fans on the roots of an ornamental olive tree in the Hobart Royal Botanical Gardens (isolate strain 1) and a *Cupressus* sp. in Cascade Brewery Garden (isolate strain 2) where *A. luteobubalina* has been present for a number of years (D. Spalding and C. Mohammed 2007, pers. comm.). Molecular analysis confirmed that these isolates were *A. luteobubalina*, have 98-100% sequence similarity with described isolates on GenBank and have seven nucleotides difference between isolates strain 1 and 2 (Morag Glen, unpublished; see Appendix 4.3).

4.2.2 Fungal isolations

Fungal isolation was conducted by growing the infected root samples on a selective medium (MAT). The root samples had first been surface-sterilised through a series of washing solutions viz 2 min in tap water, 2-3 min in 20% Chlorox™ (hypochlorite solution), and three times in sterile water. The medium was prepared by autoclaving 1% malt extract agar (MEA) for 30 min at 120°C. Fifty (50) ppm penicillin, 50 ppm streptomycin, 25 ppm polymixin and 230 ppm thiabendazole were added into the autoclaved MEA when it was cooling (at < 60°C). Hyphae that grew from the root samples were subcultured onto 2 % MEA and incubated for at least one month in the dark at 21°C.

4.2.3 Inoculum preparation and artificial inoculation of plant material

Fully colonised segments of young *Eucalyptus globulus* branches were prepared as inoculum blocks prior to the inoculation using the method described in Mansilla, Aguin *et al.* (2001) with some modification. Segments of *E. globulus* 5-6 cm length and 1-2 cm in diameter that had been taken from young branches were autoclaved for 30 min at 120°C. After cooling, 150 ml of MAT medium was poured into several 200 ml sterilised tubs. The medium was first added to occupy half of the tubs' volume and allowed to solidify. Ten branch-segments of *E. globulus* were then vertically inserted into the medium of each tub. Liquid MAT medium was then added until the rods were completely submerged in agar. After the medium in the tubs had totally solidified, seven mycelial segments (size approx. 1 x 1 cm^2) of the *A. luteobubalina* isolates were placed on the agar surface (Fig. 4.1.A). Each tub was then closed, sealed with plastic film, and placed in the dark at 21-22°C for three months. Tubs with uninoculated branch segments were also prepared to serve as controls.

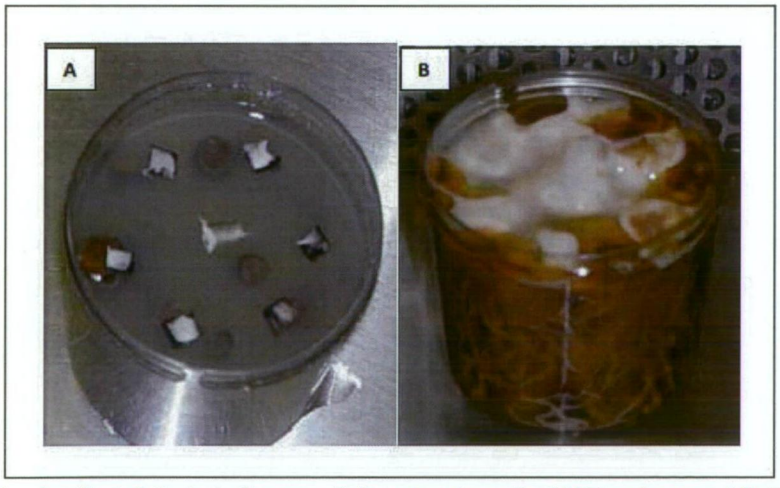


Figure 4.1. **A.** *Armillaria luteobubalina* mycelial segments on the MAT surface; and **B.** The mycelial fans after incubation at 21°C for three months.

After being fully colonised with *Armillaria* rhizomorphs and mycelium (Fig. 4.1.B), the rods were removed from the agar medium and used to inoculate the saplings by putting five colonised rods into each pot adjacent to and just touching lateral roots in close proximity to the root collar. The five lateral roots were either wounded or left unwounded. Wounding was carried out using a Swiss Army knife to remove a little bit bark (approx. 0.5- 1 cm length). All plants were saturated with water from a dripper for 15 min three times per day.

4.2.4 Experimental layout

A factorial design was used in which six treatments were tested, including combinations of two physical treatments (i.e. unwounded and wounded host root systems), and branch segments containing two different *Armillaria* inocula, (i.e. isolate strain 1 and strain 2) and an uninoculated control. The physical treatments were applied in order to examine ease of pathogen entry into the root tissue. Each treatment consisted of seven replications, resulting in a total of 42 trees across the experiment. The six treatments were: unwounded-control (UW-C), wounded-control (W-C), unwounded-isolate strain 1 (UW-1), wounded-isolate strain 1 (W-1), unwounded-isolate strain 2 (UW-2), and wounded-isolate strain 2 (W2). The *E. nitens* saplings were arranged in a randomised block design.

4.2.5 Physiological measurements

Photosynthetic capacity (A_{max}) and photosystem II yield (F_v/F_m) were assessed just prior to inoculation (T_0, 2/3 October 2008) and after the first

symptoms were observed (T_2, 29 April and 5/6 May 2009). During the six months between T_0 and T_2, an intermediate measurement (T_1, 30 January 2009) of F_v/F_m was carried out to determine if there was evidence of alterations in physiology prior to the appearance of visual symptoms. In a preliminary trial, where the same trees were inoculated under different conditions prior to this study (Appendix 4.1), no significant differences between control and treated saplings occurred in the above physiological variables over a six -month period. This may have been because of unsuccessful infection, but also suggested that extensive monitoring during the first six months after inoculation was not warranted.

Physiological assessments of maximum quantum yield of photosystem II (F_v/F_m), light-saturated photosynthetic rate (A_{max}) and relative chlorophyll content were made on three fully-expanded leaves per tree. The leaves were selected from the third or fourth leaf pair just behind the branch tip. All trees (42 saplings) were assessed. Chlorophyll fluorescence (F_v/F_m) was measured pre-dawn using a chlorophyll fluorometer (OS-30p Opti-Science). Photosynthetic rate (A_{max}) was quantified using a CIRAS infrared gas analyser (PP Systems, Herts, UK) with an artificial light source set to deliver 1500 μmol m^{-2} s^{-1} at the leaf surface and ambient CO_2 concentration (370 – 380 ppm).

A Minolta SPAD-502 chlorophyll meter was used to obtain relative chlorophyll content. For calibrating the SPAD value to estimate chlorophyll concentration, thirty of the measured leaves were collected for chlorophyll extraction and quantification. Fresh leaf discs (dry weight of each disc~ 0.020 g) were extracted for chlorophyll content with a triple extraction method (Martin, Alonso *et al.* 2007). Discs were ground in a mortar with approximately 50 μg $MgCO_3$, 50 μg washed, fine sand and a small volume of liquid nitrogen. Ground

leaf material was extracted with three small volumes of 100% cold acetone, centrifuged for 3 min. Absorbance was read at 470, 645, 663 and 710 nm with a Cary UV-VIS spectrophotometer. Total chlorophyll (Chl ***a*** and ***b***) was calculated using the equations of Lichtenthaler and Buschmann (2001). Using this data a standard curve was created (Appendix 4.4) and the SPAD values converted to chlorophyll concentration (μg/g).

4.2.6 Re-isolations and detecting *Armillaria*

Roots of all the inoculated plants and three of the un-inoculated plants were examined at the end of the experiment. To examine the entire root system, the trees were taken out of pots and the soil was shaken off. High pressure water was sprayed onto the root systems to clean off the remaining soil. The inoculum rods were then removed. Any symptoms and/or signs of infection were recorded and photographs were taken. Re-isolations were undertaken from symptomatic roots which were indicated by presence of lesions and/or fungal mycelium (Fig. 4.2). This was done to confirm the causal agent associated with the deterioration of the plants and to ensure that *A. luteobubalina* was present in the roots and remained in the inoculums rods. The re-isolations were carried out in the same way as the isolations (as described in section 4.2.2).

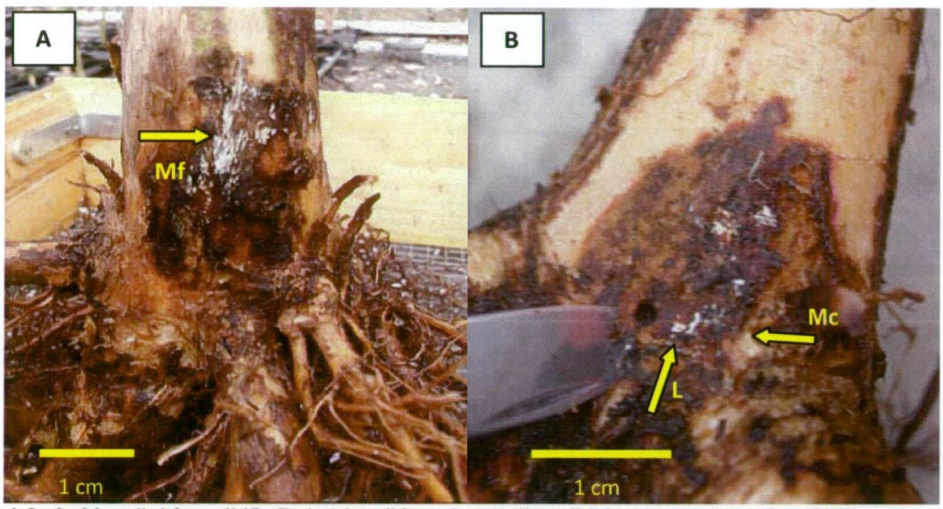


Fig. 4.2 **A.** Mycelial fans (Mf); **B**. Lesion (L) and mycelium (Mc) on the root collar of infected plants.

The presence of *A. luteobubalina* in the inoculum rods was verified by re-isolating the fungus from the rods. After being buried for about six months, most of the inoculum rods were rotten. As other secondary fungi must have been associated with the rotten rods, re-isolation was only carried out from the rods that were not rotten and showing *pseudosclerotial* plates (Fig. 4.3) indicative of an inactive stage of *A. luteobubalina*.

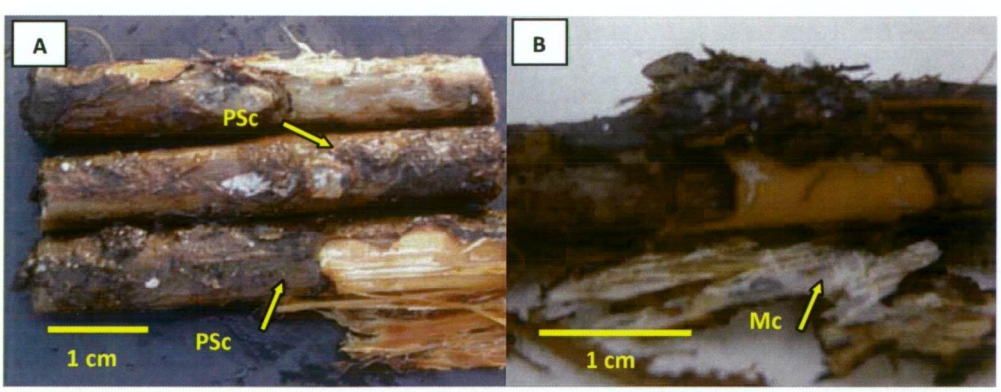


Fig 4.3. **A**. Inoculum rods with *pseudosclerotial* plates (PSc) or black crust; **B**. White mycelium (Mc) underneath the PSc.

Based on the visual appearance of fungal signs and/or root symptoms, four categories were developed to describe the infection and root condition:

1. **Positively infected by *A. luteobubalina***: either mycelial fans (Fig.4.2.A) or lesion with white mycelium (Fig.4.2.B) or both were observed visually on the excavated root; re-isolated fungal cultures confirmed a positive result.
2. **Possibly infected by *A. luteobubalina***: visual observation showed a little lesion with white mycelium; re-isolated fungal cultures confirmed a negative result.
3. **Infected by un-inoculated fungi**: visual observation showed necrotic tissue or lesion (Fig.4.4.A) but fungal isolation confirmed fungi other than *A. luteobubalina*.
4. **Uninfected**: roots were healthy (Fig.4.4.B).

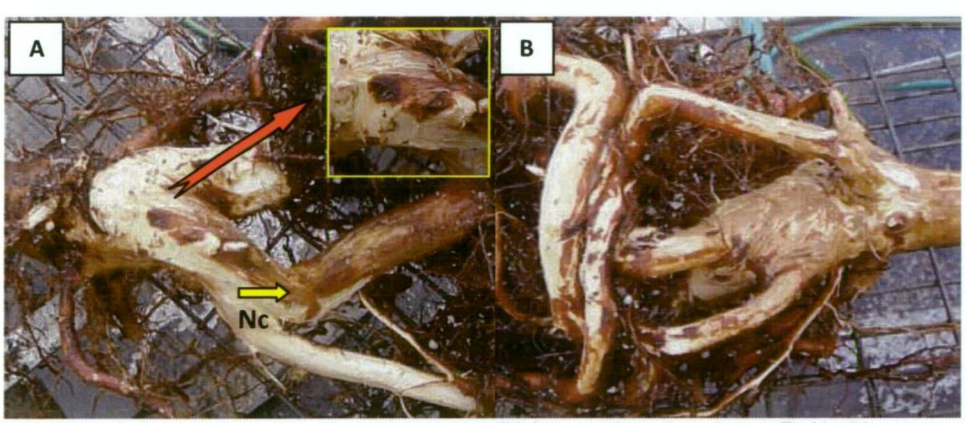


Fig. 4.4. **A**. Lesion (insert) and necrotic tissue (Nc) on un-inoculated root; **B**. Healthy root.

4.2.7 Data analysis

Two-way Analysis of Variance (ANOVA) performed in XLSTAT2009® was used to process the physiological data. Mean differences of changes in the response variables between treatments were determined by Duncan's multiple range tests.

4.3 Results

4.3.1 Changes in physiological variables

At the first measurement (T_0), just before the pots were treated with the inoculum rods, there were no significant differences between treatments in the physiological variables examined (Table 4.1). The intermediate assessment (T_1) examined the photosynthetic efficiency of PS II (F_v/F_m) only; there were no significant differences between treatments three months after inoculation (Table 4.1).

The first physiological changes were detected six months after inoculation when a significant difference in F_v/F_m between the unwounded controls (UW-C) and all other treatments was observed (Table 4.1); inoculation and wounding were associated with reductions in F_v/F_m during the six-month period of the experiment (Table 4.2). After being treated for six months, F_v/F_m of UW-C saplings increased, while in the other treatments F_v/F_m decreased; reductions in F_v/F_m were significantly greater in the inoculated saplings than in the unwounded controls (Table 4.2).

The responses of chlorophyll content (total Chl. ***a*** and ***b***) and light-saturated photosynthetic rate (A_{max}) were more variable. Six months after treatment

there was a significant difference in chlorophyll content between inoculated and UW-C saplings (Table 4.1). Chlorophyll content decreased during the six-month period of the experiment but, except for W-1 saplings, there were no differences between inoculated and control treatments; A_{max} also decreased in all treatments during this period but differences between treatments were not significant (Table 4.2).

Table 4.1. Means ± (SE) of the efficiency of PS II , chlorophyll content and photosynthetic rate of *E. nitens* saplings inoculated with *A. luteobubalina* isolates over the period of observations

Treatments / Time	Physiological variables		
	Efficiency of PS II F_v/F_m	Chlorophyll content µg/g	Photosynthetic rate (A_{max}) µmol/m^2/s
UW-C / T_0	0.78 ± 0.01 [a]	2918.8 ± 231.1 [a]	12.9 ± 2.2 [a]
W-C / T_0	0.77 ± 0.02 [a]	2772.1 ± 168.8 [a]	11.0 ± 0.5 [a]
UW-1 / T_0	0.79 ± 0.01 [a]	2824.3 ± 110.3 [a]	11.6 ± 1.5 [a]
W-1 / T_0	0.78 ± 0.01 [a]	2750.1 ± 90.0 [a]	13.5 ± 0.8 [a]
UW-2 / T_0	0.79 ± 0.01 [a]	2552.3 ± 169.5 [a]	13.0 ± 0.8 [a]
W-2/ T_0	0.79 ± 0.00 [a]	2918.8 ± 157.4 [a]	13.9 ± 1.1 [a]
UW-C / T_1	0.83 ± 0.01 [a]	NA	NA
W-C / T_1	0.82 ± 0.00 [a]	NA	NA
UW-1 / T_1	0.81 ± 0.01 [a]	NA	NA
W-1 / T_1	0.82 ± 0.00 [a]	NA	NA
UW-2 / T_1	0.82 ± 0.00 [a]	NA	NA
W-2/ T_1	0.82 ± 0.00 [a]	NA	NA
UW-C / T_2	0.81 ± 0.01 [a]	2117.7 ± 119.6 [a]	10.0 ± 0.4 [a]
W-C / T_2	0.76 ± 0.01 [b]	1939.0 ± 133.9 [ab]	8.5 ± 0.7 [ab]
UW-1 / T_2	0.74 ± 0.02 [b]	1673.7 ± 119.0 [bc]	8.0 ± 1.0 [ab]
W-1 / T_2	0.75 ± 0.01 [b]	1490.7 ± 81.3 [c]	7.7 ± 0.6 [b]
UW-2 / T_2	0.73 ± 0.01 [b]	1629.1 ± 88.9 [bc]	7.2 ± 1.1 [b]
W-2/ T_2	0.75 ± 0.01 [b]	1715.8 ± 70.5 [bc]	8.4 ± 0.5 [ab]

Note:

- **The values followed with different letter in the same column are significant at α=0.05, as determined by a Duncan's test-ANOVA for each variable at each time of observation.**
- NA = Not attempted

Table 4.2. Changes (± *SE*) of *Fv/Fm*, chlorophyll content and A_{max} of *E. nitens* saplings measured before inoculation and six months after inoculation

Treatments	Photosynthetic variables		
	Efficiency of PS II (F_v/F_m)	Chlorophyll content (µg/g)	Photosynthetic rate (A_{max}) µmol/m²/s
UW-C	0.03 ± *0.01* [a]	-801.1 ± *126.1* [a]	-3.0 ± *1.9* [a]
W-C	0.00 ± *0.02* [ab]	-833.1 ± *163.6* [a]	-2.5 ± *0.8* [a]
UW-1	-0.05 ± *0.03* [bc]	-1150.6 ± *107.4* [ab]	-3.7 ± *1.4* [a]
W-1	-0.03 ± *0.01* [bc]	-1259.4 ± *99.7* [b]	-5.8 ± *0.9* [a]
UW-2	-0.06 ± *0.02* [c]	-923.2 ± *188.6* [ab]	-5.6 ± *1.5* [a]
W-2	-0.04 ± *0.01* [bc]	-1116.9 ± *140.6* [ab]	-5.5 ± *1.1* [a]

Note:
The values followed with different letter in the same column are dignificant at significant level α=0.05, as determinate by separate Duncan's test-ANOVA for each parameter of observation separately.

Table 4.3 shows that the response of photosynthetic efficiency of PS II was affected by an interaction between time and treatments (F-ratio = 3.798, P-value = 0.005). For chlorophyll content and photosynthetic rate, the responses were more determined by the time factor (P-value <0.0001).

Table 4.3 Summary of Two-ways ANOVA at α =0.05 for all photosynthetic parameters measured (before inoculation and 6-months after inoculation)

Parameters	Source	DF	Sums of Squares	Mean Square	F-ratio	Pr>F
Photosynthetic efficiency of PS II (F_v/F_m)	Time	1	0.011	0.011	14.209	0.000
	Treatments	5	0.008	0.002	2.152	0.073
	Time * treatments	5	0.014	0.003	3.798	0.005
Photosynthetic rate (A_{max})	Time	1	330.838	330.838	60.790	< 0.0001
	Treatments	5	28.208	5.641	1.036	0.405
	Time * treatments	5	31.365	6.273	1.153	0.344
Chlorophyll content	Time	1	17901863.062	17901863.062	180.546	< 0.0001
	Treatments	5	1339278.539	267855.708	2.701	0.030
	Time * treatments	5	518757.633	103751.527	1.046	0.400

4.3.2 Reisolation from infected roots

Root excavation showed that most the inoculated trees, both wounded and unwounded, were infected by *A. luteobubalina* (Table 4.4). Wounding appeared to enhance the possibility of fungal infection by *A. luteobubalina*. Wounded trees

showed 100% and 85.7% infection of *A. luteobubalina* strain-1 and strain-2, respectively; unwounded ones, both inoculated with *A. luteobubalina* strain-1 and strain-2 showed only 71.4% infection (Table 4.4).

Fungal infection was confirmed by the presence of mycelial fans and lesions on the root or root collar (Fig. 4.2) and fungal cultures that had been isolated from the symptomatic trees and confirmed the presence of *A. luteobubalina* (Fig. 4.5). Some control saplings were infected by other fungi which were indicated by lesions and necrotic areas (Fig.4.4.A). The viability of the *A. luteobubalina* isolates on the *E. globulus* inoculum rods after being buried for six months was low. Positive reisolations of *A. luteobubalina* from these rods was only possible from three pots and all were *A. luteobubalina* strain-1; no reisolations were successful from inoculum rods carrying *A. luteobubalina* strain-2.

Table 4.4. Percentage number of trees in each of four categories based on their root condition.

Treatments	Root condition			
	Positively infected	Possibly infected	Infected by other fungi	Uninfected
UW-C	0.0	0.0	28.6	71.7
W-C	0.0	0.0	14.3	85.7
UW-1	71.4	14.3	0.0	14.3
W-1	100.0	0.00	0.0	0.0
UW-2	71.4	28.6	0.0	0.0
W-2	85.7	14.3	0.0	0.0

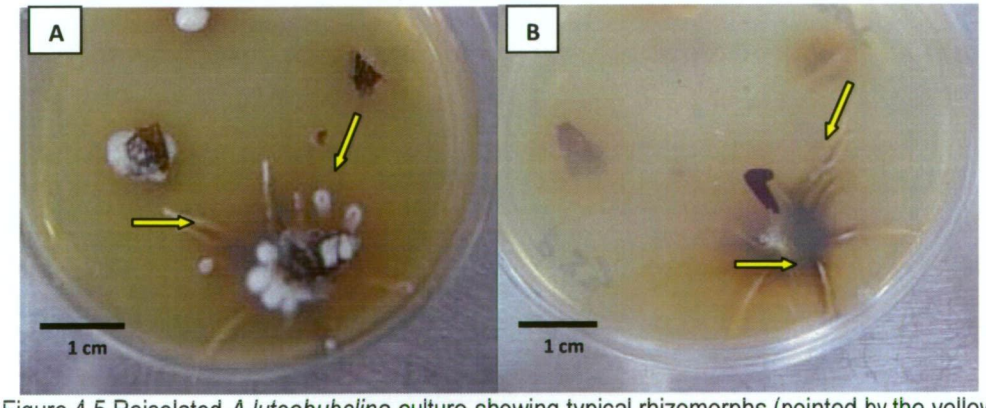


Figure 4.5 Reisolated *A.luteobubalina* culture showing typical rhizomorphs (pointed by the yellow arrows).**A**. upper side; **B**. bottom side.

4.4 Discussion

Plant physiological changes associated with root-rot disease are not easy to detect. This is because the disease needs a certain period to develop and occupy plant tissue before the plant expresses detectable physiological changes to pathogen infection (e.g. Dawson and Weste 1984). This study has shown that *E. nitens* saplings, growing under semi-controlled conditions in pots, require about six months after being inoculated by *A. luteobubalina* to express the first detectable changes in physiological performance (Table 4.1).

In this study, the efficiency of PS II (F_v/F_m) was the most sensitive physiological variable to stress caused by the root-rot pathogen; a significant reduction in F_v/F_m in response to inoculation was observed six months after treatment. Changes in F_v/F_m are widely used as a reliable diagnostic indicator of damage caused by photoinhibition in response to extreme temperatures, and water and nutrient stress (Close and Beadle 2003; Epron, Dreyer *et al.* 1992; Gamon and Pearcy 1989; Groom and Baker 1992; He, Chee *et al.* 1996; Valladares and Pearcy 1997). Since the root-rot pathogens attack the vascular system of plants, responses to the infection may be similar to those observed in response to drought stress. In drought-stressed plants, thylakoid membranes are the primary site of injury which leads to the decline of PS II activity (Dubey 1997; Mutava 2009). However there was no evidence in this study that the decline in PS II activity was associated with parallel reductions in light-saturated photosynthetic rate, A_{max} but this may be because the reductions in F_v/F_m were not yet of sufficient magnitude. Decreases of PS II activity under stress are associated with photoinhibition where free high energy radicals in the thylakoid cause photo-oxidation of chlorophyll (Havaux

1992; Mutava 2009). Differences between treatments in chlorophyll content at T_2 in this experiment suggested that this might be occurring; however reductions in chlorophyll between T_0 and T_2 were not significantly different between treatments.

While the decreases in A_{max} between T_0 and T_2 were not significant, it is probable that the reduced rate was a response to seasonal changes in incident light and temperature as the first measurement was done in mid spring, the last in late autumn. Leaves growing in high light environments attain greater A_{max} than leaves growing in the low light environments (DeJong and Doyle 1985). Reduced A_{max} also can be caused by reductions in seasonal temperatures (Battaglia, Beadle *et al.* 1996) and overnight frost (Davidson, Battaglia *et al.* 2004). Changes in photosynthetic rate are related to changes in chlorophyll content (Boardman 1977; DeJong and Doyle 1985) that may in part explain the parallel reduction in chlorophyll content in this experiment. Loss of chlorophyll was also found in *Pinus sylvestris* as seasonal temperatures declined (Ottander, Campbell *et al.* 1995).

Wounding tends to enhance the possibility of infection. Wounded saplings inoculated with both strains of *A. luteobubalina* showed a greater level of infection than unwounded saplings. In the field, wounding as well as other factors predisposing plants to stress such as poor planting, poor drainage and soil compaction are often associated with *Armillaria* root disease (Hadfield, Goheen *et al.* 1986). Outer bark may play an important role in protecting roots from invasion by pathogens (Wargo and Harrington 1991). Root movement and breakage, and associated insect feeding can potentially provide infection sites for *Armillaria* and other root pathogens (Harrington 1986; Rizzo and Harrington 1988; Whitney 1961). However, Baumgartner and Rizzo (2006) found that wounding the root collar bark and vascular cambium of grapevine rootstocks did not significantly

increase the infection rate of *Armillaria mellea* in a greenhouse trial. This suggests that wounding can induce host defence reactions, for example the production of enzymes that function in lignin synthesis which leads to reinforcement of the damaged cell wall (Baron and Zambryski 1995) and/or the release of lytic enzymes or toxic secondary metabolites that may limit hyphal penetration of the inner bark (Wargo and Harrington 1991). The possibility that wounding may have stimulated a host defence reaction in this study was not investigated.

There was a low level of successful re-isolation from the inoculum rods with pseudosclerotial plates at the end of the experiment. Such difficulties of re-isolation from pseudosclerotial plates can be understood since they are an immobile/inactive phase of *Armillaria* and had probably developed in response to the occupation of the rods by decomposing soil fungi (Dowson, Rayner *et al.* 1988).

This study has confirmed that it is very uncommon with root rot that disease expression can first be recognised by crown appearance but there was some evidence that measureable changes in at least one photosynthetic variable might occur. Root and root collar examination remain the most reliable way to judge whether or not trees are infected. For *Armillaria* root disease, the presence of mycelial fans is a characteristic that distinguishes the disease.

4.5 Conclusions

The results obtained in this study from root excavation and photosynthetic measurements can lead to the conclusion that this *E. nitens – A. luteobubalina* pathosystem was successful in demonstrating initial physiological changes due to root-rot infection. However the functional changes that led to a reduction in PS II

efficiency in the inoculated saplings require further investigation. Several months may be required following infection before any physiological changes are detected. Root-rot is known to be a latent disease that may be present in plants for an extended period without any noticeable expression of symptoms. Longer periods of observation than were possible in this experiment are recommended for further research with a similar focus of interest.

Appendix 4.1 - *Preliminary trial of artificial inoculation of* E. nitens *saplings with* A. luteobubalina *isolates*

Plants and isolates:

Forty-five one-year old *E. nitens* saplings used in this experiment were planted into 30-cm diameter plastic pots containing a mixture of perlite and vermiculite (1:1) in an open growing area. All plants were saturated with water from overhead sprayers for 15 minutes three times per day. The fungi were obtained from mycelial fans of *A. luteobubalina* growing on the root of an ornamental olive tree in the Hobart Royal Botanical Gardens (isolate strain-1) and a *Cupressus* sp. in Cascade Brewery Garden (isolate strain-2).

Experimental design:

The trial had three treatments: untreated control; pots inoculated with strain-1 and strain-2 of *A. luteobubalina.* A randomised complete block design was implemented. The pots were arranged in three blocks and each block had five plants per treatment.

Inoculation and physiological measurements:

Three fully colonised inoculum rods were inserted into the potting medium close to the root collar of the *E. nitens* saplings (Fig. 4.6). Pre-dawn water potential (ψ), chlorophyll fluorescence (F_v/F_m), light-saturated photosynthetic rate (A_{max}) and chlorophyll content) were measured three times, *i.e.* pre-inoculation, and three and six months post-inoculation.

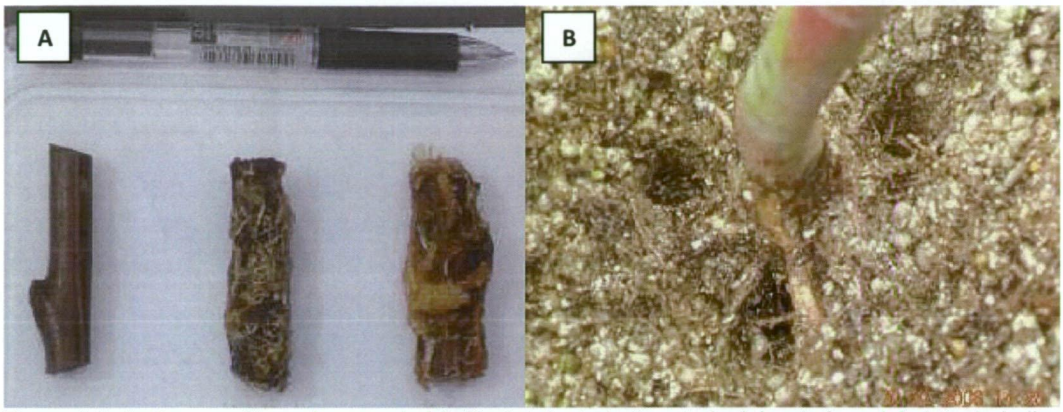


Figure 4.6. **A**. Source of inoculum: control (left), *A. luteobubalina* strain-1 (centre), and *A. luteobubalina* strain-2 (right); and **B**. Inoculation sites: three holes close to the root collar.

Results:

There were no significant differences between the treatments for either photosynthetic efficiency of PS II (F_v/F_m) or light-saturated photosynthetic rate (A_{max}) at T_0 (13/15 February 2008), T_1 (21/24 April 2008) or T_2 (2/3 October 2008) (Table 4.5). There were no significant differences between treatments for pre-dawn water potential (ψ) except at T_0 but this was not indicative of any water stress and not caused by the treatments, as they had not yet been applied.

Root excavation showed that the non-significant differences between treatments at T_2 were caused by unsuccessful infection due to poor survival of the inoculum. It was decided to repot the saplings into a soil-based medium as the perlite-vermiculite mixture was probably not a suitable environment for survival of *A. luteobubalina*. The viability of *A. luteobubalina* in the soil medium was first tested (Appendix 4.2).

Table 4.5. Means ± (SE) of water potential, photosynthetic efficiency (F_v/F_m), chlorophyll content and photosynthetic rate (A_{max}) of *E. nitens* saplings inoculated with *A. luteobubalina* isolates over the period of observations.

Treatments / Time	Water potential (ψ) MPa	Efficiency of PS II F_v/F_m	Chlorophyll content µg/g	Photosynthetic rate (A_{max}) µmol/m²/s
UW-C / T_0	-2.9 ± 0.3 [a]	0.84 ± 0.00 [a]	2322.4 ± 98.7 [a]	12.5 ± 1.2 [a]
UW-1 / T_0	-3.7 ± 0.3 [b]	0.83 ± 0.01 [a]	2150.5 ± 76.6 [a]	11.9 ± 0.9 [a]
UW-2 / T_0	-2.8 ± 0.3 [a]	0.84 ± 0.01 [a]	2102.5 ± 66.0 [a]	12.7 ± 0.9 [a]
UW-C / T_1	-1.0 ± 0.1 [a]	0.77 ± 0.01 [a]	3120.5 ± 144.5 [a]	14.5 ± 0.9 [a]
UW-1 / T_1	-1.0 ± 0.1 [a]	0.78 ± 0.01 [a]	2910.7 ± 89.8 [a]	14.2 ± 0.6 [a]
UW-2 / T_1	-0.9 ± 0.1 [a]	0.79 ± 0.01 [a]	3008.2 ± 97.7 [a]	14.2 ± 0.5 [a]
UW-C / T_2	-2.4 ± 0.2 [a]	0.78 ± 0.01 [a]	2712.7 ± 126.5 [a]	12.3 ± 0.8 [a]
UW-1 / T_2	-2.1 ± 0.1 [a]	0.78 ± 0.01 [a]	2614.8 ± 87.4 [ab]	13.0 ± 0.7 [a]
UW-2 / T_2	-2.5 ± 0.2 [a]	0.79 ± 0.01 [a]	2644.0 ± 100.5 [ab]	13.2 ± 0.7 [a]

Note:

- *The values followed with different letter in the same column are significant at α=0.05, as determined by a Duncan's test-ANOVA for each variable at each time of observation.*
- *T_0 = pre-inoculation; T_1= 3 months post-inoculation; T_2= 6 months post-inoculation.*

Appendix 4.2 – *Study of viability of* A. luteobubalina *in a mixed-soil potting medium*

Brief description of the trial:

The viability of *A. luteobubalina* inoculum in a mixed-soil potting medium was investigated. This was done because of concerns raised from the preliminary trial where the perlite and vermiculite (1:1) potting medium resulted in poor survival of inoculum (Appendix 4.1). *Armillaria luteobubalina* isolates (strain 1 and 2) that had been grown on *E. globulus* rods for three months were buried in pots containing a mixture of soil, sand and pine-bark compost (Amgrow Nu-Earth ™). Ten pieces of inoculum rod were placed into each of five pots per treatment. Sterilized *E. globulus* rods were used as controls. There was no plant material. The pots were placed in the same open growing area as used for the main trial. The viability of the isolates was confirmed by describing the visual morphology of the inoculum and quantifying the percentage survival of inoculum in the pots every month for six months.

Results:

The potting medium was suitable for supporting inoculum growth for at least three months. However, there was a marked decrease in the percentage survival of the inoculum, from 80% to 50 % and 80% to 60% for isolate strain-1 and -2 respectively, after five months in the medium (Table 4.6). This was associated with reduced mycelial growth on the surface of the rods (Fig. 4.11). This suggests that the greatest opportunity for infecting the host would occur in the first three months after inoculated rods are introduced into this medium. It was concluded that this mixed soil medium was possibly more preferable than the perlite-vermiculite for fungal survival.

Table 4.6. Percentage survival of the inoculum based on its gross morphology

Isolates	1 m.p.i	2 m.p.i	3 m.p.i	5 m.p.i	6 m.p.i
Control	0	0	0	0	0
Isolate strain 1	80	90	80	50	50
Isolate strain 2	100	80	80	60	40

***Note**: m.p.i = month post-inoculation*

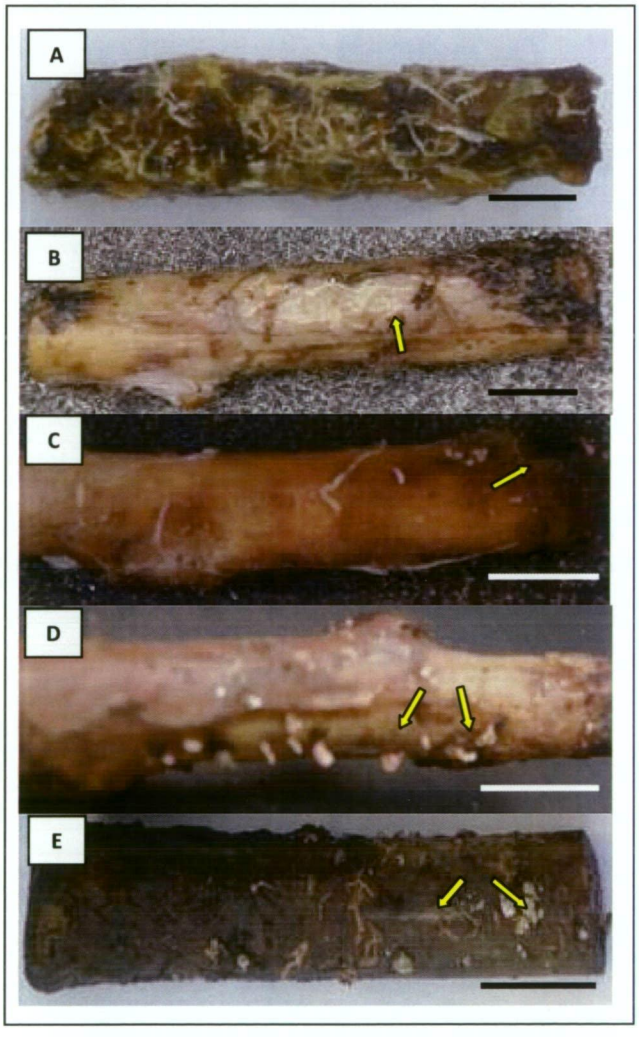


Figure 4.11. Inoculum rods. A. pre-inoculation; B. 1 month post-inoculation; C. 2 months post- inoculation; D. 3 months post-inoculation; E. 5 months post-inoculation. Bar scale = 1 cm. Arrows show the rhizomorph formation.

Appendix 4.3 – *Alignment of* A. luteobubalina *strain-1 and stain-2 sequences*

(The positions of the seven nucleotides that differ between isolates are indicated)

```
                1         11        21        31        41        51
consensus       GAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAAGC
Strain-1        ..................................................
Strain-2        ..................................................

                61        71        81        91        101       111
consensus       TTGAATCGTAGCGTTGAGAGCTGTTGCTGACCTGTTAAAGGGTATGTGCACGTTCAAAGT
Strain-1        ..................................................
Strain-2        ..................................................

                121       131       141       151       161       171
consensus       GTTGCGTTTTATTCTTTTCCCCCTGTGCACCTTTGTAGACTTGGTTAAGGATGTCGCTGT
Strain-1        ..................................................
Strain-2        ..................................................

                181       191       201       211       221       231
consensus       TGAGTGTTGCTCTTGAGCTCCCTTTGATTTT GAAGGGTTGCTTT GAGCTTCCCTTTCT
Strain-1        ..............................C.............A.............
Strain-2        ..............................T.............C.............

                241       251       261       271       281       291
consensus       TTGTCTACCAAGTCTATGTCTATAATCTCTTGTATGTGTAGAATGTCTTGTTTATTGGAT
Strain-1        ..................................................
Strain-2        ..................................................

                301       311       321       331       341       351
consensus       GCTTGCGTCCTTTAAATCTTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGA
Strain-1        ..................................................
Strain-2        ..................................................

                361       371       381       391       401       411
consensus       TGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAG
Strain-1        ..................................................
Strain-2        ..................................................

                421       431       441       451       461       471
consensus       TCTTTGAACGCACCTTGCGCCCTTTGGTATTCC AAGGGCATGCCTGTTTGAGTGTCATT
Strain-1        ..............................A.....................
Strain-2        ..............................G.....................

                481       491       501       511       521       531
consensus       AAATTCTCAACCTTGCCTTCTTTTACTAGGAGTGCGATGGATTGGATATGGGGGTTTGCT
Strain-1        ..................................................
Strain-2        ..................................................

                541       551       561       571       581       591
consensus       GGTCTCTAACGAGATCAGCTCCTCTGAAATGCATTAGCAGAAACCGTTTGACTTTGGCTG
Strain-1        ..................................................
Strain-2        ..................................................

                601       611       621       631       641       651
consensus       CTAGGCTGTGATAATATCTACGCCTTGGTGGTTGAGTCGAGTACACAAGTCCTACAACAA
Strain-1        ..................................................
Strain-2        ..................................................

                661       671       681       691       701       711
consensus       GTATC TTACTTG T CGTTTGACTTTGTATAAGGATTCAGCTTCTAACGGTCCATTGA
Strain-1        .....T.......C.T.....................................G
Strain-2        .....C.......T.C.....................................C

                721       731       741       751
consensus       TTGGACAATTTATTGACTATTGACCTCAAATCAGGTAGG
Strain-1        ....................................
Strain-2        ....................................
```

Appendix 4.4 – *Standard curve of chlorophyll content of* E. nitens *leaves estimated by SPAD*

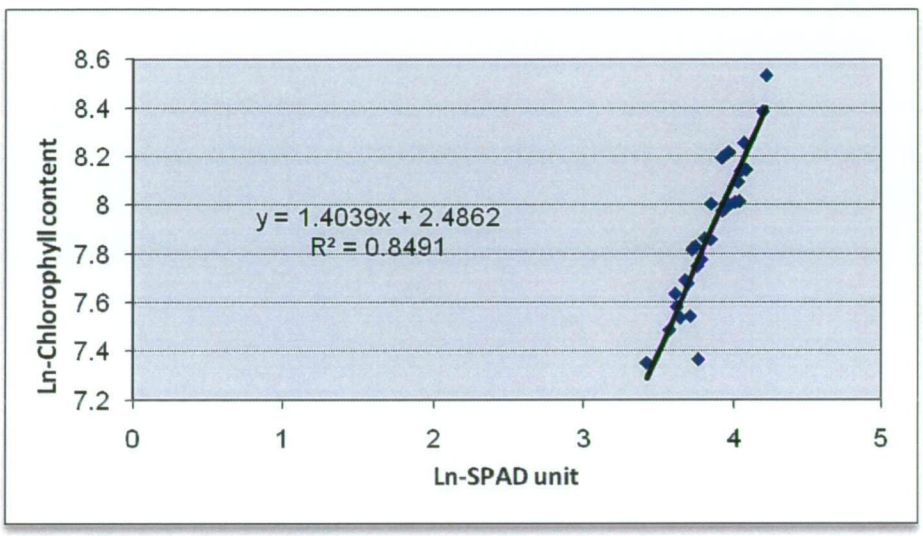


Figure 4.12. Standard curve of *E. nitens* chlorophyll content

Total chlorophyll content was quantified using the equation below (see Fig. 4.12):
Total chlorophyll = Exp (1.4039(Ln(SPAD)) + 2.4862*

Chapter 5 - General discussion

5.1 Introduction

This thesis contributes new data and information for the development of root-rot disease detection in eucalypt stands, especially *Eucalyptus pellita* plantations in Indonesia. The most significant result of this thesis is that *E. pellita* trees which are being planted as a substitute species to *Acacia mangium* in order to avoid root rot disease caused by *Ganoderma philippii* (Eyles, Beadle *et al.* 2008) are still threatened by the same fungal pathogen as well as other root-rot causal agents. This chapter summarises the main findings of the thesis and discusses them in the context of efforts to prevent severe damage and loss caused by root disease. Some aspects requiring further research are highlighted as well.

5.2 Assessment of root-rot disease in E. pellita plantations

As for many other tree crops, early detection methods for root rot caused by basidiomycete fungi remain a challenge in respect to stands of *E. pellita* . In a forest-tree plantation, where a certain degree of mortality (e.g. at establishment) is inevitable, the disease is difficult to recognise in its early stages and is only fully evident to operational staff when impact reaches significant levels. Although it may be impossible to eradicate once the disease is established, monitoring and diagnostic approaches can be taken so that any options available to reduce their establishment and spread (Garbelotto 2004) can be deployed. Even if management options are limited, a spatial and temporal understanding of incidence and severity

will assist forest managers in adjusting yield estimates and wood resource allocation.

Methods for diagnosis of root-rot disease are traditionally based on the presence of characteristic sporocarps, the appearance of the above-ground symptoms, and when other diagnostic characters are lacking, the isolation and identification of the suspected fungal causal agent into culture (Manion 1991). The work of this thesis (Chapter 2) has highlighted that it is important to isolate and identify fungal cultures from diseased roots and not to base an identification of the causal agent on sporocarp recognition alone. Even though no sporocarps of *Ganoderma philippii* were observed, molecular analyses identified 29 out of 47 samples of red root-rot as *G. philippii*. This chapter also showed the importance of combining morphological and molecular approaches to determine the taxonomic identity of the fungi especially where, as for this study, there is a wide diversity of basidiomycete wood-rotting fungi which include several fungal pathogens capable of causing root rot-rot disease.

Accurate taxonomic identification underpins effective disease management. The identification of several putative pathogens will allow us to carry out valid pathogenicity tests. The need for continued taxonomic studies is strongly supported by the identification, in this study, of a potential biocontrol for root-rot disease. Isolates identified in Chapter 2 as *Phlebiopsis* sp. and which are similar taxonomically to those used as biocontrol for *Heterobasidion annosum* root-rot in temperate forests, need to be tested to against *Ganoderma* and *Phellinus* species in tropical conditions.

Many countries such as Australia, USA, and New Zealand have applied forest monitoring or surveillance as an integral part of forest management

(Carnegie 2008). There is a critical need in Indonesia to develop a method for crown-condition monitoring that can be applied on a regular basis for recognizing any alteration in trees, including changes caused by root-rot. If there are any actions needed or if it is possible to reduce losses, these can be applied at the appropriate time.

The method developed in Chapter 3 is able to discriminate between crown-condition classes. However if this method is to be applied in a routine manner for monitoring tree condition in the plantations, well-trained assessors are required. While the crown-condition assessment method can be used to indicate health status at a site level it cannot be reliably used to predict which individual trees are infected. The difficulties in recognizing root-rot incidence from the tree's crown-condition is understandable since the most obvious symptoms are present on the roots (Filip 1986; Garbelotto 2004; Omdal, Shaw *et al.* 2004; Wallis and Bloomberg 1981). Root rot is considered as a 'sudden-death' disease in respect to the visible change in above–ground symptoms.

Chapter 4 showed the possibility of using physiological changes to indicate root rot before obvious symptoms appear. We now have a model pathosystem which can be used in future research to further investigate these physiological changes. Chlorophyll fluorescence which was the most sensitive parameter in our study offers a non-destructive alternative for the early diagnosis of stressed plants even before other physiological disruptions e.g. significant reductions in chlorophyll content and photosynthetic rate are detectable. This understanding of the physiological processes at a tree level requires further research to upscale to canopy level so this type of information can be integrated operationally into remote sensing assessment techniques to detect crown condition – such as Light Detection

and Ranging (LIDAR) (Goulas, Camenen *et al.* 1997; Saito, Saito *et al.* 2000) and hyperspectral remote sensing (Zarco-Tejada, Miller *et al.* 2000). .

5.3 Management recommendations for root-rot disease

It is recognised that root-rot fungi are rarely eliminated once they are established, and their presence may have a long-term impact on commercial forestry. A long-term vision of root-rot management therefore needs to be designed and applied, by both plantation companies and policy makers in the Indonesian Ministry of Forestry. A system to quantify and monitor disease incidence and severity is required but there are also other recommendations that can be made to reduce impact and successfully manage root-rot disease:

- ***Site-hazard rating & species/clone-site matching systems*** need to be developed and taken into consideration in the plantation expansion scheme. Plantation expansion in Indonesia was begun in the early 1980s and aimed to minimise the utilisation of natural forest. The program is targeted at converting unproductive *Imperata* grassland and secondary shrubland into productive plantation, and by the end of 2009 the program aimed to have established 5M ha of plantation (Departemen Kehutanan Republik Indonesia 2005; Rimbawanto 2002). In reality, plantations are not only established on the unproductive grassland and shrubland but also on the degraded natural forest. The plantations that were established on the sites which previously were natural forests are more likely to be prone to root-rot disease. Therefore, it is important to develop a site-hazard rating system as a complementary system to support the plantation expansion scheme. Thus, apart from considering soil

types and nutrients and planting a host that will grow vigorously on a particular site, potential diseases in the respective areas should be taken into account to decide what species should be planted where. For those plantations already established, consideration of previous land use and rotation age will also indicate risk of root-rot.

- ***Maintaining biodiversity*** (avoiding monoclonal or monoculture planting system). Forest plantations usually consist of monocultures of high-yielding exotic species or clonal materials which are likely to be more susceptible to disease problems, including root rot. Many studies show that planting timber trees in mixtures is better than monocultures and that mixtures have the potential for obtaining greater biomass per unit area (Bristow, Vanclay *et al.* 2006; Piotto, Viquez *et al.* 2004), minimizing intra-species competition (Forrester, Bauhus *et al.* 2005), improving soil fertility (Bauhus, Khanna *et al.* 2000), and reducing incidence of pest and diseases (Bosu, Cobbinah *et al.* 2006). In particular the benefits of mixed species plantation (pine and birch) on sites with root-rot caused by *Heterobasidion annosum* was shown by Lygis, Vasiliauskas *et al.* (2004). It is strongly suggested, on sites in Indonesia that are characterised with a high hazard rating for root rot, to establish a mixed planting system of *E. pellita* with other species and or clones that have different levels of susceptibility to root rot.

References

Agrios GN (2005) 'Plant Pathology.' (Academic Press: New York)

Annesi T, Curcio G, D'Amico L, Motta E (2005) Biological control of *Heterobasidion annosum* on *Pinus pinea* by *Phlebiopsis gigantea. Forest Pathology* **35**, 127-134.

Anonymous (2008) 'Report on fieldwork in Indonesia 28th January 2008 - 27th February 2008 & 26th May 2008 - 14th June 2008.' Australian Centre for International Agricultural Research [unpublished report], Canberra.

Anonymous (2009) Oxford Dictionary online. In 'Oxford English Dictionary'. (Oxford University Press: Oxford, UK).URL: http://dictionary.oed.com. [Accessed on 6th August 2009].

Ariffin D, Idris AS, Sigh G (2000) Status of *Ganoderma* in Oil Palm. In '*Ganoderma* Diseases of Perennial Crops'. (Eds J Flood, PD Bridge and M Holderness). (CABI International: Wallingford)

Baron C, Zambryski PC (1995) The plant response in pathogenesis, symbiosis and wounding: variation on a common theme? *Annual Review of Genetics* **29**, 107-129.

Battaglia M, Beadle C, Loughhead S (1996) Photosynthetic temperature responses of *Eucalyptus globulus* and *Eucalyptus nitens. Tree Physiology* **16**, 81-89.

Bauhus J, Khanna PK, Menden N (2000) Aboveground and belowground interaction in mixed plantation of *Eucalyptus globulus* and *Acacia mearnsii. Can. J. Forest Res.* **30**, 1886-1894.

Baumgartner K, Rizzo DM (2006) Relative resistance of grapevine rootstocks to *Armillaria* root disease. *American Journal of Enology and Viticulture* **57**, 408-414.

Beadle CL (2000) Physiology of euclypts in relation to disease. In 'Diseases and pathogen of Eucalyptus'. (Eds PJ Keane, GA Kile, PD Podge and BN Browns). (CSIRO Publishing: Collingwood)

Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T (2004) Complex regulation of gene expression, photosynthesis and sugar level by pathogen infection in tomato. *Physiologia Plantarum* **122**, 419-428.

Berger S, Sinha AK, Roitsch T (2007) Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interaction. *Journal of Experimental Botany* **58**, 4019-4026.

Berglund M, Rönnberg J, Holmer L, Stenlid J (2005) Comparison of five strains of *Phlebiopsis gigantea* and two *Trichoderma* formulations for treatment against natural *Heterobasidion* spore infections on Norway spruce stumps. *Scandinavian Journal of Forest Research* **2005**.

Blanchard RO, Tattar TA (1981) 'Field and laboratory guide to tree pathology.' (Academic Press, Inc.: New York)

Bloomberg WJ, Morrison DJ (1989) Relationship of growth reduction in Douglas fir to infection by *Armillaria* root disease in Southeastern British Columbia. *Phytopathology* **79**, 482-487.

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* **28**, 355-377.

Bolland L (1984) *Phellinus noxius*: cause of a significant root-rot in Queensland hoop pine plantations. *Australian Forestry* **47**, 2-10.

Bonfig KB, Schreiber U, Gabler A, Roitsch T, Berger S (2006) Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in *Arabidopsis* leaves. *Planta* **225**, 1-12.

Bosu PP, Cobbinah JR, Nichols JD, Nkrumah EE, Wagner MR (2006) Survival and growth of mixed plantation of *Milicia excelsa* and *Terminalia superba* 9 years after planting in Ghana. *Forest Ecology and Management* **233**, 352-357.

Bristow M, Vanclay JK, Brooks L, Hunt M (2006) Growth and species interactions of Eucalyptus pellita in a mixed and monoculture plantation in the humid tropics of north Queensland. *Forest Ecology and Management* **233**, 285-294.

Carnegie AJ (2008) Guest editorial and introduction to this special issue: A decade of forest health surveillance in Australia: an overview. *Australian Forestry* **71**, 161-163.

Chee KH (1990) Present status of rubber diseases and their control. *Rev. Plant Pathol.* **69**, 423-430.

Chou H, Bundock N, Rolfe S, Scholes J (2000) Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. *Mol. Plant Pathol.* **1**, 99-113.

Close DC, Beadle CL (2003) Chilling-dependent photoinhibition, nutrition and growth analysis of *Eucalyptus nitens* seedlings during establishment. *Tree Physiology* **23**, 217-226.

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Wingfield JM (2003) Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. *Mycologia* **95**: 285-293.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ (2001) The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* **10**, 387-396.

Corner EJH (1991) Ad Polyporaceas VII. The xanthachroic polypores Beih. *Nova Hedwigia* **101**, 1-175.

Crane CE, Shearer BL (2007) Hemispherical digital photographs offer advantages over conventional methods for quantifying pathogen-mediated changes caused by infestation of *Phytophthora cinnamomi*. *Australasian Plant Pathology* **36**, 466-474.

D'Souza DT, Tiwari R, Sah AK, Raghukumar C (2006) Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes. *Enzyme Microb. Technol.* **38**, 504-511.

Dai Y-C (1999) *Phellinus sensu lato* (Aphyllophorales, Hymenochaetaceae) in East Asia. *Acta Bot. Fenn.* **166**, 1-115.

Darus A, Seman IA, Hassan AH (1989) Significance of the black line within oil palm tissue decayed by *Ganoderma boninense*. *Elaesis* **1**, 11-16.

Davidson NJ, Battaglia M, Close DC (2004) Photosynthetic responses to overnight frost in *Eucalyptus nitens* and *E. globulus*. *Trees* **18**, 245-252.

DeJong TM, Doyle JF (1985) Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus*

Departemen Kehutanan Republik Indonesia (2005) Rencana strategis kementrian negara/lembaga (Renstra-KL) Departemen Kehutanan Tahun 2005-2009. In. (Departemen Kehutanan (P.04/Menhut-II/2005 tanggal 14 Februari 2005)) *persica*). *Plant, Cell and Environment* **8**, 701-706.

Dowson CG, Rayner ADM, Boddy L (1988) The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments. *New Phytologist* **109**, 423-432.

Dubey RS (1997) Photosynthesis in plants under stressful condition. In 'Handbook of Photosynthesis'. (Ed. M Pessarakli) pp. 1027. (Marcel-Dekker, Inc.: New York, USA)

Dunne CP, Glen M, Tommerup IC, Shearer BL, Hardy GESJ (2002) Sequence variation in the rDNA ITS of Australian *Armillaria* species and intra-specific variation in *A. luteobubalina*. *Australiasian Plant Pathology* **31**, 241-251.

Durand-Gasselin T, Asmady H, Flori A, Jacquemard JC, Hayun Z, Breton F, de Franqueville H (2005) Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninense* - prospect for future breeding. *Mycopathologia* **159**, 93-100.

Edgar JG, Kile GA, Almond CA (1976) Tree decline and mortality in selective logged Eucalypt forest in Central Victoria. *Australian Forestry* **39**, 288-303.

Epron D, Dreyer E, Breda N (1992) Photosynthesis of oak trees (*Quercus petraea* (Matt) Liebl.) during drought stress under field condition: diurnal course of net CO_2 assimilation and photochemical efficiency of photosystem II. *Plant, Cell and Environment* **15**, 809-820.

Eyles A, Beadle C, Barry K, Francis A, Glen M, Mohammed C (2008) Management of fungal root-rot pathogens in tropical acacia plantations. *For. Path.* **38**, 332-355.

FAO (1999) State of the World's Forests. In. (Food and Agriculture Organization of the United Nation: Rome)

Farid AM, Lee SS (2006) Root rot in tree species other than *Acacia*. In 'Heart rot and root rot in tropical *Acacia* plantations'. Yogyakarta, Indonesia. (Eds K Potter, A Rimbawanto and C Beadle). (ACIAR Proceedings No. 124)

Farid AM, Lee SS, Maziah Z, Rosli H, Norwati M (2005) Basal root rot, a new disease of teak (*Tectona grandis*) in Malaysia caused by *Phellinus noxius*. *Malaysian Journal of Microbiology* **1**, 40-45.

Filip GM (1986) Symptom expression of root diseased trees in mixed conifer stands in central Washington. *Western Journal of Applied Forestry* **1**, 46-48.

Flood J, Hasan Y, Turner PD, O'Grady EB (2000) The spread of *Ganoderma* from infective sources in the field and its implications for management of the disease in oil palm. In '*Ganoderma* diseases of perennial crops'. (Eds J Flood, PD Bridge and M Holderness) pp. 101-112. (CABI Publishing: Wallingford, Oxon, UK)

Forrester DI, Bauhus J, Cowie A (2005) On the success and failure of mixed-species tree plantation: lessons learned from a model system of *Eucalyptus globulus* and *Acacia mearnsii*. *Forest Ecology and Management* **209**, 147-155.

Fox ED, Curry SJ (1980) Notes on the truat tree (*Eucalyptus gomphocephala*) in the Perth area. *The Western Australian Naturalist* **14**, 174-186.

Gamon JA, Pearcy RW (1989) Leaf movement, stress avoidance and photosynthesis in *Vitis californica*. *Oecologia* **79**, 475-481.

Garbelotto M (2004) Root and butt rot diseases. In 'Forest pathology'. pp. 1-9. (Elsevier Ltd.: California, USA)

Gardes M, Bruns T (1993) ITS primer with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**.

Glen M, Bougher NL, Francis AA, Nigg SQ, Lee SS, Irianto R, Barry KM, Beadle CL, Mohammed CL (2009) *Ganoderma* and *Amauroderma* species associated with root-rot disease of *Acacia mangium* plantation trees in Indonesia and Malaysia. *Australasian Plant Pathology* **38**, 1-12.

Glen M (2006) The use of DNA techniques to identify fungi. In 'Heartrot and rootrot in tropical Acacia plantations'. Yogyakarta, Indonesia (Eds K Potter, A Rimbawanto and C Beadle). (ACIAR Proceedings No. 124, 7-9 Feb 2006).

Goodwin N, Coops NC, Stone C (2005) Assessing plantation canopy condition from airborne imagery using spectral mixture analysis and fractional abundances. *International Journal of Applied Earth Observation and Geoinformation* **7**, 11-28.

Goulas Y, Camenen L, Guyot G, Cerovic Z, Briantais JM, Schmuch G, Moya I (1997) Measurement of laser-induced fluorescence decay and reflectance of plant canopies. *Remote Sensing Reviews* **15**, 305-322.

Greig BJW, Strouts RG (1977) Honey fungus. *Arboricultural Leaflet* **2**, 2-11.

Grieg JW (1976) Biological control of *Fomes annosus* by *Peniophora gigantea*. *European Journal of Forest Pathology* **6**, 65-71.

Groom QJ, Baker NR (1992) Analysis of light-induced depression of photosynthesis in leaves of wheat crop during the winter. *Plant Physiology* **100**, 1217-1223.

Guest D, Brown J (1997) Infection process. In 'Plant pathogens and plant diseases '. (Eds JF Brown and HJ Olsen) pp. 245-262. (University of New England: Armidale, NSW,Australia)

Gunthardt-Goerg MS, Vollenweider P (2007) Linking stress with macroscopic and microscopic leaf response in trees: New diagnostic perspectives. *Environmental Pollution* **147**, 467-488.

Guyot J, Flori A (2002) Comparative study for detecting *Rigidoporus lignosus* on rubber trees. *Crop Protection* **21**, 461-466.

Hadfield JS, Goheen DJ, Filip GM, Schmitt CL, Harvey RD (1986) 'Root diseases in Oregon and Washington conifers. R6-FPM-250-86.' USDA Forest Service, PNW Region, Washington, D.C.

Hall FG, Hilker T, Coops NC, Lyapustin A, Huemmrich KF, Middleton E, Margolis H, Drolet G, Black TA (2008) Multi-angle remote sensing of forest light use efficiency by observing PRI variation with canopy shadow fraction. *Remote Sensing of Environment* **112**, 3201-3211.

Haniff MH, Ismail S, Idris AS (2005) Gas exchange responses of oil palm to *Ganoderma boninense* infection. *Asian Journal of Plant Sciences* **4**, 438-444.

Hardiyanto EB (2003) Growth and genetic improvement of *Eucalyptus pellita* in South Sumatra, Indonesia. In 'Eucalypts in Asia'. Zhanjiang, Guangdong, People's Republic of China. (Ed. JW Turnbull). (ACIAR)

Harrington TC (1986) Growth decline of wind-exposed red spruce and balsam fir in the White Mountains. *Canadian Journal of Forest Research* **16**, 232-238.

Harwood CE, Alloysius D, Pomroy P, Robson KW, Haines MW (1997) Early growth and survival of *Eucalyptus pellita* provenance in range of tropical environments, compare with *E. grandis, E. urophylla* and *Acacia mangium. New Forest* **4**, 203-219.

Haskell BD, Norton BG, Costanza R (1992) What is ecosystem health and why should we worry about it? In 'Ecosystem health'. (Eds R Costanza, BG Norton and Haskell) pp. 3-20. (Island Press: Washing D.C)

Havaux M (1992) Stress tolerance of photosystem II *in vivo*: antagonistic effects of water, heat and photoinhibition stresses. *Plant Physiology* **100**, 424-432.

He J, Chee CW, Goh CJ (1996) 'Photoinhibition' of *Heliconia* under natural tropical condition: the importance of leaf orientation for light interception and leaf temperature. *Plant, Cell and Environment* **19**, 1238-1248.

Hilker T, Coops NC, Hall FG, Black TA, Wulder MA, Nesic Z, Krishnan P (2008) Separating physiologically and directionally induces changes in PRI using BRDF models. *Remote Sensing of Environment* **112**, 2777-2788.

Hood IA, Redfern DB, Kile GA (1991) Armillaria in planted hosts. In 'Armillaria root disease'. (Eds CG Shaw III and GA Kile). (Forest Service United States Department of Agriculture: Washington, D.C)

Hseu R, Wang H, Wang H, Moncalvo JM (1996) Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. *Applied and Environmental Microbiology* **62**, 1354-1363.

Innes JL (1993) 'Forest Health: Its Assessment and Status.' (CAB International: Wallingford, UK)

Irianto RSB, Barry K, Hidayati N, Ito S, Fiani A, Rimbawanto A, Mohammed C (2006) Incidence and spatial analysis of root rot of *Acacia mangium* in Indonesia. *Journal of Tropical Forest Science* **18**, 157-165.

Irianto RSB, Barry KM, Santoso E, Turjaman M, Widyati E, Sitepu I, Mohammed CL (2003) Heart rot and root rot disease of Acacia mangium plantation in Sumatra Indonesia. In 'Proceedings of the International Congress of Plant Pathology'. Christchruch, NZ, Feb 2-8 th

Issac S (1992) 'Fungal-Plant Interaction.' (Chapman & Hall: London)

Ivory MH (1996) Diseases of forest trees caused by the pathogen *Phellinus noxius*. In 'Forest trees and palms: diseases and control'. (Eds SP Raychaudhuri and K Maramoroch) pp. 336. (Oxford and IBH Publishing Co.: New Delhi, India)

Johnson EW, Wittwer D (2008) Aerial detection surveys in tne United States. *Australian Forestry* **71**, 212-215.

Kallio T, Hallaksela AM (1979) Biological control of *Heterobasidion annosum* (Fr.) Bref. (Fomes annosus) in Finland. *European Journal of Forest Pathology* **9**, 298-308.

Kavanagh K (2005) 'Fungi: biology and application.' (John Wiley & Sons: West Sussex, UK)

Kececioglu D (1991) 'Reliability engineering handbook.' (Prentice Hall, Inc.: Englewood Cliffs, New Jersey)

Kile GA (2000) Woody root rots of Eucalypts. In 'Diseases & Pathogen of Eucalyptus '. (Eds PJ Keane, GA Kile, PD Podger and BN Browns) pp. 293-306. (CSIRO Publishing: Melbourne)

Kile GA, Watling R, Malajczuk N, Shearer BL (1983) Occurence of *Armillaria luteobubalina* Watling & Kile in Western Australia. *Australian Plant Pathology Society* **12**, 18-20.

Klecka WR (1980) 'Discriminant Analysis.' (Sage Publication: Beverly Hills,CA)

Kolb TE, Wagner MR, Covington WW (1994) Utilitarian and ecosystem perspective: concepts of forest health. *Journal of Forestry* **92**, 10-15.

Kornerup A, Wanscher JH (1961) 'Methuen handbook of colour.' (Methuen & Co. Ltd.: London, UK)

Lachenbruch PA (1975) 'Discriminant analysis.' (Hafner Press: New York)

Lim TM (1977) Production, germination and dispersal of basidiospores of *Ganoderma pseudoferreum* on *Hevea. J. Rubb. Res. Inst. Malaysia* **25**, 93-99.

Logan BA, Adams III WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. *Functional Plant Biology* **34**, 853-859.

Loguercio-Leite C, Wright JE (1995) The genus *Phellinus* (Hymenochaetaceae) on the Island of Santa Catarina, Brazil. *Mycotaxon* **54**, 361-388.

Lopes DB, Berger RD (2001) The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology* **91**, 212-220.

Lucas GB, Campbell CL, Lucas LT (1992) 'Introduction to plant disease: identification and management.' (Springer Publishing: New York, USA)

Lucas JA (1998) 'Plant Pathology and Plant Pathogens.' (Blackwell Science Ltd.: Cambridge)

Luyssaert S, Raitio H, Vervaeke P, Mertens J, Lust N (2002) Sampling procedure for the foliar analysis of deciduous trees. *J. Environ. Monit* **4**, 858-864.

Lygis V, Vasiliauskas R, Stenlid J (2004) Planting *Betula pendula* on pine sites infested by *Heterobasidion annosum*: disease transfer, silvicultural evaluation, and community of wood inhabiting fungi. *Can. J. Forest Res.* **34**, 120-130.

Manion PD (1991) 'Tree disease concepts.' (Prentice-Hall: New Jersey)

Mansilla JP, Aguin O, Sainz MJ (2001) A fast method for production of *Armillaria* inoculum. *Mycologia* **93**, 612-615

Martin I, Alonso N, Lopez MC, Prieto M, Cadahia C, Eymar E (2007) Estimation of leaf, root, and sap Nitrogen status using the SPAD-502 chlorophyll meter for ornamental shrubs. *Communication in Soil Science and Plant Analysis* **38**, 1785-1803.

Maurel M, Robin C, Capdevielle X, Loustou D, Deprez-Loustau M-L (2001) Effects of variable root damage caused by *Phytophthora cinnamomi* on water relations of chestnut saplings. *Ann.For.Sci* **58**, 639-651.

Menge JA, Ploetz RC (2003) Disease of avocado. In 'Disease of tropical fruit crops'. (Ed. RC Ploetz) pp. 35-72. (CABI Publishing: Wallingford, UK)

Meyer S, Saccardt AK, Rizza F, Genty B (2001) Inhibition of photosynthesis by *Colletotrichum lindemuthianum* in bean determined by chlorophyll fluorescence imaging. *Plant Cell Environ.* **24**, 947-955.

Mizoue N (2002) CROCO: semi-automatic image analysis system for crown condition assessment in forest health monitoring. *J. For. Plann.* **8**, 17-24.

Mizoue N, Dobbertin M (2003) Detecting differences in crown transparency assessments between countries using the image analysis system CROCO. *Environmental Monitoring and Assessment* **89**, 179-195.

Mizoue N, Masutani T (2003) Image analysis measure of crown condition, foliage biomass and stem growth relationships of *Chamaecyparis obtusa*. *Forest Ecology and Management* **172**, 79-88.

Moncalvo JM, Wang HF, Hseu RS (1995) Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. *Mycological Research* **99**, 1489-1499.

Morrison D, Merler H, Norris D (1991) 'Detection, recognition and management of *Armillaria* and *Phellinus* root diseases in the Southern Interior of British Columbia.' B.C. Ministry of Forestry Research Branch, 179, Victoria, B.C.

Morrison DJ, Pellow KW, Norris DJ, Nemec AFL (2000) Visible versus actual incidence of *Armillaria* root disease in juvenile coniferous stands in the southern inferior of British Columbia. *Can. J. For. Res.* **30**, 405-414.

Morrison DJ, Williams RE, Whitney RD (1991) Infection, disease development, diagnosis, and detection. In 'Armillaria root disease. Agricultural handbook no.691.'. (Eds CGI Shawn and GA Kile). (USDA Forest Service: Washington DC)

Mutava RN (2009) Charachterization of grain sorghum for physiological and yield traits associated with drought tolerance. Kansas State University.

Newsam A (1964) Root disease infection and treatment. In 'Planter's Bulletin'. (Rubber Research Institute of Malaysia: Kuala Lumpur)

Nicolotti G, Gonthier P (2005) Stump treatment against *Heterobasidion* with *Phlebiopsis gigantea* and some chemicals in *Picea abies* stands in the western Alps. *Forest Pathology* **35**, 365-374.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary bioinformatics online* **4**, 193-201.

Njambere EN, Attanayake RN, Chen W (2010) Aplication of molecular marker and DNA sequence in identifying fungal pathogen of cool season grain legumes. In 'Molecular identification of fungi'. (Eds Y Gherbawy, K Voigh) (Springer: Berlin, Newyork).

Nobles MK (1948) Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. *Canadian Journal of Research* **C 26**, 281-431.

Nobles MK (1958) Cultural characters as aguide to the taxonomy and phylogeny of the Polyporaceae. *Canadian Journal of Botany* **36**, 883-926.

Nobles MK (1965) Identification of cultures of wood-inhabiting Hymenomycetes. *Canadian Journal of Botany* **43**, 1097-1139.

Nunez M, Ryvarden L (2000) East Asia polypores - Ganodermataceae and Hymenochaetaceae, Vol. 1. *Synop. Fungorum* **13**, 1-168.

Old K, Coops N, Tongway D, Stone C, Smith I (1999) 'Scoping Study for Montreal Process National Indicator of Forest Health and Vitality 3.1.c.' Departement of Agriculture Fisheries & Forestry Australia.

Olson Å, Stenlid J (2000) Functional units in root diseases: Lessons from Heterobasidion annosum. In '*Ganoderma* diseases of perenial crops'. (Eds J Flood, PD Bridge, M Holderness). (CABI Publishing: Wallingford, New York).

Omdal DW, Shaw CG, III, Jacobi WR (2004) Symptom expression in conifers infected with *Armillaria ostoyae* and *Heterobasidion annosum. Can. J. For. Res.* **34**, 1210-1219.

Onsando JM (1997) Distribution, severity and spread of *Armillaria* root disease in Kenya tea plantation. *Plant Disease* **81**, 133-137.

Ottander C, Campbell D, Oquist G (1995) Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. *Planta* **197**, 176-183.

Park DS, Sung JM, Kim YS, Yoo YM, Ryu YJ, Cha DY (1994) Analysis of interspecific allozyme variation within the genus Ganoderma by polyacrylamide gel isoelectric focusing. *RDA Journal of Agricultural Science* **36**, 212-221.

Parrotta J (1992) The role of plantation forests in rehabilitating degraded tropical ecosystem. *Agricultural Ecosystem and Environment* **41**, 115-133.

Percy KE (2002) Is air pollution an important factor in international forest health? In 'Effects of air pollution on forest health and biodiversity in forest of the Carpathian Mountains'. (Eds RC Szaro, A Bytnerowicz and J Oszlanyi) pp. 23-42. (IOS Press: Amsterdam)

Percy KE, Ferretti M (2004) Air pollution and forest health: toward new monitoring concepts. *Environmental Pollution* **130**, 113-126.

Peries O (1965) Recent development in the control of the diseases of the Hevea rubber tree. *Q.J. Rubber Res. Inst. Ceylon* **41**, 33-46.

Piotto D, Viquez E, Montagnini F, Kanninen M (2004) Pure and mixed forest plantation with native species of the dry tropics of Costa Rica: a comparison of growth and productivity. *Forest Ecology and Management* **190**, 359-372.

Ploetz RC, Schaffer B (1987) Effect of flooding and Phytophthora root rot on photosynthetic characteristics of avocado. *Proc. Fla. State Hort. Soc.* **100**, 290-294.

Podger FD (1972) *Phytopthora cinnamomi* - a cause of letal diseasemin indigenous plant communities in Western Australia. *Phytopathology* **62**, 972-981.

Poulsen J, French A (2009) (Ed.)^(Eds) 'Discriminant function analysis (DA).' (URL: http://userwww.sfsu.edu/~efc/classes/biol710/discrim/discrim. [Accessed on 18th May 2009])

Pratt JE (2000) Effect of inoculum density and borate concentration in a stump treatment trial against *Heterobasidion annosum*. *Forest Pathology* **30**, 277-283.

Putra EI, Sutisna U, Soekotjo (2001) 'Assessment of biodiversity indicator in forest health monitoringfor sustainable forest management: Tree species diversity.' SEAMEO BIOTROP, Bogor, Indonesia.

Reader U, Broda P (1985) Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**, 17-20.

Redfern DB, Boswell RC (2004) Assessment of crown condition in forest trees: comparison of methods, sources of variation and observer bias. *Forest Ecology and Management* **188**, 149-160.

Redfern DB, Filip GM (1991) Inoculum and infection. In 'Armillaria root disease'. (Eds CG Shaw and GA Kile) pp. 233. (USDA Forest Service: Washington, D.C)

Rimbawanto A (2002) 'Plantation and tree improvement trends in Indonesia.' ACIAR Technical Reports No. 51e, Canberra.

Rimbawanto A (2006) Heart rots in plantation hardwoods: the background to this ACIAR project. In 'Heart rot and root rot in tropical *Acacia* plantations'. Yogyakarta, Indonesia. (Eds K Potter, A Rimbawanto and C Beadle). (ACIAR Proceedings No.124, 7-9 Feb 2006)

Rizzo DM, Harrington TC (1988) Root movement and root damage of red spruce and balsam fir on subalpine sites in the White Mountains, New Hampshire. *Canadian Journal of Forest Research* **18**, 991-1001.

Robert C, Bancal M, Nicolas P, Lannou C, Ney B (2004) Analysis and modelling of effects of leaf rust and *Septoria tritici* blotch on wheat growth. *Journal of Experimental Botany* **55**, 1079-1094.

Rolando CA, Little KM (2003) Using chlorophyll fluorescence to determine stress in *Eucalyptus grandis* seedlings. *Southern African Forestry Journal* **197**, 5-12.

RRIM (1961) Root disease control. In 'Planters' Bulletin' pp. 177-181. (Rubb. Res. Inst. Malaysia: Kuala Lumpur, Malaysia)

RRIM (1974) Root diseases control part II. In 'Planters' Bulletin' pp. 157-164. (Rubb. Res. Inst. Mallaysia: Kuala Lumpur, Malaysia)

Ryvarden L (1994) Can we trust morphology in *Ganoderma*? In 'Proceedings of contributed symposia 59A, *Ganoderma* - Systematics, Phytopathology and Pharmacology. Fifth International Mycological Congress'. Vancouver, August 14-21 1994. (Eds PK Buchanan, RS Hseu and JM Moncalvo) pp. 19-24

Ryvarden L, Johansen I (1980) 'A preliminary polypore flora of East Africa.' (Fungiflora: Oslo, Norway)

Saito Y, Saito R, Kawahara TD, Nomura A, Takeda S (2000) Development and performance characteristics of laser-induced fluorescence imaging lidar for forestry application. *Forest Ecology and Management* **128**, 129-137.

Sariah M (2000) A control strategy for basal stem rot (*Ganoderma*) on oil palm. In '*Ganoderma* diseases of perennial crops'. (Eds J Flood, PD Bridge and M Holderness). (CAB International: Oxon, UK)

Schomaker ME, Zarnoch SJ, Benchtold WA, Latelle DJ, Burkman WG, Cox SM (2007) 'Crown-condition classification: A guide to data collection and analysis.' Forest service - USDA, Asheville, NC.

Seo GS, Kirk PM (2000) *Ganodermataceae*: nomenclature and classification. In 'Ganoderma Diseases of Perennial Crops'. (Eds J Flood, PD Bridge and M Holderness) pp. 3-22. (CABI International: Wallingford, Oxon, UK)

Shafri HZM, Hamdan N (2009) Hyperspectral imagery for mapping disease infection in oil palm plantation using vegetation indices and red edge techniques. *American Journal of Applied Sciences* **6**, 1031-1035.

Sharma PK, Hall DO (1992) Changes in carotenoid composition and photosynthesis in sorghum under high light and salt stress. *J. Plant Physiol.* **140**, 661-666.

Shaw CG, III, Stage AR, McNamee P (1991) Modeling the dinamics, behavior, and impact of *Armillaria* root disease. In 'Armillaria root disease'. (Eds CG Shaw, III and GA Kile). (USDA Forest Service Washington D.C)

Shaw CGI, Kile GA (1991) 'Armillaria root disease.' (Forest Service United States Departement of Agriculture: Washington, D.C.)

Shearer BL, Crane CE, Fairman RG, Grant MJ (1998) Susceptibility of Plant Species in Coastal Dune Vegetation of South-western Australia to Killing by *Armillaria luteobubalina*. *Australian Journal of Botany* **46**, 321-334.

Sierota ZH (2003) Costs and effects of biological control of root rot in Poland. In 'Proceedings of the 10th International Conference on Root and Butt Rots. IUFRO Working Party 7.02.01'. Québec, Canada. (Eds G Laflamme, JA Bérubé and G Bussières) pp. 194-196

Sigh AK, Dubey RS (1995) Changes in chlorophyll *a* & *b* contents and activities of photosystem 1 & 2 in rice seedlings induced by NaCl. *Photosynthetica* **31**, 489-499.

Sigh G (1991) *Ganoderma* - the scourge of oil palms in the coastal areas. *The Planter* **67**, 421-444.

Smith RR, Mc Crary SW, Callahan RN (2007) Gauge repeatability and reproducibility studies and measurement system analysis: A multimethod exploration of the state of practice. *Journal of Industrial Technology* **23**, 1-12.

Soekotjo, Sutisna U (1997) 'Vegetation structure indicator: Present status of free species diversity.' SEAMEO BIOTROP, Bogor, Indonesia.

Solberg S, Strand L (1999) Crown density assessments, control surveys and reproducibility. *Environmental Monitoring and Assessment* **56**, 75-86.

Stalpers JA (1978) Identification of wood-inhabiting Aphyllophorales in pure culture. *Centraalbureau Voor Schimmelcultures, Baarn. Studies in Mycology* **16**, 1-248.

Stone C (1998) Assessment and monitoring of decline and dieback of forest eucalypts in relation to ecologically sustainable forest management: a review with a case study. *Australian Forestry* **62**, 51-58.

Stone C, Coops N, Culvenor D (2000) Conceptual development of a Eucalypt Canopy Condition Index using high resolution spatial and spectral remote sensing imagery. *Journal of Sustainable Forestry* **11**, 23-45.

Stone C, Haywood A (2006) Assessing canopy health of native eucalypt forests. *Ecological management & restoration* **7 S1**, S24-S30.

Stone C, Matsuki M, Carnegie A (2003) Pest and disease assessment in young eucalypt plantations: field manual for using the Crown Damage Index. In. (Ed. M Parsons). (National Forest Inventory, Bureau of Rural Sciences: Canberra)

Stone C, Old K, Kile G, Coops N (2001) Forest health monitoring in Australia: National and regional commitments and operational realities. *Ecosystem Health* **7**, 48-58.

Stone C, Turner R, Verbesselt J (2008) Integrating plantation health surveillance and wood resource inventory systems using remote sensing. *Australian Forestry* **71**, 245-253.

Supriyanto, Soekotjo, Justianto A (2001) 'Assessment of production indicator in forest health monitoring to monitor the sustainability of Indonesian tropical rain forest.' SEAMEO BIOTROP, Bogor, Indonesia.

Sutisna U, Putra EI, Soekotjo, Marsono D (2001) 'Assessment on the modification of FHM vegetation quadrates to address tropical species diversity of trees.' SEAMEO BIOTROP, Bogor, Indonesia.

Tiarks A, Nambiar EKS, Cossalter C (1998) 'Site management and productivity in tropical forest plantations.' Centre for International Forestry Research, Bogor, Indonesia.

Turner PD (1965) The oil palm and *Ganoderma* IV. Avoiding disease in new plantings. *The Planter* **41**, 331-333.

Turner PD (1981) 'Oil palm diseases and disorders.' (Oxford University Press: Oxford and Kuala Lumpur)

UNEP (2007) Forest loss. In. (United Nation System-Wide Earthwatch)

Valladares F, Pearcy RW (1997) Interaction between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. *Plant, Cell and Environment* **20**, 25-36.

Vollenweider P, Gunthardt-Göerg MS (2006) Erratum to "Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage" [Environ. Pollut. 137 (2005) 455-465]. *Environmental Pollution* **140**, 562-571.

Wagner T, Fischer M (2002) Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. *Mycologia* **94**, 998-1016.

Wago PM, Shaw CG, III (1985) Armillaria root rot: the puzzle is being solved. *Plant Disease* **69**, 826-832.

Wallis GW, Bloomberg WJ (1981) Estimating the total extent of *Phellinus weirii* root rot centers using above- and below- ground disease indicators. *Can. J. For. Res.* **11**, 827-830.

Wardlaw T (2000) Armillaria root and collar rot of *Eucalyptus nitens*. In 'Forest Health Bulletin'.

Wargo PM, Harrington TC (1991) Host stress and susceptibility. In 'Armillaria root disease'. (Eds CG Shaw, III and GA Kile). (USDA Forest Service: Washington, D.C.)

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenesis. In 'PCR protocols: a guide to methods and applications'. (Eds MA Innis, DH Gelfand, JJ Sninsky and TJ White) pp. 315-322. (Academic Press: San Diego, CA)

Whitney RD (1961) Root wounds and associated root rots of white spruce. *Forestry Chronicle* **37**, 401-411.

Wulff S (2004) Nonsampling errors in ocular assessments - Swedish experiences of observer influences on forest damage assessments. In 'Environmental monitoring'. (Ed. GB Wiersma) pp. 337-346. (CRC Press: Boca Raton, Fla)

Zarco-Tejada PJ, Miller JR, Mohammed GH, Noland TL, Sampson PH (2000) Chlorophyll fluorescence effects on vegetation apparent reflectance: II. Laboratory and airborne canopy-level measurements with hyperspectral data. *Remote Sensing and Environment* **74**, 598-608.

Zarnoch SJ, Bechtold WA, Stolte KW (2004) Using crown condition variables as indicators of forest health. *Can. J. For. Res.* **34**, 1057-1070.