From: Gordon, J. and Davis, J. (eds) 2007. Adoption of ACIAR project outputs: studies of projects completed in 2002–2003. ACIAR: Canberra.

Assessment of eucalypt rust as a pathogen of *Eucalyptus* spp. and other Myrtaceae, and development of sensitive methods for its detection in germplasm in Australia (FST/1996/206)

Ken Old

Collaborating organisations	CSIRO Forestry and Forest Products and CSIRO Plant Industry, Australia; Federal University of Vicosa (UFV), Brazil; The Forestry and Agriculture Biotechnology Institute (FABI); University of Pretoria, South Africa
Project leaders	Ken Old (CSIRO Forestry and Forest Products) July 2000 – June 2002, Dr Inez Tommerup (CSIRO Forestry and Forest Products) July 2002 – June 2003
Related projects	FST/1993/118, FST/1994/041
Principal researchers	Dr Inez Tommerup (CSIRO Forestry and Forest Products), Dr Trevor Booth (CSIRO Forestry and Forest Products), Prof. Acelino Alfenas (UFV), Dr Jeremy Burdon (CSIRO Plant Industry)
Duration of project	1 July 2000 to 30 June 2003
Total ACIAR funding	$598,373

Project objectives	■ Test the susceptibility of a comprehensive range of Australian Myrtaceae, drawn from many native genera tree and shrub species, to *P. psidii*. Particular emphasis was given to the economically important genera *Eucalyptus, Melaleuca and Syzygium* ■ Assess pathogenic and life-cycle variation in collections of the rust from various hosts through detailed cross-inoculation trials and population studies of the rust using DNA-based genetic markers ■ Prepare maps indicating eucalypt-growing areas of high, medium and low risk from *P. psidii* using climate interpolation and disease hazard mapping methods ■ Develop molecular diagnostic techniques for detection of *P. psidii* in seed, tissue-cultured plants or pollen
Location of project activities	Australia and Brazil

Overview

All plants, including trees, are subject to infection by many species of fungi, some of which cause diseases of roots, stems and leaves. In most instances the plant species that make up native vegetation have evolved in company with their major pathogens, and in undisturbed plant communities epidemic disease is unusual, although not unknown. *Eucalyptus* spp. belong to the large and diverse Myrtaceae family, and with a few exceptions are native only to Australia. Some eucalypts have proved to be very successful plantation species and are grown as exotics in many parts of the world, especially in the southern hemisphere, for pulp, paper and, increasingly, solid wood products. Genera of Myrtaceae are major components of Australia's native vegetation and occur as indigenous species and plantation crops throughout South-East Asia, where they are often grown for wood, spices, medicinal oils and fruits.

South America also has a diverse myrtaceous flora. Unlike Australia, where eucalypts have no known rust pathogen, a major disease of Myrtaceae is present in South America. *Puccinia psidii* (often called guava rust) has probably evolved on indigenous species but has somehow transferred to eucalypts and other exotic Myrtaceae. Over the latter half of the 20th century Brazil became pre-eminent in the southern hemisphere as a producer of pulp and paper based on intensively managed, very fast growing eucalypt species. Although *P. psidii* had been formally identified on eucalypts in Brazil decades earlier, the first serious outbreak in plantations occurred in the 1970s. The problem has been largely solved for the pulp and paper companies, however, through selection of clones that are highly resistant to rust.

The rust has been found to have a wide host range across many genera of Myrtaceae. It has also shown a capacity for international spread, for example to Jamaica in the 1930s, where it disrupted the allspice industry based on *Pimenta dioica*. The rust was also reported on this host in Florida in 1979 and spread to other species, including the important woody weed *Melaleuca quinquenervia*, a species native to Australia.

For many years *P. psidii* has been regarded as a threat to Australian native vegetation, and quarantine measures have been in place to reduce the chances of incursions by the pathogen. Pathologists have recognised the danger of a situation where Australian native Myrtaceae have evolved in the absence of a potentially serious and damaging pathogen. Information on the host range and biology of the fungus and possible incursion pathways was, however, limited to outcomes of research and field observations made in South America, primarily Brazil, rather than being tailored to Australia's needs. For example, there was no information on which parts of Australia and countries to our north would be at greatest risk from rust epidemics following an incursion of the pathogen. Identification of the rust in the event of an incursion into Australia would be based on spore morphology and host symptoms, whereas recent advances in molecular taxonomy have made possible the development of a rapid diagnostic test.

The project was initiated in 1999 through a request by CSIRO Forestry and Forest Products to ACIAR to support a research team from Australia, South Africa and Brazil to assess *P. psidii* as a threat to Australian plant communities and to eucalypts grown as plantations in Australia and elsewhere. A further objective was to develop a reliable DNA-based detection technique for this rust. Research was carried out in three main areas:

- The susceptibility of species of Myrtaceae native to Australasia (mostly eucalypts and *Melaleuca* spp.) and a small number of South African species to *P. psidii* was investigated. Seed samples were sent to Brazil in accordance with that country's importation regulations, and seedlings were inoculated under controlled conditions at the Federal University of Vicosa (UFV), Minas Gerais province.

The late Dr John Fryer, ACIAR Forestry Research Program Manager January 1995 to December 2004, discusses the eucalypt rust project with a graduate student in the greenhouse facilities of the Federal University of Vicosa, Brazil.

- A DNA-based 'fingerprint' was developed for the unequivocal identification of *P. psidii* and its detection at very low concentrations in plant material and as a contaminant of other substrates. This method was successfully used to detect the rust at low levels in plants and samples of pollen and seed.
- Through bioclimatic modelling, regions were identified in Australia and other parts of the world where the rust could be expected to cause severe epidemics in native vegetation and plantations of susceptible species. In Australia the areas most at risk include high rainfall areas of the east coast from the Victorian border to Cape York with some outliers in the Northern Territory.

At the time of inception of the project, this disease was known to occur only in the Americas; however, during 2005, *P. psidii* was reported in Hawaii, an indication of its capacity for international spread and the timeliness of these investigations.

Project achievements

The susceptibility or resistance to *P. psidii* of 58 species of Myrtaceae, including major plantation species of *Eucalyptus and Melaleuca* and a range of other economically and ecologically important species, was tested in Brazil. Many species were represented by several seed lots from different provenances. Reliable data obtained for more than 120 seed lots indicated a range of resistance or susceptibility to rust (Tommerup et al. 2003). Seed lots of some genera, including both rainforest trees and understorey species, proved to be difficult to germinate in sufficient numbers to provide enough seedlings for rigorous testing, but some indication of their likely susceptibility to rust was obtained.

For eucalypts, large differences in susceptibility to rust were found between and within species; for example, a higher proportion of *E. grandis* seedlings from New South Wales provenances were rust resistant than were those from Queensland. *Eucalyptus brassiana* provenances from Queensland and Papua New Guinea were consistently rust resistant, as were several species of *Corymbia*. The commercially important *Melaleuca alternifolia* and *M. cajuputi*, sources of tea-tree and cajuputi oils, were found to be highly susceptible, whereas *M. ericifolia* proved to be highly resistant to *P. psidii*. Rust susceptibility varied greatly across other genera found in Australia; for example, brush box (*Lophostemon confertus*) was highly resistant, whereas *Syncarpia glomulifera* (turpentine tree) proved to be relatively susceptible (Tommerup et al. 2003). *Heteropyxis natalensis*, native to South Africa and a non-myrtaceous species, was also found to be susceptible (Alfenas et al. 2005).

The highly sensitive polymerase chain reaction (PCR)-based detection assay developed for *P. psidii* was able to detect a single rust spore on its own or four spores on small pieces of plant tissue. The validated assay reliably detected the rust in naturally infected leaves, flowers, fruits, pollen, seeds, stems and wood, and in asymptomatic plants, as cryptic infections. A survey of 77 commercially prepared pollen and seed sources from various regions of Brazil detected rust contamination in samples form all locations using the above assay.

Earlier work had suggested that, as with many other fungi, pathogenic variation was present in rust populations. Three distinct races of *P. psidii* were identified using a standard series of test clones, confirming significant pathogenic variation. This finding has important implications for plant quarantine

and disease management in the event of an incursion by the rust, as pathogen variability makes it difficult to predict impacts on natural vegetation and plantations. In addition, the success of selection for rust-resistant clones or families of trees becomes less certain.

Disease hazard models for eucalypt rust were developed to map potentially high risk areas for *P. psidii* throughout eucalypt-growing regions of Australia, Africa and Asia (Booth et al. 2000; Glen et al. 2007b). The maps are based on climatic data from localities in South America where rust epidemics occur matched with similar climates elsewhere. In Australia the region most at risk encompasses most of the higher rainfall areas of the east coast, especially north of Sydney. These forested areas include national parks, state forests and hardwood plantations, coastal *Melaleuca* stands and tea-tree plantations.

Following the recommendation of an external reviewer of the project in 2003, an international workshop was held in Bangkok in October 2004, supported by ACIAR and FAO. The theme was 'Development of an Asia–Pacific strategy for eucalypt rust' and the event was organised through the Asia Pacific Forestry Commission. The workshop provided an opportunity to pass on the outcomes of the ACIAR/CSIRO project to a wider audience. Most of the research team made presentations, with further contributions from staff of the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), concerned with quarantine, biosecurity and plant protection. The workshop, generally judged as successful, was attended by tree health specialists from most South-East Asian countries and the Indian subcontinent.

Young *Eucalyptus grandis* heavily infected with eucalypt rust in Brazil

The difference the project has made

Unlike most projects to receive ACIAR funding, the benefits cannot be immediately identified and attributed to one or a few collaborating countries. In Brazil, the major partner, *P. psidii* has been a problem for eucalypt plantations for decades, but has largely been solved through the efforts of forest pathologists and tree breeders working with the pulp and paper companies. For Australia, South Africa and many other southern hemisphere and South-East Asian countries, future invasion of eucalypt plantations and susceptible components of native plant communities by *P. psidii* would have major and damaging economic and environmental impacts.

The ACIAR project brought together an international team of leading eucalypt pathologists to share their experience, knowledge and skills. The research outcomes have increased knowledge of the genetics and epidemiology of eucalypt rust in Brazil, and have provided tools to assist in the exclusion of *P. psidii* from parts of the world which are free from the pathogen. In addition, awareness campaigns, especially in Australia, based partly on project outcomes and conducted by research team members and the Australian Quarantine Inspection Service (AQIS), have raised the appreciation of the threat to a high level of readiness. An additional incentive has been the discovery in 2005 of *P. psidii* in Hawaii on the indigenous tree, ohia (*Metrosideros* sp.), the first authentic record of the pathogen outside the Americas.

Project findings have been communicated at many levels during and since the completion of the project. As a consequence, DAFF and Plant Health Australia (PHA), the peak NGO concerned with pest and disease impacts, have been quick to use outcomes and assist with their implementation. A National Diagnostic Standard for *P. psidii*, prepared by CSIRO, has been partially funded by PHA. Although not yet formally accepted, the standard will be incorporated into the National Plantation Timber Biosecurity Plan. The Office of the Chief Plant Protection Officer (an office within DAFF) has also been proactive in the publication of an awareness leaflet and the development of an Incursion Contingency Plan for *P. psidii*. Aspects of the plan were workshopped with a wide range of stakeholders from state and federal agencies and the plantation industry in April 2006. The plan would be activated in the event of a future incursion by the rust.

Australia's enhanced capacity to respond to the threat has already been tested through the detection by AQIS in 2005 of rust spores on a consignment of wood from Brazil. Samples provided to CSIRO for testing with the newly developed diagnostic DNA sequences proved positive for *P. psidii*. Furthermore, a proportion of the spores were still viable, presenting a real threat of incursion. AQIS responded immediately by imposing a ban on timber from Brazil and other countries harbouring the pathogen, thereby closing a potential loophole in our quarantine barrier.

Responses to the outcomes of the project have not been limited to Australia. Following information presented by the project team at an International Congress of Plant Pathology in Christchurch in 2003, the European Plant Protection Organisation has prepared a pest risk assessment (PRA) for *P. psidii*. In New Zealand there is concern that the rust may be able to infect indigenous myrtaceous plants such as pohutukawa and rata, and a PRA for *P. psidii* is being undertaken by Biosecurity New Zealand. Diagnostic laboratories have also collated the available information for use in case of a suspected incursion. In South Africa Professor Wingfield has published an awareness leaflet and web message on behalf of the South Africa Tree Protection Cooperative Programme.

Project impacts

The project outcomes have already assisted in the prevention of incursions by *P. psidii* into Australia and in the planning of feasible and cost-effective responses. The interception of eucalypt rust by AQIS in 2004 has shown the project's value in terms of rapid diagnosis and response. By keeping the pathogen out of Australia and other countries where Myrtaceae are present, very large economic and environmental benefits will accrue. Such benefits, even if limited to Australasia, are extremely difficult or impossible to quantify. They would, however, be significantly large for a pathogen with such a wide host range (Glen et al. 2007a). Impacts on other countries would vary depending on the status of Myrtaceae in native vegetation and their importance as plantation crops.

Immediate social impacts in countries where the rust is not present are limited to heightened quarantine vigilance at airports and docks, and bans on the importation of risky materials, as in the case of suspension by AQIS of all trade in commercial Myrtaceae timber from 'guava rust' countries.

Until the initiation of this project, there was little or no firsthand knowledge of *P. psidii* among pathologists in Australia. Assembly of the international team with ACIAR support has profoundly changed this situation, through assisting in the reciprocal flow of expertise from Brazil to Australia and South Africa. The CSIRO team made regular visits to Brazil and gained an in-depth knowledge of the rust, its impacts and its biology. In return, the high level of expertise of the CSIRO group in detection and diagnosis of extremely small amounts of fungal biomass, and their skills in disease hazard mapping, have been transferred to Professor Alfenas's team at UFV.

A young UFV postgraduate student, Edival Zauza, was given major responsibility for the research carried out in Brazil. This was a significant career opportunity for him, achieving co-authorship, so far, on five research articles and a UFV–supported visit to Bangkok via Portugal to participate in the FAO/ACIAR-sponsored workshop.

Following the return of Dr Langrell to Europe, project funds were used to support a Murdoch University postgraduate student, Dr Morag Glen, who has since moved to Dr Mohammed's lab in Hobart, now a part of Ensis. Dr Glen has worked on the National Diagnostic Standard and the Incursion Contingency Plan, and has recently published a comprehensive review of *P. psidii* (Glen et al. 2007a).

The scientific impacts of the project have been considerable. Prior to 1998, research on *P. psidii* was largely restricted to scientists based in Brazil, with papers often published in Portuguese and not readily accessible to Australian scientists. The ACIAR project was the first to direct sufficient funds and scientific expertise to carry out key research in Brazil aimed primarily at *P. psidii* as an exotic threat to Australia and other countries where Myrtaceae are grown. Through the research and linkages established within the team, Australian, Brazilian and South African scientists who participated in the project have published a series of journal articles, with additional papers currently being submitted to prestigious international journals. Other researchers have picked up on gaps in the research; for example, a detailed study has recently been published on the taxonomy of rusts of Myrtaceae by research staff of Biosecurity Australia (Simpson et al. 2006).

The science of DNA-based detection and diagnosis of plant pathogens in germplasm or small amounts of host tissue, or as spores, has been advanced to a high level and applied to several tree pathogens in addition to *P. psidii*, for example leaf blotch of eucalypts caused by *Mycosphaerella* spp. (Glen et al. 2007a). Similar techniques are being used by the FABI group to determine the phylogenetic position of *P. psidii* within the Uredinales.

Of particular importance is the demonstration that rust spores are commonly present within seed and pollen samples. There is an international trade in such materials as plantation companies worldwide seek to improve their planting stock to increase growth rates and improve wood quality. Although risks from pollen had been suggested previously, this is the first unequivocal evidence with regard to this form of eucalypt germplasm.

It could be argued that increasing global movement of passengers and commodities and the widespread cultivation of Australian native species, especially eucalypts, as exotic plantations will inevitably result in future incursions of *P. psidii* into Australia. This project has provided knowledge and tools to assist in preventing such an occurrence, as well as information on those species, plant communities and geographic areas most at risk from rust epidemics. There is also an enhanced state of preparedness in organisations and agencies with responsibility for maintaining the health and ecological sustainability of Australian forests and plantations of susceptible species.

References

Alfenas, A.C., Zauza, E.A.V., Wingfield, M.J., Roux, J. and Glen, M. 2005. *Heteropyxis natalensis*. Australasian Plant Pathology 34, 285–286.

Booth, T.H., Old, K.M. and Jovanovic, T. 2000. A preliminary assessment of high risk areas for *Puccinia psidii* (*Eucalyptus* rust) in the Neotropics and Australia. Agriculture, Ecosystems and Environment 82, 295–301.

Glen, M., Alfenas, A.C., Zauza, E.A.V., Wingfield, M J. and Mohammed, C. 2007a. *Puccinia psidii*, a threat to Australia's environment and economy: a review. Australasian Plant Pathology 36, 1–16.

Glen, M., Smith, A.H., Langrell, S.R.H. and Mohammed, C.L. 2007b. Development of nested polymerase chain reaction detection of *Mycosphaerella* spp. and its application to the study of leaf disease in *Eucalyptus* plantations. Phytopathology 97, 132–144.

Simpson, J.A., Thomas, K. and Grgurinovic, C.A. 2006. Uredinales pathogenic on species of Myrtaceae. Australasian Plant Pathology 35, 549–562.

Tommerup, I.C., Alfenas, A.C. and Old, K.M. 2003. Guava rust in Brazil—a threat to *Eucalyptus* and other Myrtaceae. New Zealand Journal of Forestry Science 33, 420–428.