Tree Protection News

tpcp

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 threat of diseases and pests to trees in South African forests, both in plantations and natural ecosystems, has certainly never been as evident as it is at present. In the last few years, pine plantations have been ravaged by the onslaught of the Sirex wood wasp. The unbelievably rapid spread of the bronze leaf bug, *Thaumastocoris perigrinus,* has been nothing short of shocking.The pitch canker fungus, *Fusarium circinatum*, has made its first appearance in established plantations and as I write this note, I am looking over an area of self sown white stinkwood (*Celtis africana)* that are dying of an undetermined cause.

Home owners in various parts of South Africa have been devastated by the rapid wilt of Palm trees that is apparently caused by an introduced beetle that kills the growing tips. In the case of the latter problem, there are at least hundreds of magnificent old palm trees dying in Pretoria. At a cost of some R3000 to remove a single tree (personal experience with three trees), the total loss to home owners and municipalities will surely run to millions of Rand.

A humbling, though certainly not pacifying reality is that the incidence and the impact of tree pests and disease is likely to continue to grow in years to come. While we all recognize that crop plants are affected by diseases and pests, what is seldom realized is that trees act rather differently to annual crops. When a disease or pest problem emerges, trees that have grown for decades, if not centuries (some palms in Pretoria) are lost and never to be

Keeping trees healthy is a team effort, the more eyes out there, the quicker we can act on new incursions.

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replaced. Other than the losses (economic or aesthetic) to existing trees, the impact on future tree planting must be considered. Some highly desirable species may well never be planted again. In the case of forest plantations grown for timber or fibre, the costs of developing new species or hybrids for future planting can be enormous. Examples that need to be considered as very real are the potential loss of key species (eg *P. patula* or *E. grandis)* as pure planting stock or even hybrid parents, to forestry programmes.

The research team of the Tree Protection Co-operative Programme (TPCP) and the Centre of Excellence in Tree Health Biotechnology (CTHB) are commonly amazed by the expectation that there will be easy and short-term solutions to tree pest and disease problems. The truth is that these sorts of problems are amazingly complex and that the solutions typically lie in long-term studies and investment. The actions taken by the Australian Government in order to deal with the impact of the Sirex wood wasp in the 1970's provide a fine example. While it is reasonable to expect that in some cases (Sirex a case in point) we will be able to apply control measures developed elsewhere, other problems will be unique to South Africa and they will demand serious investment. *Thaumastocoris* provides a perfect example. This is an insect about which virtually nothing is known, neither life cycle information nor methods to manage its impact. Yet it appeared in South Africa unexpectedly and it spread in a manner nothing short of shocking. An apt question might be "*Thaumastocoris* quo vadis?" Certainly the answer to dealing with this problem will lie in a serious investment in studying and developing a biological control strategy for it.

What strikes me, when thinking about these many new and emerging problems, is the fact that the only manner in which to view tree protection activities is a process. There will most likely never be rapid answers to problems and a long-term view will be the only option open to us.I believe that the most important asset in our arsenal against these problems is a base of human capital with an interest and experience to study and deal with these problems. In this regard the establishment of the TPCP by the South African forestry industry must be regarded as one of its most forward thinking actions. While this programme now enjoys a substantial international recognition as a leading force in forest pathology and forest entomology, it has significant limitations. Perhaps the greatest of these is a lack of permanent full-time staff that can deal with new and rapidly emerging problems.
While the contents of this note might appear to be rather "doom and gloom", I retain the view that there is much to feel positive about regarding the long term sustainability of forestry. Perhaps the greatest advantage that South African forestry has, is that it is not overly reliant on a single species of tree. The situation in countries such as New Zealand and Chile is rather different. In these countries, a heavy reliance on a single species must be a significant cause for disquiet.

The New Year is well on its way with four months behind us. In this short time, some very significant gatherings have been held and there is much activity (perhaps more than ever in the past) on the tree health front. The Sirex steering committee has already had several rigorous meetings and what must certainly be the largest deployment of any biological control agent in recent times is underway as I write. A "brain storming" session has been held to consider the "way forward" in dealing with new and threatening pests and diseases and TPCP field trips and monitoring activities have been active since very early in the year. We are moving rapidly to the annual research meeting of the TPCP, which this year, will be held back to back with a major international symposium on Sirex. This will include some 30 scientists from other parts of the world and some of them will also be involved in the TPCP meeting. Later in the year, a significant meeting on tree health issues will be held at FABI as a satellite meeting to the IUFRO Eucalyptus meeting to be held in Durban. This certainly must be declared the "Tree Pest and Disease Year". Let us hope that it will lead us in the direction of solutions, or at least a deeper understanding of the many problems that challenge us.

Bacterial Blight of Eucalyptus

There are an increasing number of emerging bacterial pathogens of plants recognized worldwide. One of these pathogens is *Pantoea ananatis*, the causal agent of bacterial blight and die-back of eucalypts.The disease was first observed in a nursery in KwaZulu/Natal. The pathogen subsequently spread to other nurseries and currently occurs in recently established eucalypt plantations with devastating results. Repeated die-back leads to multi-branched, stunted trees.

Efforts have been made by the TPCP to gain an understanding of the epidemiology and biology of bacterial blight in South Africa. *P. ananatis* is a known epiphyte on other plant species, including those plants which are known hosts of the pathogen. Our initial efforts were to establish whether or not this bacterium occurred on healthy eucalypt leaf surfaces. Leaves were thus collected from a GN hybrid, known to be very susceptible, and our results conclusively showed that the pathogen resides on the surfaces of these healthy leaves. We believe that *P. ananatis* is an opportunistic pathogen which infects the host, either through natural openings or wounds, when environmental conditions are favourable for infection. The ideal conditions would appear to be high humidity and warm temperatures.

Our next research question revolved around attempting to understand the pathogenicity of *P. ananatis* in eucalypts. Questions asked were, for example; does an opportunistic pathogen such as *P. ananatis* have similar elicitors of pathogenicity as related, non-opportunistic pathogens such as the causal agent of Stewart's wilt, *P. stewartii?* Similarities at both the phenotypic and genotypic level were found. However, difficulties are being experienced in locating these factors on the genome.Due to the recent developments in DNA sequencing it is now possible to sequence entire genomes relatively quickly and cheaply. The TPCP has thus acquired additional funding from the NRF and the University to sequence the genome of a pathogenic strain of *P. ananatis* from eucalypts. This will be the first plant pathogen sequenced in Africa. Once we have the sequence it will create tremendous research opportunities for the TPCP such as being able to

conduct comparative genomics with related sequenced pathogens. Ultimately, we should have a clear understanding of the major molecular mechanisms used by *P. ananatis* to invade its host. This in turn will provide valuable information for the future management of this disease.

CONGRATULATIONS

A number of TPCP/CTHB students received degrees in the last few months. We take this opportunity to congratulate them again and celebrate with them. Their success, is the success of the entire programme and the forestry industry as a whole.

B.Sc. Honours

Boithumelo Mashangoane: Generation of polymorphic markers and analyses of the population biology of the pitch canker fungus.

Marcele Vermeulen: Chrysoporthe species in Africa.

Nicolette Fouche: Identification and pathogenicity of selected wound associated Ceratocystis spp. from broad leaved trees in Australia, Europe and Africa.

Quentin Santana: Characterization of the mitochondrial genes of Armillaria fuscipes.

Magister Scientiae (M.Sc.)

Rebecca Makhado: Epi- and endophytic members of the Enterobacteriaceae associated with healthy Eucalyptus leaves.

Philosophia Doctorae (Ph.D.)

Gavin Hunter: Taxonomy, phylogeny and population biology of Mycosphaerella species occurring on Eucalyptus.

INTERNATIONAL NEWS
Portugal Takes Drastic Measures to Stop Tree Killer

http://www.planetark.com/dailynewsstory.cfm/newsid/40969/story.htm

PORTUGAL: March 21, 2007

Story by Henrique Almeida

ALCACER DO SAL, Portugal - Fernando Silva shook his head as he watched two lumberjacks cut through what remained of his small pine forest in the south of Portugal.

"Those trees were a good source of income and now they are gone," 60-year-old Silva said, looking away. Silva is unlucky. His valley lies in the way of the government's efforts to cut a 430 km (267 mile) corridor through Portugal's central and southern forests to contain a plague of microscopic worm-like organisms that are killing pine trees and threatening to spread throughout the nation's forests and beyond.Pine Wilt Nematode was first detected in Portugal in 1999, but has spread at an alarming rate recently. The EU has offered Portugal up to 8 million euros (US$10.64 million) to control the plague and Portugal's government has said that the drastic measures should help control the spread of nematodes.

The corridor is meant to contain the pests inside 2.47 million acres (1 million hectares) of land, or about one ninth of Portugal's total forest, and stop them from spreading elsewhere in Europe. European Union inspectors visited the country a few weeks ago to check the situation."If only they would have spared the healthy ones," said Silva, adding that he had planned to sell some of his pine trees to a wood panel factory. But the government has blamed landowners for failing to destroy infected trees in time to stop the disease from spreading further. Recent droughts have also helped spread the blight as it weakened trees' defences.

And some landowners have not been as resigned to the government measures as Silva. "One pointed a rifle at me and told me to leave immediately," said Rogerio Rocha, a lumberjack who was hired by the government to help cut the forest corridor. Despite the resistance, the government expects to complete the corridor by the end of the month before insects emerging in the warm spring months begin to spread the organisms again. "Doing nothing would result in an embargo on Portugal's pinewood exports to the rest of European Union, which is an extremely important source of revenue for Portugal," Agriculture Minister Jaime Silva told Reuters.Portugal's cork, pulp and pine wood exports account for about 2.5 billion euros (US$3.30 billion) a year.

The corridor will also help Portugal control fires that have raged across the country in recent years, he said. But it will also cost this small Iberian country about 500,000 pine trees to create the corridor. Landowners complain that the 4-10 euros the government is offering for every healthy pine tree it destroys is not enough.

"I have pine trees 45 years old that are worth about 24 euros. The money is just not enough," said Pedro Silveira, who heads an association of forest owners in the south of Portugal and has three of his properties affected by the corridor.

Bursaphelenchus xylophilus feeds on the cells lining the resin canals of the tree and multiplies very rapidly. As they destroy the resin canal cells, the tree's water-moving system becomes clogged.

The tree then wilts and dies within weeks. The pest is thought to have arrived in Portugal by sea eight years ago, and caught authorities by surprise when it infected more than 300,000 trees last year, twice the number in 2005.

The government says the compensation offered to landowners is fair and that it is better to sacrifice some pine trees now rather than lose them all in the future. "Landowners complain they are losing their pine trees but we say it is better to lose some trees now than lose all of them later," Jaime Silva said.

Fabians attend the 15th International Conference on Nitrogen Fixation

The 15th International Conference on Nitrogen Fixation was held in Cape Town from 21-26 January 2007. In this picturesque setting at the foot of Table Mountain the delegates (most of whom were from Europe and the USA) agreed that South Africa was an appropriate location for hosting a conference themed "Biological Nitrogen Fixation (BNF) Applications for Poverty Alleviation". The keynote address by Lamourdia Thiombiano (Senior Soil Resources Officer for the FAO Regional Office in Africa), was very aptly entitled "Biological Nitrogen fixation and poverty alleviation in Africa". Numerous other presentations also addressed this issue, as well as the role of BNF in food security and its uses and limitations in sustainable agriculture and forestry.

Among the plants of agronomic importance, legumes are most diverse. The keynote address by Noel Ellis from the John Innes Centre in Norwich in the United Kingdom, touched on some of the problems this diversity and species richness pose for breeding programmes. In his presentation entitled "Gene Discovery and Marker Development in Crop Legumes", he addressed issues such as the evolution and domestication of crop species, as well as strategies for the exploitation of information from model systems for application in less intensively studied target crops. Presentations on similar issues such as genomics of nitrogen fixers and genetics/regulation of nitrogen fixation were also included.

A significant number of the sessions at the conference focused on the bacterial symbionts responsible for BNF. This interaction between plant and bacterium usually results in the formation of specialized root structures known as nodules, within which BNF occurs. The majority of these BNF bacteria apparently form part of the class *Alphaproteobacteria*, but an increasing number of authors are now reporting that members of the Betaproteobacteria are also capable of nitrogen-fixation and nodulation. Under the auspices of the CTHB, Emma Steenkamp and Chrizelle Beukes presented their work on the root nodule bacteria from the enigmatic legume tribe *Hypocalypteae*. This tribe includes the single genus, *Hypocalyptus*, with only three known species (*H. sophoroides, H. oxalidifolius and H. coluteoides)* all of which are endemic to the Western Cape. Their preliminary data suggests that the bacteria responsible for BNF in these plants forms part of the Betaproteobacteria genus *Burkholderia.* Together with other presentations on the bacterial symbionts of various legume species, this talk highlighted the importance of understanding BNF for agricultural and conservation management.

This conference provided the delegates with the opportunity to meet the world leaders in the field of BNF and to discuss with them some of the specific issues of BNF pertaining to the South African situation. However, the conference did not only entail work and no play, and delegates were taken on a number of different mid-conference tours to view some of the spectacular Cape scenery. One of these, the Peninsula tour, included visits to Maiden's Cove, The Cape of Good Hope Cape Point and a wonderful but windy visit to the penguin colony at Boulders Beach.

From left to right, Chrizelle Beukes, Emma Steenkamp and Francina Phalane (ARC-PPRI, Roodeplaat) on a very windy day at Cape of Good Hope in the Table Mountain National Park.

Pitch Canker Update

Pitch canker is one of the most important diseases of *Pinus* species in the world. The causal agent, *Fusarium circinatum* (Pitch Canker Fungus = PCF), is responsible for a serious root and root collar disease of pine seedlings in nurseries across South Africa. In other parts of the world such as USA, Mexico, and Spain, the fungus causes typical resin-soaked cankers on trunks and lateral branches of pines. Until recently, these symptoms on established plantation trees, have never been observed in South Africa. As reported in the November 2006 issue of *Tree Protection News*, the first outbreak of pitch canker on mature pine trees occurred in the Tokai plantation near Cape Town, and it has been shown to be caused by PCF.

To monitor the spread of the disease in the Tokai plantation, researchers at the TPCP have randomly selected three sites within the 70 ha affected area. At each of these sites, 50-tree plots were established and the trees were evaluated based on a disease rating score of 0-3, where 0 = healthy and 3 = resinous stem cankers. In addition to monitoring the spread and severity of the disease, the PCF and insects (specifically, the deodar weevil *Pissodes nemorensis*) are also collected on a regular basis at these and other sites. Results of the analyses of the population biology of the pathogen and its association with the weevil, will be extremely valuable for understanding the etiology of the disease on plantation trees in South Africa. Current research projects are also aimed at determining the potential origin(s) of the pathogen in Tokai and whether it is caused by new genotypes

of the fungus. Overall, this will allow the researchers to evaluate the extent to which PCF threatens pine plantations in other parts of the country. Indications are, however, that the use of disease tolerant trees and good site and silvicultural practices remain the most appropriate means for management of pitch canker in nurseries and plantations.

As part of an agreement with some forestry companies, the TPCP has been providing screening facilities to industry to select PCF tolerant *Pinus* spp. for future planting. The Pine *Fusarium* screening program forms a part of the management strategy to reduce the impact that *Fusarium circinatum* has on *Pinus* forestry in South Africa. For the past two years, the screening programme has been managed by Kgosi Mongwaketsi. As part of his duties he has also been responsible for performing the diagnostic PCR used to identify *Fusarium circinatum* from samples received by the clinic. Kgosi recently accepted another career opportunity at Komatiland Forests. His duties will be taken over by Izette Greyling, who will be managing the TPCP/CTHB diagnostic clinic and the *Fusarium* screening programme on a full time basis. We wish Kgosi all the best with his new position and hope to continue interacting with him. We also welcome Izette to the screening programme and wish her all success with her new responsibilities in FABI.

Left: Internal, resin soaked lesion caused by PCF infection of three-year-old P. patula *tree.*
Right: External symptoms of pitch canker showing copious exudation of resin on stem of tree.

SA - USA connections in the fight against Sirex

The woodwasp *Sirex noctilio* is native to Eurasia. A number of related Siricid wood wasp species,some almost indistinguishable from *S. noctilio*, are native to North America. Most of these native Siricids are rare and attack already dead or dying trees. In 2004, a *S. noctilio* female was caught in a trap in New York State. A massive survey effort followed this discovery, and the wasp was found to be well established and spread throughout New York. More recently it was also reported from Canada.

The extent of the distribution of *S. noctilio* in the USA and Canada most likely means that the wasp has been present for a number of years. This is typical for new introductions, which often have a lag phase during which it is very difficult to detect small populations. The presence of the pest often only becomes apparent once it is well established in an area and the damage starts out from the background levels of damage.

As a non-native attacking living trees, *S. noctilio* poses a serious threat to native *Pinus* in North America. North America has many native *Pinus* species that are an important part of the economy and ecology of the region. To date the damage has been limited.

The fear is, however, that the wasp population would build up and spread to areas where it can become as aggressive a killer of *Pinus* trees as in other countries, such as South Africa.

The control of *S. noctilio* has both challenges and a 'helping hand' in the USA. One of the challenges lie in the presence of many native Siricids that might be affected by control mechanisms aimed at *S. noctilio.* This concern has been raised regarding the introduction of the parasitic nematode, *Deladenus siricidicola*. None of the native Siricids, however, carry the fungus *A. areolatum* (they are symbiotic with *A. chailletii*), which is essential for *D. siricidicola* survival.

On the positive side, there are a number of native parasites of Siricids already present in the environment. In fact, a number of the early introductions of parasitoid wasps into New Zealand originated from North America. These native parasites might help control *S. noctilio* naturally.

In response to the *S. noctilio* threat, the US government (through USDA APHIS and USDA Forest Service), has launched a wide scale monitoring program. Together with Universities, they are also engaged in research to better understand the ecology of this wasp in the North American context and to explore control options. As part of this process, a Sirex Science Advisory Panel has been put together to advise research, legislature and control efforts of this wasp. TPCP researchers have had the opportunity over the past two years to work with this panel. In December 2006, Bernard Slippers attended the Sirex panel meeting in Indianapolis on behalf of the group, as well as the National Entomological Society of America meeting that preceded it. Apart from contributing to the work in the USA, this involvement has given us the opportunity to present our research and control program to a panel of international experts for their input. It has also forged collaborative links on projects such as chemical ecology, trap design and more (see 'Sirex research: A sticky business'), which is providing information and tools to apply in the fight against Sirex in the SA forestry industry.

Prior to the USA Sirex Panel meeting, Bernard visited the USDA APHIS labs. This photo taken during that visit includes (l to r) Dr. Robin Bedding (CSIRO, Australia), Kelly Downer, Dr. David Williams (both from USDA APHIS) and Prof. Ann Hajek (Cornell University), involved in a demonstration on B. siricidicola rearing and study techniques.

SIREX RESEARCH – A STICKY BUSINESS!

Besides the mainstream research of improving the biological control of the woodwasp, *Sirex noctilio*, the TPCP is also involved in other projects aimed to further our understanding of *S. noctilio* and develop more effective management tools. One of these research projects is to determine the relationship, if any, between resin flow and resin chemistry of pine trees, and *S.noctilio* attack.This project is in collaboration with Dr. Kier Klepzig and Dr. Brian Strom of the Southern Research Station, USDA. Another project, in collaboration with Mr. Vic Mastro of APHIS-USDA, is to test various lures and traps for their attractiveness to *S.noctilio*.

It is well known that *S.noctilio* primarily attacks stressed and suppressed trees. However, the mechanism behind this preference is poorly understood. An important factor that could play a role in this regard (as has been shown for bark beetle – pine interactions) is the resin flow and chemistry of the pine trees. In order to test this, two *S.noctilio*-infested sites were chosen in the KZN Midlands in 2006, and the resin flow of 100 trees per site was measured over the Sirex flight season (October 2006 to January 2007). The pattern of *S. noctilio* attack on these trees will be noted. Resin samples from attacked and unattacked trees will then be sent to the USDA for chemical analysis and comparison. Results from this trial can assist us to measure variation of 'resistance' in individual trees and in different compartments, to better understand the population dynamics of *S. noctilio* within different areas, and to help design lures for *S. noctilio* (see below).

Finding an effective monitoring tool is an essential step for any new insect introduction. For the *S. noctilio* woodwasp, trap trees (trees stressed with herbicide to attract *S. noctilio*) have generally been used for such tools. However, the use of trap trees as a monitoring tool is limited for two reasons. Firstly, the degree to which *S. noctilio* is attracted to trap trees is greatly influenced by the degree of 'natural' stress within that compartment. Secondly, trap trees give you an indication of whether the tree was attacked or not, but they do not give you an indication of the *S.noctilio* population size.

A widely used and effective monitoring tool used for many insects is artificial traps that use specific, attractive lures. Much effort is being made, especially in the USA and Canada, to develop such a trap system for *S. noctilio*. These efforts are focussed on lures containing chemical compounds, known as kairomones, which are released by stressed trees. In collaboration with APHIS we have tested different trap designs and lures. Results this past *S. noctilio* flight season were encouraging, with some trap and lure combinations catching over 100 female wasps per trap in a month. Thus far, sticky panel traps were the most effective (albeit a messy affair). Trials this coming flight season will test an improved trap design and improved lures, based on the trials described here, and ongoing research in the USA on kairomones that most strongly attract *S. noctilio*. The aim is to provide an effective trap and lure that can be used on a national scale to monitor current *S. noctilio* infestations and, importantly, detect new infestations.

An update on *Thaumastocoris* research in South Africa

It was recently discovered that it is *Thaumastocoris peregrinus,* and not *T. australicus*, that infest *Eucalyptus* trees in South Africa, Australia and Argentina. Little is still known about the biology, natural enemies and control of this pest. Most work on *T. peregrinus* has been done in Australia, but even there no suitable control measures have been found for plantation situations. Apart from our own work, we at the Tree Protection Co-operative Programme (TPCP) are keeping contact with these researchers to keep abreast of any new developments.

Thaumastocoris peregrinus is a small (2 mm in size), sap-sucking, gregarious bug. This pest is now found in all *Eucalyptus* growing regions and feeding on the majority of commercially available *Eucalyptus* species and clones. The sap feeding behaviour of these bugs results in a reduction of the leaves' photosynthetic ability and often results in leaf loss and even death of severely infested trees. Rapid population dispersal and build up of *T. peregrinus* occurs when optimal conditions are present for growth and

reproduction, with each female being capable of producing 60 eggs within a 35 day life cycle, resulting in several generations a year.

Currently, no effective control strategies exist to control *T. peregrinus* on a large scale, neither in South Africa or elsewhere. Biological control is a long term solution that might take years to develop. It is at present still in the early stages of development even in Australia, based on parasitic insects. We are also exploring biological control options at the TPCP, such as isolating potential pathogenic fungi.

One control option is using chemical pesticides. To date there are no chemicals registered for spraying *T. peregrinus* in South African forestry plantations. Australian researchers are undertaking chemical control studies using trunk injected systemic insecticides that appear to be effective in controlling *T. peregrinus.* There are concerns, however, about the efficiency of systemic insecticides in large *Eucalyptus* trees, as well as the negative impacts it might have on several beneficial insects. There is a need to test pesticides in the South Africa situation under guidance of chemical companies in South Africa, and considering FSC and legislative requirements. The TPCP is exploring options of using semiochemicals (chemicals used in plant-insect or insect-insect communication) to control or monitor the pest. This is a longer term option, but is seen as a positive alternative to pesticides in an integrated pest management system. Monitoring tools for *T. peregrinus* are currently being tested in Pretoria and in Tzaneen with the help of Sonia du Buisson of Hans Merensky. The aim is to develop a standardised monitoring strategy that could be applied country wide. This is a critical step to help understand factors affecting the build-up and decline of *T. peregrinus* populations across different sites, and associated with different levels of damage. Effective monitoring tools are also useful in testing effects of control measures. Amongst other things, this information can be used to develop risk models to devise future control strategies.

TPCP/CTHB FIELD ACTIVITIES 2006

A crucial component of disease management and monitoring is communication with foresters and farmers. Without such interaction, any disease and pest management programme would fail. The TPCP thus conducts regular field visits to forestry areas and presents talks at industry field days, or on special request. These field visits also form an important part of the training of TPCP students, especially providing them with practical exposure to the problems they are trying to solve through their laboratory work. During 2006, researchers and students from the TPCP spend a total of 531 person days in the plantations of South Africa. These included presentations on various topics, including forest pathology and forest entomology at six field days.

Field visits can be requested by any member of the TPCP. We, however, do try to schedule these events in such a manner that they can be combined with visits to different companies, and also include field work for specific projects. In this manner, we are able to reduce the very high costs of working in the field.

Plantation tree health requires a team effort. Field work and surveys of the TPCP would not be possible without the assistance of foresters and farmers, the people who spend time in their plantations on a regular basis. It is, therefore, imperative that any disease and pest problems are reported to us. We encourage any interaction and feed back from foresters and farmers, irrespective of whether this is by phone or e-mail. This can be done through the following channels:

Jolanda Roux: 0829093202; jolanda.roux@fabi.up.ac.za
Field Extension, Pathology, Diagnostic Clinic
Brett Hurley: (012) 4203938/9; brett.hurley@fabi.up.ac.za Entomology Extension and Sirex programme
Bernard Slippers: (012) 4203938/9 Bernard.slippers@fabi.up.ac.za Entomology and Sirex programme
Izette Greyling: (012) 4203938/9; izette.greyling@fabi.up.ac.za Diagnostic Clinic and Fusarium Screening Programme
Teresa Coutinho: 012 4203934; teresa.coutinho@fabi.up.ac.za Fusarium Screening Programme and Pine Fusarium Working Group
Mike Wingfield: 012 4203938/9; mike.wingfield@fabi.up.ac.za Director of TPCP

Foresters discussing Thaumastocoris during a TPCP field visit to KwaMbonambi.

Contractors learning about Eucalyptus diseases.

Contractors and foresters at a tree health field day

TREEHEALTHNET

What is Treehealthnet?
TreeHealthNet is an e-mail List Server that has been set up to provide a discussion forum concerning tree and forest health issues.The server is set up in such a way that only those seriously involved in forestry in South Africa are involved and it is carefully maintained by a moderator (Wilhelm de Beer). The attractive part of a List Server is that all participants receive messages written to it. This means that it serves as an excellent forum for discussion and announcements (meetings, new web sites, etc.)

Why is Treehealthnet necessary?
With the increasing pressures on forestry and forests due to pests and diseases, we all benefit from a communication platform treating tree health issues, which reaches a broader community, including forestry staff at grassroots level.

Who is responsible for the listserver?
The list server is maintained at FABI at the University of Pretoria.

Who can subscribe and participate?
Staff of the TPCP member-companies and scientific and technical staff linked to Tree Protection Co-operative Programme (TPCP) and the DST/ NRF Centre of Excellence in Tree Health Biotechnology (CTHB). At present the list server has about 120 subscribers.

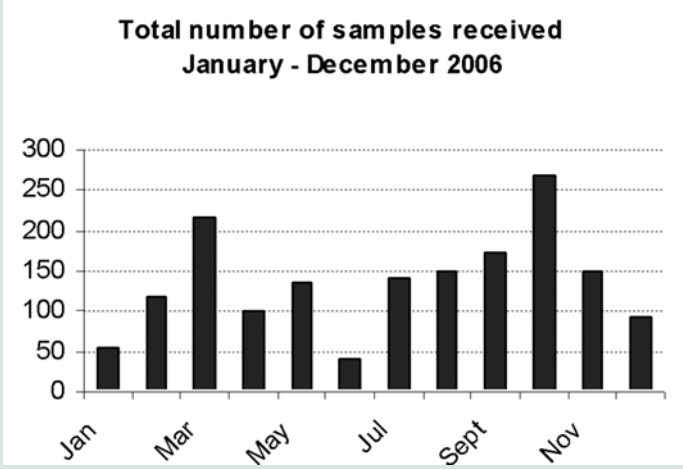
What is discussed on Treehealthnet?
News relevant to tree health issues, important announcements, feedback about current research efforts and field work. Field trips are announced in advance, enabling foresters, forest managers and other stakeholders to request visits from TPCP and CTHB researchers.

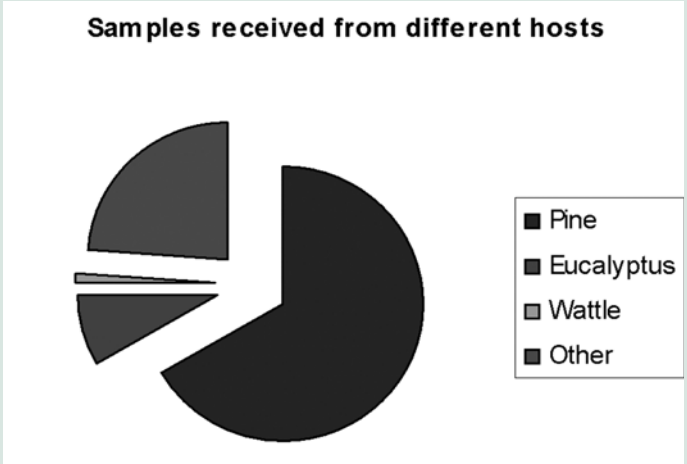
About subscription
The listserver is SPAM secure and every effort is made not to overburden subscribers. It is also open only to those who have subscribed and it is possible to unsubscribe as easily as it is to subscribe.

How does one subcribe?
Send an e-mail to the list manager, Wilhelm de Beer, at wilhelm.debeer@fabi.up.ac.za, requesting subscription to Treehealthnet.

TPCP/CTHB DIAGNOSTIC CLINIC

The past year has been a very busy one for the TPCP/CTHB Diagnostic Clinic. 1635 samples were processed, of these 67% were *Pinus* samples and 8% were *Eucalyptus*. Other samples, which included Petri dishes with mycelial growth for *Fusarium circinatum* confirmation, insect, water, soil and seed samples, as well as samples from non-forestry trees, made up 24% of the samples received. Black wattle samples made up the remaining 1% of samples processed.





The majority (94%) of pine samples received were *Fusarium circinatum* related. These included asymptomatic samples from nurseries for *Fusarium* screening as well as diseased material for diagnoses. Of these samples, 42% tested positive for *Fusarium circinatum*.

Although *P. radiata* and *P. patula* were found to be the most susceptible Pinus species, we have noticed an increase in incidence of the pathogen on more tolerant species like *P. elliottii* and *P. taeda*.

The fact that almost half of all pine samples received tested positive for *F. circinatum* is worrying, especially since the first report of Pitch canker disease on mature trees was reported for the first time in South Africa in May 2006.

The disease clinic also saw an increase in the number of *Diplodia pinea* samples. Diplodia *pinea* is a stress associated opportunistic pathogen that occurs as an endophyte. Infection occurs following stress conditions such as hail, frost or high temperatures. The majority of samples were from the Mpumalanga province with disease symptoms becoming apparent after severe cold and frost conditions were experienced in various forestry regions of the province. Off-site planting, poor planting techniques and localized soil conditions were also found to be involved in disease development.

The diagnostic clinic is currently managed by Izette Greyling, with the assistance of Jolanda Roux. In 2006, Izette was assisted by Kgosi Mongwaketsi in the molecular identification of *F. circinatum*, and by Bianca Hinze and Happy Maleme in the general running of the clinic. Each year, however, we try to expose new students to forestry diseases and have thus appointed new students in the clinic for 2007. They are Marcele Vermeulen, Guillermo Perez and Boithumelo Mashangoane. We would like to take this opportunity to thank the outgoing clinic members and to welcome the new ones.

If you have any questions regarding possible diseases or if you would like to send samples to the clinic please contact:
Izette Greyling izette.greyling@fabi.up.ac.za or
Jolanda Rouxjolanda.roux@fabi.up.ac.za

This would enable us to offer advice on what samples to send as well as the best way to package and send the samples. We would also be able to discuss the problem in more detail and determine if a site visit is necessary. Please also join our online tree health forum, Treehealthnet, for regular updates on pests and diseases, fieldtrip advertisements and other issues related to tree health. You can also find more information of sample submission on our website (http://www.fabinet.up.ac.za/tpcp/contact_info).

The 2007 diagnostic team assisting Izette and Jolanda.
Boithumelo Mashangoane, Guillermo Perez and Marcele Vermeulen

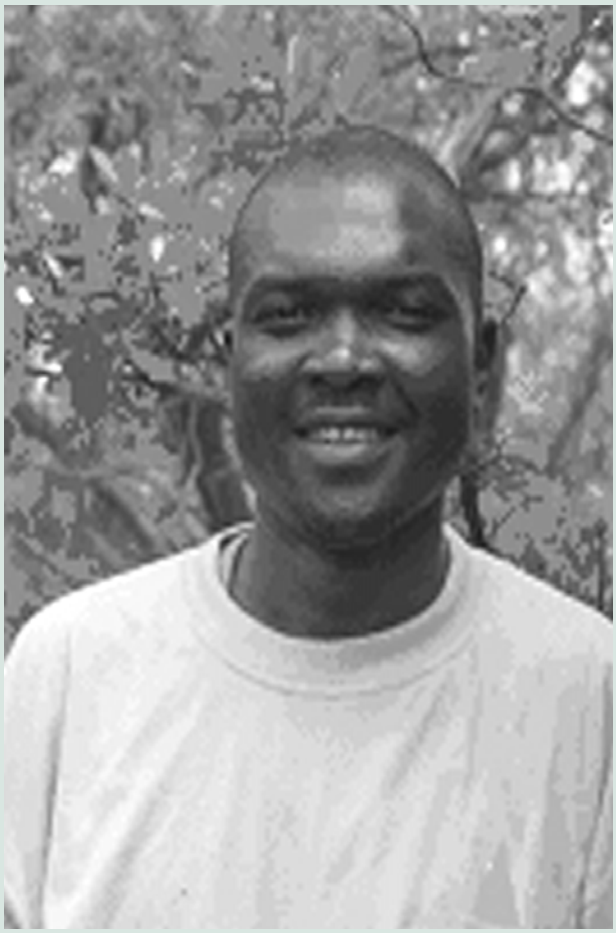
WELCOME TO THE TPCP AND CTHB

A number of new people have joined the research programmes of the TPCP and CTHB in the last few months. We would like to welcome you all to our family and trust that you will enjoy your experiences with us.

Didier Begoude is a Cameroonian who has joined the CTHB for his Ph.D. Didier will be working on the diseases of *Terminalia* species in Africa.

He holds a degree in Agriculture from the Faculty of Agronomy and Agricultural Sciences at the University of Dschang in Cameroon and a masters degree in tropical and subtropical crop protection from the University of Gembloux (Belgium).

Before starting his Ph.D, Didier started working for the Cameroonian Agricultural Research Institute on biological control of cocao black pod disease caused by *Phytophthora megakarya.*



Guillermo Perez, from Uruguay, joined the TPCP in 2007 to start his Ph.D. which will deal with *Mycosphaerella* leaf diseases of *Eucalyptus* spp. in South Africa and Uruguay. Guillermo obtained his B.Sc. degree (2004) from the Facultad de Ciencias, Universidad de la República, Uruguay, working on wood decay fungi on *Eucalyptus*. Thereafter he obtained a M.Sc. degree focused on the population biology of *Inocutis jamaicensis* a basidiomycete fungus that produces heartwood decay in standing trees, especially in *E. globulus* plantations in Uruguay, causing important economic losses.

Riikka Linnakoski is doing four months of her Ph.D.work at the TPCP as part of the collaborative project between the University of Joensuu (Finland) and FABI.The focus of her research is the bark beetle associated fungi in the boreal forests of Finland and Russia. She is doing her PhD in the faculty of Forestry (University of Ecosystems).

Jane Njuguna is a Kenyan, currently busy with her Ph.D. degree at the Swedish University of Agricultural Sciences in Upsala. She is working on "The causes, impacts and epidemiology of stem cankers on five tree species in Kenya" and will be spending three months in FABI to learn more about forestry diseases and do some phylogenetic analyses on the Botryosphaeriaceae that she collected from diseased trees.

THE RESEARCH TEAMS OF THE TREE PROTECTION CO-OPERATIVE PROGRAMME AND DST/NRF CENTRE OF EXCELLENCE 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.

Contact numbers and web address:
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Fax.: 012 420 3960
http://www.fabinet.up.ac.za

In order for us to coordinate services to you please help us by using the following contact address:
FABI, Lunnon Rd, University of Pretoria, Hillcrest, Pretoria Gauteng.

Postal Address
Tree Protection Co-operative Programme (TPCP)
Att: Prof. Mike Wingfield, FABI, University of Pretoria
Pretoria, 0002

Abstracts of presentations at the 45th Congress of the Southern African Society for Plant Pathology, January 2007,Benoni

Identification of *Ceratocystis* species isolated from hardwood trees in Namibia, Australia and Norway

Nicolette Fouche, J. Roux, H. Solheim, R.N. Heath, G. Kamgan Nkuekam, P. Chimwamurombe, G.S. Pegg & M.J. Wingfield

Fungi in the genus *Ceratocystis* include some of the most serious tree and herbaceous plant pathogens known to science. Knowledge pertaining to the species diversity and impact of *Ceratocystis* spp. on hardwood tree species is, however, limited in many countries. This is especially true for Africa, Australia and Scandinavian countries. In recent years, reports of *Ceratocystis* spp. causing diseases on hardwood trees in forestry plantations have increased dramatically. *Ceratocystis fimbriata* sensu lato has been reported on *Eucalyptus* in South America and Africa, while *C. albifundus* is considered the most important pathogen of commercially grown *Acacia mearnsii* trees in South Africa. *Ceratocystis pirilliformis*, is a relatively newly described species found on *Eucalyptus* trees in Australia and South Africa, which has been shown to be pathogenic in field tests. As part of collaborative research projects including Australia, Namibia, South Africa and Norway, surveys were conducted for *Ceratocystis* spp., aimed at collecting new species and to consider their potential to cause disease. Samples were collected from mechanical and harvesting wounds on several hardwood tree species, as well as from trees on which sap stain was evident. Isolates of *Ceratocystis* spp. were identified based on morphology and DNA sequence comparisons for various gene regions. Sequence data for the ITS, Beta-tubulin and Elongation factor 1-a gene regions were used and comparisons made via phylogenetic analyses. Pathogenicity trials were conducted with some of the collected *Ceratocystis* spp. to determine their potential to cause disease. *Ceratocystis* spp. collected during this study were identified from *Populus tremulae* trees in Norway and from *Eucalyptus* spp. in Australia and Namibia. A new species, most closely related to *Ceratocystis savanae prov. Nom.,* recently described from native South African trees, is reported for the first time from *E. camaldulensis* near Rhundu in Namibia. Greenhouse inoculations gave rise to very small lesions suggesting that it is not pathogenic on *E. camaldulensis*. The surveys near Brisbane Australia showed the presence of *C. moniliformi*s, *C. pirilliformis* as well as an apparently undescribed species. Several *Thielaviopsis* isolates were collected from harvesting and artificial wounds in Norway. These isolates are most closely related to *Thielaviopsis thielavioides*. A potential pathogen of *Populus tremulae* was also collected from stains in the xylem of stumps and logs in Norway. This *Ceratocystis* sp. is also new to science and is currently being described. In inoculation trials on young *P. tremulae* trees in Norway it produced relatively large lesions, suggesting that it might be an important pathogen. Results of this study clearly emphasize the fact that many undescribed and potentially pathogenic species of *Ceratocystis* remain to be discovered and they also contribute to the understanding of this important group of fungi.

First report of the tree pathogen *Chrysoporthe austroafricana* from Namibia

M. Vermeulen, J. Roux, M. Gryzenhout, P. Chimwamurumbe & M.J. Wingfield

Chrysoporthe cubensis and *Chr. austroafricana* are important canker pathogens of commercially grown *Eucalyptus* spp. in tropical and sub-tropical parts of the world. *Chr. austroafricana* is known only from Africa, where it infects non-native *Eucalyptus* spp. and *Tibouchina* spp. as well as native *Syzygium* spp. *Chr. cubensis*, has been reported from South America, Asia, Australia and Africa. In Africa, it is known only to infect non-native *Eucalyptus* spp. and *S. aromaticum*. The origin of both *Chr. cubensis* and *Chr. austroafricana* is unknown. It is believed that *Chr. cubensis* was introduced into Africa, as it has not been found on native plants on this continent. *Chr. austroafricana* is thought to have a centre of origin in Africa because it has a highly diverse population in the region and has been found on native African trees. The geographical distribution and host range of *Chrysoporthe* spp. in Africa is, however, incompletely understood. The aim of this study was to survey native and non-native Myrtales in the Caprivi region of Namibia for the presence of *Chrysoporthe* spp. A further aim was to determine the virulence of the collected fungi and to analyze the population diversity of isolates collected. Isolates were identified based on morphology and DNA sequence comparisons after having been assigned to morphologically similar groups. For DNA sequence comparisons, the internal transcribed spacer regions (ITS1, ITS2) and the conserved 5.8S gene of the ribosomal RNA (rDNA) operon and the â-tubulin 1 and 2 regions of the â-tubulin gene were used. Preliminary consideration of the population diversity of isolates was achieved using vegetative compatibility group's (VCGs). Use was made of Stoddart and Taylor's genotypic diversity and Shannon index to determine the Genotypic and Phenotypic diversity of the population. Only two species of Myrtales, non-native *Eucalyptus* sp. and a native *S. guineense* were encountered in the survey. No symptoms of *Chrysoporthe* infection were found on the *Eucalyptus* sp., however, *Chrysoporthe* infection was common on the *S. guineense*. *Chrysoporthe* infections were found on the stems and branches of *S. guineense* at Popa Falls and on the above ground roots of *S. guineense* at Katima Mulilo. DNA sequence comparisons and morphological observations revealed that *Chr. austroafricana* was present on the samples. Results of the VCG tests on the isolates showed that *Chr. austroafricana* in Namibia has a high genotypic diversity. This is the first report of any fungus in the genus from Namibia. The presence of *Chr. austroafricana* on native trees and the absence on non-native trees, as well as the high VCG diversity in Namibia further supports the hypothesis that *Chr. austroafricana* is native to Africa.

Identification and characterization of Botryosphaeriaceae associated with native Marula trees in South Africa

B. Hinze, B. Slippers, D. Pavlic and M.J. Wingfield

Sclerocarya birrea subsp. *caffra* (Marula) belongs to the family Anacardiaceae and is native to South Africa. The Marula tree is a dominant species in indigenous plant populations and is also economically important, especially in rural communities. Little is known regarding the diseases of Marula or even the fungi that occur on these trees. Some fungal groups such as the

Botryosphaeriaceae are, however, well-known on Mango, which is a non-native relative of Marula. Botryosphaeriaceae are common endophytes and latent pathogens of many woody hosts and they provide a useful model to explore the potential ecology and evolution of pathogens present on related native and introduced tree species in South Africa. The objective of this study was, therefore, to identify and characterize Botryosphaeriaceae on Marula. Trees were sampled in north eastern parts of Limpopo, Mpumalanga and Zululand. Botryosphaeriaceae were isolated from asymptomatic and diseased leaves and twigs. Seventy-five isolates were obtained, of which most were from asymptomatic tissue. Isolates were induced to sporulate in culture and grouped based on conidial morphology. ITS and EF regions of representatives of each morphological group were sequenced. MSP-PCR was used to distinguish groups of isolates with fusoid to ellipsoid conidia. Based on morphology and DNA sequence comparisons, isolates could be grouped in the genera *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*. The *Neofusicoccum* isolates, representing at least two undescribed species, were the most abundant and widely distributed. These two species were morphologically indistinguishable. These species were isolated from Marula in the northern and eastern tropical regions of South Africa and are most likely host specific. *Lasiodiplodia gonubiensis,* which was recently described on native *Syzigium cordatum,* was isolated from Marula in Zululand. This species is also thought to be native to Southern Africa. *Lasiodiplodia theobromae* was also identified from Marula in this survey, and it is the only species known to also occur on non-native Mango. This illustrates a stronger influence of the environment (sub-tropical) on host infection by *L. theobromae* than the host itself. Future work will entail a more detailed isolation and comparison of Botryosphaeriaceae from 'domesticated' Mango and the 'Wild Marula, in areas where they grow in close proximity.

Fusarium species in the *Gibberella fujikuroi* complex associated with *Syzygium cordatum* floral malformation

M. Kvas, E.T. Steenkamp, B.D. Wingfield, W.E.O. Marasas and M.J. Wingfield

Waterberry (*Syzygium cordatum*) is a tree native to Southern Africa that is commonly found near streams, forest margins and in swampy areas. Throughout its geographic range, trees suffer from a type of floral malformation. The affected inflorescences are abnormally enlarged, excessively branched and do not bear fruit. Similar symptoms have been observed on mango (*Mangifera indica*) where the disease is associated with a number of *Fusarium* species in the *Gibberella fujikuroi* complex (GFC). The aim of this study was to identify the *Fusarium* species, especially those in the GFC, associated with malformed waterberry inflorescences. *Fusarium* isolates were obtained from symptomatic and asymptomatic flowers collected in different regions of South Africa. The isolates were identified using morphological characteristics and DNA-sequence comparisons. For the morphological identifications, fungi were examined microscopically and characteristics such as the spatial arrangement of microconidia shape and size of macro- and microconidia and presence of mono-, polyphialides and chlamydospores, were evaluated. For the DNA-based identifications, isolates were characterised using restriction fragment length polymorphism (RFLP) analysis of the ribosomal RNA intergenic spacer (IGS) region. The first ~700 and ~500 bases of the genes encoding translation elongation factor 1a (EF1a) and b-tubulin, respectively, were then sequenced for a representative of each unique IGS-RFLP-type. The resulting EF1a sequences were compared to those in the *Fusarium* identification database (http://fusarium.cbio.psu.edu/) using the basic local alignment search tool (BLAST). The EF1a?and b-tubulin sequences were also subjected to phylogenetic analyses. The results indicated that the majority of *Fusarium* species associated with malformed waterberry inflorescences represent saprophytic species such as *F. oxysporum, F. equiseti, F. solani, F. pallidoroseum*. Several isolates that are closely related to known GFC species such as *F. sacchari, F. proliferatum, F. udum* and species that appear to be undescribed were also found. An unusually large number of *Fusarium* species are associated with waterberry floral malformation and the relationship between these fungi, as well as their role in the development of the disease remains to be determined.

Identification of *Botryosphaeriaceae* from *Eucalyptus* planted to feed Koala bears at the Pretoria Zoo

H. Maleme, D. Pavlic, B. Slippers, B. D. Wingfield, M. J. Wingfield

The *Botryosphaeriaceae* are endophytes and latent pathogens of numerous woody hosts, including *Eucalyptus*. These fungi cause canker and die-back diseases in exotic *Eucalyptus* plantations in South Africa. Such disease symptoms were observed on the *Eucalyptus* trees grown in Pretoria for feeding Koala bears at the Pretoria Zoo. The aim of this study was to identify and characterise the *Botryosphaeriaceae* from 20 *Eucalyptus* species maintained by the Pretoria Zoo. Isolations were made from diseased and asymptomatic twigs and leaves. All isolates resembling *Botryosphaeriaceae* were induced to sporulate in culture. Four groups of isolates were distinguished based on anamorph morphology and their identity was confirmed using comparison of ITS rDNA and translation elongation factor1-á (EF- 1a) gene regions sequence data with known *Botryosphaeriaceae*. PCR- RFLP technique was also used for identifying isolates in the *N. parvum* and *N. ribis* complex, using Primers BOT 15/16 and RE *HIha*. Isolates that are difficult to induce in culture were also distinguished with this technique. Four species of *Botryosphaeriaceae* were identified, and that include *N .parvum, N. australe*, *B. dothidea* and an undescribed species. These species were identified mostly from leaves of 17 different *Eucalyptus* spp., except on *E. coniocalyx*, *E. uiminalis* and *E. obliguao.* No host specificity was, however, evident amongst the species of *Botryosphaeriaceae* for specific *Eucalyptus* species.