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Contents

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2 Executive summary

The switch from growing mainly acacias to eucalypts on mineral soils in Indonesia was prompted by the devastating effect of Ceratocystis wilt and canker disease which resulted in large productivity losses, making the species commercially unviable. Eucalypts, however, are not as adaptable and their susceptibility to Ceratocystis is also increasing, so the development of Ceratocystis-tolerant acacias would be welcomed by industry and community forest growers. Screenings carried out under this project reinforced the low levels of genetic tolerance to Ceratocystis in *A. mangium,* with only 6.7% survival after testing over 1000 *A. mangium* clones. This has prompted a search for resistance or tolerance in other acacia species and the adoption of a hybrid breeding strategy. To this end, alternative acacia species were screened for tolerance and a hybridising orchard established that includes the most tolerant of the *A. mangium* and *A. auriculiformis* clones. Seeds from this orchard will be available to community growers and to industry partners. Seed of additional acacia species was also imported into Indonesia and seedlots of *A. auriculiformis* were exchanged between Indonesia and Vietnam, making improved germplasm available to Indonesia and increasing the genetic diversity available to Vietnamese growers.

A rapid preliminary screening protocol, based on detached phyllodes, was developed and has been adopted by industry partners. Subsequent field trials will still be needed to confirm disease tolerance under environmental conditions though the new protocol is expected to accelerate acacia breeding programs by allowing the rapid, initial screening of a larger numbers of clones than can be achieved using the current, potted plant protocol.

A small burning trial conducted in Vietnam indicated that there was some reduction in pathogen inoculum but it is uncertain whether this will be sufficient to reduce the incidence of Ceratocystis in subsequent rotations. VAFS will monitor the disease incidence in this trial.

Root rot continues to reduce productivity in Indonesian plantations though disease progression is slower in eucalypts than in *A. mangium*. As well as potentially increasing the yield in eucalypt plantations, biological control of root rot will be essential for the viability of hybrid acacias in mineral soils, where the pathogen is widespread. Field trials provide some indication that the selected biological control agent, a *Phlebiopsis* sp., has the potential to reduce incidence of root rot though the highly variable distribution of inoculum makes it difficult to provide statistical support for this conclusion.

Application of BCA inoculum needs further development; application of oidia to freshly cut stumps of harvested trees presents some WHS challenges and was abandoned in this project to explore alternative strategies based on inoculation of seedlings. In pot trials, oidia of *Phlebiopsis* sp. were unable to colonise seedling rhizospheres. The addition of woodblock inoculum allowed rhizosphere colonisation by BCA mycelium The *Phlebiopsis* was able to survive in the woodblock, the rhizosphere and the soil and overgrow woodblocks colonised by *Ganoderma phillippii,* that were introduced a month later. The presence of *Phlebiopsis* did not, however, measurably reduce the incidence of seedling infection and death in this trial. The *A. mangium* seedlings were infected by *G. philippii* before the pathogen inoculum was overgrown by *Phlebiopsis* sp.

Industry partners continue to use woodblock inoculum operationally to reduce root rot incidence in eucalypt plantations. Their current BCA is a *Cerrena* species, but they plan to begin using a combination of *Phlebiopsis* and *Cerrena.*

Three workshops were held during this project; a *Ceratocystis* workshop, a Biological Control workshop and a Remote Sensing workshop. Internationally recognised experts were invited to speak at each of the workshops, all of which were filled to capacity and feedback was enthusiastically supportive. Participants came from partner countries as well as from additional SE Asian countries, expanding opportunities for networking and collaboration.

3 Background

What is the problem: In tropical Southeast (SE) Asia, plantation forests of Australian acacia and eucalypt species now exceed seven million hectares. They are managed on short rotations, typically 5–8 years, for wood production. Their main purpose is to supply wood for the wood processing industries throughout the region, so their productivity is of critical importance to the region. Global trends indicate that non-native hardwood species established as plantations will be challenged by increasing numbers of disease and pest problems.

Indonesia's forest industries are dominated by the pulp and paper sector. As part of the need to place these industries onto a more sustainable footing and become less reliant on sourcing pulpwood from native forest, Indonesia's Ministry of Forestry has promoted policies that encourage the development of a plantation-based wood supply. Three species, *Acacia mangium* (~0.6M ha), *A. crassicarpa* (~0.3M ha) and *Eucalyptus pellita* and hybrids (>0.3 Mha) currently account for the majority of the plantings because of their superior performance; *A. mangium* has been preferred because of its good growth and satisfactory pulp yield. Growth rates of *A. mangium* plantations in Sumatra ranged between 22 and 35 m^3/ha/yr before being impacted by fungal diseases (*Ganoderma* and *Ceratocystis*), which has reduced growth on infected sites to 15 m^3/ha/yr or lower.

Vietnam has approximately 0.2M ha eucalypt and 1.2M ha of acacia plantations. About half of the plantation estate is managed or co-owned by smallholder farmers. Recently, some plantations of Acacia in Vietnam have experienced a serious disease problem from *Ceratocystis* with up to 20% mortality.

Harwood and Nambiar 2014 state that efficient systems for monitoring productivity are still evolving. Spatial mapping to analyse the relationships between growth and mortality on the one hand and variation in site characteristics and management inputs on the other is urgently required to better understand and manage environmental and biotic risks to productivity.

Knowledge underpinning the problem:

Australian acacias and eucalypts planted as non-natives in various parts of the world are increasingly threatened by pests and pathogens as plantation estates age, especially in SE Asia (Wingfield, Roux et al. 2011). These include those that are introduced accidentally from the same areas of origin as the trees, as well as 'new encounter' pests and pathogens that are undergoing host shifts to infect the non-native trees. Pests and pathogens do not recognise national borders and shared host species in SE Asia implies shared pest and pathogens.

One of the two most important diseases currently confronted by Indonesian and Malaysian pulpwood industries is root-rot disease caused by basidiomycete facultative parasites. Studies have identified *G. philippii* as the main species consistently associated with infected *A. mangium* roots displaying symptoms of red-root disease (Glen, Abou Arra et al. 2009, Coetzee, Wingfield et al. 2011). Mohammed, Rimbawanto et al. (2014) has reviewed root-rot diseases in tropical hardwood plantations; root-rot fungi are originally present in the native forest which when cleared for plantations are left in infected stumps and roots as inoculum sources. Disease build-up in woody debris left behind after harvest in short pulpwood rotations of five-to-seven years is associated with an accelerated development of disease such that tree death can exceed 50% in some areas within < 20 years of establishing the first rotation.

Root diseases caused by basidiomycetes are among the most studied diseases of trees in the world, in particular for the genera *Armillaria* and *Heterobasidion*, and there are many potential approaches to their management (Mohammed, Rimbawanto et al. 2014).

Screening of genetic trials in the field and artificial inoculation in pot experiments with acacia have not yet shown any exploitable trends in resistance to *Ganoderma* root-rot disease (Budi Tjahjono, Mardai Unen and Heru Indrayadi, pers. comm.). In the northern hemisphere bio-control of *H. annosum s.l.* by application of a fungal competitor (*Phlebiopsis gigantea*) to stump surfaces to prevent pathogen spore colonisation is a true success story in the management of a basidiomycete root-rot disease. This BCA has been effective because (i) it is highly competitive against *H. annosum* and (ii) the production of large numbers of oidia facilitate application to stumps at the time of felling. It is a strategy more likely to be deployed with success over the many thousands of hectares involved in tropical plantations, especially in South-east Asia where topography and climate often prohibit other management approaches such as stump/root removal or the use of chemicals.

Results from ongoing ACIAR research supports the further exploration of biocontrol for *Ganoderma* root-rot disease (Mohammed, Beadle et al. 2012 and unpublished data);

- Root-rot pathogen spread in acacia was previously considered to be predominantly vegetative (infected root/debris - healthy root contact) but preliminary research in FST2003/048 indicated that *G. philippii* spores may play a role in initiating new disease foci. During FST2003/048 we isolated several isolates of *Phlebiopsis* species closely related to *P. gigantea* from eucalypt and acacia plantations in Sumatra. In FST/2009/051 isolates of this *Phlebiopsis* species have been shown to have similar properties to *P. gigantea in vitro* and oidia production has been demonstrated.
- We are also working in FST/2009/051 on additional isolates of a *Cerrena* species that are conspecific with an isolate developed by the plantation company Arara Abadi in the previous project (FST2003/048) as a BCA. Laboratory tests at Arara indicated that this species is effective against *G. philippii* and the company is now producing large quantities of colonised wood blocks for operational use at planting. This application method is labour-intensive and energy-consuming - to prepare the wood blocks as well as to transport and apply them in planting holes or where a tree has died and is dug out. It is not a method that can be transferred to small-holder farmers. In the current project (FST/2009/051) we have produced oidia from *Cerrena*, though not as abundant as those produced by the *Phlebiopsis* sp. isolates.
- Pot and field trials were set up in FST/2009/051 to test BCA activity of both *Cerrena* and *Phlebiposis* and to ensure that the BCAs do not demonstrate pathogenicity towards the hosts. Although results were variable these BCA candidates remain promising and field trials have been carried out. FST/2009/051 finished in 2015 and FST/2014/068 allowed continued monitoring of field trials set up during FST/2009/051.

The second devastating disease of plantation *A. mangium* is caused by a fungal pathogen belonging to the genus *Ceratocystis*. This fungal pathogen causes stem cankers and rapid wilting in trees. Disease symptoms were first recognised approximately a decade ago in Indonesia and Vietnam and *Ceratocystis* has also been reported as causing considerable losses in Malaysia. *Ceratocystis* isolates from infected plants were identified as *C. manginecans*, a serious pathogen of mango trees in Oman and Pakistan.

The fungal genus *Ceratocystis* includes many economically important tree pathogens (Kamgan, Jacobs et al. 2008). Until the 1980s, this genus of plant pathogens was not known from non-native plantation-grown forestry species (Roux, Meke et al. 2005). However, a number of reports have been made of *Ceratocystis* species causing death of non-native plantation-grown forestry species from several locations worldwide (Roux, Meke et al. 2005). Affected trees include species of Australian acacias and eucalypts.

Infection is often associated with pruning wounds such as singling wounds in acacia (Tarigan, Wingfield et al. 2011) or other mechanical damage (monkeys and squirrels in acacia). *Ceratocystis* species are vectored by wood-and bark-associated insects, typically those that visit fresh wounds acting as infection courts (Roux, Meke et al. 2005). The fungi infect woody tissue and produce strong aromas (often like a brewery), which are attractive to insects. Although it can be assumed that the biology of *C. manginecans* will be similar to other *Ceratocystis* species there have been no studies to confirm this or the significance of the different dissemination mechanisms usually associated with *Ceratocystis*. In addition these fungi can easily spread between countries and continents, thus posing a considerable quarantine threat. It is imperative to better understand the specific biology of this *Ceratocystis* species.

We do not know the risk factors that drive either of the two diseases. Definition of high-risk areas will assist the effective deployment of management strategies e.g. planting tolerant acacia, targeting biocontrol. Inventory information in SE Asia is seldom linked to soils, terrain and other productivity variables such as abiotic or biotic damage, except in a general way (Harwood and Nambiar 2014). In FST/2009/051 the influence of soil and topography on *A. mangium* survival and cause of mortality was explored in Central Sumatra using a range of GIS and statistical procedures with the plantation company PT RAPP. Data included Pre-Harvest Inventory surveys, 1:10000 soil mapping, and 5 meter digital elevation models. The dominant cause of tree mortality was wind, followed by *Ganoderma* and *Ceratocystis*. Partition analysis indicated mortality by *Ganoderma* and *Ceratocystis* was related to a number of topographic and soil attributes, whilst mortality caused by wind was more closely related to topographic attributes specifically, topographic position index and valley depth. This study is constrained by limited spatial coverage and individual tree information. Significant developments in micro-electromechanical hardware and image processing systems permit the capture of low-cost digital imagery suitable for local-scale investigations. Operators of Unmanned Aerial Vehicles (UAVs) have the potential to deliver processed products more cheaply and faster than from satellite or aircraft commissioned imagery and hence are attractive for repeated, localised deployment. UAVs are being flown by some forestry companies in Indonesia for to capture digital imagery and their use for pathological investigations is consistent with existing regulation.

The research approach:

There is an urgent need to reduce the mortalities in acacia caused by *Ceratocystis* and *Ganoderma* in Indonesia and Vietnam through strategies underpinned by previous research and which will be likely to generate feasible management strategies. This new project specifically builds on research on biological control agents (BCAs) and the use of GIS-based disease risk assessments established in FST/2009/051, disease tolerance screening research carried out by CSIRO (Dr Brawner) and acacia breeding by VAFS in collaboration with UTAS (Prof. Griffin) and CSIRO (Dr Harwood).

The approach was developed over several years by discussions with Indonesian, Vietnamese and Australian colleagues, it is demand driven Research Development and Extension. At the 2014 IUFRO Working Party 2.08.07:Genetics and Sivilculture of Acacia International Conference in Vietnam the participants' conclusions support the approach;

- *A sound understanding of basic science, epidemiology and ecology of every target organism is critical. Genetic solutions will be appropriate in many cases.*
- *No single solution is available and a belief in quick fix approaches can be counter-productive. Current research efforts in biological control show promise but need further field testing.*
- *As technologically advanced solutions require more grower knowledge to implement, and as more smallholder farmers get involved, the adoption pathway is less clear. The forestry sector needs to learn from agriculture, and involve social scientists more directly to continue and improve the adoption of technology.*

The clear message from the IUFRO conference was that there are many unresolved technical issues with acacia plantation forestry and there is a need for a more integrated and inter-disciplinary approach to defining management systems which deliver sustainable and profitable plantation productivity.

The development of the Research, Development and Extension (RDE) objectives and activities:

The current and historical context to *Ganoderma* and *Ceratocystis* as outlined above underpinned the development of the RDE for this project. The main research question used to formulate objectives and activities was:

What are the strategies to quickly, sustainably and cost effectively reduce the impacts of Ceratocystis *and* Ganoderma*?*

To address this question, a research strategy was developed involving: studying the biology of the diseases; extending research on biocontrol of *Ganoderma*; exploring the potential for deploying genetic resistance to *Ceratocystis*; and understanding the spread of diseases within the *Acacia* plantations.

The basic concept of effective disease management is always "know your enemy". Relatively little is understood about acacia *Ceratocystis*. This justifies a fundamental scientific investigation of its biology and ecology especially factors which may influence disease management options and their deployment (e.g. the mechanisms of disease dissemination such as insect vectors). The use of genetic resistance to limit disease impact has been proven cost effective, operationally feasible and sustainable in other major plantation forestry systems and is an option that warrants investigation for *Ceratocystis* in acacia although work in Malaysia has shown that finding genetic resistance could be very challenging (Brawner, unpublished). It is less likely that resistance to a generalist pathogen like *Ganoderma* root-rot will be found by screening, the option of biological control had shown promise in a previous ACIAR project and justified activity to further develop this strategy.

The development of successful methods to control diseases is the first step in the reduction of their impacts. The deployment of any control measure in extensive plantations will inevitability involve targeting high risk areas and an ability to define such locations. In summary the strategies adopted in the new project to quickly, sustainably and cost effectively reduce the impacts of *Ceratosystis* and *Ganoderma* are:

- explore the biology of *C. manginecans,*
- screen for resistance in *A. mangium* and *A. auriculiformis* (the other parent species of acacia hybrid clones widely planted in Vietnam) to *C. manginecans,*
- further develop promising BCAs to apply against *Ganoderma,*
- promote improved assessment of disease risk and impact to underpin successful disease management.

Balance between research, extension and capability building:

As threats from pests and diseases on non-native tree hosts in SE Asia increase, the capability to deal with future threats must be improved and a regional approach to forest protection established. The carriage of the research activities will be planned in such a way to provide maximum capability building within the scope of the budget e.g.

- a workshop with international experts will summarise regional progress in acacia *Ceratocystis* research and gaps in knowledge,
- a regional training workshop will be organised to explore the myths and realities of biological control in forestry and how to critically evaluate success or failure,
- a workshop (based on the use of UAV borne sensors to acquire data) that highlights the need for inventory operations to also consider and correlate biotic (and abiotic) factors with productivity,
- a scientific writing workshop that combines training in journal writing and the use of social media to promote science.

4 Objectives

Aim: To reduce the impacts of current productivity limiting diseases of Acacia plantations and build capacity and collaboration on forest health in Indonesia, Vietnam and neighbouring SE Asian countries.

Objectives and activities:

1. **To reduce the impact associated with *Ganoderma* root rot disease in *A. mangium* plantations[1].**
    - BCA spores and pathogen spores experimentally co-inoculated onto stumps,
    - BCA field testing (stump inoculation trials) and semi-commercial deployment of BCAs,
    - Improvement of BCA formulation and refinement of application methods.

2. **To reduce the impact associated with *Ceratocystis* canker and wilt disease in *A. mangium* plantations[2].**
    - Investigation of aspects of disease biology important to disease management,
    - Development of screening protocols,
    - Screening entire breeding populations (not just deployment populations),
    - Revising breeding strategies to incorporate disease screening and deployment of resistant material.

3. **To build capacity in forest health management in SE Asia**
    - *Ceratocystis* workshop - to summarise regional progress in acacia *Ceratocystis* research and gaps in knowledge. To highlight how regional and international collaboration and joint RDE between the public and private sector can support effective forest protection,
    - Biological control workshop - to explore the myths and realities of biological control in forestry and how to critically evaluate success or failure,
    - Disease risk and impact analysis workshop.The analysis of cost effective remotely (UAV) acquired sensed data will assist the mapping of disease spread, the understanding of impact and risk, allow the more effective deployment of management strategies e.g. planting tolerant acacia, targeting biocontrol (depending on the efficacy of this approach) in areas of highest risk or treating areas of lowest risk and abandoning high risk areas.
    - Publication writing workshop. To encourage SE Asian scientists to promote their research through journal publication and the social media.

[1]The reduction in impact can be validated 5-10 years after “proof of concept” in the field in the new project.
[2]The reduction in impact can be estimated from the data obtained from screening trials (if tolerance is found) and validated 3-5 years after the deployment of genetic resistance in the field.

5 Methodology

Forestry-based livelihoods that have developed as a result of ACIAR's support for the domestication of Australian trees (acacias and eucalypts) in SE Asia are threatened by an increasing number of diseases. The research was conducted under three major objectives which focussed on mitigating the immediate disease threats to acacia plantations in SE Asia; *Ganoderma* and *Ceratocystis* and capability building in forest health management.

ACIAR has been supporting research on *Ganoderma* for more than a decade; it is a pathogen that also affects agricultural crops. Previous research has identified promising candidate biocontrol options and carried out a GIS based study of risk factors associated with *Ganoderma* incidence and severity. The methodology for the first objective therefore focussed on the further development of biocontrol options for *Ganoderma*.

The rapid and recent spread of *Ceratocystis*, a new fungal pathogen of acacia, in SE Asian countries presents a serious risk to productivity. The methodology adopted for the second objective therefore seeks to increase our understanding of the biology of *Ceratocystis* canker and wilt in *Acacia*. While biological control offers the best option for the management of a root-rot disease such as *Ganoderma*, genetic resistance offers more chance of success for this type of disease. The methodology adopted for screening resistance against *Ceratocystis* will build capability in the application of genetic resistance to disease management.

The third capability building objective is closely linked to the first two objectives and was delivered by 4 workshops.

Objective 1: To reduce the impact associated with *Ganoderma* root rot disease in *A. mangium* plantations.

Isolates of the fungi *Phlebiopsis* and *Cerrena* have been shown to have significant potential as biological control agents against *Ganoderma* in confrontations carried out in the laboratory. Pot and field trials (stumps inoculated with BCA oidia (spores) and planting holes with BCA carried on wood blocks) that were set up at the end of FST/2009/051were monitored during FST/2014/068. The pot trials did not give as much information as hoped due to the problems of growing woody block inoculum. However it has been established that stumps can be inoculated with BCAs in the field and it is this methodological approach that was pursued.

1.1. *BCA and pathogen spore co-inoculation onto stumps* (successful inoculation of pathogen spores must be achieved *ab initio*). This trial was set up at CFBTI in Yogyakarta and involved a limited number of stumps (50 stumps). BCA and pathogen isolates that had been characterised in FST/2009/051 in laboratory pairing tests on cultural media and wood blocks and were selected for this and other trials. Destructive sampling was used to obtain information about the extent of colonisation and competition. Culturing and DNA analysis (species specific PCR developed in FST/2009/051) were used to detect colonisation by the pathogen and BCA.

1.2. *Methods for producing, formulating and field application of the biocontrol agent* were refined. Different spore carriers and application methods were tested (e.g. spray, painting).

1.3. *The most promising BCAs were applied to acacia and eucalypt stumps* in field trials across two different rotations, 3 locations (two in Central Sumatra and one in southern Sumatra), two times of the year (dry and wet seasons), two levels of root-rot disease in previous rotation (high and low). Each of the treatments included at least 100 stumps for each of the BCA agents. Design allowed for destructive sampling to obtain information about the extent of BCA colonisation. Cultural isolations and molecular tools will be used to detection colonisation.

1.4. *Trials at PT RAPP set up during FST/2009/051 with different BCAs applied in the planting hole will be monitored* for root rot disease incidence and the effectiveness of BCA agents applied.

Objective 2: To reduce the impact associated with *Ceratocystis* canker and wilt disease in *A. mangium* plantations.

There are significant gaps in knowledge about this new disease which need to be filled for successful disease management and the successful deployment of any genetic resistance which may be found during this project. This initial Ceratocystis workshop (see objective 3) brought together the disparate knowledge of various research groups of the biology of Ceratocystis. The two main areas proposed as activities for this project at the workshop (see 2.2 and 2.3) were the investigation of insects associated with the disease and host responses to infection.

To develop resistant acacia varieties we focussed on developing rapid and cost- effective screening methods that may be applied across South East Asia at the scale required. Existing screening methods (tree inoculations and the observation and measurement of resultant cankers) were used to screen deployment populations at the start of the project and to validate the protocols developed.

A common germplasm was provided from the Australian Tree Seed Centre for a regional trial network to be set up through other private and public initiatives. In addition, new germplasm of species known to naturally hybridise with the commercially planted acacia species (A. auriculiformis, A. crassicarpa and A. mangium) was planted to provide a possible alternative route to developing resistant germplasm through interspecific hybridization, as has proved valuable in eucalypt breeding.

In parallel with our resistance screening work, new breeding strategies that emphasise the importance of early and extensive disease resistance screening were developed with partners. Results from this work will ensure that resistance is continually assessed and multiple resistance mechanisms are available as (and if) changes in pathogen virulence arise.

2.1 *To identify the insects associated with C. manginecans,* insects were collected as they emerged from stems of Ceratocystis-infected trees at three different stages of infection and healthy trees. Trapped insects were identified, and fungi isolated from the insects using carrot baiting and by plating them onto malt extract agar. Fungi were identified using morphological characteristics and DNA sequence comparisons.

2.2 A field trial planned to test the biochemisty of response to infection with two clones of *A. auriculiformis*, one of *A. mangium* and five hybrid clones was abandoned because of concerns about spreading inoculum in plantations.

2.3 *Screening acacia deployment populations to develop disease resistant breeds (Indonesia, Vietnam and SE Asia)*: the project inception meeting formally confirmed the availability of acacia and *Ceratocystis* germplasm for use in resistance screening and agreement on the screening protocols to be developed. In Indonesia, acacia germplasm managed by the lead Indonesian partner (CFBTI) was screened, using the agreed screening protocol, for *Ceratocystis* resistance in pot trials. Making it

operationally feasible to screen massive populations was managed by industry partners. The locations were selected on the basis of biosecurity precautions (to minimise the transport of fungal strains to new locations. A series of experiments, using consistent host and pathogen germplasm, was evaluated quantitatively using the dissected measures of disease impact on the host. The use of common germplasm will allow for estimates of the accuracy of alternative screening systems and the genetic correlation among various assays.
In Vietnam, existing hybrid acacia clones and those developed by ACIAR project FST/2008/007 are of critical importance to the Vietnamese industry and were the initial populations screened for resistance. Screening of advanced generation selections from *Acacia mangium* and *A. auriculiformis* populations took place in southern Vietnam where risk of the disease is highest.

2.4 *Developing robust disease screening systems*; in order to develop a disease resistant breed of acacia and understand the genetic architecture of the resistance, large numbers of individuals must be screened and efficient systems are required to integrate into existing breeding programs. The existing protocol to be used for initial screenings uses artificial inoculation of stems in 9-month-old trees to induce lesions that are measured to evaluate host tolerance. Increasing the accuracy of the assessments and the speed with which they are undertaken is important and a range of variables must be better understood.

2.5 *Breeding and deployment strategy revision with technology transfer*; the current breeding strategy for acacias focuses on increasing productivity and wood quality traits with attention given to risk mitigation opportunistically. Revision of the breeding strategies for the major acacia species was undertaken with project partners in Vietnam and Indonesia to provide a systematic approach to developing disease resistance. The project partners held a side-meeting at the IUFRO working party 2.08.07 meeting held in Indonesia in July of 2017. We took this opportunity to share ideas on restructuring acacia breeding strategies with colleagues working in the breeding and forest pathology. The merits of a co-ordinated regional disease screening approach as applied in other tree improvement programs faced by serious disease problems was evaluated. VAFS expertise in interspecific hybrization and polyploid breeding will be used to explore the utility of hybridisation for the infusion of resistance into breeding populations. Following this meeting, project partners drafted new acacia breeding strategies and shared results on the genetic control of disease resistance via publication in peer reviewed journals.

Throughout the project, the forest companies were engaged to test the screening systems. Considerable time was spent by Australian project partners during the initial protocol development stage to develop a large scale and cooperative approach to disease screening. While in-house screening undoubtedly takes place within companies, the emphasis of the cooperation was to provide FORDA and VAFS information and germplasm to increase the diversity of the resistant breeds the project intends to develop.

Objective 3. To build capacity in forest health management in SE Asia

The workshops were used as a vehicle to evaluate and promote how regional collaboration and joint RDE between the public and private sector can support effective forest protection. Workshops were a platform for presenting the big picture in forest health and disease management e.g. comparison of principles in similar biocontrol systems in other parts of the world, management of root infecting basidiomycetes in forestry in general, constraints and opportunities of breeding for resistance in fast growing plantation species, diversity, biology and impact of *Ceratocystis* in other systems, application of evolutionary principles in disease management. Workshop Presentations were shared on-line among participants.

3.1 *Ceratocystis workshop* - focusing on the state of knowledge for *C. manginecans,* summarised regional progress in acacia *Ceratocystis* research and gaps in knowledge.

3.2 *Biological control workshop* - to explore the myths and realities of biological control in forestry and how to critically evaluate success or failure. Input invited from all those in the region working on different systems e.g. endophytic *Trichoderma* or bacteria as BCAs respectively for *Ganoderma* and *Ceratocystis*, the biocontrol of putative insect vectors for *Ceratocystis*. This workshop was run in collaboration with FST/2011/028 Biological control of Eucalypt pests overseas and in Australia.

3.3 *Disease risk and impact analysis workshop*. Understanding the impact and the definition of high risk areas by using cost effective remotely sensed data will assist the effective deployment of management strategies e.g. planting tolerant acacia, targeting biocontrol. The workshop and field demonstration was organised in the later stages of the project with invitees from Vietnam and other SE Asian countries.

- The activity was limited to a single case study focused on the methodology required to determine risk factors associated with *Ganoderma* and *Ceratocystis* mortality and will extend the research carried out in FST/2009/051. The studied estate occupies around 11,000 ha. The mean annual rainfall (1996 - 2003) is 2880 mm in which average rainfall in all months exceeds 130 mm. Geologically most of the estate consists of acid Quaternary sedimentary blueish grey sandstone in places superimposed with claystone, and recent to sub recent alluvium sub-recent terrace and alluvial-colluvial landforms. Relief in the estate is generally rolling (slope 8-15%) to hillocky (slope 15-30 %) with moderately to strongly dissected terrain.
- Initial discussions with project partners were held to agree on what can and cannot be undertaken for this activity i.e. platform design, UAV availability, permission to fly UAVs, data availability/sharing, access to ground-based reference measurements and eventual publication of results.
- While there are few published examples of UAV acquired imagery being used for detecting and mapping unhealthy tree crowns, numerous classification methodologies have successfully been applied to high spatial resolution imagery acquired by satellite and light aircraft platforms, including the use of object-based classifiers operating at the individual tree-scale. It is reasonable to assume that these methodologies could successfully be applied to imagery acquired by UAVs.
- The manipulation, processing and analyses of the very large data sets that result from the acquisition of high spatial resolution digital imagery was a major challenge. Attention was directed to developing operational protocols for the acquisition, processing and integration of these large geo-spatial datasets and software development to automate the process.
- Data acquired during the field day were made available to workshop participants to take home and use as a practice dataset, following the procedures as outlined in the workshop and subsequently published illustrating methodology and analytical procedures.
- The workshop included other possible applications of UAV sensor data (disease impact and risk assessment, monitoring the success of management strategies, the detection of new diseases or other problems) and presentations from workshop participants.

3.4 *Publication writing workshop.* To encourage SE Asian scientists to promote their research through journal publication and the social media.

6 Achievements against activities and outputs/milestones

Objective 1: To reduce the impact of Ganoderma to Acacia mangium

PC = partner country, A = Australia, VN=Vietnam, IN=Indonesia, AM=Acacia mangium, AH=Acacia hybrid

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1 BCA and pathogen spore co-inoculation onto stumps (IN)				
1	**Revised activity in 2016:** Achieving artificial colonisation of wood blocks and simulated stumps with Ganoderma spores (= Stump trial 1).	Method for obtaining *Ganoderma* asexual spores (oidia).	**Revised date:** 1/6/17	**Completed** The artificial colonisation of sterilised wood samples by the pathogen has been achieved using asexual spores from *Ganoderma* isolates but trials using unsterilized, simulated stumps in the greenhouse have been unsuccessful.
2	**Revised activity in 2016:** Co-inoculations of the pathogen and BCA on wood blocks and simulated stumps (= Stump trial 2).		**Revised date:** 31/12/17 **Revised date:** 15/12/2018	**Discontinued** Co-inoculations of the pathogen and BCA on wood blocks and simulated stumps have been attempted but mite infestation in the laboratory led to contamination. Repeated at CFBTI but colonisation of non-sterile stumps by pathogen oidia was not demonstrated.
1.2 Methods for producing, formulating and field application of the biocontrol agent (IN)				
1	Production of BCA inoculum in the laboratory	A reliable technique for producing BCA inoculum in large quantities	**Due date:** 31/3/18	**Completed** We are confident of obtaining a good yield of BCA from a single oidial culture and of storing viable oidia, but the development of commercial-scale inoculum for stump application has been discontinued (see 1.2.2) in favour of methods better suited to inoculations of small plants at the nursery stage. Initial problems with contamination of grain cultures for the inoculation of small plants have been resolved. Desy and Watik visited Arara PT in December 2019 for training in alginate bead production. Pot trials using oidia to inoculate seedling roots were unsuccessful. While rhizosphere colonisation has been observed following inoculation with woody inoculum, the lack of a woody substrate precludes establishment in the rhizosphere. Inoculation with BCA in the nursery will therefore need to be based on small pieces of colonised wood. The size of the wood for inoculum will need to be optimised to ensure survival of the BCA until planting out of the seedling.

2	**Additional activity for 2018-2019 suggested for after mid-term review:** To inoculate the nursery potting soil or roots with *Phlebiopsis* and examine the roots of acacia as seedlings grow for the survival of the BCA agent (either in the rhizosphere or endophytically)	Investigation into feasible application methodology initiated.	**Revised date:** 15/06/20	**Issues flagged in 2017-18:** Spraying after harvesting is very difficult as access to the site is limited while heavy machinery is operating. Two methods of harvesting are used and application methods need to be optimised for both: • Large harvesting machines are increasingly in use, and additional stump-spraying equipment that fits these machines is in operational use in Europe. • Where site access for these machines is difficult, harvesting is done by hand-held chainsaws. Addition of oidia to chainsaw oil has been discussed, but overheating may result in loss of viability and stump coverage with oil is patchy at best. Spraying immediately after felling, either by the chainsaw operator or by a co-worker, working alongside the chainsaw operator, is the only way that thorough and timely inoculation can be achieved. This will add to the cost of harvesting, as well as OHS issues. **Additional activity on track after delays and will be completed by April/May 2020:** Given the barriers to application in the field a third application method should be considered i.e. the colonisation of seedling roots with BCA oidia in the nursery before planting OR by mycelium infected grain. In the previous ACIAR project, pot trials investigating BCA activity against *Ganoderma* indicated that species of *Phlebiopsis* colonise the rhizosphere of young acacias and eucalypts. Although freshly cut stumps maybe infected by spores, *Ganoderma* in successive rotations increasingly infects living trees from underground inoculum. It is difficult for any BCA applied to debris on the surface to reach this source of inoculum. Rhizosphere colonisation of the host with BCA may be a better option. Two preliminary pathogenicity tests have been conducted. While tree deaths did not occur within 6 months of inoculation, symptoms of infection were observed, infected root systems were seen and the pathogen re-isolated from infected roots, so the main experiment has been commenced. Seedling roots have been inoculated with BCAs, maintained in the greenhouse for a month to allow root colonisation to occur and then inoculated with pathogen.

1.3 BCAs application to acacia and eucalypt stumps in the field in a series of trials across different rotations, locations, times of the year, levels of root-rot disease in previous rotation, different BCAs and pathogen isolates (IN).				
1	**Revised activity in 2016:** Intensive trials set up at three location with several BCA agents and regular monitoring and destructive sampling (= Stump trial 3)	Three trials at 3 different sites to trial and compare BCA agents (understand colonisation ability)	1/5/18 Write up: 31/3/19 Revised date of write up: 30/06/2020	**On track (experimental lab work nearly complete) and will be completed by April/May 2020** All three sites have been set up; eucalypt stumps at Arara, Riau, *A. mangium* stumps at RAPP, Riau and MHP, South Sumatra). Three trials have been sampled twice and two have been sampled a third time. Problems have arisen due to likely cross-contamination in the lab meaning that results are not reliable. Results of PCR tests for the third sampling need to be confirmed using next-generation DNA sequencing direct from wood for a subset of samples because of contamination issues.
2	**Revised activity in 2016:** Compartment size stump inoculation trial set up with most promising BCA agents with regular monitoring to vet BCA efficacy (= Stump trial 4)	A compartment size stump inoculation trial to test BCA agents at a semi-operational level.	Trial to be completed 31/11/18: Write up: 30/5/19	**Completed** This trial was monitored for the third time in January 2019 when the trees were 30 months old Root rot incidence was lower in all treatments compared to controls but not significantly so. Spatial variability of inoculum load appears to be a limiting factor in demonstrating statistical significance. Publication drafted.
1.4 Trials set up during FST/2009/051 with different BCAs applied in the planting hole will be monitored for root rot disease incidence and the effectiveness of BCA agents applied (IN)				
1	**Additional activity 2017:** Continue monitoring of trial until 2018 with different BCAs applied in the planting hole	Testing of BCA agents when applied as block inoculum in planting hole	**Date of additional activity:** 15.12.2018	**Completed** Analysis of data from monitoring at 4 years revealed no significant differences in level of root rot between treatments and controls. Patchy distribution of *Ganoderma philippii* inoculum may complicate statistical analyses. Data presented at project final review in April 2019

Objective 2: To reduce the impact of Ceratocystis to Acacia

PC = partner country, A = Australia, VN=Vietnam, IN=Indonesia, AM=Acacia mangium, AH=Acacia hybrid

No.	Activity	Outputs/ milestones	Delivery dates	
2.1 To identify the insects associated with *C. acaciivora*				
1	Field trapping set-up to identify insects involved in the dissemination of *Ceratocystis*.	Trial plan and establishment report	(Insects identified : 30/5/19)	**Completed** Trials have been established, insects collected and reports being drafted. RAPP and Sinarmas have made collections and submitted samples to LIPI. Barcoding has been completed for the first set of samples, though RAPP has subsequently declined to continue this aspect of the project. In Vietnam, VAFS have collected beetles, mainly *Euwallacea fornicatus* and *Xyleborus crassisculus* and isolated *Ceratocystis* from these insects. (But DNA sequencing to confirm *Ceratocystis* species identity and DNA barcoding of a subset of the *Euwallacea fornicatus* are required before this work can be published in an international journal).
2.2 The biochemistry of response to infection				
1	Field trial (VN) inoculated with *Ceratocystis* to investigate the biochemistry involved in host defence response	Report on inoculation procedures of field trial and chemical analysis of samples harvested	**Due date:** 1/01/16 **No revised date (decision made in 2016)**	**Discontinued** The field trial in VN with different AH clones and originally targeted for biochemical investigations is now 7 years old but has not been inoculated. This activity was a) given low priority at the inception workshop and b) it was agreed that we would not artificially inoculate in the field and we have abandoned the trial. **Research by JAF Scholar** (Tran Trang Thanh)**:** The chemical response to *Ceratocystis* has been investigated using a pot trial in VN and artificial inoculations and a journal paper has been published. Research also presented at IUFRO · INAFOR Joint International Conference 24 – 27 July 2017 Yogyakarta, Indonesia (Promoting sustainable resources from plantations for economic growth and community benefit).
2.3 Screening acacia deployment populations to develop disease resistant breeds (Indonesia, Vietnam and SE Asia)				
1	Meeting in Indonesia involving VN partners and industry	Meeting minutes with list of trials & pathogen strains	30/09/15	**Completed** Meetings and discussions held at project induction workshop in September 2015 and the *Ceratocystis* workshop in February 2016.
2	Develop and disseminate protocols for inoculum production VN/IN	Protocol for CA production	**Due date:** 30/12/15 :	**Completed** (30/4/2016) Standard protocol available for use across SE Asia. This protocol was developed with all partners including review by Wingfield. **2017 update:** Issue over inoculation with scalpel (VN) or needle (IN) resolved. Needle is used.
3	Selection of top AH clones from FST/2008/007 and collect seed from forward selections VN	Material available for initial screening trials in VN	30/03/16	**Completed** New clones are available for deployment and F3 germplasm available for screening in VN.

4	Establish untested AM in field trials VN/IN	Trial establishment reports	**Revised date:** 30/8/2016	**Completed** The issues over Am seed being imported into Indonesia were not resolved and therefore this activity will only apply to Vietnam (see revised objective 2017). Trials with Am have been established by VAFS and assessments are being undertaken 2017. These trials will provide initial data to identify sources with tolerance for further importation of seed if it shows resistance. Data and seed will be available 2019-2020 with trials assessed at age 3 in N VN, age 1 in S VN
5	Establish screening trials with AH clones (VN) and seed from AM selections in VN & IN acacia breeding programs **Additional activity 2018-2019 suggested after mid-term review**: Establish screening trials with AA	Trial establishment reports completed Results from screening trials with *A. auriculiformis*	**Due date:** 30/08/16 **Revised date:** 30/10/2016 **Additional activity due date:** 15.12.2018	**Completed** **Completed** Partners agreed to undertake additional screening trials for the project. VAFS will soon sow AA and AM seed of individuals related to those in the hybrid screening. Indonesian partners agreed to screen a large population of *A. auriculiformis*. Screening of *A. auriculiformis* has been completed by APP and RAPP. VN – AA have been inoculated artificially in shade house by VAFS in June 2018. Experiment 1 evaluated clones commonly used in Vietnam and new selections from the VAFS clonal development program. Ten of the clones have been established over large areas in Vietnam for many years, however the majority were new selections from the IFTIB trial network. Experiment 2 evaluated 86 selections from across IFTIB's AA progeny trial network. For the progeny trials, open-pollinated seed was collected from selections made in first and second-generation seedling seed orchards as well as from selections that had been grafted into clonal seed orchards.

6	Summarise results	Initial reporting on the genetic control of *Ceratocystis*: potential and constraints	Due date: 31/5/17 *(Final report 30/11/18)*	**Completed** **Screening AH clones (VN) – artificial inoculation** The data from the VAFS hybrid (and other species) artificial inoculation screening trial has been collected and summarised. Presented at the project mid-term review and IUFRO meeting. **Screening AM (IN) – artificial inoculation** The data from the RAPP and APP artificial inoculation screening trials of AM has been collected and was presented at the project mid-term review and IUFRO meeting. The initial MHP screening trial failed as the wrong inoculum was used. This screening was repeated and has been provided and will be summarised in a publication. **Screening of AH clones (VN) – natural inoculation** The four field trials set up in different locations in Vietnam (north and south) were designed to have two trees per plot. There is no disease at 3 sites and low levels of infections at one site. All trees are being wounded every 6 months to promote infection.
7	**Extension 2019-2020 activity** Screening of new *Acacia auriculiformis* germplasm to select the most genetically diverse populations	Raise seedlings of 150 seedlots and sample for DNA PCR and genotyping Selection of seedlots and establishment of seed orchards in Ba vi and Dongha provinces	30/6/19 30/10/2019 Revised date 30/3/2020 31/12/19 Revised date 30/6/2020	**Completed** Genetic diversity of AA in Vietnam is low, so VAFS and CFBTI have exchanged seedlots of AA to improve genetic diversity in both countries in efforts to increase pest and disease resistance. Experience in Brazil with hybrid eucalypt breeding indicated that the likelihood of finding resistance to pests and diseases is determined more by the genetic diversity in the hybrid combinations tested than by the total number of clones tested. Thus, breeding programs for pest and disease resistance in Acacia needs to consider not just the number of clones and families but the levels of genetic diversity in the material tested. Half of the required funding to do this work was provided by the Vietnamese government, the other 50% from ACIAR
2.4 Developing robust disease screening systems				
1	Establish host age trial with similar genetics every 3 months IN	Trial design, establishment report	**Due date:** 30/09/15 **No revised date**	**Abandoned** Given the project team’s decision not to complete inoculations in the field, this trial was abandoned as impossible to complete.

2	Stem sections from VN FST/2008/007 clonal trials inoculated with multiple strains	Breeding/clonal values for CA damage and pathogen - host interactions	**Due date:** 30/06/16 **No revised date**	**Discontinued** The phyllode inoculation protocol proved immensely popular with all who saw Aswardi's presentation at the *Ceratocystis* Workshop. As the phyllode protocol has now been verified as a useful preliminary screening tool in multiple studies in both Indonesia and Vietnam, it is not considered worthwhile to continue with the stem inoculation protocol which was prone to problems of desiccation and contamination.
3	Evaluate phyllode inoculation assay in VN and IN	Protocol for phyllode assay	**Due date:** 30/12/17	**Completed** See 2.4 6. Phyllode inoculations carried out in Indonesia show significant promise. A publication has been submitted to the European Journal od Plant Pathology. Phyllode inoculations of Vietnamese hybrids have been less promising, however a relationship with seedling mortality and lesion length has been observed and heritability has been confirmed. **Completed - Additional activities** • AA testing based on phyllode inoculation in VN has confirmed the utility and ease of this technique • IN: Utility of phyllode inoculation has been confirmed in two laboratories using multiple isolates of *Ceratocystis*. A manuscript has been prepared and will shortly be submitted to Plant Pathology.
4	Fresh vs cultured inoculum trials	Understanding of inoculum requirements	**Due date:** 30/06/18	**Completed** A preliminary study showed that an isolate stored in culture for a year and a freshly isolated culture (the same stored culture re-passaged through a living plant) have been compared. There were no significant differences in pathogenicity. The results of this activity have been included in a manuscript (submitted) which presents the potential of using phyllodes for testing tolerance to *Ceratocystis*.
5	IN Mixed vs single inoculum with multiple assessment times	Experimental plan methods and results documented	**Due date:** 30/12/18	**Completed** Different types of inoculum have been compared (spores vs mycelium). There are only differences in the early stages of infection.
6	Evaluate log, phyllode and stem inoculation relationships VN and IN	Estimates of heritability & genetic correlations	**Due date:** 31/5/19	**Completed** Phyllodes, small stem sections and pot plants have been inoculated in IN and results indicate that phyllode inoculation will prove an informative and rapid inoculation technique. A manuscript has been prepared. Phyllode inoculations have been enthusiastically adopted by VAFS pathologists. This work has been included in the manuscript describing the tolerance screening of acacias in Vietnam. (see 2.4.2)
2.5 Breeding and deployment strategy revision				
1	Review of breeding strategy for AM, AH and AC in VN and IN	Meeting minutes documents strategies	**Due date:** 30/09/15	**Completed** Meetings and discussions held at project induction workshop in September 2015, the *Ceratocystis* workshop in February 2016 and during visits.

2	Alternative acacia plots established within AM plantations for hybridisation VN and IN	Maps of alternative species plots	**Due date:** 30/03/16 **Revised date:** 30/05/2018	**Completed** **IN:** Monitoring of alternative species is on-going. Also, every 3 months seed is being collected to evaluate the proportion of hybrids that will be useful for further screening. **VN:** Selections have been made from material in alternative species plots established with seed of alternative species in the VAFS seed store. Material has been centralised into the Bao Bang research centre and will be monitored for *Ceratocystis* infection. Flower crops are being monitored as part of the polyploid development project that has been developed from prior ACIAR work and is currently supported by MARD.
2.6 Extension 2019-2020 activity: Obtain germplasm of alternative species for assessment of tolerance to *Ceratocystis* and suitability for incorporation into breeding programs				
1	Obtain seed of alternative *Acacia* species for progeny trials and future hybridisation IN	Report on seed available in Indonesia	15/12/19	**Completed**
2	Obtain pollen of alternate Eucalyptus species for hybridising with CFBTI *E. pellita*. IN	Report on pollen available in Indonesia	15/12/19	**Completed**
2.7 Extension activity 2019-2020: Determine whether burning of slash may reduce *Ceratocystis* inoculum between rotations.				
i	Determine the relationship between the presence of *Ceratocystis manginecans* inoculum in the soils of Acacia hybrid plantations and slash management VN	Select location and establish plots, survey disease incidence. Take soil samples, harvest trees and apply treatments. Isolations, DNA extraction and sequencing Second soil sampling qPCR on soil samples Report and recommendations	31/5/19 Revised date: 15/6/2020	**Completed without qPCR** Current recommendations are to retain slash for improved soil stability, but many small-holder farmers prefer to burn as they consider that this helps to reduce disease levels. There is no data available to support or refute this hypothesis so this trial will provide evidence to assist VAFS in confirming or changing the guidelines. Field work completed but lab work experienced delays, now back on track. Travel restrictions prevented completion of qPCR and soil samples could not be appropriately stored for later analysis.

3	Review of resistance breeding *Draft and final breeding strategy*	Report (to coincide with mid-project review)	**Due date:** 30/05/17 *(Due dates: 30/03/18 and 30/03/19)*	**Completed** Kien, Thinh, Harwood et al. wrote "A revised tree breeding strategy to help Vietnam to meet the challenge of *Ceratocystis* stem wilt and other diseases". This document was published in a Vietnamese journal This publication content was presented at the IUFRO meeting in Yogyakarta and used as a model to discuss with Indonesian Project Partners at the IUFRO meeting. During the project mid-term review meeting in Yogyakarta, the project partners met to discuss the initial findings of this project and how resistance breeding is best integrated into their tree improvement programs. It was agreed that a central screening centre in Indonesia was required. Strategy for such a centre developed by CFBTI as part of the July 2018 project review meeting in Indonesia but has not been formalised.

Objective 3. To build capacity in forest health management in SE Asia

PC = partner country, A = Australia, VN=Vietnam, IN=Indonesia, AM=Acacia mangium, AH=Acacia hybrid

No.	Activity	Outputs/ milestones	Delivery dates	Comments
3.1 Ceratocystis workshop – gap analysis (IN)				
1	Organisation of workshop	Date set and invitees lined up	**Due date:** 30/8/15 **Complete:** 30/9/15	**Completed** The *Ceratocystis* workshop was in part organised at the Inception workshop. The workshop was intended to run back to back with the inception workshop, but this was not possible as contracts had not been signed and funding was not available.
2	*Ceratocystis* workshop (IN)	Workshop	**Due date:** 30/9/15 **Complete:** 20/2/16	**Completed** *Ceratocystis* workshop involved 70 participants from 7 SE Asian countries, Australia and internationally renowned invited speakers from Brazil, South Africa and Hawaii. This workshop included a field trip to view both *Ganoderma* and *Ceratocystis* damage.
3	Publishing of workshop proceedings **Revised activity 2017:** To write a review of acacia *Ceratocystis*	Special Issue on *Ceratocystis*	**Due date:** 30/8/16 **Revised date:** 30/5/17 **Revised date:** 15.12.2018	**Completed** There are too many conflicting IP issues and time pressures on workshop participants to publish a special issue (i.e. a series of journal articles) although all the power point slides have been made available to participants. However, *Ceratocystis* review published in ACIAR special issue of Australian Forestry.

3.2 Biological control workshop (VN)				
1	Organisation of workshop	Date and location set and invitees lined up	**Due date:** 31/3/17 **Revised date:** 31/7/17 *(Workshop write-up: 30/8/18)*	**Completed** Held 21-22nd July coinciding with the IUFRO Acacia conference "Towards a sustainable future for Acacia plantations". Yogyakarta, IN in 24 - 28 July 2017. Crawford funding ($15K) was award to Caroline Mohammed and Morag Glen to support this ACIAR workshop. This enabled the workshop to span two ACIAR forestry biocontrol projects; one that focuses on BC of insects *FST/2012/091 Biological control of galling insect pests of eucalypt plantations in the Mekong region*, led by Simon Lawson and this project that focuses on the application of fungal BCAs for a fungal disease). We also invited an international expert from outside Australia to the workshop - Marc Kenis of CABI. Presentations are on project website, media coverage in Indonesia – see report to Crawford Fund.
3.3 Disease risk and impact analysis workshop (IN)				
1	Scoping meeting (IN)	Scoping report for activity	**Due date:** 30/6/2017 **Revised date:** 31/8/2016	**Completed** A scoping visit was carried out in August 2016 and is reported in the trip report. A training seminar was given at each of the 3 companies by Christine Stone, NSW DPI. We agreed with RAPP to use a site for the RS case study that is a biological control trial site (acacia) – see 1.3.2 – and acquired images of this site but tree canopies were too close to construct an accurate DEM.
2	Data acquisition (IN) by industry partner under the guidance of Australian experts	Data set acquired	31/12/2017 *(Analysis of data for case studies = 1/7/2018* *Organisation of workshop = 31/12/2018)* 15/12/2019	**Completed** This activity i.e. data acquisition was carried out (i.e. the first fly over) in September 2017 Acacia trial plot (see 1.3.2) was used to detect unhealthy trees but not for inventory assessment of mortalities - RAPP could not provide a good DEM (too low resolution). However, the case study has clearly demonstrated constraints to collecting reliable data and allowed improvement of methodology! A eucalyptus site has been surveyed that has better ground visibility (hence DEM can be created from RS data) – this site was intended for use as a case study for the workshop but poor image quality resulted in difficulty identifying individual tree crowns. A mini case study was conducted on the field day in real time during the workshop. Images obtained were of excellent quality, permitting accurate tree measurements and identification of diseased trees from the orthophoto and point-cloud. A paper has been published in Australian Forestry. The process of detection of diseased trees from UAV acquired data has been automated.
3.4 Publication writing workshop(s) IN/VN				
1	Project partners less experienced in publishing attend a workshop (VN and/or IN)	Workshop.	TBA – timing to be left flexible	**Completed** Writing workshop run in Yogyakarta in March 2018. 7 attendees – focused on publications arising from ACIAR projects. Scientific publications about *Ceratocystis* published in Vietnamese are being translated. One has been published in *Australasian Plant Pathology* and a second is undergoing editing prior to submission for publication.

7 Key results and discussion

7.1 BCA inoculum production and formulation

Stump trials, planned to commence early in the project to allow time for monitoring, were dependent on adequate production of oidia and the ability to maintain their viability during transport to the field sites. Oidia were initially harvested by adding sterile water to the surface of BCA isolates growing on agar media, scraping with a sterile glass 'hockey stick' and then pouring off the oidial suspension (Pratt, Niemi et al. 2000). Refinement of production techniques by selection of isolate, growth media, and age of culture was frustrated by a high level of unexplained variation in oidial production and an apparent decline in oidial production over time.

After much experimentation, it was determined that isolates grown from a single oidium had a greater ability to produce abundant oidia on agar medium and that wood blocks were a better substrate for oidia production than agar medium. This made it possible to grow sufficient inoculum for the planned field trials. Oidia were counted using a haemocytometer and by counting viable colonies after plating of a small aliquot of the suspension. Haemocytometer counts were approximately four times greater than figures obtained from viable counts, Figure 7.1. This discrepancy may be due to; counting of unviable mycelial fragments as oidia, damage to oidia during harvesting or lack of separation of oidia when plated.

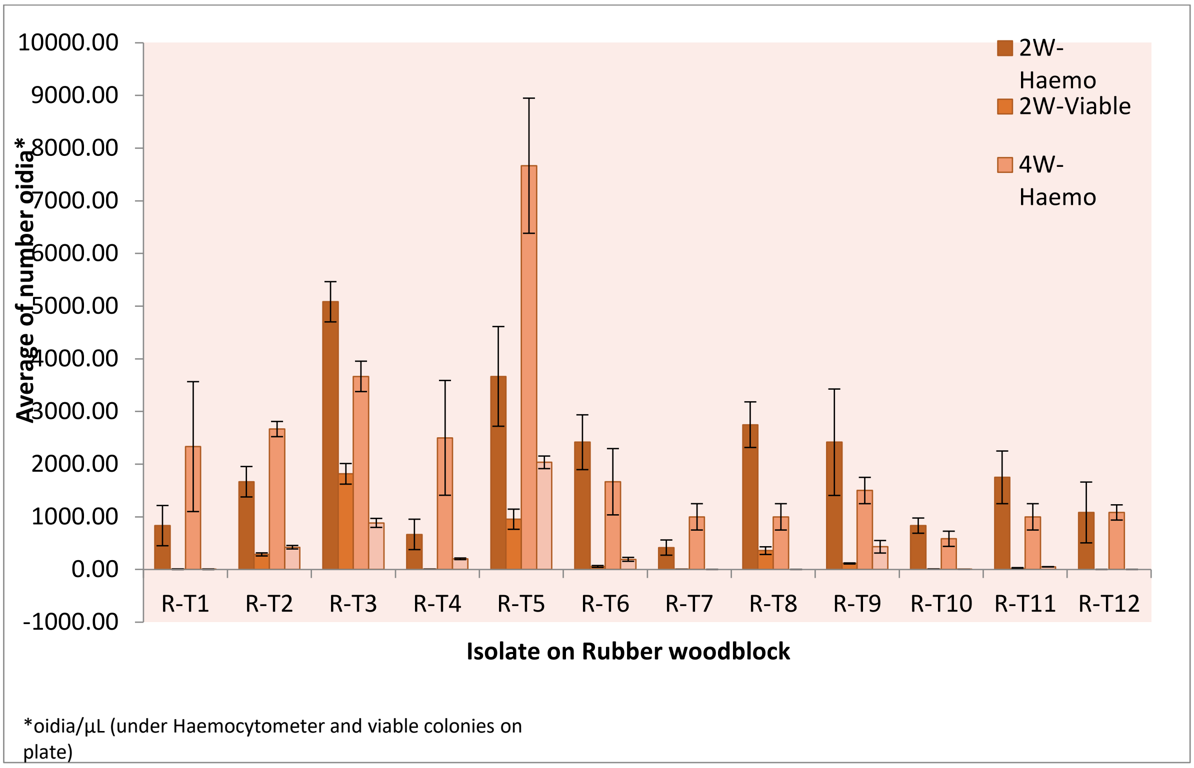

Figure 7.1: Oidia production by 21 potential BCA isolates grown on rubber wood blocks for two to four weeks, as determined by haemocytometer and viable counts.

Oidia stored in water germinated very quickly at room temperature, though refrigeration allowed preservation for up to a week. Solutions investigated for longer term room temperature storage included storage in glycerol or sucrose solutions, ethanol solutions, mineral oil and vegetable oil. Glycerol and sucrose solutions were very prone to contamination while ethanol solutions tended to reduce viability of oidia. Storage in oils may extend the viable life of oidia but haemocytometer counts were not possible and viable counts were extremely low. This may have been due to the clumping of oidia in small oil droplets after spreading onto agar so that a group of germinating oidia were counted as a single colony.

As field trials had to be established before methods for long-term storage of viable inoculum were developed, wood block inoculum was transported and oidia resuspended in the on-site accommodation before application to stumps. The first field large-scale field trial, based in logas South compartment B110, highlighted some logistical problems in timely application of stump treatments. This, combined with several other research findings from this project and the previous one, FST/2009/051, prompted an exploration of alternative methods for BCA application. The new method was based on potential rhizosphere and/or endophytic colonisation by BCAs of seedling roots in the nursery - before seedlings are planted in the field. Recourse to this method was justified because

- of the logistical barriers to BCA stump application in the field
- BCAs applied to stumps may invade stumps but not persist in them over extended periods of time
- Stump inoculation with *P. gigantea* is known to be effective in preventing colonisation of new sites by *Heterobasidion annosum,* but is not so effective at infested sites
- BCA agents need to be applied at sites that are highly infested as very few uninfested locations remain
- BCA block inoculum in the planting hole, carried out by Sinarmas Forestry, has been the most effective and persistent method of BCA application against the root rot pathogen on highly infested sites

Since the ability of BCAs to colonise the rhizosphere was first documented by Heru Indrayadi, Arara PT have conducted experimentation to achieve reliable rhizosphere colonisation in the nursery. But having achieved colonisation of the rhizosphere it is necessary to establish a trial to investigate if this colonisation reduces the likelihood of *Ganoderma* infection. A pot trial to test this was planned to take place at CFBTI in Yogyakarta.

Before the trial could proceed it was necessary to demonstrate pathogenicity of the *G. philippii* isolates under this experimental system. Pathogenicity trials with root rot pathogens require significant time, but a lack of host deaths has been problematic in previous BCA trials. We obtained fresh isolates of *Ganoderma philippii* in case ours had lost pathogenicity over the years in culture, but these were not any more aggressive than the those that had been maintained in the laboratory for up to a decade. While seedling death did not occur, symptoms of root rot (yellowing and wilting) were observed and, on harvesting, root infections were seen. *Ganoderma philippii* was re-isolated from the infected roots. Due to time constraints, the experiment had to proceed on the basis that disease could be demonstrated in controls even if seedlings did not die. Two different BCA isolates, Pb8 and Pb11, were used to create three different forms of inoculum; 10ml oidia suspension, 10g colonised barley and 10g alginate bead inoculum. Each treatment combination was replicated seven times and seedlings inoculated on 5th December 2019 by uncovering roots, applying inoculum then recovering with soil (Figure 7.2).

Figure7.2: Application of BCA inoculum to roots of acacia seedlings; left, oidia suspension; middle, colonised barley; right, alginate beads.

One month after inoculation, the root system of one seedling in each treatment was checked for fungal colonisation but no mycelial covering was evident on the roots. Pathogen inoculation was considered pointless as the rhizosphere colonisation had failed. Rather than completely abandoning the experiment, woodblock inoculum of the two BCA isolates was prepared and applied to the seedling roots in August 2020. Two weeks after application of woodblock inoculum, rhizosphere colonisation was confirmed for Pb8 but not for Pb11. The Pb8 woodblock inoculum was also covered in thick white mycelium whereas the Pb11 woodblock inoculum was not. Re-isolation confirmed the identity of the white mycelium as *Phlebiopsis* sp.

Pathogen inoculum was applied in September 2020; roots were uncovered and four woodblocks, colonised by *Ganoderma philippii*, were placed next to the roots before recovering with soil. Harvesting was conducted in December 2020, 3 months after pathogen application. By the time of harvesting, 22 of the 60 seedlings had died (Table 7.1) with no apparent treatment effect.

Table 7.1: Number of dead/infected trees with symptoms of red root rot in different BCA/pathogen treatments.

	Pb8	Pb8 control	Pb11	Pb11 control
Rep 1	2	0	3	3
Rep 2	2	4	3	3
Rep 3	1	0	0	1

7.2 BCA field trials

7.2.1 Wood block field trial established during FST/1009/051

Monitoring of a large field trial established by RAPP near the end of FST/2009/051 was continued during FST/2014/068. This trial tested nine potential BCA fungi applied as wood block inoculum in the planting hole. Plot size was 25 trees (5x5) with 20 reps per treatment, established in 2014 in a 6ha compartment in Logas South. Surveys were conducted at 13, 25 and 37 months after planting. At the final survey, root rot incidence ranged from 10 to 19.8% but there were no significant differences among treatments. Untreated control trees had a 25% incidence of root rot. The patchy distribution of root rot inoculum makes it very difficult to achieve statistical significance in field trials.

7.2.2 Stump trial to follow establishment and growth of BCA in root systems

As field sites for the new stump trials were a day and a half's travel from the laboratory, wood block inoculum was transported and oidia resuspended in the on-site accommodation. The first field trial was established at three sites to test the ability of different BCA isolates to colonise stumps of acacia and eucalypts and to grow down into the root systems. Trials were revisited and destructively sampled to track the growth of the BCAs (Figure 7.3). We used a combination of culturing and PCR species-specific detection to confirm the extent of stump colonization over time by the BCA species. PCR results are promising but the positive results are infrequent. The low frequency of detection may be due to the small size of fragments taken for re-isolation and overgrowth by more rapidly growing fungi. There were also a few positive results from root systems that were not inoculated with the target fungus. These may be spurious results caused by cross-contamination in the molecular laboratory. This incident provided an opportunity for essential training in contamination control for CFBTI scientists in the molecular lab. Rectification of the problem has required servicing of the laminar flow cabinet. The third set of samples are being tested using freshly prepared reagents and a subset of samples is being analysed using a different technique (amplicon sequencing of DNA extracted directly from the stump or root samples). Results are not yet available, due to delays caused by the impact of CoVID-19 on international transport.

Isolates that morphologically resembled those of a species of *Neonothopanus* were obtained from a high proportion of stumps/root systems in the stump inoculation trials. Isolates of a *Neonothopanus* sp. were very aggressive against *G. philippii* in laboratory tests, but this species was not tested as a biocontrol agent in the stump trials because it did not produce a high number of oidia and was therefore not suitable for the proposed stump inoculation method.

Figure 7.3. Eucalypt root system uncovered for sampling.

7.2.3. Field trial to determine efficacy of BCAs in preventing root rot

The semi-commercial trial (compartment sized) in which BCAs were applied directly after harvest to stumps was assessed for root rot disease for a third time in January 2019. Root rot was still at low levels (Figure 7..4) but slowly increasing as was the rate of *Ceratocystis* infection (but at a faster rate than *Ganoderma*). Differences between BCA treatments are not statistically significant though the spatial variability of inoculum may be a major impediment (Figure 7.5).

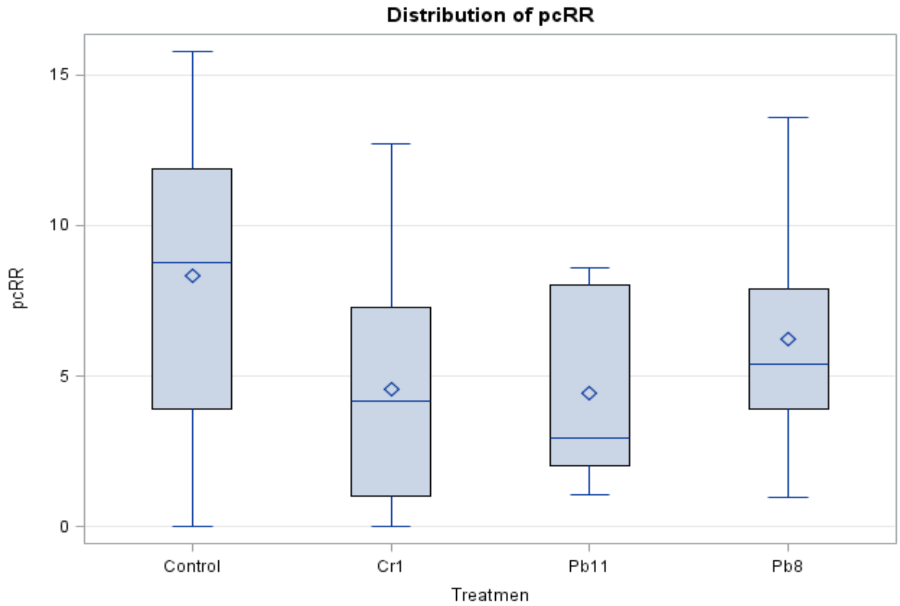

Figure 7.4. Incidence of root rot (%) after stump treatment with one isolate of *Cerrena* (Cr1) and two isolates of *Phlebiopsis* (Pb8 and Pb11) compared to control, uninoculated stumps.

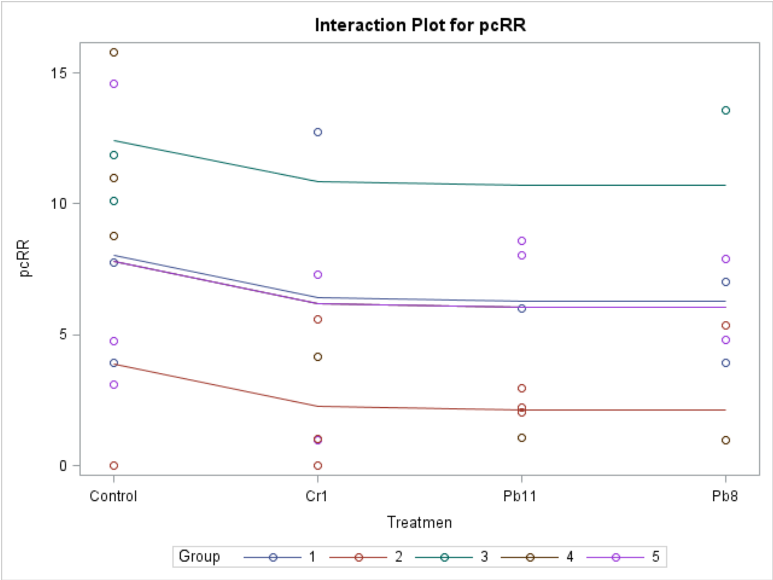

Figure 7.5. Incidence of root rot (%) in treated and control plots, grouped by spatial proximity. In each group, root rot incidence is higher in control plots than in treated plots, regardless of which isolate was used.

The most significant result of this trial in terms of scientific impact was that it raised considerable concerns about the feasibility of treating stumps under manual harvesting conditions in the field due to cost and OH&S issues. Testing the efficacy of stump treatment using machine harvesters (which are becoming more prevalent in Indonesia) is beyond the scope of this project but can be a recommendation for industry.

7.2.3 BCA application to older acacia plantations

A small trial was established in Gunung Kidul to assess the ability of *Phlebiopsis* sp. to colonise the rhizosphere of established acacias. Woodblock inoculum of Pb8 was applied to ten X-yo trees, 5 *A. mangium* and 5 *A. auriculiformis.* Trees were harvested one month later and white mycelium characteristic of *Phlebiopsis* sp. was observed colonising the soil, including the rhizosphere, and the root system. Re-isolation confirmed the identification as *Phlebiopsis* sp.

7.3 Insects involved in the spread of *Ceratocystis manginecans* in Indonesian acacia plantations

Emergence traps were used to collect insects from acacias with different levels of Ceratocystis disease (Table 7.2). Trees were harvested and stems cut into sections of 50-80 cm, the logs were sealed at each end with paraffin liquid then placed into mesh bags with collection jars placed at the top and bottom and monitored periodically.

Table 7.2: Trees harvested for insect trapping and (in brackets) the number of beetles collected from harvested logs in each disease class.

Tree species	**Location**	**Infection stage**			
		Healthy	Early	Medium	Dead
A. mangium	Hoa Binh province	3 (0)	3 (77)	3 (65)	3 (71)
Acacia hybrid	Yen Bai province	3 (0)	3 (67)	3 (54)	3 (62)

The majority of beetles were identified, based on morphology, as belonging to the *Euwallacea fornicatus* species complex, with a smaller percentage identified as *Xylosandrus crassiusculus* and a few *Xyleborinus aff. artestriatus.*

These are all species of ambrosia beetle and carry symbiotic fungal propagules in mycangia, though auxiliary fungi may also be present (Saragih et al. 2020). Isolation of *Ceratocystis manginecans* was attempted from a subset of beetles in this study and was successful for 17 beetles representing all three species that emerged from *A. mangium* but for only two collections of *X. crassiusculus* that emerged from acacia hybrid.

In Indonesia, a more limited sampling of beetles was obtained and identification was based on sequencing of a fragment of the cytochrome oxidase (COX1) gene (Table 7.3). No fungal isolations were made from the beetles collected in Indonesia.

Table 7.3: Insects collected emerging from Ceratocystis-infected acacia in Indonesia

Insect species	**Common names**	**Location**	**Host**
Xylosandrus compactus	Shot hole borer, black coffee borer, tea stem borer	1	*Acacia auriculiformis*
Tribolium castaneum	Red flour beetle	1	*A. mangium*
Euwallacea similis		1	*A. mangium*
Rhizopertha dominica	Lesser grain borer	1	*A. mangium*
Euwallacea perbrevis	Tea shot hole borer	2	*A. mangium*

7.3 Screening acacia deployment populations

A large screening trial was conducted in Indonesia to select *A. mangium* progeny with the greatest tolerance to infection by *C. manginecans.* The same seedlots from CFBTI's second-generation seedling seed orchard were divided among the three participating forestry companies, Arara Abadi, RAPP and MHP. Additional families from each company's internal breeding programs were also included. Seedlings were germinated and clonally replicated for the tests. Over 5,000 ramets from more than 1,000 clones were screened.
Each company screened several of their isolates of *C. manginecans* and selected the most aggressive, thus each company used a different isolate. The identities of these three isolates were confirmed by sequencing of the ribosomal DNA internal transcribed spacers and a fragment of the β-tubulin gene. Inoculation protocols were standardised across the three companies, using a needle to wound the stem at a specified height before application of a mycelial plug from a 7-day-old culture plate and sealing with parafilm. External lesions and ramet survival were monitored until 50% of the seedlings had died, when internal lesions on surviving plants were measured.

Screening results confirmed the low likelihood that pure *A. mangium* tolerant to *Ceratocystis* will be available for deployment. It appears that parents selected for tolerance will always have some progeny that are susceptible. The genetic signal for tolerance to *Ceratocystis* in *A. mangium* could be used for the selection of *A. mangium* for hybridisation.

Field trials have been established by two of the companies and a set of 20 tolerant clones was provided to CFBTI for establishment of a seed orchard to produce seed for industry and smallholders. Meetings in July 2018 were used to propose a public-private partnership that will help ensure this seed orchard is intensively managed to provide seed as quickly as possible to industry and smallholders. In April 2019, *Ceratocystis* was detected in the vicinity of the seed orchard but has not yet affected these plantings.

This seed orchard has been augmented by *A. auriculiformis* (AA) clones identified as part of additional screening work being undertaken by the industry partners using seed that the project provided to CFBTI. These screenings have shown that *A. auriculiformis* has comparatively high levels of tolerance to *Ceratocystis.* VAFS identified 86 superior forward selections of AA progeny trials and collected seed to develop advanced generation seed orchards. These families were screened for *Ceratocystis* tolerance and identified parents with greater potential to produce disease tolerant offspring.

Seeds from five species of *Acacia - A. aulacocarpa, A. crassicarpa, A. leptocarpa, A. midgleyii* and *A. peregrinalis* – has been imported into Indonesia from Australia and distributed to the three partner companies. These species are being tested for tolerance to *Ceratocystis*. Eucalyptus pollen has also been imported and a portion sent to Arara PT for controlled crossing and screening trials as eucalypts are becoming increasingly attacked by *Ceratocystis*.

A rapid, preliminary screening protocol using detached phyllodes was developed and validated (Nasution et al., submitted) as a replacement for screening of small, potted plants. Uptake of this method by industry was rapid.

In Vietnam, the main commercial acacia clones and a pedigreed *Acacia auriculiformis* trial were screened for tolerance to Ceratocystis (Brawner et al. 2020). This study also confirmed the heritability of the level of phyllode damage.

More than 100 seedlots of *Acacia auriculiformis* unrelated to the Vietnamese population (according to pedigree records) were imported into Vietnam from Indonesia to enrich the genetic diversity of the breeding population (Table 7.4). Progress is being made in determining the genetic diversity and resistance to pests and diseases, especially *Ceratocystis* (Fig 7.6). In addition, the seedlings from this activity will be planted out for genetic conservation.

Figure 7.6. Propagation and testing of germplasm imported into Vietnam from Indonesia: Seed germinated (left), DNA extracted (middle), using the Tissuelyser (right) supplied by ACIAR.

Table 7.4. List of *A. auriculiformis* seedlots tested for genetic diversity.

Index	CSIRO NO.	Provenances	No. Family
Infusion materials			
1	18601	Melville Orchard	11
2	16608	Bandabern of Bulla WP, PNG	2
3	16609	Belamuk WP, PNG	1
4	17553	Bensbach WP, PNG	37
5	17966	Boggy Creek, QLD	10
6	16756	Enormamby river, QLD	1
7	18359	Lower poscoe river, QLD	14
8	16606	Morehead R Rouku WP, PNG	8
9	18246	Morehead River WP, PNG	1
10	17961	Oliver River, QLD	2
11	18247	Wenlock River, QLD	13
Existing breeding population			
12	19326	Sakaerat SSO Thailand	24
13	19255	Melville Orchard	16
14	18998	Pascoe Cape Work, QLD	2
15	17966	Boggy Creek, QLD	2
16	18854	Archer R & Tribs, QLD	2
17	19246	Wenlock River, QLD	2
18	19250	Coen River, QLD	2
	Total		**150**

Microsatellite analysis was conducted as described in Le et al. (2016) using 16 markers. Population genetic parameters for each marker and species (N_a = Number of different alleles, H_o = Observed heterozygosity, H_e = Expected heterozygosity, F = Fixation index), and Genetic distance were estimated using GenAlEx V6.5 (Peakall and Smouse 2012).

The seed sources of the existing Vietnamese breeding population of *A. auriculifomis* originated mainly from natural provenances in Queensland - Australia and selected families from Melville Orchards (Australia) and Sakearat orchard (Thailand). After introduction of the Indonesian material, the genetic diversity available in Vietnam was improved (Table 7.5):

Table 7.5. Genetic diversity of Vietnamese *A. auriculiformis* breeding population before and after infusion.

Population	Na	Ne	I	Ho	He	F
Existing	10.2	4.9	1.63	0.77	0.75	-0.03
After Infusion	14.3	6.4	1.87	0.82	0.78	-0.05

(Ne = No. of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity, F = Fixation Index)

Despite the greater number of alleles per loci and number of effective alleles in thePNG population compared to the QLD population, there is no remarkable difference in genetic diversity level between QLD and PNG populations in other parameters (Table 7.6). The results showed that the greatest genetic variability was among progenies within populations, as also noted by Ratnam and Norwati (1993) using isozymes markers.

Table 7.6. Genetic diversity of *A. auriculiformis* from different countries of origin (excluding seedlots derived from seed orchards).

Population	No. Family	Na	Ne	I	Ho	He	F
QLD	50	8.1	2.8	1.32	0.75	0.72	-0.04
PNG	49	11.3	4.6	1.75	0.76	0.72	-0.05

The seedlings that germinated from imported seedlots were planted for genetic conservation and seed orchards in Bavi (Hanoi) in March 2020 for further assessments and potential deployment.

7.4 Management of Ceratocystis inoculum

The trial commenced in 2019 in an experimental site in the *Acacia* hybrid plantation in Tuyen Quang/Hoa Binh Province, Vietnam. The aim is to determine if there is a relationship between the levels of *Ceratocystis* vascular wilt and canker disease inoculum in the soils of *Acacia* hybrid plantations under different slash management regimes. Slash retention is promoted in Vietnam over burning especially on steep sites but since the advent of *Ceratocystis* disease farmers have returned to burning.

Treatments were designed to determine if slash removal or burning can reduce the presence of *Ceratocystis* in the soils of *Acacia* hybrid plantations. The treatments are:

•Treatment 1: slash retained

•Treatment 2: slash removed and added to Treatment 3

•Treatment 3: slash burnt (Addition of slash from Treatment 2 is to increase the fuel load and potentially the intensity of the burn).

The treatments were applied to 9 plots (3 reps of each treatment) at the trial site (Figure 7.7).

Figure 7.7. The trial site (left) and the burning trial (right)

Soil samples were taken for isolation of Ceratocystis by carrot baiting before harvest (June 2019) and after the application of treatments (September 2019) (Table 7.7). An additional 24 samples were taken in August 2019, each sample was divided into three sub-samples by depth; 0-5, 5-10 and 10-15 cm. Ceratocystis was recovered from the 24 sub-samples of 0-5cm depth but none of the deeper sub-samples.

Table 7.7: Ceratocystis isolation from soil samples

	Before Treatment (June 2019)		**After Treatment**	
	# soil cores	# with Ceratocystis	# soil cores	# with Ceratocystis
Treatment 1	60	7	135	12
Treatment 2	60	8	135	7
Treatment 3	60	9	135	4

Sections of wood infected with Ceratocystis were buried in trial plots before the treatments and recovered for isolation of Ceratocystis in August 2019 after the treatments were applied. Each of the nine plots had five wood samples randomly located within the plot. Wood samples were recovered following the treatment and isolation of Ceratocystis attempted. Of the 45 samples, Ceratocystis was isolated from 35 with a 90% recovery rate from treatments 1 and 2 and only 53% from treatment 3 (burnt plots).

DNA was extracted from 24 of the isolates from soil and a fragment of the β-tubulin gene was amplified and sequenced; the sequence was consistent with *C. manginecans.* A pathogenicity test was also conducted for 21 of these isolates. They were inoculated into 8-month-old *A. hybrid* trees (commercial hybrid) with a stem diameter ranging from 0.7-0.9 cm, and height between 80-90 cm.

The experiment was set up as a randomized complete block design, with 24 treatments (22 *C. manginecans* isolates, mock control (inoculated with agar only) and un-wounded control). Five replicates, in each replicate, 4 *A. hybrid* trees were inoculated with one of 21 *C. manginecans* isolates. Therefore, a total of 20 trees were inoculated for each of 21 *C. manginecans* isolates).

The over bark lesion length was measured after 35 days (15/9/2020). The control plants did not develop lesions so were excluded from the statistical analysis. Data were analyzed by Genstat version 12 and the results shown in Table 7.8. All isolates produced lesions indicating pathogenicity against *A. auriculiformis*.

Table 7.8: Lesion length produced by C. manginecans isolates, isolated from soil in Hoa Binh, on A. hybrid 35 days after inoculation. Different letters show sig. differences at p < .001, N =20 trees.

Isolate	OB Lesion length (cm)	Grouping
1	12.30	ab
2	13.80	abc
3	13.70	abc
4	15.55	abcde
5	14.50	abc
6	11.85	ab
7	12.65	abc
8	20.40	fg
9	16.00	bcde
10	21.90	g
11	21.95	g
12	15.30	abcd
13	12.85	abc
14	13.80	abc
15	19.00	defg
16	16.85	cdef
17	12.60	abc
19	19.80	efg
20	12.50	ab
21	11.55	a
22	11.85	ab

p<0.001, lsd = 4.33

From the results to date, it appears that burning of slash may reduce the level of inoculum in the soil. It is unclear if this degree of inoculum reduction will be sufficient to reduce infection in subsequent rotations. Following the trial, the site was replanted with alternating rows of *Acacia* hybrid and *Chuckrasia tabularis* which will be monitored for disease development.

8 Impacts

8.1 Scientific impacts – now and in 5 years

In this project we have shown that detached phyllodes can be used to screen acacia clones for genetic tolerance to Ceratocystis, providing an opportunity to accelerate breeding programs. We have shown a greater level of genetic tolerance in *A. auriculiformis* than in *A. mangium,* thus demonstrating the necessity of developing acacia hybrids that incorporate the best attributes of both species. We have also demonstrated that Ceratocystis inoculum can persist in soil and is pathogenic to acacia. Burning of slash may reduce inoculum but whether this is sufficient to reduce disease levels in subsequent rotations is yet to be determined.

We have shown that *Ganoderma philippii* is genetically diverse and there are no barriers to sexual recombination across Indonesia, however clonal spread is common in acacia plantations, particularly in later rotations. *Phlebiopsis* sp. can reduce inoculum of *G. philippii* in woody residues. It can also colonise the rhizosphere of seedlings and older plants from a woody base but not from oidia, grain inoculum or alginate beads. Rhizosphere colonisation may not prevent root infection by *G. philippii,* as *Phlebiopsis* may need a wood base to be competitive.

We have demonstrated the viability of UAVs in detecting diseased trees for more effective targetting of pest surveys. The following papers have been published;

Brawner J, Chi NM, Chi N, Glen M, Mohammed CL, Thu PQ, Kien ND. Tolerance of Acacia populations following inoculation with the Ceratocystis canker and wilt pathogen in Vietnam. *Tree Genetics and Genomes.* **16,** 77.

Chi NM, Thu PQ, Mohammed C. 2019. Screening disease resistance of *Acacia auriculiformis* clones against *Ceratocystis manginecans* by artificial and natural inoculation methods. *Australasian Plant Pathology.* **48,** 617-624.

Gill, W., Eyles, A., Glen, M. and Mohammed, C. (2016), Structural host responses of *Acacia mangium* and *Eucalyptus pellita* to artificial infection with the root rot pathogen, *Ganoderma philippii*. For. Path., 46: 369–375

Nasution A., Glen M, Beadle C, Mohammed C, 2019. *Ceratocystis* wilt and canker - a disease that compromises the growing of commercial Acacia-based plantations in the tropics. *Australian Forestry* **82**, 80-93.

Page DE, Glen M, Puspitasari D, Rimbawanto A, Ratkowsky D, Mohammed C, 2018. Sexuality and mating types of *Ganoderma philippii, Ganoderma mastoporum* and *Ganoderma australe*, three basidiomycete fungi with contrasting ecological roles in south-east Asian pulpwood plantations. *Australasian Plant Pathology* **47**, 83-94.

Page D.E, Glen M, Puspitasari D, Prihatini I, Gafur A, Mohammed C. 2020. Acacia plantations in Indonesia facilitate clonal spread of the root pathogen, *Ganoderma philippii*. *Plant Pathology* in press https://doi.org/10.1111/ppa.13153.

Puspitasari D., Glen M., Indrayadi H, Gafur A., Prihatini I., Hidayati, Rimbawanto A., Husna Nurrohmah A., Mohammed C. 2017. *Phlebiopsis* sp.1 and *Cerrena* sp. – two basidiomycete fungi with high potential as biocontrol agents for root rot disease in Indonesian *Acacia mangium* plantations. IUFRO · INAFOR Joint International Conference 24 – 27 July 2017 Yogyakarta, Indonesia (Promoting sustainable resources from plantations for economic growth and community benefit).

Puspitasari, D., Wibowo, A., Rahayu, S., Prihatini, I., and Rimbawanto, A. 2016. Karakter morfologi isolate *Phlebiopsis* sp.1 Jamur pengendali hayati yang potensial untuk *Ganoderma philippii*. Jurnal Pemuliaan Tanaman Hutan, Vol.10 No.1, p. 51 - 61[1]

Rimbawanto A., Hai V. D., Mohammed C. 2017. Management strategies for Acacia plantation diseases in Indonesia and Vietnam. IUFRO · INAFOR Joint International Conference 24 – 27 July 2017 Yogyakarta, Indonesia (Promoting sustainable resources from plantations for economic growth and community benefit).

Trang T., Eyles A., Davies N., Glen M., Ratkowsky D., Mohammed, C. 2017. Screening for host responses to a canker and wilt pathogen, *Ceratocystis manginecans*, in Acacia plantation genotypes. IUFRO · INAFOR Joint International Conference 24 – 27 July 2017 Yogyakarta, Indonesia (Promoting sustainable resources from plantations for economic growth and community benefit).

Trang TT, Eyles A, Davies N, Glen M, Ratkowsky D, Mohammed C. 2018. Screening for host response in *Acacia* to a canker and wilt pathogen, *Ceratocystis manginecans. Forest Pathology* **48**, e12390.

Trang T., Glen M., Beadle C., Eyles A., Ratkowsky D., Mohammed, C. 2017. Fungal agents associated with stem defects in plantations of *Acacia* hybrids grown for sawlogs in South Vietnam. IUFRO · INAFOR Joint International Conference 24 – 27 July 2017 Yogyakarta, Indonesia (Promoting sustainable resources from plantations for economic growth and community benefit).[2]

Tran TT, Glen M, Beadle C, Ratkowsky D, Mohammed C, 2019. Wood-rotting basidiomycetes are a minor component of fungal communities associated with Acacia hybrid trees grown for sawlogs in South Vietnam. *Forest Pathology* **49** e12498.

Nasution A, Glen M, Ratkowsky D, Evans K, Indrayadi H, Brawner J, Gafur A, Mohammed CL. 2020. Phyllode inoculation provides a rapid protocol for resistance and tolerance screening of *Acacia* species against *Ceratocystis* wilt and canker disease. Submitted to European Journal of Plant Pathology.

Another four papers are at an advanced draft stage and submission is anticipated before June 2020. These include;

Brawner J, Nirsatmanto A, Indrayati H, Dianto VD, Tarigan M, Yuliarto M, Hardiyanto E, Glen M, Mohammed CL, Rimbawanto A. Screening clonally replicated *Acacia mangium* breeding populations for tolerance to Ceratocystis canker and wilt disease.

Nasution A, Glen M, Evans K, Gafur A, Mohammed CL. Diversity of bacterial endophytes in *Acacia mangium* in Indonesian plantations.

Nasution A, Glen M, Evans K, Gafur A, Mohammed CL. Potential for bacterial endophytes of *Acacia mangium* as biological control against *Ceratocystis manginecans*.

8.2 Capacity impacts – now and in 5 years.

A focus of the project has been on developing a sustainable research capacity within the collaborating organisations. We have worked with collaborators to develop their research skills in recent workshops (including scientific communication) and as part of project activities on an individual basis. Collaborators have been encouraged and supported to on-train others within their own and other organisations, and in the broader community. Specific training activities and capacity building tasks within the project are listed below:

- Mr Trang from VAFS has returned to Hanoi after successfully completing his PhD at UTAS, all 3 ensuing publications adding to the scientific impacts of this or a previous project. Data exists for a 4th publication which we are working on
- Aswardi Nasution has been awarded his PhD and, in the process, expanded his skills in laboratory work, field work, data analysis and critical thinking.

- Completion of David Page's PhD investigating the population genetics and dissemination strategies of *G. philippii.*
- Commencement of 1 JAF scholar (Mr Heru Indrayadi) to research host specificity and resistance to *Ceratocystis*.

Objective 1

- Training in alginate beads to produce biocontrol inoculum
- Training in contamination control in the molecular lab

Objective 2

- Hybridisation and tissue culture techniques
- Experimental design, trial assessment and analysis of taxa and progeny trials
- Germplasm transfer guidelines
- Protocols for *Ceratocystis* inoculum production
- Methodology for screening tolerance to *Ceratocystis.*

Objective 3

- In responses to the survey carried out at the end of the Biological Control workshop it would appear we had a significant capacity impact by raising the understanding of biological control across project participants, invitees from other countries in SE Asia and students studying forestry at Gadja Mada University.
- UAV workshop covering flying, data acquisition and data processing for novices attended by approximately 70 participants from all over SE Asia. The training data set was distributed to workshop participants, providing a step-by-step procedure or workflow for skill development. We have had follow-up from several asking about the next workshop and what further training can be provided.
- Field monitoring using UAV photogrammetry
- Management of a diagnostic laboratory.
- The trained are now becoming the trainers e.g. Desy Puspitasari has trained Watik in the mycology lab and Istiana Prihatini has trained Nindi in the molecular lab.

Other training activities

- A visit to Vietnam by scientists from Australia and Indonesia to learn about VAFS progress in acacia hybrid development. This visit included a day of meetings with presentations by both Indonesian and Vietnamese scientists followed by a field trip to visit trials. Visitors included Anto Rimbawanto, Inung Hidayati, Arif Nirsatmanto, Sri Sunarti and Husna Nurrohmah from CFBTI, Heru Indrayadi and Valerianus Dianto from Sinarmas, Caroline Mohammed and Morag Glen from UTas and Jeremy Brawner (USC).
- Diagnostic training for two CFBTI scientists, Istiana Prihatini and Desy Puspitasari, at the University of Florida. This training introduced everything from sample reception and documentation to morphological and molecular diagnostic techniques including quantitative PCR and DNA sequencing using the latest Nanopore technology. Istiana Prihatini has received further in-house training in qPCR since returning to Yogyakarta.
- Training of Sri Sunarti and Husna Nurrohmah on hybridisation techniques in Malaysia.
- A visit to Sarawak Forestry Corporation and Sabah Softwoods by Anto Rimbawanto, Inung Hidayati and Arif Nirsatmanto to learn about their approaches to tree breeding, resistance screening, conservation of genetic resources and alternative plantation species and to discuss potential future collaborations in these areas.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The economic impacts from research on pest and pathogen are difficult to quantify accurately because of the stochastic nature of biotic agents. Between them, Indonesia and Vietnam have about 2M ha of acacia plantations. Most of this area is susceptible to damage and death from fungal diseases (*Ganoderma* and *Ceratocystis*), and in Central Sumatra and Kalimantan which account for the greater proportion of the estate in Indonesia, the plantations are highly susceptible.

An ex-ante indication of the potential lost economic benefits can be derived from the information given below:

Country	Area of Acacia Plantation	Assumed Infection Area[1]	Reduced Productivity[2]	Stumpage Value of Healthy Plantation	Loss/Ha	Potential Economic Loss[3]
Indonesia	0.8 m Ha	0.5 m Ha	45%	$1,500/Ha[4]	$675	$337 m
Vietnam	1.2 m Ha	0.2 m Ha	25%	$2,300/Ha[5]	$575	$115 m
Total	2.0 m Ha	0.7 m Ha				$452 m

1. *Assumes 62.5% of Indonesia's acacia plantations (currently high infection spread) and 16.7% of Vietnam's acacia plantations (currently low infection spread) will be infected over next 8 years.*

2. *Average of 32-57% loss of productivity assessed (reductions from 22-35m^3/ha to15m^3/ha) in Indonesia and estimated 25% loss of productivity in Vietnam where infections are less severe.*

3. *Calculated from assumed infection areas and loss values and expressed as the total loss over one rotation (5-8 years).*

4. *Estimated stumpage value for pulpwood sold to large companies in Indonesia.*

5. *Estimated stumpage for pulpwood sold for export markets in Vietnam (from Byron, 2014 – unpublished report for ACIAR of the Acacia economy in Vietnam).*

From this ex-ante assessment, the potential economic loss from diseases of acacias in Indonesia and Vietnam is at least $56 million per year (assuming 8-year rotations). This is only the direct effects on the plantation growers - it will be multiplied through the impacts on harvesting, transport and downstream processing e.g. installed pulp mill capacity and burgeoning furniture industries in Vietnam. Failure of plantations estates will also lead to further reliance on imports or native forests to meet shortfalls in wood supply.

8.3.2 Social impacts

An outcome from the project is an improved understanding and assessment of how a regional approach (between Indonesia and Vietnam) to a shared problem such as *Ceratocystis* can facilitate more effective outcomes. We have gained greater understanding of social and institutional barriers to this type of collaboration that can be applied to future regional research.

Increased economic gains at the individual smallholder household and industrial levels will have flow-on effects to the wider community in rural areas, including development of roads, service provision, schools and other infrastructure e.g. distributed down-stream processing for sawn timber, which would see the creation of local industries and employment opportunities. The creation of wealth at the individual smallholder household level will facilitate greater gender equality and the sustained uptake of educational opportunities by women and children.

Plantation companies also provide direct funding to local communities for schools, health and infrastructure projects. In Indonesia the companies assist smallholders with community and garden farms. *Ceratocystis* is a disease which attacks trees in the horticultural sector. Safeguarding these community and garden initiatives through increased knowledge about *Ceratocystis,* its potential host range ensures that both large scale plantation companies and the smaller scale farmer plantings and community farms will continue to benefit long term.

This project specifically targets, in Indonesia and Vietnam, the professional development of young female scientists. The majority of the CFBTI scientists working on this project are female and all have received further training in relevant disciplines. Desy Puspitasari and Istiana Prihatini received training in diagnostics at the University of Florida, Desy Puspitasari and Watik were trained in alginate bead production at Sinarmas, Sri Sunarti, Husna Nurrohmah and Inung Hidayati visited both Vietnam and Malaysia to increase their knowledge of tree breeding and forest pathology. The project co-ordinator in Vietnam was female.

8.3.3 Environmental impacts

Environmental impacts have been minimal, but we anticipate that the project will provide greater wood security for the large processing mills, thus reducing pressure on the native forest resources.

8.3.4 Policy impacts

FOERDIA is promoting the development of acacia hybrid clones since there is such low levels of tolerance in *A. mangium*. FOERDIA is also discussing the need for a forest disease screening centre supported by both the public and private sectors. This centre would be proactive in quickly screening for new biotic threats

8.4 Communication and dissemination activities

- *Ceratocystis* workshop and field trip provided training in scientific background to *Ceratocystis* for 40 participants. Talks were given by 4 international experts, M. Wingfield, A. Alfenas, I. Barnes and P. Cannon as well as local experts. Training given in the identification of Ceratocystis and Ganoderma in the field. Awareness of the importance of biosecurity and threats such as *Austropuccinia psidii* was also raised.
- The Biological Control workshop was held on 21-22nd July 2017 to coincide with the IUFRO Acacia conference "Towards a sustainable future for Acacia plantations". Yogyakarta. Approximately 50 participants from all over SE Asia and from Fiji attended. Crawford funding ($15K) was obtained to support this ACIAR workshop.
- A writing workshop, targeted to project collaborators at CFBTI, was held in Yogyakarta in March 2018, run by Dr Chris Beadle. Initial drafts for six papers were written at this workshop, with writing still in progress.
- We have been training in solving contamination problems arising in molecular or mycological research carried out in the laboratory to empower others to systematically solve these issues.
- Remote Sensing workshop, July 2018 gave theoretical and hands-on training to 70 enthusiastic participants from throughout SE Asia. The preparation for this workshop has required good communications and engagement with diverse public and private sector stakeholders across SE Asia.
- Website, newsletters (internal project communications and other stakeholders)
- 3 project staff exchange visits outside of workshops (internal project communications and other stakeholders)

- The project final review was held in Yogyakarta in April 2019. ACIAR Forestry research program manager, Nora Devoe was present with two reviewers, Su See Lee and Ken Hobson. In addition to Caroline Mohammed and Morag Glen from UTas and Jeremy Brawner, currently of UFI, it was attended by eight collaborators from CFBTI, six from VAFS, three from RAPP, four from Sinarmas two from MHP and two from the University of Gadjah Mada. Simon Lawson and Madaline Healy from the University of the Sunshine Coast also attended the review and a further morning of planning for a new project.
- All presentations from the final review can be viewed at; https://www.dropbox.com/sh/p9krngurcqyx370/AADFzj-qH52j0KV-0HuionFZa?dl=0
- Following the final review of FST 2014/068, Caroline Mohammed, Morag Glen and Anto Rimbawanto attended the final review of FST 2012/091 in Vientiane, Laos. This gave an opportunity to engage with forestry, research and quarantine agencies in Laos and to discuss a potential future project.

9 Conclusions and recommendations

9.1 Conclusions

Genetic selection and breeding for tolerance remain the best options for control of Ceratocystis. The low level of tolerance in *A. mangium* means that this species is unsuitable for regions where Ceratocystis is present unless a hybrid breeding approach is adopted. The deployment of hybrid acacia clones has been adopted in Vietnam and this project has facilitated the integration of selection for disease tolerance into the breeding program. In Indonesia, this project has facilitated the selection of disease tolerant germplasm to initiate a hybrid breeding program and established a hybridising seed orchard for this purpose.

Over-reliance on a single species has resulted in large monoculture areas that facilitate disease spread and increase the potential impact of new diseases. The growth and pulping characteristics of *A. mangium* make this a very profitable plantation species if disease can be controlled. While a hybrid strategy may enable the retention of *A. mangium's* favourable characteristics in combination with the disease tolerance of another species such as *A. auriculiformis,* the adoption of a clonal forestry approach should not be at the cost of genetic diversity in planting stock.

Silvicultural approaches may also make a contribution to control of Ceratocystis, e.g. treatment of slash between rotations. Further research is needed to support a recommendation for burning of slash.

The Ceratocystis workshop brought together experts from four continents all faced with endemic or emerging disease problems caused by a range of *Ceratocystis* species. The role of insects in spreading Ceratocystis inoculum was a common factor and investigations in this project implicated a range of insect species in Indonesia and Vietnam. The ability of Ceratocystis to adapt to new host species was identified as a biosecurity threat for horticulture as well as forestry.

The BCA field trials have shown some promise in reducing root rot inoculum, though results are not statistically conclusive. There is no clarity about the best application method, though at least one industry partner is continuing this research independently. It is likely that the best application method will vary depending on site and harvesting methodology, e.g. stump application of oidia where mechanical harvesting machines are used and woodblocks applied at planting where stump application is not feasible. The ACIAR work has optimised oidia production but further development of mechanised application incorporated into harvesting machinery will require greater industry investment and may be continued in-house.

The remote sensing case study and workshop highlighted the growing interest in this area and clearly demonstrated the benefits for inventory management as well as detection of disease hot spots for better targeted health surveillance. Forestry companies in SE Asia are starting to use remote sensing operationally but the opportunity for further training in this area was embraced enthusiastically. Additional in-house skill development in this area is a high priority for the industrial plantation sector and there is potential for local service providers to work with community growers. This could enhance the early detection of invasive pests, especially as equipment becomes more sophisticated.

9.2 Recommendations

The acacia breeding program in Indonesia should focus on the creation of hybrids that combine the growth and pulping characteristics of *A. mangium* with the disease tolerance of alternative species.

Large monoculture areas should be avoided, especially where clones are deployed. Alternating species in each rotation may also help to disrupt disease cycles.

Better integration of the forestry sector with biosecurity agencies may encourage a more proactive approach to biosecurity.

The root rot biological control isolates and potential application methods developed in the ACIAR projects should be made available to small-holders.

BCA application methodologies suitable for large industrial plantations need further in-house development to obtain a protocol that suits each company, their sites and harvesting methods.

Further training in remote sensing and particularly photogrammetry and expansion of this service to accommodate the needs of small-holders for early detection of disease.

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11 Appendixes

11.1 Appendix 1:

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