

## tree pathology

NEWSLETTER OF THE TREE PATHOLOGY COOPERATIVE PROGRAMME UNIVERSITY OF PRETORIA news

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#### CONTENTS

From	th	e D	irec	tors
desk.				

#### SASPP Congress....2

#### FABI opening and Annual TPCP Meeting.......3

#### SASPP Congress abstracts......5

### DNA fingerprinting in the new millennium......7

#### Fingerprinting congress abstracts..8

Field	d	aı	75	a	n	đ					
										8	
lectu	re	92					8		8	Q	æ

#### Diagnostic clinic...10

TPCP develops links with W. Australia 11

#### TPCP graduandi....11

Visitors to the TPCP from abroad.......12

#### Farewell.....12

#### Mycosphaerella leaf blotch disease.....13

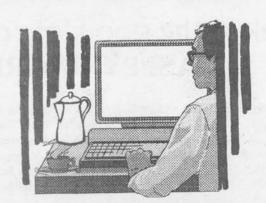
#### Symposium on molecular genetics of Eucalyptus......14

Forest entomology		
and the TPCP	5	į

## FROM THE DIRECTOR'S DESK

sit and write this note from the corner of my office, which some of you will know, has walls built from the most beautiful blue-stained pine logs. I have to wonder whether the producers of this product realise that the beauty of these logs is due to the activities of some of the most fascinating fungi known to us. Sadly also a group of fungi that kill living trees and reduce the value of saw timber. So we again have the situation of "one person's meat....".

A few weeks back, many readers of Tree Pathology News will have attended the annual meetings of the Pathology Tree operative Programme (TPCP) which was held alongside the inauguration the Forestry Agricultural Biotechnology Institute that now houses the TPCP. For the TPCP team and the FABI family. organising this event that was attended by about 200 people, was a major undertaking. Three weeks later, we are only starting to come to the end of the process of tying up the loose ends. As part of this process, we would like to thank all those that were able to share these two special days with us. We have also received so many



letters of appreciation that it is wholly impossible to respond to each of these individually – but we do want our forestry friends to know that these sentiments are sincerely appreciated.

Tree Pathology News and ICFR newsletter.....

When you received this copy of Tree Pathology News, you might have been surprised to find that it arrived in the same package as your ICFR newsletter. Indeed, our biennial newsletter will in future always be dispatched together with the ICFR newsletter, and the TPCP will contribute to the remaining ICFR newsletters. This is part of a new initiative in which we will strive to bring the TPCP and ICFR closer to each other. The ICFR has been a member of the TPCP since the formal initiation of the cooperative programme in 1990, so the two groups have always worked in close association. However, opportunities to expand this relationship and particularly to establish joint research ventures are great. Together with Prof. Colin Dyer, the new director of the ICFR, we will explore a wide range of routes to exploit these opportunities in coming months and years.

In this newsletter, you will read of various activities of the TPCP during the last six months. For a group as large as the TPCP, there is always a great deal of news to share with our members. We, however, also do not wish Tree Pathology News to be overly long. We have thus chosen a "sampling" of news items that relate to all aspects of the TPCP. I hope that you will enjoy this selection, which ranges from social events to various research initiatives.

Visit our Web page ......

One of the more colourful routes to explore the activities of the TPCP is by visiting our Web pages. During the course of the last

six months, these have been totally revamped by the most capable Albe (Meteorite) van der Merwe. Albe is also known for his fascinating research on the genetics of the Cryphonectria canker pathogen – clearly a person of many talents. The TPCP Web pages are now "housed" alongside the Web pages of FABI, which you might also find interesting. You can now see our newsletter, the diagnostic pamphlets, the 'faces" of most of the team members, and many other interesting components of this unique programme.

New forest entomologist......

Many readers of Tree Pathology News will be aware that Prem Govender, forest entomologist from the ICFR, has recently joined FABI. Prem is now closely linked with the TPCP team and our intention is that he will provide Forest Entomology input into the Programme. Although the focus has tended to be on interactions between forest insects and pathogens, various TPCP team

members have worked in the field of forest entomology for many years. Prem's arrival will not only consolidate but also expand these activities. The ultimate aim is to build a much stronger base in forest entomology in South Africa, particularly through the training of students, and thus to ensure long term stability of this very important field. To accommodate this new development, we envisage a name change, which would replace the "PATHOLOGY" in TPCP with "PROTECTION". The scope of the Programme would then be to provide the forestry industry with support in the fields of both pathology and entomology.

I hope that you will enjoy this issue of Tree Pathology News. Please let us have your comments on improvements that you feel we might make to this informal line of communication. Ultimately we focus on a single aim encompassed in our slogan, which also serves as our Mission Statement "KEEPING TREES HEALTHY".

#### Back to the good 'ole days-SASPP CONGRESS 1999

In the heart of the Natal midlands, a congress was held, the 37<sup>th</sup> congress of the Southern African Society of Plant Pathology (SASPP), to which a party of enthusiastic Fabians went. Last year, we marched in our masses to Edinburgh to expand our knowledge during the International Congress of Plant Pathology (ICPP) '98. This time the march was closer to home, to "Maritzburg", where we were treated to warm

Sharks hospitality (and much humidity and thunderstorms). Much knowledge was acquired, many a new acquaintance was made, and much mirth and laughter was exchanged during this annual event.

The FABI team presented excellent posters and talks on their particular line of interest, and much was learnt from other fields of interest. We were treated by even more excellent talks by prominent key note speakers, such as

Dr. Mary Palm on quarantine measurements in the USA, Prof. Gerry Adams on Cytospora canker, Dr. David Guest on Phytophthora canker, Dr. S.V. Thomson on control strategies for fire blight, and more about endophytes, by Dr. Dennis Wilson. More pay-offs were the release of a new Global Plant & Pest Information System (GPPIS) for the detection and identification of most of the plant diseases and symptoms known throughout the world (which will prove valuable in the disease

clinic). We were also given a scintillating and thoughtprovoking talk by Brian Garman about science, the public and the press (or rather science, mysterious to the public, unpopular with the press, and unwilling to mix with both these elements).

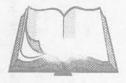
However, all things intellectual had to be relieved by things more ...well...worldly, and we had to do strange things. The Mildenhall stakes turned out to be backbreaking work (as always). (For those of you unfamiliar with this strange

exercise, start thinking about how to get a bottle of beer from your forehead to your mouth without using your hands. This usually involves an unusual tilting of the head, extensive strain on the lower back and legs, no sense of balance and no clue as to where up or down is. Prizes will be given at the next TPCP meeting for the most innovative contestant from the forestry sector. It usually helps to have a few beers in already to figure out how to do this). The last night we were also treated with a sumptuous meal, and could become really naughty and giggly because everyone had to wear his/her school or university blazer.

As is the case in many schools, there had to be a function for the distribution of prizes. Most noteworthy, was that Jolanda Roux has yet again won the woman's division of the renowned Mildenhall stakes. FABI also had the most contestants and won the overall competition. But even more to our fame, Mike won yet another award, the prestigious Persoon medal, for all of his achievements. We really are proud of our own Dux scholars. What an ending to a very enjoyable contents.



As soil is the material substrate of life, water is literally its essence. Water is life. From "Out of the earth – civilization and the life of the soil" by Daniel Hillel





A first and a last.....the first official TPCP meeting at the Forestry and Agricultural Biotechnology Institute (FABI), our exciting new home at the University of Pretoria, but also the last meeting this millennium. Nowadays we are bombarded with so many "lasts of this millennium/first of the next", that it is fast becoming a hollow cliché. But maybe in this case it is fitting. The move to Pretoria and all the initial growth pains are now behind us. And ahead of us, the challenges and excitements now lay waiting in a new millennium.

This year the Annual TPCP Meeting was combined with the official opening of FABI. And what an auspicious occasion. But hard work it was for sure. For weeks before, many of us often had very little sleep and a couple of good workouts. The big day itself started At 6, things were already buzzing around Picking up the waste around the building.

This year the Annual TPCP Meeting was combined with the start of the property of the

the last decorations into place, testing the projection system one

last time, etc.

Around 8:30 the ~ 170 delegates attending the opening were all seated in the beautiful Sanlam Auditorium. The delegates included friends, old and new; colleagues from other academic institutions in South Africa and abroad; representatives of funding organizations.

"You are
present at the
coming of age
of a rather
sturdy yout",
whose influence
is going to be
increasingly felt
in the future"

agricultural and forestry industries, as well as the government. Wilhelm de Beer, the master of ceremonies, had the honour of introducing some very important

speakers. The first of these was the Dean of the Faculty of Agricultural and Biological Sciences, Prof. Robin Crewe, who welcomed the delegates and gave a very brief overview of the

history of the idea that, today, is FABI. "You are present at the coming of age of a rather sturdy youth, whose influence is going to be increasingly felt in the future." Prof. Johan van Zyl, the Vicerector and Principal of the University (also the father of the fabulous FABI idea) did some more welcoming and gave us a background as to the philosophy behind the programmes of excellence at the UP, of which FABI is a proud example. "It

"Basic research must come first in order to solve problems of agricultural importance...."

excites us at the UP to see what can be achieved when everybody works together towards this dream...of excellence." Next was Mike's turn as Director of FABI to introduce Fabulous FABI. And as always, the passion and excitement that is Mike (and FABI) was clearly visible (and not to badly contained in his 30 minutes either) as he explained what FABI is, what it looks like, how it grew and why it is so important for the future of Agriculture and Forestry. "...the fears and the opportunities, the excitements and the problems...ahead of us there is 100 years of fighting, arguing and a total reformation of what agriculture is all about, and that is what FABI has to be part of." Prof. Hauke Hennecke from the ETHZ in Switzerland (no top-secret documents involved) concluded the first session of the morning with a more formal scientific lecture about beneficial plant microbe symbiosis. From this it was clear that basic research is crucially important in plant biotechnological research. "Basic research must come first in order to solve problems of agricultural importance, providing short-term and long-term

benefits to the industry....I am, therefore, pleased to see

that thi



Prof. Mike Wingfield and Prof. Johan van Zyl at the opening of FABI

philosophy is applied and a specific objective of FABI." The session was then fittingly concluded by a very popular musical interlude.

Mr. Neville Denison, Manager of Tree Improvement Mouding Forests tried to recall the music team to take over his allocated time, but when this failed, continued to entertain us himself with

the history and role of the TPCP in South African Forestry. He also gave honour to the vision and passion of Mike, his team and all the industry role players that worked together to make this programme the most successful of it's kind in the world. "The perpetuation of the tremendously productive industry-academia alliance...is pivotal to the SA position in the forefront of forest pathology....TPCP = True Partnership Commitment and Passion"



One could not help to want more after the fascinating 45 minutes that followed as Prof. Callie Pistorius, Codirector of the Institute for Technological Innovation of the UP explained 'the dynamics of technological change' and the urgent need to manage innovation (= Invention + exploitation) to stay ahead in today's

foundations for our

and

highly competitive world. "Companies and industries will rise and fall by the ability of their managers to manage the wave of creative destructive that is innovation." This talk was followed by the fabulous 'Inside FABI' video. This excellent and very professional documentary of the doings and happenings inside FABI was put together by Dr. Dennis Wilson, currently a postdoctoral fellow in FABI. The video was thoroughly enjoyed by everyone and is sure to be a very valuable asset for many years to come, and perhaps a wonderful look into the 'old' FABI somewhere deep into the next millennium.

After lunch, the delegates were invited by the programme to "spend a lively afternoon exploring "living" laboratories, discussing results with students and staff, being baffled by biotechnology, come face to face with fungi and discover a hidden world that forms

future forests food." That was the intention and was very

Kime (CTC) and Rodney Hearne (Banana Growers Association). Mike also announced that five of the labs will be named in honour of the five people who helped to establish the TPCP, namely Mr. John Tew (HL&H), Mr. Neville Denison (Mondi), Mr. Neville Wessels (SAFCOL), Prof. Piet Lategan (UOVS) and Dr. Mike Shaw (Sappi). As usual a wonderful dinner closed the day (for some). Apart from the lively atmosphere and interaction amongst delegates that always characterizes these dinners, we also had the privilege to listen to Dr. Viktor Hesse (Chairman of the Board of the UP), to give thanks to the people involved in preparing the day and lastly to be entertained by all the boo-boos-\$#\*&^%!& captured during the filming of the "Inside FABI" video. As I said, the day ended there for some, but others (it is being said) from there went to FABI to finish the last of the wine

and some excess energy at the beat of ....how should I know? Annual Meeting of the TPCP the next day followed the opening FABI. programme was shortened compared previous years due to shortened time, but nevertheless filled to the brim exiting with developments and results. Mike (TPCP). Brenda (FMBC) and Teresa (Diagnostic Clinic) gave reviews of the year that had past and some insights into what is an exiting next year. Prem Govender, who joined the team in January, gave summary of what is

planned

for entomology at the TPCP and the importance of entomology to the health of our plantations. There were also presentations

from visiting international

researchers, Prof. Gerard

Adams and Aaron Maxwell,

clearly

again

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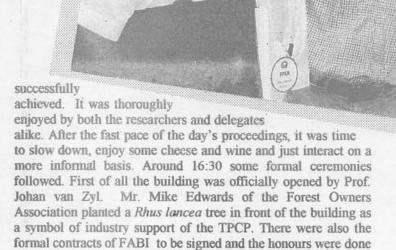
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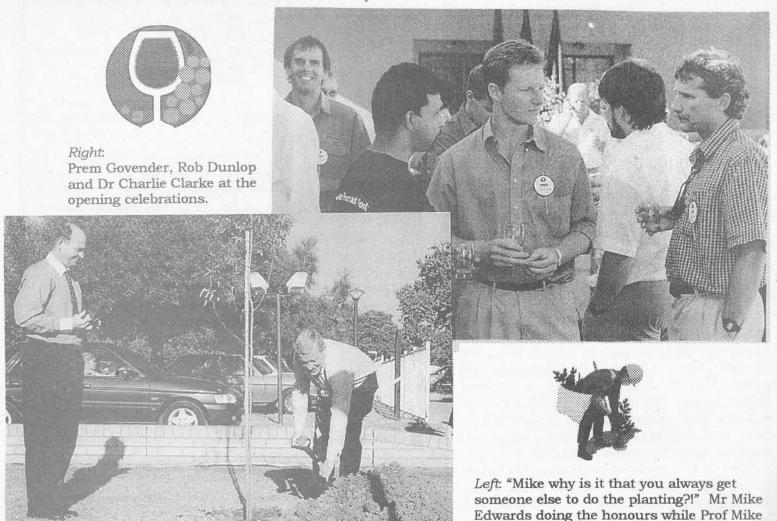
the importance of and need demonstrated for international collaboration regarding research on plant pathogens. From the students side there were project reports on blue stain (Wilhelm de Beer), Armillaria root rot (Martin Coetzee), the Amylostereum symbiont of the Sirex wasp (Bernard Slippers). Ceratocystis wilt of Acacia mearnsii and eucalypts (Jolanda Roux), Cryphonectria canker (Schalk van Heerden),

that



by Dr. Andrew Morris (Sappi), Waldo Hinze (Safcol), Dr. Themba Simelane (DWAF), Andrew Thompson (Mondi), Patrick biological control of Sphaeropsis (Oliver Preisig) and Endothia canker (Marieke Venter).

An exciting and productive two days were ended with a meeting of the TPCP board. We would like to thank all our industry colleagues and friends who shared these two days with us. Thank you for your support and input, without this co-operation from all parties this programme would not be what it is today. TRUE PARTNERSHIP, COMMITMENT AND PASSION from all involved.



#### RELATIVE SUSCEPTIBILITY OF NORTHERN AND SOUTHERN PROVENENCES OF *PINUS GREGGII* TO POST-HAIL ASSOCIATED *SPHAEROPSIS SAPINEA* DIE-

Wingfield looks on.

#### BACK

H.Smith, M.J. Wingfield, T.A. Coutinho & P.W. Crous

Sphaeropsis sapinea is a notorious post hail-associated die-back pathogen of Pinus spp., in South Africa. The search for pines able to grown vigorously on marginal sites has recently focussed on Pinus greggii, which is a closed cone pine and indigenous to Mexico. The Central American and Mexico Coniferous Resources Co-operative (CAMCORE) has, therefore, established provenance trials of this pine species in Brazil, Colombia and South Africa. Sixty-five families of both the southern and northern provenances of P. greggii were evaluated for their tolerance to infection and subsequent die-back caused by S. sapinea. Families were evaluated for tolerance following natural infection after hail damage, as well as based on artificial inoculations. Southern provenances of P. greggii were considerable more susceptible to infection by S. sapinea than were northern provenances of this species. Considerable potential thus exists to capitalize on these disease tolerant trees in future breeding programmes.

#### TAXONOMY AND PHYLOGENY OF THE GENUS AMYLOSTEREUM

B. Slippers, M.J. Wingfield, B.D. Wingfield & T.A.

Coutinho

The genus Amylostereum is best known for its mutualistic association with the Siricidae, a family of wood wasp that has a wood boring larval state. The study of this Basidiomycete is complicated by the rarity, or absence in some regions, of the fruiting structures. Furthermore, the morphological characteristics, especially in culture, are similar the different between species. Many misidentifications have resulted due to these problems. The influence of convergent evolution also makes these morphological characters unsuitable to deduce phylogenetic relationships. In this study, the phylogeny of the genus was investigated using sequence analysis of the mt-SSU-rDNA and nuc-IGS-rDNA of 20 isolates representing all four species of Amylostereum. The mt-SSU-rDNA sequences of the genus were also compared to the related sequence of 87 other Basidiomycetes obtained from TREEBASE. The sequence data showed that A ferreum and A. laevigatum are most closely related to each other. A. chailletii was also closely related to these two species, while A. areolatum was the most distantly related and clearly defined species in the genus. Phylogenetically, the genus Amylostereum was more closely related to Echinodontium tinctorium than to any other Basidiomycete included in the analysis. One isolate from Sirex areolatus in the USA did not cluster with any of the known species of Amylostereum. It did. however, clearly cluster within the genus Amylostereum and might represent a new species. Sequence and RFLP analyses of the nuc-IGS-rDNA region showed that this region contained paralogous sequences in A. areolatum. In this study we thus successfully tested previously unanswered taxonomic and phylogenetic hypotheses concerning the genus Amylostereum.



When conductor Leonard Bernstein took the New York Philharmonic on tour, he found this note under his hotel-room door one night: "I think you should know that the fellow in your band who plays the instrument that pulls in and out only bothered playing during the odd moments you were looking straight at him"

#### MOLECULAR RELATEDNESS OF GEOGRAPHICALLY DIVERSE ISOLATES OF CONIOTHYRIUM ZULUENSE FROM SOUTH AFRICA AND THAILAND

L.M van Zyl, M.J. Wingfield, T.A. Coutinho, B.D. Wingfield and K. Pongpanich.

Conjothyrium canker caused by Conjothyrium zuluense Wingfield, Crous & Coutinho is a serious stem canker disease of various Eucalyptus spp. in sub-tropical parts of South Africa. A Conjothyrium sp. associated with similar symptoms on E. camaldulensis was observed for the first time in Thailand in 1996. It was previously thought that C. zuluense is native to South Africa. The aim of this study was, thus, to compare South African isolates of C. zuluense, together with isolates of the Contothyrium sp. from Thailand using DNA sequence data. The variable ITS1 and ITS4, as well as, conserved 5.8S gene of the rRNA operon were amplified. Products were then sequenced and the DNA sequences compared. Analysis of the data showed that all Coniothyrium isolates associated with stem cankers on Eucalyptus spp. grouped together in a single major clade. This clade was clearly distant from isolates of other Coniothyrium spp included for comparative purposes. Although fungi from South Africa and Thailand could be distinguished from each other, we believe that the latter isolates represent C. zuluense. This would be a first record of C. zuluense outside of South Africa

#### MOLECULAR CLONING OF THE PGIP GENE OF EUCALYPTUS GRANDIS

Chimwamurombe PM, Wingfield BD, Oberholster A-M, and Wingfield MJ

Fungal polygalacturonases (PGs) have been implicated as one of the important factors linked to infection of plants by Polygalacturonase-inhibiting proteins (PGIPs) inhibit these PGs specifically. In this process, they have a role in halting ingress of pathogens. PGIPs are also part of the plant defence protein super family of leucine-rich repeat proteins, which are important in signal transduction pathways in plant defence responses. The objective of this study was to clone the gene encoding for the PGIP in Eucalyptus grandis. Genomic DNA was isolated from young Eucalyptus grandis leaves and used as template in a degenerate PCR. A 909 bp fragment was cloned, sequenced and then used to probe a Eucalyptus grandis DNA genomic digest in a Southern blotting experiment to enable cloning of the full length PGIP gene showing the promoter and termination regions. The cloned PGIP sequence was translated to amino acid sequence and compared to other published sequences by CLUSTALW (EBI). Eucalyptus grandis PGIP falls within the leucine-rich repeat proteins and its biological importance must now be determined.

### dna fingerprinting in the new millennium

The year 1982 saw the light on the first paper published on the use of DNA fingerprinting.

Since then this technique has become increasingly popular to use in distinguishing and identifying organisms on DNA level. Today, the development of DNA fingerprinting techniques such as RFLPs (restriction fragment length polymorphisms), AFLPs (amplified fragment length polymorphisms) and SNPs (single nucleotide polymorphims) is considered as a highly advanced research area with applications relating to identification and biological relationships, ranging from humans to animals and plants to fossils.

This year the 5<sup>th</sup> International DNA Fingerprinting Conference was held in Port Elizabeth. Delegates included researchers at institutes and universities from Europe, Asia, United States of America and Africa. The TPCP was never before represented at an international fingerprinting conference, but since it was held in South Africa we had the opportunity to attend and present research results relevant to this field.

Sir Alec Jeffreys and co-workers on DNA fingerprinting.....

Sir Alec Jeffreys and co-workers were the first group to publish a paper in Nature on the use of DNA fingerprinting where they proved the identity of a young boy from Africa. The identity of the child was disputed after the mother immigrated to the UK and she later applied for citizenship for the boy. Sir Jeffreys was consulted to ascertain the mother-child relationship. It was possible to convince the court that the mother was indeed telling the truth. This paper revolutionized the whole of the scientific community and led to the publication of many hundreds of papers

with DNA fingerprinting applications. For this reason it was not surprising that Sir Alec Jeffreys was invited to address the attendees at the plenary opening lecture. At this lecture, he enthusiastically reported on human minisatellites, repeat DNA instability and meiotic recombination.

DNA fingerprinting is based on the detection of differences (polymorphisms) on the DNA level between organisms. Profiles are obtained from identified (known) and unknown samples. The differences and similarities observed are then used to reveal relationships between organisms or to determine the unknown organism's identity.

....impact in the human and animal research areas....

It was evident from the conference that DNA fingerprinting has its most significant impact in the human and animal research areas. It has thus far been used with great success by forensic scientists in the investigation of crimes relating to human

identification and biological relationship determination. We saw the presentation of talks where scientists used fingerprinting techniques to identify aeroplane crash victims, criminals in law suits, paternity tests and population studies. The technology could even be used on highly degraded (ancient) aDNA or DNA from very old samples.

Several talks and posters focused on the use of fingerprinting in the microbial, and specifically, fungal world. Examples of organisms of interest included Cryphonectria, Armillaria, Mycosphaerella, Verticillium and Phytophthora. The DNA fingerprints could be successfully used for analysis of genetic variability of populations, epidiomology, identification of specific

individuals, development of useful markers, and detection of meiotic recombination.

......the new kid on the block: microsatellite fingerprinting.......

The star of the show was definitely the new kid on the block: microsatellite fingerprinting. Microsatellites are dispersed repeats of a few DNA bases, that display a very high rate of change. This marker system can be used for the identification of specific alleles, enabling the TPCP and FMBC groups to study pedigrees of trees and phytopathogenic fungi. These high-resolution data is becoming increasingly important in disease management strategies vital to the survival of the South African Forestry Industry.

#### THE RESEARCH TEAM OF THE TREE PATHOLOGY CO-OPERATIVE PROGRAMME

DNA

fingerprinting is

based on the

detection of

differences

(polymorphisms)

on the DNA level

between

organisms.

The research team of the Tree Pathology Cooperative Programme is varied. It includes full time staff of the University of Pretoria (Prof M.J. Wingfield, Director and Mondi Professor, Prof. B. Wingfield, Dr. T.A.Coutinho, Dr. A. Viljoen and Dr. J. Roux), Rosemary Visser, Celeste Marais, Marveline Molema and Sonja de Beer, colleagues and students attached to other organisations such as the ICFR, technical assistants funded by the University or through membership fees and post graduate students who are mainly funded by the FRD. Staff from various Departments in the University provide advice and support where this is required.

# Genetic analysis of sexual recombination in Cryphonectria cubensis isolates from Colombia

N.A. van der Merwe, B.D. Wingfield and M.J. Wingfield

Cryphonectria cubensis is a fungal pathogen that causes a serious stem canker disease of Eucalyptus. It has a wide distribution and is a serious threat to forestry industries in tropical and subtropical countries. It has been suggested that C. cubensis from Brazil, Venezuela and

Indonesia is homothallic, based on the fact that perithecia contain ascospores that are not the product of sexual recombination. In this study, we reconsider reproduction in C. sexual cubensis using Colombian isolates of the fungus. Various techniques can be used to analyse the genetic variation in sexual spores in fungi. Fungal isolates that represent discrete genetic entities are cytoplasmically compatible. Thus, a common. approach is to identify Vegetative Compatibility Groups (VCGs) amongst isolates when attempting to determine whether sexual outcrossing has occurred prior to the production of perithecia. Single spore isolates originating from perithecia on Eucalyptus cankers in Colombia prepared and tested for vegetative

compatibility within each perithecium on two synthetic media Results of these tests were confirmed at the molecular level by using Randomly Amplified Microsatellite Sequences (RAMS). The latter approach was used because vegetative compatibility is a phenotypic trait governed by a number of codominant loci. Therefore, if isolates were of the same VCG, they would not necessarily represent the same genetic entity. Our results confirmed the fact that homothallism is common in C. cubensis. Thus, single ascospores from a single perithecium need not necessarily represent sexual outcrossing. We also found good evidence to show that sexual outcrossing is common in the larger population of the fungus.

# PCR/RFLPS as a quick diagnostic tool for Armillaria species from different parts of the world

M.P.A. Coetzee, B.D. Wingfield & M.J. Wingfield

Fungi belonging to the genus Armillaria (Basidiomycetes, Tricholomycetales) are considered to be amongst the most important plant pathogens. This fungus is cosmopolitan and causes root rot on a broad range

of hosts which includes both native and planted conifer and hardwood trees as well as shrubs in gardens and parks. Rapid identification of species within this genus is problematic since classification is based on the basidiocarps (mushrooms). These structures are only produced during certain times of the year under specific environmental conditions. In some species basidiocarps have rarely been seen in nature. Identification methods based on PCR-RFLPs chain (polymerase reaction fragment length restriction polymorphisms) have resolved this problem. In this study we PCR-RFLP have compiled profiles from data available in

literature for Armillaria species from Europe, North America, Japan and China. PCR-RFLP profiles were obtained for the African. Australia and New Zealand Armillaria species. PCR- RFLP profiles were based on interspecific polymorphisms detected in the transcribed sequences (ITS1 and ITS2) between the small subunit (SSU) and large subunit (LSU) as well as the intergenic spacer region (IGS) between the LSU and 5S gene on the ribosomal DNA (rDNA) operon. availability of a this diagnostic key makes it rapid and reliable identification of Armillaria isolates from different parts of the world possible.



A seemingly tranquil tree standing in the field is really a highly restless organism in continuous motion: its leaves orienting themselves to intercept sunlight and fluttering in the breeze while absorbing carbon dioxide and releasing water vapour

from: "Out of the earth - civilization and the life of the soil" by Daniel Hillel

## tpcp extension services......

As part of the TPCP's extension services we regularly attend field days and take guests to the field to present talks to foresters and researchers. This is an important learning opportunity for all parties involved. During the first part of 1999 we were fortunate to welcome a colleague from abroad to our Programme for a week in January. During this week, Drs. David Guest and Gerry Adams were shown forestry practices in Sabie and Zululand and had the opportunity to talk to foresters from some of our forestry companies. Dr. David Guest is a leader in *Phytophthora* research in the world and is currently situated

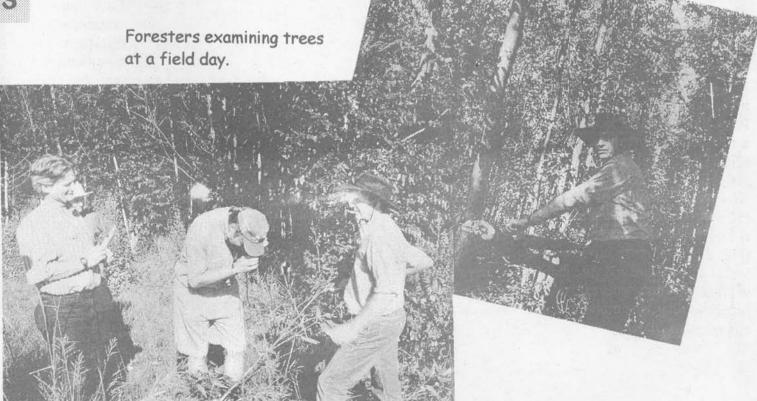
at the School of Botany at Melbourne University in Australia. He has done extensive research into Jarrah dieback in Australia and visited South Africa to attend our annual Congress for the Southern African Society of Plant Pathology. Dr. Gerry Adams, a leading plant pathologist and mycologist, from Michigan State University has been a sabbatical visitor in our group for the past 6 months, conducting research on Cytospora spp.

This visit included field stops to look at diseases, visit nurseries and breeding programmes and also to show our guests some healthy trees and successes. Both visitors presented talks on forest pathology at a field day organized by Mondi in Kwambonambi. We would like to thank our forestry colleagues from SAFCOL and Mondi for providing us with excellent accommodation for our guests. Both were highly impressed by your forestry operations and your hospitality.

#### Sawgu black wattle field day – 18 march 1999

On the 18<sup>th</sup> of March the South African Wattle Growers Union hosted a field day, dealing mostly with black wattle. At this field day the TPCP presented a talk on the diseases affecting these trees. The importance for sound breeding strategies and correct management of plantations were highlighted in the strive for a competitive industry. The black wattle industry has lagged behind in this aspect, but through renewed funding by SAWGU and the industry as well as co-operation between the ICFR, industry and the TPCP it is hoped that this will soon be corrected. Through co-operation Acacia

mearnsii can be the tree of the future!



### DIAGNOSTIC CLINIC -

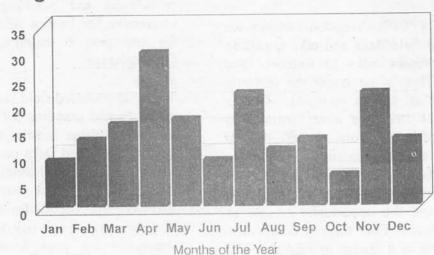
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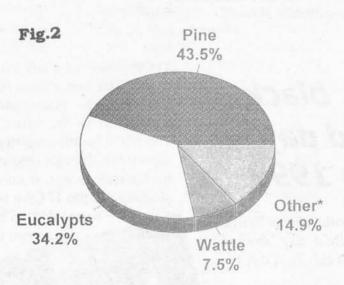
One of the important services offered to members of the TPCP the diagnostic laboratory. The diagnostic services function through foresters submitting samples the to Programme for analysis. **Isolations** pathogens аге conducted from plant material, soils and water, using state of the art techniques.

From January to the

end of November 1998 the clinic received record a number of 155 samples. This is substantially more than the 126 samples we received for 1997. In April alone, the month that we moved into the Forestry and Agricultural Biotechnology Institute (FABI) at the University Pretoria, we received a total of 30 samples (Fig. 1). In 1998, the majority of samples from pine (50.9%) followed by eucalyptus (29.8%) (Fig. 2). In previous years we had tended to receive an equal proportion of pine and eucalypt samples. majority The

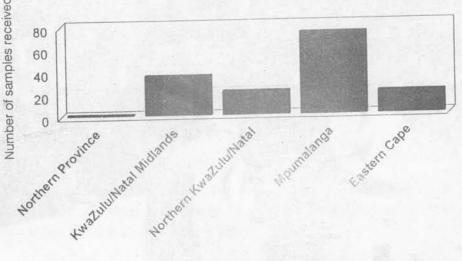
Fig. 1





\*include water, growth media and other tree samples

Fig. 3



samples were received from the Mpumalanga Province, followed by the KwaZulu-Natal Midlands (Fig. 3). We also conducted analysis of a number of water, growth media and seed samples for the presence of pathogens.

> The services of the Diagnostic Clinic is available free of charge to members of the TPCP. All samples should preferably be send to the clinic within 2-3 days after collection to ensure successful the of the analysis material. For this purpose material should be kept from drying out and stored in a refrigerator until dispatch. Use should also be made of courier services to ensure speedy door door delivery. The older the material we receive. the less likely successful diagnosis of the problem is. Please contact Teresa Coutinho for more information regarding the submission of samples. This also helps in the timely preparation specialised media for isolation of the pathogens. certain Please feel free to make use of this service, since it also helps in maintaining a detailed database regarding occurrence OI forestry diseases in South Africa.

The TPCP Develops **Co-operative Links** with Western Australia

In 1997 Mike Wingfield attended the biennial meeting of the Australasian Plant Pathology Society. It was during this time that many West Australian foresters first became aware of the potential threat posed by Mycosphaerella Leaf Blotch (MLB) disease to their eucalypt plantations. An interest was established in pursuing aspects of this disease as part of a PhD project and Mike invited Aaron

Maxwell, a Ph.d. student from Murdoch University, over to South Africa to investigate aspects of MLB in this country. Aaron became the first person to be exchanged in an initiative established between Mike and Professor Bernie Dell and Dr Giles Hardy at Murdoch University in Western Australia.

Much of the research effort has historically been on Phytophthora disease problems but this research base is broadening. Murdoch University also hosts the State Agricultural Biotechnology Centre (SABC) which is similar in many ways to FABI, but the focus at the SABC is on agricultural crops. However, students in the forest pathology group are increasingly using the modern

Murdoch University has a small but growing forest pathology

group with strong links to industry groups in Western Australia.

and well-equipped facilities of the SABC to conduct research with a molecular basis

Researchers from these two groups on both sides of the Indian Ocean are keen to develop co-operative links on a number of disease problems, which we have in common. One of these diseases is Sphaeropsis on pine and it is upon this that another Australian, Dr Treena Burgess, has work at FABI. Hopefully in the coming

been invited to from FABI will be able to spend some vear a student time at Murdoch University, probably to investigate aspects of Phytophthora.

#### **1998/1999 TPCP GRADUANDI**

1998 and the first half of 1999 has been very successful for the TPCP with regards to the number of students who completed their degrees in some aspect of forest pathology. Three Ph.D's, two M. Sc.'s and five honours degrees were conferred on students from the TPCP/FMBC. A number of other students will also, within the next few months, be handing in their theses. Bernard Slippers was awarded the S2A3-Billiton Bronze Medal from the South African Association for the Advancement of Science for a masters degree student who completed an exceptionally meritorious masters degree dissertation.

#### Ph.D: Celeste Linde (UOFS)

Population structure of Phytophthora cinnamomi in South Africa.

#### Jolanda Roux (UOFS)

Diseases of Acacia mearnsii in South Africa, with particular reference to Ceratocystis wilt.

#### Corli Witthuhn (UOFS)

Molecular taxonomy and mating type genes in Ceratocystis sensu stricto.

#### M. Sc.:

#### Bernard Slippers (UOFS)

The Amylostereum symbiont of Sirex noctilio in South Africa.

#### Schalk van Heerden (UP)

Pathogenicity and variation amongst South African isolates of Cryphonectria cubensis

#### Honours:

Lieschen Bahlmann (UP)

Oliver Dickens (UP)

Carin Dunn (UOFS)

Nonnie Geldenhuis (UP)

Andre Pretorious (UP)

Johannes van der Merwe (UOFS)

Francois Wessels (UP)



## visitors to the tpcp from abroad

Professor Gerry Adams joined the TPCP in September 1998 on an eight months sabbatical. He is from the Department of Botany and Plant Pathology at Michigan State University. He has very broad interests which include fungi that cause cankers on trees, nursery diseases and mushrooms.

Dr. Anupama Gaur, a postdoctoral fellow, from India joined the TPCP in August 1998. She completed her PhD at Gwalior University and the subject of her thesis was on endomycorrhizae-plant interactions.

She has now changed direction and is working on the population genetics of Ceratocytis spp. associated with

eucalypts.

**Dr. Treena Burgess**, a postdoctoral fellow, from Australia joined the TPCP at the end of February for a two

year period. Her PhD thesis which she completed at Murdoch University was on ectomycorrhizae. After her PhD, she spent 3 years doing a



Postdoctorate under Prof. Giles Hardy at Murdoch University on waterlogging and *Phytophthora*. Her current project is concerned with the population genetics of *Sphaeropsis sapinea* in Australia and South Africa.

Mr. Xudong Zhou, a PhD student, from China joined the TPCP in September 1998. He completed his MSc at the University of Yunnan on blue stain fungi associated with *Tomicus piniperda*. He is currently working on Ophiostomatoid fungi associated with bark beetles in South Africa.



Mr. Aaron Maxwell from Murdoch University, Australia, joined the TPCP in January 1999 for four months. He is in the first year of his PhD under Professor Giles Hardy and is working on Mycosphaerella cryptica (Mycosphaerella leaf blotch of Eucalypts).

#### **FAREWELL**

The TPCP has to say goodbye and farewell to a number of its team members. It has been a wonderful experience having these people in our midst and we share many happy memories. We wish you all success in the future and hope to see you soon. Our doors are always open to you.

**Prof. Gerard Adams** who spend 8 months in our labs on his sabbatical from Michigan State University in the U.S.A. Gerry spent his time in South Africa working on species of *Cytospora* and gave a number of talks to the forestry industry. He has made a lot of good friends amongst the students and will certainly be remembered as one on the coolest Professors ever to spent time in our group. He spent hours teaching and talking to students and made many fascinating new mycological discoveries. He has sorted out the taxonomy of *Cytospora* spp. on various hosts, from a number of different countries and has broadened the South African host range considerable. Even bug weed was not left alone! Gerry goes back to his teaching and work responsibilities in the middle of May.

**Dr. Dennis Wilson** who spend 14 months in our labs on a post-doctoral fellowship from Arizona State University. Dennis came to us to work on the endophytes of *Eucalyptus* and got

sidetracked into doing a number of other things, apart from his research. He quickly gained reputation as an exciting lecturer of science and biology as well as a film producer of note. Dennis leaves us in May to start an educational trek through Africa back to London, before he goes back to Arizona to resume his teaching and research responsibilities there.

**Karin Jacobs** who has been part of the TPCP family for 7 years, starting as a laboratory assistant in her 3<sup>rd</sup> year at the Free State University. She has now completed her Ph. D and will be spending the next two years, freezing, in Canada where she will be doing a post-doc at the Biological Resources program of the Eastern Cereal and Oilseed Research centre (until recently known as the Biosystematics Research Centre).

Karin will be doing a taxonomic study on sporodochial (produces cushion-like fruiting bodies known as sporodochia) genera of hyphomycetes. The eventual products of her research will be taxonomic revisions or monographs of several sporodochial genera and an identification key to the genera of sporodochial fungi for use in studies of pathogens and the biodiversity in agricultural and forest ecosystems.

**Len van Zyl** who has also been part of the TPCP family for 7 years. He has nearly completed his Ph. D. and will be spending the next two years as a post-doc at North Carolina State University. He will be joining the group of Prof. Ron Sederoff who is currently the director of the Forest Biotechnology Group Len will focus his research on the lignin comp sition in s<sub>1</sub> compression wood.





Whether setting off across Australia's Nullarbor Plain, winching a land cruiser across a flooded river in northern Queensland, meandering between wineries around Margaret River or commuting the leafy suburbs of Sydney one cannot avoid the presence of Eucalypt trees.

# Mycosphaerella leaf blotch disease : a question of origin

This uniquely Australian genus containing over 450 species is ubiquitous in the Australian landscape and may even be found gathered along waterholes and dry creek beds of some of the more parched regions of the continent. The leaves of these trees play host to entire fungal communities consisting of endophytes, parasites and pathogens which are in turn interconnected with various mycoparasite and insect players, each struggling to earn a living in this competitive environment. In healthy indigenous forests this often means that although pathogens and parasites are present they do not cause significant levels of disease. Where disease is apparent it is often due to a combination of disturbed habitat and introduced pathogens.

European colonisation of Australia not only brought with it a concomitant invasion of plant and animal species but also led to the dispersal of eucalypts throughout the world. These trees may be found in botanical gardens, private property, in plantations naturalised in many regions around the globe including Africa, Brazil, China, India, south-east Asia, Spain, Portugal, California USA. An Australian may easily feel at home in parts of the Western Cape and other regions of South Africa. past forty years there has been an enormous increase Eucalypt establishment of plantations world wide. current estimates in excess of 12 million hectares. This changing distribution of eucalypts and other plants across the globe raises a of bio-geographical questions. Some plants shifted to new ideal environments, leaving behind constraints imposed by pests and diseases. They are

thus able to proliferate wildly and become 'weeds'. Other plants may introduce new diseases to a susceptible indigenous plant population, and still others may succumb to pests or diseases present in their new environment.

#### "Were they pushed or did they jump"?

Plant material brought into both South Africa and Australia has no doubt harboured pathogens previously absent in these countries. A classic example of this is the disease caused by Phytophthora cinnamomi, a pathogen that originates in Indonesia and has wreaked havoc in native plant communities of South Africa and Australia. These native plants which had not co-evolved with the pathogen have not had the chance to develop a degree of resistance to these foreign pathogens. Another question of interest, but which remains unanswered is that of the origins and role of various Mycosphaerella spp. associated with plantations. Members of the eucalypt Mycosphaerella are associated with a leaf blotch disease of eucalypts with different species causing varying amounts of damage. Some species prefer different eucalypt hosts over others or even one leaf developmental stage to another Perhaps most interesting is the apparent distribution of various Mycosphaerella species. Some species have been recorded only in eastern Australia, with another suite being present in western Australia, while some species are recorded only in southern Africa and another only in Portugal and the USA. This raises the question 'were they pushed or did they jump?" In other words were these Mycosphaerella species brought across to new continents with eucalypt material or have they jumped from other host plants indigenous to the environment to which eucalypts have been introduced? If they have been introduced from Australia then why are they present at such low levels in Australia as to remain un-recorded? Although these are fascinating questions in themselves, they also have implications to the potential management of this disease.

#### Mycosphaerella leaf blotch.....

The story in South Africa is an interesting one. Mycosphaerella leaf blotch (MLB) first made an impact here in the 1930's when it caused severe damage in Eucalyptus globulus plantations. Foresters responded to this by ceasing to plant E. globulus in many regions. The outbreak of this disease was originally attributed to Mycosphaerella molleriana but later workers suggested that the culprit was Mycosphaerella nubilosa. In 1996 Pedro Crous established that the destructive species present in South Africa was in fact Mycosphaerella juvenis. Neither M. molleriana nor M. nubilosa are present in South Africa. The former has only been confirmed in Portugal and the USA and the latter occurs only in eastern Australia and New Zealand. Interestingly M. juvenis has only been recorded in South Africa. The reason behind the distribution of these species may have potential implications for the control of this disease in South Africa. If M. juvenis is present in Australia it would be instructive to know why it is not causing

noticeable levels of disease. Whereas, if M juvenis has jumped from a native southern African plant then control solutions may be sought here in South Africa.

#### Foresters must remain vigilant.....

Cultural practices and breeding programmes play a critical role in any control strategy. It has been shown that NSW provenances of E nitens are more resistant than Victorian provenances. It is also known that once E. nitens is planted below a given altitude in South Africa then MLB becomes a potential problem. So, by careful provenance selection and appropriate site selection South African foresters have (for the present) been able to manage the severity of MLB. However, if the range of plantations is to increase then further work may need to be done in controlling Mycosphaerella in its more favourable environment at lower altitude. Also, if M. juvenis is native to southern Africa then there may be potential for it to develop increased levels of pathogenicity through the greater genetic variation present in an indigenous pathogen population. Foresters must remain vigilant to the threat of this disease and maintain genetic diversity in their breeding programme as well as continuing to select for resistance to MLB.

#### Symposium on **Molecular Genetics** of Eucalyptus

In early February Brenda Wingfield visited Australia to attend a Symposium on the molecular Genetics of Eucalyptus in Hobart, Tasmania and to visit with colleagues in Canberra. She had the following to tell upon her return.

I started my trip to Australia by attending the symposium. The first morning I learnt more about Eucalyptus phylogeny than I think I actually wanted to know. I knew that there were a lot of Eucalyptus species but "that" many? And then there are a variety of related genera. I think the take home message here is that there is a lot more that I could learn about Eucalyptus and that there is much more to this group than I had appreciated. interesting fact though, is that from a molecular phylogeny

standpoint not a great deal has yet been published. In many respects we are much further ahead with some of our fungal molecular phylogenies. So maybe the mycologists are ahead of the Botanists in some respects?

The afternoon of the first day contained many molecular talks. A couple of particularly interesting ones regarding the use of the chloroplast genome were presented. Some of this information may be important in the South African context. Chloroplast genomes are generally passed through the female line. This has implications for the choice of parent species used in hybrid crosses.

This could be more important than I had initially appreciated.

The Second day of the symposium was much more "up my street" and there were a number of very interesting talks given about the use of RAPDs, microsatellites and RFLPs in the identification of Eucalyptus. All of these are very familiar acronyms for the molecular tools that many of the students and researchers in my lab use every day. These terms will also be very familiar to those of you more closely linked to the Forest Molecular Biology Cooperative Programme (FMBC).

The final meeting of the day was perhaps the highlight of the meeting as this was a Round Table Discussion where decisions

> were made on some of the next steps in Eucalyptus molecular genetics and genomics. The first international collaboration that will be

between research groups in Brazil, Europe, Australia and South Africa (FMBC) will be aimed at establishing a set of microsatellite primers to be used for the identification of the widest possible range of Eucalyptus species. The main focus from the FMBC viewpoint will be on the more important commercial species, thus enabling clonal identification. Another initiative will be to map a single Eucalyptus genome using

microsatellites. This will form the backbone for chromosome maps of other species. The current investigate genome organization in Eucalyptus.

suggestion is that the focus should be on a commercially important species. Other suggested projects are the mapping and sequencing of the chloroplast genome, the production of a cDNA library to produce ESTs (expressed sequence tags) and possibly sequencing a mega base of DNA to

After the meeting in Hobart I went on to visit a couple of research labs at the CSIRO in Canberra. It is our intention to do our next sabbatical in Canberra and so with this visit I have made some inroads into

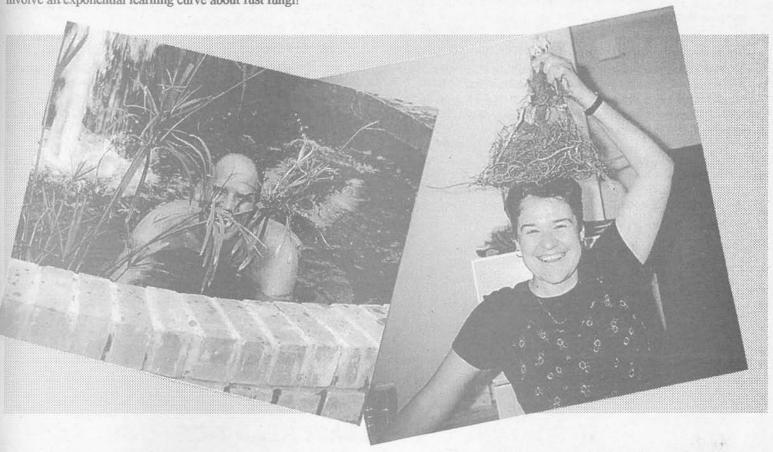
pranning the sabbatical. My visit in Canberra was very successful and my host (Dr. Jeremy Burton) spared me a great deal of his

microsatellites, RFLPs.....these are very familiar acronyms for the molecular tools that many of my students and researchers in my lab use every day.

RAPDS.

very valuable time in showing me around and discussing some details of the project that we hope to undertake together. This will be working with a variety of rust fungi. Jeremy is an internationally recognized expert in this area. This project may sound a little unusual, but one of the most important disease threats to the South African Forestry Industry is the introduction of Guava Rust which is a devastating disease in *Eucalyptus* plantations. Also the most significant gene for gene disease resistance work has been done using rusts which infect corn and wheat, and more recently the work done in South Carolina on the White Pine Blister rust. Our proposed sabbatical will therefore, involve an exponential learning curve about rust fungi!

As you can gather I did not see much of Australia – just a few Kangaroos and no Koalas. But the trip has established some firm collaborations and the number of E-mail addresses that I now regularly communicate with ending in ".au" (which indicates Australia) has increased dramatically. My impressions of Australia......? A nice enough place to visit, has some Anglo-Saxon overtones that as an English speaking South African I could relate to but I missed the BUZZ and "Genetic Diversity" of South Africa. Maybe I will get to see more of the place on my next visit – it is however, VERY far away from South Africa – and for that matter anywhere else in the world.



## forest entomology in the tpcp

In January this year Prem Govander, previously from the ICFR, joined the University of Pretoria and the TPCP. He has been appointed in the Department of Zoology and Entomology as a lecturer and will be responsible for starting a Forestry Entomology Programme at the TPCP. At the annual TPCP meetings, held on the 10<sup>th</sup> of March Prem presented a lecture on the current status, as well as the future, of forestry entomology. This article presents some extracts of this presentation.

#### 1. Primary objectives of forest protection

- · To sustain the future forests and food of our new nation,
- To train future scientists, thus ensuring that a source of expertise in the specialist field of forest entomology is available in South Africa,
- To undertake goal-directed research on priority pest problems experienced by major players in the South African Forestry

Industry and generate practical products and solutions to these problems,

 To monitor pest outbreaks in plantations, permanent sampling plots and countrywide surveys.

#### 2. Bill Dyck report

In March 1995, Bill Dyck reviewed forestry research in South Africa for the Department of Water Affairs and Forestry (DWAF) and the Forest Owners Association (FOA). He identified various threats to forestry Research and Development in South Africa. Currently the situation appears to have deteriorated even further with regards to the issues he raised about Forest Entomology. The Dyck report is therefore used as a point of reference to further explain the situation regarding forest entomology in South Africa.

#### 2.1 Factors that threaten Forestry Entomology in South Africa

- Scientist mobility scientist movement out of forestry research into research management, education, other research fields or overseas.
- Succession Planning this is incomplete partly because of the fragmented structure and lack of depth in most organisations. Many programmes are vulnerable to staff

losses and insufficient training through university graduate degree programmes.

- Funding Uncertainty the consistently declining budget, especially in forest protection: this country requires considerable skills to ensure that introduced insects and diseases do not destroy the forest resource. Dependence on uncertain industry funding has a destabilising effect on scientists and their associated research institutes.
- Absence of collaboration and duplication of effort this
  often leads to inefficiencies and is the outcome of inadequate
  funding and planning. Collaboration is the vehicle whereby
  the skills of a combined team effort can make greater
  progress than can individual effort.

#### 2.2 Some recommendations to realise this opportunity

- Strategic research there is a need for longer-term strategic focus and research planning. This would ensure that research programmes are designed to enable results to be modelled and extrapolated beyond the immediate research project (process research) as opposed to the necessary but short term, less expensive, annual-type applied research (empirical research). This type of strategic focus is one of the cornerstones of the success of the TPCP.
- Collaboration and co-operation it is critical that scientists do not work in isolation of either the end-user of research or of other scientists (especially scientists in the same field of research).

#### 2.3 Forest entomology recommendation

The TPCP as a centre of excellence is working extremely well, whereas forest entomology research is relatively uncoordinated. Therefore, and because forest entomology and pathology skills are interchangeable, a logical solution would be to include forest entomology research with the TPCP. The Department of Zoology and Entomology at Pretoria University is integrally associated with the TPCP and is the only university in South Africa with Forest Entomology expertise.

#### 3.0 Perks of a university association

- Funding from organisations such as the Foundation for Research Development (FRD) and Department of Trade and Industry (DTI) THRIP.
- Opportunities for interaction and networking with international universities and institutions. This includes student exchange programmes.
- Interaction with postdoctoral fellows from other countries opens the door to an international scientific network and foreign expertise becomes readily available.
- Establishment of foreign contacts to assist in the search and introduction of biological control agents.
- There are also strong working relationships with the National Insect Collection and several quarantine establishments.
- Most postgraduate students are highly motivated, dynamic and gifted. Their expertise is made available to the forest industry at a fraction of the cost of hiring consultants or employing full time personnel. Their research focus is intense (undivided attention) and represents a commitment to a specific area of research within a defined time frame.
- Build up of a critical mass of high calibre postgraduate students. Pretoria University also has an "UP with Science Program" where the cream of the crop of high school students are mentored and introduced to science. These students can be informed of forestry and be attracted to forestry research at an early age.

It is of utmost importance to support and actively encourage forest entomology in South Africa. It is clear from field situations that insect pests can yearly have a dramatic impact on yield and lead to large-scale losses. FABI and the TPCP is an active partner in South African forestry and is committed to the promotion of forest entomology in South Africa and thus the protection of our forests from pests and diseases.

#### IMPORTANT : PLEASE READ THIS

In order for us to coordinate our services to you please help us by using the following contact address:

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