



# Tree Protection News



Newsletter of the Tree Protection Co-operative Programme and  
the DST/NRF Centre of Excellence in Tree Health Biotechnology.

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## FROM THE DIRECTOR'S DESK

The Tree Protection Co-operative Programme (TPCP) is rapidly moving towards the end of its 17th year of operation. In this period, the Programme has changed gradually to accommodate the needs of its members. In many ways, it has matured. First a small initiative, focused on a single new disease, important to the emerging Eucalyptus clonal programme (Chrysoporthe canker), and now an established applied research entity, providing various services and solutions to pest and disease problems.

Dealing with disease and pest problems has been an important element, ever since the onset of plantation forestry in South Africa more than 100 years ago. I have made the point in the past that these agents have shaped the nature of forestry in this country. Forestry practices and the species that have been chosen for planting have in many cases emerged from efforts to avoid pest and disease problems. Yet, I believe that it is true to say that we have been quite fortunate in not having had to face the onslaught of some of the most devastating problems that have challenged forestry in other parts of the world. This is a situation that was bound to change, with increasing global trade moving pests and pathogens around the world. Certainly, I don't think that there would be any argument against the statement that disease and pest pressures on forestry have increased dramatically in recent years.

While I choose to be optimistic and prefer not to be the bearer of bad tidings, it seems likely that we will face increasing pressures due to pest and disease problems in the future. The more important question must be how we should approach this apparent threat. This is clearly a battle that we cannot afford to lose. My view is that it will require a greater team effort and closer alignment between the TPCP research team and field activities of its members. This is a process that has been emerging during

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the course of the last seven years, starting with much closer operational ties between the TPCP and the ICFR. Here we can also consider the emergence of the Fusarium working group and the Sirex Steering Committee, both residing outside the TPCP. Some members are already establishing programmes within company, to deal with the applied side of pest and disease management issues, and this direction seems likely to grow in the future. It is logical that applied pest and disease management must be led from the operational business. The role of the TPCP must be to provide research solutions, especially those that are of a somewhat longer term nature. Diagnostics and support of company monitoring efforts should also logically remain an important role for the TPCP.

To effectively deal with serious pest and disease incursions, huge financial investments will be needed. These will most likely be substantial beyond the realms of reasonable investment by companies. Thus, I believe strongly that Government will need to provide significant support for protection research. Here, it is logical to argue that the Government has the responsibility to enable industry to be effective. I am not implying that it is Government's role to subsidize private enterprise, but rather to support business, in the interests of increasing GDP and providing employment and opportunities for all South Africans. In this regard, I have attempted to respond to requests for contributions to the Forestry Charter that is in the formative stages of development. Here we need to especially acknowledge Mike Edwards of FSA for ensuring that there is a clearly articulated requirement for support for forest protection, in the draft Charter. We must now also ensure that this document gains the support it deserves and that it is eventually approved. I am convinced that the future of forestry in South Africa will rely on it many times in the future.

Working from the base of an academic environment has reinforced in my mind the rapidly emerging shortage of highly skilled and effective researchers in South Africa. As member of appointment committees for various organizations, I have come to realize that one often times cannot find suitable candidates to fill positions. This I fear is a problem that is going to increase in the future and it is one that needs urgent attention. From the TPCP perspective, one of the defining indicators of success is a reasonable output of highly skilled graduates in the field of forest protection. Here we have been very

effective and I was especially delighted to learn that the programme had been awarded the annual prize for Human Capital Development by the Government supported THRIP programme. This is a feather in the cap of the members of the TPCP as much as it is an award that has come to the research team. My hope is that we will be able to initiate and encourage similar processes, in other areas of science, important to forestry.

The TPCP year is rapidly drawing to a close, although I must also add that the programme never stops completely. This has also been a year with many challenges, some of which have not necessarily been simple to deal with. The Mega Sirex biocontrol experiment, undertaken in an amazing team effort between TPCP and forestry groups, has certainly been a source of considerable pressure for all involved. We now wait with interest to see the results, which hopefully will help us to understand this perplexing problem more clearly. Then we have had to face a first field outbreak of pitch canker, growing infestations of *Thaumastocoris* and a suite of other important problems. When I look back 17 years, I am amazed at how much the TPCP activities have changed during this period. Yet change is normal and change drives us to do more and to do it better.

Thinking of the Christmas period, just two months away, I realise also that it is time to thank members of the TPCP, the staff of the member companies, for the fantastic support that we have had during the course of this year. Given some of the factors mentioned above, this has been a year where huge team effort has been required on various fronts. And these various teams have operated most effectively, getting the job done, but also leading to close friendships between foresters, forest managers and TPCP team members. Students have had many opportunities to engage with operational forestry activities in the plantations and many often forgotten benefits have emerged from these liaisons. On behalf of the entire TPCP team, the management including Teresa Coutinho, Brett Hurley, Jolanda Roux, Bernard Slippers, Emma Steenkamp, Martin Coetzee and Brenda Wingfield, as well as the technical staff and the students of the TPCP research team, I thank all members of the Programme for their friendship and support. We wish you a blessed festive season and hope that 2007, the eighteenth birthday of the TPCP will be a great year for you.

# CONGRATULATIONS!!!

The TPCP and CTHB would like to congratulate a number of current students who obtained their degrees in 2006.

## PhD

Marieka Gryzenhout: Taxonomy and phylogeny of *Cryphonectria* and allied genera.

Mesfin Bogale: Molecular characterization of *Fusarium* isolates from Ethiopia.

Grace Nakabonge: A study of *Chrysosporthe* and *Cryphonectria* species on Myrtales in Southern and Eastern Africa.

## MSc

Almuth Hammerbacher: The biology and epidemiology of *Fusarium circinatum*.

Brett Hurley: Fungus gnats in forestry nurseries and their possible role as vectors of *Fusarium circinatum*.

## BSc Honours

Bianca Hinze: Identification of *Botryosphaeria* spp. occurring on *Schizolobium parahybum* in South Africa.

Chrizzelle Beukes: Determining the biodiversity of protists found in freshwater sediment.

## BSc

Marcele Vermeulen  
Nicolette Fouche

The year has also been very productive in a number of other fields. Amongst these, several TPCP/CTHB members become proud parents in 2006. We wish to congratulate all

of you with the new family members and wish you lots of happiness (and sleep).

**Dina Paciura** (MSc student) gave birth to Daniel David on the 27<sup>th</sup> of February.

**Marieka Gryzenhout** (post doctoral fellow) gave birth to Sietske on the 3<sup>rd</sup> of April.

**Tania Hurley** (wife of Sirex programme leader, **Brett Hurley**) gave birth to Joshua David on the 8<sup>th</sup> of August.

**Renschia Marais** (wife of CTHB/CAMS team leader, **Gert Marais**) gave birth to Amerissa on the 13<sup>th</sup> of August 2006.

**Julia Kreiss** (wife of post doctoral fellow, **Wolfgang Maier**), gave birth to Noë Kreiss on the 30<sup>th</sup> of August.



Three proud TPCP/CTHB dads and their young ones. FLTR: Brett Hurley, Gert Marais and Wolfgang Maier with Joshua, Amerissa and Noë.

## Welcome to the TPCP/CTHB



Kershney Naidoo joined FABI in the second half of 2006 to conduct her PhD studies under the supervision of Mike and Brenda Wingfield. Kershney was born and raised in Pietermaritzburg and completed all her previous degrees at the University of Natal, now known as the University of KwaZulu-Natal. She holds a BSc majoring in Genetics, a BSc Honours degree in Genetics and obtained her MSc working on bacterial antibiotic resistance and protein-protein interactions. Kershney's PhD will be focused on the complete characterization of the mating type genes of the important *Acacia mearnsii* wilt pathogen, *Ceratocystis albifundus*.

## TPCP/CTHB Annual research meeting

8 & 9 May 2007 at FABI in Pretoria

This annual meeting presents a forum at which TPCP/CTHB researchers and students present feedback on some of the research projects of the programmes. Yearly, international speakers are also invited to present research on a topic relevant to the TPCP/CTHB. It is also an important venue at which researchers and foresters can interact and discuss tree health issues and future projects. If you are interested in attending this meeting, please contact the research director/co-ordinator of your company.

# International Congress on Plant Pathogenic Bacteria

The International Congress on Plant Pathogenic Bacteria was held at the Royal College of Physicians of Edinburgh in Scotland from the 10-14th July 2006. The Congress was attended by Teresa Coutinho, her collaborator at the University of Pretoria, Professor Fanus Venter, and two of their PhD students. They were Pieter de Maayer, and Carrie Brady, both conducting research on bacterial blight and die-back of *Eucalyptus*. The TPCP/CTHB team made various contributions to the meeting.

These included one poster presentation:

De Maayer P, Venter SN & Coutinho TA (2006)  
The virulence factors of *Pantoea ananatis*.

and two paper presentations:

Brady CL, Venter SN, Cleenwerck I, Vancanneyt M, Swings J & Coutinho TA (2006)  
Examining the taxonomy of the genus *Pantoea*.

Coutinho TA, Goszczynska T, de Maayer P & Venter SN (2006) *Pantoea ananatis*: an emerging pathogen?

The congress was attended by 160 scientists from various parts of the world. The majority of presentations were by established researchers i.e. few presentations by postgraduate students. The congress covered the following topics: diagnostics and taxonomy, functional genomics and systems biology, emerging pathogens and diseases and finally molecular plant pathogen interactions.

Two keynote presentations took place each day by scientists publishing in highly rated journals. These included, for example, Alan Collmer from Cornell University, USA, who spoke on "Functional genomics of *Pseudomonas syringae* virulence: from hope to gene ontology". The head of the NCPPB (National Collection of Plant Pathogenic Bacteria) presented a keynote address on "Classification and identification of plant pathogenic bacteria – a review". In bacterial nomenclature changes have been dramatic and drastic, for example, the genus *Erwinia* was divided into six species. Accepting these changes has not been easy for plant pathologists (non-taxonomists) who feel that taxonomists are messing with a system that has previously worked,

so why change.... The whole pathovar system was also heavily debated. A pathovar is a pathogenic variety of a species and is based solely on whether or not the microbe is able to cause disease in a particular host.

We met many scientists who wish to have *Pantoea* strains identified, including the authors who described *P. agglomerans* pv. *betae* and *gypsophila*. Our collection of *P. ananatis* and strains of other *Pantoea* spp. in FABI is also growing with offers of new strains from countries such as Mexico and Denmark. Of interest to the TPCP is a researcher from INRA in France who is conducting research on the ecology of *Pseudomonas syringae* and who is interested in testing the ice nucleating ability of *P. ananatis* strains. Ice nucleating strains are those that cause frost damage to plants at higher temperatures than what would normally occur if the pathogen was absent. The results should be interesting to *Eucalyptus* growers.



The Congress in Edinburgh was a very useful experience for both the students and staff who attended the meeting. New techniques were acquired and our network of collaborators expanded considerably. The next Congress of Plant Pathogenic Bacteria will be held in Reunion in 2010 and Teresa Coutinho has been elected to the organizing committee.

# FABI Wins a THRIP Excellence Award

At a function held on 5 October 2006 at the Midrand Conference Centre, the 2005/2006 THRIP Annual Report was launched. Dr Willem Barnard, Chairman of the THRIP Board, said that THRIP is unique in its ability to stimulate the kind of collaboration needed to create an integrated science system in South Africa. One measures the real value of public investment in scientific research through the academics who conduct it, the investors who pay for it and the individuals who benefit from it.

In recognising the efforts of the project leaders, students and industry partners, the THRIP Excellence Awards winners were announced at this function. Each winner was presented with a trophy designed by a student from the Central University of Technology, who had gained experience in rapid prototyping and design while working on one of the THRIP projects.

Nine award winners were announced for the following categories:

- 1. Human Resource Development**
  - Quality and quantity of students
  - Best black or female student
- 2. Research collaboration**
- 3. Competitiveness of the industry partner**
- 4. Technology**
  - Advanced hi-technology
  - Social development
- 5. SMME development**
- 6. Outstanding black researcher/project leader**
- 7. Best exhibition**

It was with great pride that we heard that the Tree Protection Co-operative Programme team at FABI were the winners of the "Quality and Quantity of Students" sub-category of the Human Resource Development Category. Their project title, in partnership with the South African forestry industry is "Sustainable fibre production/enhanced wood quality". The criteria for judging the winner in this category is based on Project Leaders who have trained a high number of students and have a high number of graduates. The award was presented by The Minister of Trade and Industry, the honourable Mandisi Mphahlele and received by Prof. Brenda Wingfield and Dr. Bernard Slippers of the TPCP team.

This award to the TPCP Team recognises not only the students trained, but the contribution that the TPCP members, most forestry companies in South Africa, are making to human capital development in the country. While world-class research on tree health issues is being conducted, and member companies are receiving services and advice linked to their financial contributions, the human resource development component of THRIP is often overlooked. In all ways, this award comes to the South African Forestry Industry, as much as it does to the TPCP research group.

Congratulations and thanks to the South African Forestry Industry members of the TPCP and to the whole TPCP/THRIP team at FABI on yet another prize for excellence in science and service to South Africa.

## First Outbreak of Pitch Canker on Mature Pines in South Africa

*Fusarium circinatum*, the causal agent of pine pitch canker disease in the USA, was first reported in South Africa in 1990 on *Pinus patula* seedlings in a nursery. The pathogen subsequently spread throughout South African pine nurseries causing a serious root and collar rot disease of various *Pinus* spp. The stem canker disease on plantation trees, that is typical of pitch canker in the USA, has never been observed in South Africa. However, the nursery disease caused by *F. circinatum* has led to thousands of rands of losses to forestry companies and private nurseries in the country. It also affects plantation establishment as infected, but seemingly healthy plants, die shortly after transplanting. A few cases have also been observed where 3-4 year-old *P. patula* trees have died in the field as a result of *F. circinatum* infection. Furthermore, naturally regenerated *Pinus* seedlings in compartments have been found to become infected with the fungus as it is spread from infected transplanted trees. The nursery infections, problems with establishment, and spread into the field have made *F. circinatum* one of the most serious and important constraints to the establishment of especially *P. patula* in South Africa.

As predicted by a number of experts in the field, the first outbreak of pitch canker has recently been identified in South Africa. This outbreak was on five and nine-year-old *P. radiata* trees in the Western Cape, near Cape Town. Symptoms observed are typical of pitch canker disease as seen in the USA. Branches on trees start dying, creating "red flags" in the crowns of the trees. This is associated with stem cankers exuding large amounts of resin which runs down the stems of the trees. Chopping into the wood reveals resin/pitch soaked wood. Other than the stem cankers, dying trees were infested with the weevil, *Pissodes nemorensis*. Researchers from the TPCP made isolations from infected tissue, weevil galleries and from adult insects. *Fusarium circinatum* was consistently isolated from both pine tissue and insects. The fungus was characterized based on morphological characteristics and using DNA sequence comparisons.

This is the first outbreak of pitch canker on plantation trees in South Africa. The fungus can thus no longer be considered only as a nursery pathogen in the country. It now seriously threatens the future of plantation forestry in South Africa. Any management strategies should be based on the selection and breeding of disease tolerant trees, backed up with good site and silvicultural management to reduce the impact of the disease.

# North American Forest Insect Workshop Conference

Brett Hurley of FABI was invited to speak at the North American Forest Insect Workshop Conference (NAFIWC), which was held from 22-25 May this year in Asheville, North Carolina, USA. The conference focused specifically on forest entomology issues relevant to North America, drawing people primarily from USA, Canada and Mexico, but also from other countries. Brett was asked to present on the current situation of the *Sirex* woodwasp in South Africa. *Sirex noctilio* has recently become established in the USA and Canada and is of serious concern to these countries.

The conference was very well attended with over 350 delegates present. The delegates were mainly from the USDA Forest Service, the Canadian Forest Service, State and Provincial forestry organizations, universities, NGOs and industry. A diverse range of topics were covered, with the six main topics being invasive insects, management and silviculture, scale and interactions, change, direct tactics in forest insect management, and biodiversity and natural heritage. There was also a discussion session specifically dealing with the new introduction of *Sirex noctilio* in North America, where strategies to deal with this new invasive pest were discussed. The conference provided a great opportunity to build collaborative research relationships with people working in forest health issues and to learn from the various approaches used to address forest entomology issues. Brett and the TPCP team are most grateful to the USDA for providing funding for this visit and thus to enable the team (as well as the South African forestry community) to expand its experience in dealing with *Sirex* and other alien invasive forestry pests.

## ***Symbiosis between *Sirex noctilio*, *Amylostereum areolatum* and *Deladenus siricidicola*: A South African perspective.***

B.P. Hurley, B. Slippers and M.J. Wingfield  
Forestry and Agricultural Biotechnology Institute (FABI),  
University of Pretoria

*Sirex noctilio* Fabricus was first detected in South Africa in 1994, in the Western Cape. It has since spread to the Eastern Cape and KwaZulu-Natal provinces. The parasitic nematode, *Deladenus siricidicola*, has been released in all three provinces, but with very limited success. Despite the absence of well established biological control agents in South Africa, *S. noctilio* populations remain low in the Western Cape, in contrast to rapidly increasing populations in the Eastern Cape and KwaZulu-Natal. In these two provinces *S. noctilio* causes significant damage to *Pinus* plantations, with some sites having over 50 % mortality. The interactions between *S. noctilio*, its fungal symbiont (*Amylostereum areolatum*), its biocontrol agent (*D. siricidicola*), and its host (*Pinus*), was examined in an effort to understand the differences between provinces and the reason for the low success with *D. siricidicola*. Differences in silvicultural practices between provinces, such as thinning, are likely to be the main factor responsible for differences in *S. noctilio* populations. Moisture content of the tree at time of inoculation, loss of virulence of *D. siricidicola* and incompatibility between *S. noctilio* and *D. siricidicola* strains, or between *D. siricidicola* and *A. areolatum* strains, are important factors that can contribute to the success of *D. siricidicola* as a biocontrol agent. A better understanding of these factors is needed to ensure sustainable control of *S. noctilio* in South Africa and elsewhere.

## TREEHEALTHNET

*An online forum where  
tree health issues are discussed*

About two years ago, the TREEHEALTHNET listserver was established to enable fast and effective communication between scientific and technical staff linked to the Tree Protection Co-operative Programme (TPCP), the DST/ NRF Centre of Excellence in Tree Health Biotechnology (CTHB), and staff of the TPCP member-companies. News related to tree health, feedback about current research efforts and field work, as well as important announcements are circulated among subscribers to this listserver. Field trips are announced in advance, enabling foresters, forest managers and other stakeholders to request visits from the TPCP and CTHB researchers.

At present the TREEHEALTHNET listserver reaches about 120 subscribers, mainly staff of South African forestry companies. Most are decision-makers or research staff of companies. Given the increasing pressure on forestry and forests due to pests and diseases, we would all benefit from the listserver reaching a broader community, including forestry staff at grassroots level. If you wish to subscribe or you wish to have your staff/ colleagues participating, just send an e-mail to the list manager, Wilhelm de Beer, at wilhelm.debeer@fab.up.ac.za. The listserver is SPAM secure and we will make every effort not to overburden subscribers. It is also open only to those who have subscribed and it is possible to unsubscribe as easily as one subscribes to the list.

# Ophiostomatoid fungi: expanding frontiers

*A symposium on North Stradbroke Island, Brisbane, Australia*

*16-18 August 2006*

The ophiostomatoid fungi are a group of fungi that include important tree pathogens, bluestain fungi, and bark-beetle associates that are of huge quarantine importance for the global wood and timber trade. In 1990 a symposium was organized in Bad Windsheim, Germany, where almost all the researchers working on these fungi gathered from all over the world to share their experience and discuss future focus areas for research. The result of the 1990 meeting was a book that has served as major reference work for the research community, the forestry industry and quarantine authorities. The face of research in this field changed rapidly, partly because of the foundation laid at the 1990 meeting, but also as a result of the rapid advance of DNA based techniques during the past decade. The time was ripe for consolidating current knowledge, and to contemplate the direction of future research in this field.

The TPCP has the largest number of people in any single research institute in the world working in this field, at present 18, and we are collaborating with almost every other group. For this reason the initiative to organize another 'Ophiostomatoid fungi' meeting came from FABI. Under the leadership of Mike Wingfield and Keith Seifert (Canada), the meeting was organized as a satellite meeting of the 8<sup>th</sup> International Mycology Congress (IMC) held in Cairns during August 2006.

The Ophiostomatoid meeting took place on North Stradbroke Island, just off the coast of Brisbane. Forty delegates attended, representing South Africa (11), USA (7), Canada (6), Australia (3), Spain (2), UK (2), Norway (2), New Zealand (2), and one each from China, Japan, Colombia, Finland and Austria. Thirty-five oral presentations and 10 poster presentations were delivered over a 3 day period, covering the taxonomy, phylogeny, genetics, control, population biology, insect and mite associations, and global distribution of these fungi.

Delegates agreed that this was one of the best academic meetings they have ever attended, because there

was such a high degree of focus. The outcome of this meeting will be another multi-authored book, currently in preparation, that will cover all aspects related to these important fungi. Apart from the academic discussions, many new friendships and future collaborations were established. Certainly a highlight in all of the attendees' careers.

For more information and images, visit the meeting website at:  
<http://www.fabinet.up.ac.za/ophiostoma/index>



FlTR: Keith Seifert (Canada) and Mike Wingfield (South Africa), the organizers of the Ophiostomatoid meeting in Australia

In July this year, two members of the TPCP programme, Prof. Jolanda Roux and Ronald Heath, attended the International Union of Forestry Research Organizations (IUFRO) meeting focusing on alien invasive species and international trade. This meeting was hosted by unit S7.03.12 of IUFRO and held at the Ecological Education and European Integration Centre of State Forests in Jedlnia, Poland from the 3<sup>rd</sup> to the 7<sup>th</sup> July 2006. The meeting was attended by 62 delegates representing 17 countries, including three delegates from Africa. This IUFRO working group was only established in 2005, focusing on global forestry issues related to the unwanted international movement of alien invasive species, including fungi, insects, nematodes, and plants. Focus topics of the meeting were: (1) The process of invasiveness and (2) nursery stock as a pathway for invasions. Under the topic of "The process of invasiveness" individual topics arose such as predicting invasiveness, ecological factors, interactions with the forest landscape and genetic considerations.

The meeting was divided into four sessions and a day of field visits. The sessions were:  
Session I – Alien invasive species in Europe  
Session II – World ecological impact  
Session III – Detection and policy  
Session IV – Prevention measures and treatments

Both talks presented by members of the TPCP formed part of Session II and focused on the situation in Africa. Jolanda gave a talk entitled "Invasive pathogens and pests: an African perspective", discussing the problems Southern Africa has

experienced due to the introduction of non-native pests and pathogens onto the continent. Ronald talked about "*Ceratocystis albifundus*, an African fungus threatening flora internationally", which highlighted the threat of African fungi to international flora. Other TPCP projects were represented by posters. One by Slippers *et al.* considered the interactions between woodwasps, their symbiotic fungi and biological control agents and its implications for the control of *Sirex*. Another poster by Wright *et al.*, entitled "Pine Pitch canker – the South African situation", discussed some new developments regarding another well-known disease problem in South African forestry.

Of particular interest to the TPCP group was a session on ISPM15, a new regulation for the movement of wooden packaging material such as pallets and crates. Specific talks in this session dealt with whether insects infest wood packaging material after heat-treatment, and what effect left over bark on the packaging material has on the survival of bark and wood boring insects. A number of talks on this topic presented figures from studies indicating the amount of introductions, how many of these introductions established and how many stay unidentified.

A major concern discussed at the meeting was the lack of detailed and correct information on insect and fungal pest invasions into countries. Several talks thus dealt with new projects, such as DAISIE and FORTREATS, which aim to collate this information for specific regions.

### ***ISPM No.15 "Guidelines for Regulating Wood Packaging Material in International Trade"***

***International standards for Phytosanitary Measures drawn up by the International Plant Protection Convention (IPPC) (<https://www.ippc.int/IPPC/En/default.jsp>) to reduce the risk of introduction and/or spread of pests associated with wood. ISPM15 deals specifically with unprocessed timber products and impacts on forestry companies. ISPM15 and other guidelines can be downloaded from the IPPC website and also from the website of the South African National Plant Protection Organization (<http://www.nda.agric.za/docs/npposa/wood.htm>). The South African plant protection organization has also drawn up specific guidelines for South Africa which can be downloaded from their website.***



# Annual TPCP/CTHB Research Meeting

The annual meeting of the Tree Protection Cooperative Programme (TPCP) and the DST/ NRF Centre of Excellence in Tree Health Biotechnology (CTHB) was held at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria on the 9<sup>th</sup> and 10<sup>th</sup> of May this year. During this meeting, the research progress of the TPCP and CTHB was presented to the various stakeholders and attendees, including forest managers, field foresters and forestry researchers. As in the past, this provided a unique opportunity for interaction and discussion of forestry pest and disease problems between forestry colleagues and TPCP / CTHB students and staff. Scientists from abroad with whom the TPCP / CTHB collaborates were also invited to give the audience a global perspective of forestry pest and disease problems. This year the visiting scientists included: Dr. Diana Six (University of Montana, USA), Dr. Kier Klepzig (USDA, Southern Research Station, Louisiana, USA) and Dr. Rich Hoffstetter (Northern Arizona University, USA), who have great experience in insect-fungal interactions; Dr. Paula Klasmer (INTA, Argentina), who works specifically on Sirex; and Mr. DB Chetri who works in pathology and entomology in the Kingdom of Bhutan.

After the TPCP meeting, the visiting scientists were taken to various field sites to give them a better understanding of the pests and diseases in South African plantations and activities of the TPCP and CTHB. The last field stop in this field trip was to the

Sirex-infested pine plantations in the Bulwer-Underberg area. This was of particular interest to the USA scientists, as the Sirex woodwasp has only recently been introduced into that country. The USA is in the process of developing a control strategy and comparisons between the USA and South African situations were especially useful. The field visits gave rise to various discussions, ideas and even future collaborative projects, all which will add to a better understanding and management of forestry pests and diseases in South Africa.



***Dr. Paula Klasmer explains the procedures used in Argentina to inoculate Sirex-infested trees with the parasitic nematode, *Deladenus siricidicola*. From left: DB Chetri (Bhutan), Stuart Charlton (Singisi Forest Products Pty Ltd, Hans Merensky), Philip Croft (Mondi Shanduka), Prof. Jolanda Roux (FABI), Dr. Diana Six (USA), Dr. Bernard Slippers (FABI), Dr. Paula Klasmer (Argentina), Marcel Verleur (Sappi).***

## IMPORTANT VISITORS TO FABI

Tuesday 25 April 2006 was a red letter day for the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) at FABI, University of Pretoria, when the Deputy Minister of Science and Technology, the Honourable Derek Hanekom, Mr Joseph Tshikomba, Deputy-Director: Human Capital at DST and Ms Heibre Roos, also of DST, paid a visit to FABI.

Staff and students of the CTHB and the Tree Health Biotechnology Programme (TPCP) welcomed the guests to tea in the FABI courtyard. Prof Mike Wingfield introduced the Minister and his colleagues to the students and staff of the CTHB/TPCP. An informal tea followed allowing the members of DST to have some time to meet the students in the programme. This was followed by a smaller meeting of the CTHB Project leaders, Prof Wingfield and the DST guests. Prof Wingfield presented a short talk about the research being done at FABI and, in particular, the challenges and contribution the CTHB had made over the two years since its inception. Mr Hanekom and Mr Tshikomba asked very pertinent and in-depth questions about these research areas and were later taken on a tour of the FABI laboratories and other facilities. During the tour there were a number of opportunities for the DST guests to discuss projects with the students in the laboratories and observe the workings of the state-of-the-art facilities and equipment in FABI.

It was a great pleasure and privilege to have Mr Hanekom and colleagues visit FABI and Prof Wingfield issued an open invitation to the Deputy Minister and his colleagues to visit FABI again in the future.



***Bianca Hinze shows some of her work to Deputy Minister Hanekom and visitors.***

## Progress in understanding and handling of the *Sirex* biocontrol agent, *Deladenus siricidicola*

Various nematode species of the genus *Deladenus* (= *Beddingia*) parasitise siricid woodwasps. One species, namely *D. siricidicola*, proved to be an effective biological control agent of *Sirex noctilio* in Australasia, parts of South America and the Cape Province of South Africa. The nematode sterilizes the female wasps, but without significantly impairing their natural fitness and oviposition behavior. This facilitates the spread of the nematode to other trees possibly containing still uninfected wasps.

*Deladenus siricidicola* has not been successful in all areas where it has been deployed, including some areas of South America and Kwazulu-Natal in South Africa. After low levels of success in 2004 in Kwazulu-Natal, increased and detailed attention was given to the handling and deployment of the nematodes during 2005, which was a logical first step. The results were still very disappointing (less than 10% parasitism, when above 95% has been achieved elsewhere). To further refine the focus on techniques, but also to test potential biological barriers to inoculation and ways to improve it, a trial was initiated by FABI via the *Sirex* Steering Committee. This trial was seen as one of the central research foci for 2006 regarding *Sirex* control.

Some of the main aims of the trial are to test the influence of the following variables.

1. Nematode source. The sources included are (a) the strain used thus far in FABI, (b) directly from Australia, (c) isolated from Kwazulu-Natal wasps, and (d) isolated from Cape wasps. This would identify potential problems with loss of virulence of strains currently in use, as well as different routes of nematode supplies. It would also give a chance of identifying strains that have been 'positively selected' in the environment, and have passed through a recent parasitic cycle.
2. Moisture content. Nematodes need a certain moisture threshold in the tree to survive. Because of the lack of rain during the winter season in Kwazulu-Natal and consequent drying out of inoculated trees, low tree moisture content may be a major contribution to the lack of success in this province. Its monitoring and correlation with actual parasitism rates are thus crucial.
3. Position of the tree. Trends in 2005 inoculations suggested that the bottom part of the log had a higher percentage infected wasp females compared to the top of the tree.
4. Standing trees. In an attempt to preserve moisture and because the bottom part of the log appeared to have higher parasitism rates in 2005, inoculation of standing trees was investigated as an alternative technique.

The trial has been refined and established with significant inputs from various industry members, particularly those associated with the *Sirex* Technical Committee. The trial has

been conducted at two sites, both managed by Mondi Shanduka. Phillip Croft, the new *Sirex* operations coordinator, has been one of the main drivers of the inoculations, readings, co-ordination of tree felling and collection, and setting up a very large emergence facility in PMB. In total, 504 trees were inoculated. These are divided into standing and felled lots, with the trees further sub-sampled into bottom, middle and top sections. This correlates to a total of 1512 logs, in 504 drums from which tens of thousands of wasps are expected to emerge. These wasps will be sent to FABI where they will be dissected and microscopically examined for parasitized eggs, as well as other measurements. The wasps are expected to emerge from about October to January, after which the results will be analyzed to determine factors which influence parasitism success.

The rearing and storing of the nematode has been largely based on techniques developed in Australia. FABI has had to experiment with various modifications in these techniques in an attempt to increase their efficiency under our conditions. Two important aspects where we have had recent success are with the long term storage of the nematode and testing its ability to develop a parasitic phase. Long term storage is done in liquid nitrogen (-196 degrees C) in a glycerol medium. This stores the nematode in an inactive state, from which they can be rejuvenated when needed for mass rearing. The alternative would be to continuously rear them on the fungus (*Amylostereum areolatum*), which is known to lead to a loss of virulence over time.

After initial problems, Hardus, Brett and the team have also optimized the so-called 'virulence test' for *D. siricidicola* for our conditions. This common description of the test is confusing. The test aims to reproduce the conditions the nematode experiences in the tree when in the vicinity of a *Sirex* larva, namely low pH and high CO<sub>2</sub>, which stimulates the production of the parasitic phase of the nematode. The test, however, is an artificial reproduction of the natural situation and it is not clear how specific 'values' from the test might correlate to actual 'virulence' in the field. It is thus more a 'conversion test', rather than a 'virulence test'. This 'conversion test' is nevertheless important. If the nematode has lost the ability to change to the parasitic phase it can not infect *Sirex* larvae. The test might thus in future be useful as a direct comparison between strains to help point to potentially defective strains (i.e. be used as an early screening or quality control measure). More work is now needed to make a connection between the lab based results and 'virulence' in the field.

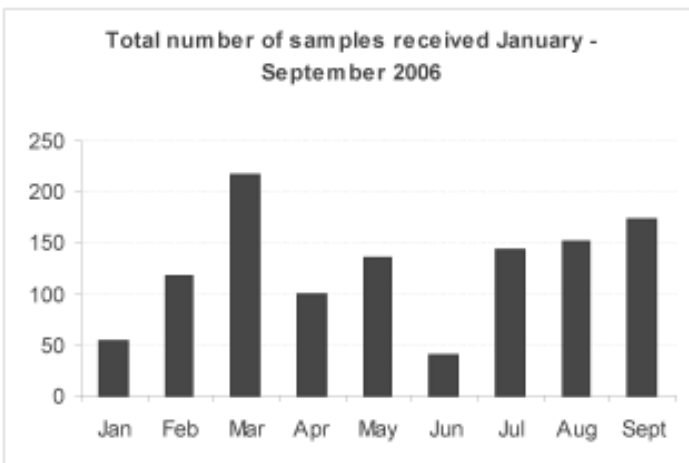
# Diagnostic Clinic 2006

The TPCP/CTHB diagnostic clinic is a service provided to the industry to assist in the identification and management of disease and pest problems of trees. This service is available to all member companies of the TPCP. Foresters and farmers are encouraged to contact the TPCP/CTHB to discuss possible disease and pest problems. Information gathered through the diagnostic clinic forms an important component of the early detection of new pest and disease problems, tracking of changes in pest and disease scenarios and provide information for the database kept by the TPCP/CTHB.



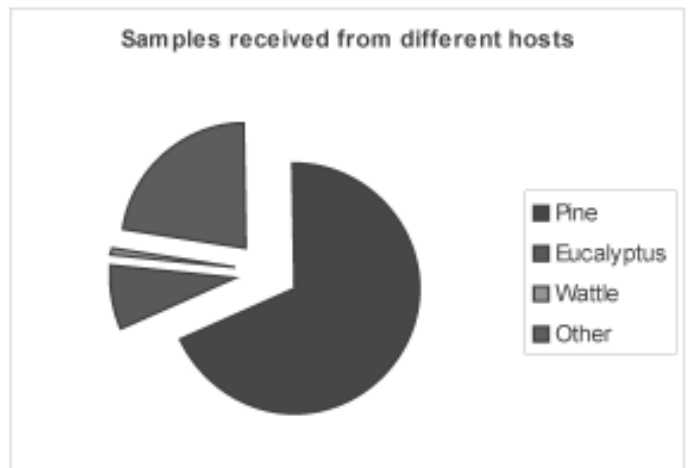
From February 2006 the clinic has been under new management, with Izette Greyling taking primary responsibility for the day to day running of the clinic and the sending of reports. She is assisted and guided by Prof. Jolanda Roux and Brett Hurley, both of whom are also responsible for managing the field services of the TPCP/CTHB. This is crucially important as the diagnostic clinic and field services are closely connected. For example, where samples cannot be couriered, or something new or different is reported by the clinic, Brett or Jolanda will arrange a field visit to follow up on the problem. Izette was assisted in her task by Kgosi Mongwaketsi (*Fusarium* molecular diagnostics), Bianca Hinze and Happy Maleme. With the appointment of a person to the clinic on a full-time basis, we believe that our services have improved greatly in 2006. Any feedback or suggestions by industry are, however, welcome.

The diagnostic clinic had a very busy nine months up to end of September 2006. A total of 1125 samples were processed during the period January to September 2006. Pine samples, including both nursery samples for *Fusarium* screening and disease analysis samples, comprised approximately 67.8% of the total number of samples received. Only 8 samples were received from Black wattle. 8.6% of samples received were from *Eucalyptus* and 22.8% of samples were classified as other. Samples classified as "other" include water, soil, seed, insect and Petri dish samples. Samples received from native or non-forestry tree species were also classified as other.



## CONTACTING THE TPCP/CTHB FOR DIAGNOSTICS

- Izette Greyling (izette.greying@fab.up.ac.za); 012 420 3938/9/3933 as clinic manager should be your first stop in sending samples to FABI. She will provide you with a reference number for your sample and keep you updated with progress. She will also send out a letter with the results of the analyses.
- Jolanda Roux (jolanda.roux@fab.up.ac.za); 082 9093202 guides Izette in the clinic and manages field extension for the TPCP. If Izette is thus not available, you can talk to Jolanda. Jolanda will also most likely be the one to do field visits in cases where it is not possible to send samples.
- Entomological samples are logged by Izette who then liaises with Brett Hurley and Bernard Slippers for identification and report writing. Brett Hurley (brett.hurley@fab.up.ac.za); 082 9093211
- We will respond to the receipt of samples (preferably by e-mail), but please always check that samples have been received. These are sometimes lost during dispatch. If you are unsure of the problem being encountered or need advice on collection of samples, please phone Izette, Jolanda or Brett and discuss the problem before collecting and sending the samples.



# The 8<sup>th</sup> International Mycological Congress (IMC8)

The 8<sup>th</sup> International Mycological Congress (IMC8), hosted by the Australian Mycological Society, was held in the Cairns International Conference Centre from 21-25 August 2006. This was the first time ever that this congress took place in the Southern Hemisphere. It was attended by almost 800 delegates (students, researchers, academics, practitioners and industry representatives) from all around the world. Although most delegates were from Australia, New Zealand, Thailand, Indonesia, Singapore and Japan, a significant number represented countries from North and South America, Europe and Africa. Fourteen Fabians also presented their research. They included MSc students (Joha Grobbelaar and Gilbert Kamgan Nkuekam), PhD students (Wilhelm de Beer, Magriet van der Nest, Marelize van Wyk and Draginja Pavlic), post-docs (Drs Martin Coetzee and Xudong Zhou), project leaders (Drs Bernard Slippers and Emma Steenkamp and Prof Jolanda Roux) and Professors Mike and Brenda Wingfield.

A wide spectrum of mycological topics were addressed, ranging from ecology, evolution and biosecurity to fungal pathology and medical and veterinary mycology. In most of these, it was demonstrated how new technologies such as fungal genomics and proteomics will increase our knowledge of the evolution and biodiversity of fungi, ultimately enabling us to control fungal disease and to utilize fungi optimally in agriculture, food and medical sciences.

Below are abstracts of some of the presentations by TPCP/CTHB researchers at the IMC meeting.

## ***Global distribution and evolution of the pine pitch canker fungus, *Fusarium circinatum****

Emma T. Steenkamp, J. Wright, R.J. Ganley, E. Iturrirxa, R. Ahumada, B.D. Wingfield, W.F.O. Marasas and M.J. Wingfield

*Fusarium circinatum* is an important pathogen of *Pinus* spp. that represents a significant threat to native and commercial forests, worldwide. This fungus causes large resinous cankers accompanied by pitch-soaked wood, crown die-back and stunted growth of established trees. The fungus can also be a very serious pathogen of nursery plants, where the symptoms of infection include root and root collar rot. Introduction of *F. circinatum* to new locations may be facilitated by insect vectors, although the majority of new introductions have probably resulted from trade in seed. Pitch canker is known in the USA, Mexico, Chile, Haiti, South Africa, Spain, and Japan, with unconfirmed reports from Italy, Iraq, South Korea and China. The primary objective of this study was to improve our understanding of the global distribution and spread of *F. circinatum* by studying its overall evolution and population biology. We, therefore, conducted a phylogenetic analysis that included representatives of *F. circinatum* populations from California, Florida, Mexico, Chile, Spain and South Africa. After DNA extraction, several housekeeping genes and non-coding regions were PCR-amplified and sequenced. These sequences were then aligned and subjected to phylogenetic analyses. Results revealed the presence of many nucleotide polymorphisms

between the six populations, with fewer differences within populations. These data also allowed us to identify sequence signatures that differentiate some of the populations from others. Our preliminary data further suggest that isolates representing the Chilean isolates are more closely related to isolates from Mexico than to those representing the other populations examined. Ultimately the results of this study will provide valuable insight into the origin of the pitch canker fungus and its global spread.

## ***Genotypic Diversity of *Armillaria fuscipes* in South African Pine Plantations***

Martin P. A. Coetzee, Brenda D. Wingfield, Michael J. Wingfield

*Armillaria fuscipes* (Basidiomycetes, Agaricales, Tricholomataceae) is the causal agent of Armillaria root rot on a variety of economically important *Pinus* species in Southern African plantations. The taxonomy of this fungus has been well established but its population structure in pine plantations has not yet been determined. The aim of this study was to ascertain the genetic diversity of *A. fuscipes* in *Pinus elliottii* and *P. patula* plantations in South Africa. Isolates were collected from plantations in the Mpumalanga and Limpopo provinces. RFLP analyses of the IGS-1 region were performed on the fungal isolates to confirm their identity. The genotypes of the isolates were assessed using vegetative incompatibility. These tests were conducted by crossing

all the isolates with one another in all possible combinations on malt extract agar. Genetically identical isolates were identified by the absence of a demarcation line between two crossed isolates. AFLP analyses were employed to further assess the genetic diversity of the isolates. Banding patterns for these analyses were obtained from a variety of primer sets. RFLP profiles typical of *A. fuscipes* were obtained for all isolates indicating that they all represent the same species. Isolates collected within discrete infection centers were found to represent single clones of *A. fuscipes*. Preliminary results on the isolates from different centres suggest that each represents a different genotype. Assessment of the total number of clones and their genetic relationships to one another will provide information on the genetic history of *A. fuscipes* in South Africa and assist in the management of this pathogen in pine plantations.

***Comparison of molecular ecological patterns in populations of different woodwasp fungal mutualists***

Bernard Slippers, Vasiliauskas, R., Van der Nest, M.A., Stenlid, J., Wingfield, M.J

There are two common factors in many mutualistic relationships, which appear to stabilise them. These are vertical transmission of symbionts between host generations and genotype uniformity in the symbiont. The same factors can also have detrimental effects, if the symbiont population loses its adaptive ability or where deleterious mutations arise. The consequences of symbiont transmission and reproductive modes have not been extensively explored in the well known mutualism between *Amylostereum* fungi (Basidiomycotina) and various wood wasps in the family Siricidae (Hymenoptera). Female wasps carry asexual *Amylostereum* spores in internal mycangia and inoculate the fungus into softwoods when they oviposit. Sexual fruiting structures of these fungi, however, occur frequently in some areas. It has traditionally been thought that the transmission of these fungi between wasp generations is only vertical, from mother to female offspring. Our studies support this hypothesis in some populations. However, the fact that various wasp species share the same fungal symbiont, implies that horizontal transmission occurs, at least on evolutionary time scales. Recent experimental studies and field observations have,

furthermore, suggested ways in which horizontal transmission might occur on ecological time scales. In this study we combine data generated using various molecular markers, including RAPDs, microsatellites, nuclear and mitochondrial sequence data, and PCR-RFLPs, to determine patterns of spatial distribution and ecological transmission in populations of *A. areolatum* and *A. chailletii*, associated with various wood wasp species. These data suggest that there are discrete differences in the molecular ecological patterns of populations of the *Amylostereum* spp. Overall *A. areolatum* appears to be more genotypically uniform with greater geographical and host associated structuring of populations, reminiscent of a strong influence of vertical transmission and asexual reproduction. In contrast, *A. chailletii* appears to be more genotypically diverse, with less structured populations, suggesting greater levels of horizontal transmission and sexual reproduction at ecological time scales. However, in both species, some local population patterns differ from the overall patterns. We thus explore the differences in molecular ecological patterns of populations of *Amylostereum* spp. associated with Siricidae and the possible factors that affect them.

***Emerging fungal diseases threaten world forests***

Michael J. Wingfield

The first recorded examples of epidemic diseases resulting in the destruction of forest ecosystems date back to the beginning of the 20<sup>th</sup> Century. This coincides closely with a time where trade and the movement of wood and wood products, particularly between northern hemisphere countries, increased substantially. It was also a time when forestry industries were being established in many parts of the world and where germplasm required for the establishment of plantations was moved between countries, without control. Diseases such as Dutch elm disease, chestnut blight, white pine blister rust thus emerged in native woody ecosystems, where they have caused irreparable damage. While these classic examples of tree diseases are well-known amongst mycologists and pathologists, it is important to realize that the emergence of similar new diseases has continued virtually unabated. New fungal pathogens have not only been introduced into new native forest ecosystems but pathogens of non-native plantation trees have been moved widely. In the latter case, the impacts have been variable and often times, it has been possible to manage disease problems through silvicultural practices and tree breeding. This is,

## The 8<sup>th</sup> International Mycological Congress (IMC8)... from page 13

however, substantially depleting the profitability of intensive plantation forestry and in some cases businesses based on this practice are failing. To complicate matters, examples of fungal pathogens undergoing anthropogenic host-jumps are emerging. World-wide trends are clearly focused on attempts to stem the flow of tree pathogens to new environments. While success is achieved in some situations, this is an enormously complex problem and it seems likely that many new fungal diseases will continue to threaten world forests and forest industries in the foreseeable future.

### ***Cryphonectria* canker of *Eucalyptus*: A little-known disease caused by an assemblage of fungi of extreme quarantine relevance**

Brenda D. Wingfield & Marieka Gryzenhout

*Cryphonectria* canker causes one of the most serious diseases of *Eucalyptus* in plantations grown in the tropics and sub-tropics. Its impact has thus been responsible for shaping the nature of *Eucalyptus* forestry industry in many

countries. DNA-based phylogenetic inferences have, in recent years, made it possible to review the taxonomy of the pathogen responsible for *Cryphonectria* canker of *Eucalyptus*. This substantially changed our understanding of the disease, its causal agent and its likely origin. *Cryphonectria* canker of *Eucalyptus* is now known to be caused not by a species of *Cryphonectria* but by a suite of species residing in the genus *Chrysosporthe*, which has been newly described to accommodate them. These fungi including *C. cubensis*, *C. austroafricana* and *C. doradensis* all have unique biological characteristics and they have clearly originated in different parts of the world. Interestingly, their hosts of origin are not species of *Eucalyptus*, but rather various tree species, not only the Myrtaceae, but more broadly in the Myrtales. *Chrysosporthe cubensis*, the best-known of the species is common in South East Asia and in South America and probably represents two different but closely related species. These fungi are all serious pathogens, some of which have already moved intercontinentally. They represent pathogens of great economic importance in countries where they are already known. Perhaps more importantly, in terms of quarantine, they appear to represent a highly threatening assemblage of fungi, which until very recently have virtually been overlooked.



Some of the TPCP/CTHB researchers and all the luggage required for an international meeting at the Brisbane International Airport.

THE RESEARCH TEAMS OF  
THE TREE PROTECTION  
CO-OPERATIVE  
PROGRAMME AND DST/  
NRF CENTRE OF EXCEL-  
LENCE IN TREE HEALTH  
BIOTECHNOLOGY

The research teams of the two programmes are varied, but overlap in many cases. It includes full time research and lecturing staff of the University of Pretoria [Prof. M.J. Wingfield (Director and Mondi Professor), Prof. B.D. Wingfield, Prof. T.A. Coutinho, Prof. J. Roux, Dr. B. Slippers, Dr. M.P.A. Coetzee, Dr. G. Marais, Dr. E. Steenkamp, Mr. B. Hurley] and technical staff (H. Hatting, R. Visser, P. Khumalo, V. Nkosi, K. Mongwaketsi, M. Mahlangu, L. Twala, M. van Zyl, H. Doman, E. Müller, V. Clarence, J. Hale, H. Roos, A. Shumba, M. Fouche, M. Mbonani, T. Mojela). Colleagues and students attached to other organizations such as the ICFR and post graduate students also form part of these teams. Staff from various departments of the University provide advice and support where required.

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Tree Protection Co-operative

Programme (TPCP)

Att: Prof. Mike Wingfield

FABI

University of Pretoria

Pretoria

0002

## The CTHB offers opportunities for students to do their experiential training

FABI and the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) gave Penelope Mahlangu, Project Assistant at AfricaBio, the opportunity of doing her in-service training at the University of Pretoria to enable her to obtain her National Diploma in Biotechnology. Penelope worked in our lab for three weeks during which she completed a small project on identifying samples of the fungus *Ganoderma*. Her project formed part of establishing a foundation for future work within the CTHB on plant pathogenic *Ganoderma* species. During her in-service training she gained experience in extracting DNA, doing PCRs, sequencing and using some of the bioinformatics tools available for taxonomic purposes. She also acquired an understanding about the different groups of fungi, their characteristics and the fact that some of them cause disease leading to the death of trees. She worked under the supervision of Dr Martin Coetzee, research fellow at FABI and the CTHB. She has just informed us that she passed her training and will be graduating in September this year.

Congratulations to Penelope on her success. Her closing words were "thank you very much, please if you can, give other students the same opportunity – it has really bettered my life".



# TPCP STUDENT “HABLA ESPAÑOL” IN ECUADOR

By Lorenzo Lombard

During December 2005 and January 2006 I had the opportunity to visit the Republic of Ecuador to further my Ph.D. studies. The aim of the visit was to establish plantation trials and collect diseased plant material and soils for isolation of possible fungal plant pathogens. This was also an opportunity for me to learn more about forestry practices applied in this country, as well as to help with the education of forestry personnel in tree diseases.

Fundacion Forestal Juan Manuel Durini (FFJMD), my hosts during my visit, is a prominent forestry company in Ecuador. They produce veneer and block boards from indigenous hardwood tree species grown in plantations. These plantations are mixed stands with pachaco (*Schizolobium parahybum*), chuncho (*Cedrelinga catenaeformis*), jacaranda, *Terminalia* spp., *Cordia* spp. and *Eucalyptus deglupta*. With the exception of *E. deglupta*, these tree species are all indigenous to the Ecuadorian region, thus highly susceptible to pests and diseases present in natural forests.

Inoculation trials with *Lasiodiplodia theobromae* were established in pachaco stands. This pathogen causes die-back of pachaco in the stands, resulting in large financial losses to FFJMD. Chuncho trees were also inoculated with *Fusarium* isolates obtained from bark cankers of these trees. This is a “new” disease in Ecuador that is already devastating all chuncho trees commercially planted. Wounding trials were established to bait

for *Ceratocystis* spp. and *Ophiostoma* spp. in nine different tree species. Soil samples were collected for *Cylindrocladium* baiting, an important pathogen not only in Ecuador, but South Africa as well. It is believed that *Cylindrocladium* spp. originates from tropical South America.

During my stay in Ecuador I met with a wide variety of cultural experiences. The integration of Spanish colonialism and indigenous Indian culture is clearly reflected everywhere in Ecuador. The people are always friendly and willing to help in all aspects. Some beautiful Catholic Cathedrals and other monuments are spread throughout the cities and towns reflecting the proud historic heritage of Ecuador.

These research visits are crucially important in establishing and strengthening collaboration between different forestry countries, especially since it is well known that pests and pathogens are moved around very easily. Gaining first hand experience in another country is thus important in helping us understand potential problems before they may arrive in our own country. I am thus very grateful for the opportunity provided to me to broaden my experience base and meet new people. I would like to thank the personnel and management of FFJMD for taking good care of me during my stay. Also my thanks to the TPCP and people at FABI for their support.



Above: Lorenzo Lombard, Ing. Fernando Montenegro and Colon Zambrano collecting wood samples from wounded pachaco trees.

Left: The FFJMD team and Lorenzo in a pachaco stand. FLTR: Lenny, Lorenzo, Bladimer and Lois. Back FLTR: Fernando and Johan