

The Forestry and Agricultural Biotechnology Institute (FABI) is located at the University of Pretoria. The primary objectives of the Institute are to:

- Promote the broad field of plant biotechnology through an interdisciplinary approach and with close linkage to a wide range of academic departments
- Undertake research of the highest possible caliber, while at the same time providing short and longer term benefits to the forestry and agricultural sectors of South Africa
- Establish partnerships with industries linked to agriculture and forestry, both nationally and internationally, to produce new and improved products and thus to promote competitiveness in trading
- Promote the education, particularly of South Africans in the fields of forestry and agriculture

The association of FABI with the University of Pretoria, one of the largest residential Universities in South Africa, provides access to a wide range of human and technological resources. Currently, academic staff and postgraduate students from research programmes in the Departments of Biochemistry, Botany, Genetics, Microbiology and Plant Pathology, Zoology and Entomology and Plant Production are associated with the Institute. This affords FABI the opportunity to build future resources in biotechnology which will be crucial to the future of forestry and agriculture in South Africa.

In every way, FABI represents an amalgamation of a tremendous base of expertise in forestry and agriculture from different universities and research organisations in South Africa. The Institute has been operational since 1998. This document represents the fourth FABI biennial report covering the period from May 2003 to May 2005.

Forestry and Agricultural Biotechnology Institute (FABI)

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Forestry and Agricultural
Biotechnology Institute
FUTURE FORESTS and FOOD

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DIRECTOR'S REPORT

The approximately two years since the appearance of our last biennial report, has rushed by with so many events and activities that it is difficult to capture the essence of these in a single document. Yet, just in saying this, we recognize substantial growth and achievement in FABI. During the past two years, the Institute has celebrated great achievement in all of its core activities; research, education and community service. FABI has unquestionably become one of Africa's premier plant and microbial biotechnology research groups. This is clearly also being recognized worldwide. The fact that the Institute is only seven years old makes this fact even more remarkable.



Many times during the year, I am asked to what I might attribute the remarkable growth and success of FABI. This is a complex question which probably has a multitude of answers. It is a question that was interrogated deeply as part of the FABI external review at the end of 2003. It was also considered in a study by the Human Sciences Research Council, who sought to better understand the remarkable relationship between certain FABI researchers and their commercial industry partners. In reading the various documents emerging from these and other studies, my overall conclusion is that it is the people of FABI, postgraduate researchers, students, academic and support staff that make the Institute what it is. Having worked in more institutions than the average academic, I can only marvel at the tremendous team spirit of the FABI family.

The FABI team now incorporates approximately 150 people. The majority of the group represent postgraduate (M.Sc. and Ph.D.) students, with a smaller core of postdoctoral scientists, permanent academic and support staff. At last count, the FABI "list server" and major conduit of communication between us, included some 180 people. This is a number that changes continuously, with visitors and students coming and going. We retain the remarkable statistic of having some 30 languages (give or take a few depending on the month) spoken in the Institute, which must be one of the most culturally diverse groups of its kind in the world. As noted above, our group is often referred to as the FABI family and we clearly cherish the positive aspects of "family" (positive team spirit, mutual respect, support of a common goal) that characterize us. On the other hand, we use this word cautiously because we do not adhere to a hierarchical management style. In fact, our operating system is quite the opposite of hierarchical. FABI is led by all of the FABI team, with some guidance from a relatively small Management Committee (MANCOM). The latter group includes all of the research group leaders who have also contributed brief communications in this report, and a student representative. We also rely on the wisdom of an Advisory Committee including the Dean of the faculty of Natural and Agricultural Sciences and the heads of the six Faculty Departments with which FABI is aligned.

One of the most exciting FABI events during the last two years was the inauguration of the new FABI SQUARE and BIOINFORMATICS building. Many people reading this report will have participated in the day of science and celebration, held to mark the completion of the remarkable new three story building that we occupied in early 2004. Amongst others, this facility includes a world class bioinformatics centre, one of South Africa's only plant quarantine facilities, a new DNA sequencing facility to house our high throughput sequencers and associated equipment, new seminar and board rooms, a modern positive pressure tissue culture facility and a suite of new laboratories. It is remarkable that this new building was required so soon after completion of the first FABI building. But this also illustrates the very rapid and somewhat unexpected growth and accomplishment of FABI during the first few years of its existence.

The inauguration of FABI SQUARE was attended by about 250 people including more than one hundred from outside Pretoria and a very large number of those from countries other than South Africa. We all enjoyed an exciting suite of scientific lectures, tours of the new facilities, tree planting ceremonies, signing of contracts, a formal inauguration ceremony and last but in no ways least, a wonderful dinner celebration. Perhaps the only difficulty was the fact that the 14th annual meeting of the Tree Protection Co-operative Programme (TPCP) followed the next day and this left little time for recovery from the Inauguration.

I am greatly tempted to note some of the great accomplishments of the FABI groups of researchers during the last two years. Yet, this is a perilous path due to the fact that I will inevitably fail to mention some achievements and I will also fall into the trap of judging what I consider to be the most noteworthy. Aside from a growing output of outstanding publications and a remarkable number of M.Sc. and Ph.D. graduates (I remember nine Ph.D.s at the April 2004 graduation!) FABI team leaders have excelled in bringing exciting new projects to the Institute. The exceptional Royal Society/ NRF award for legume improvement in collaboration with the CSIR, the University of Limpopo and Rothamsted Research, UK; the finalization of new contracts underpinning the Forest Molecular Biology group and the award of one of South Africa's first six Centres of Excellence (the Centre of Excellence in Tree Health Biotechnology) by the Department of Science and Technology (DST) and the National Research Foundation (NRF) are amongst some of the notable achievements linked to FABI.

Many people enquire as to the source of funding for FABI. Again this is not an easy question to answer. Clearly the University of Pretoria contributes a substantial part of the funding that allows FABI to exist. This comes mainly in the form of salaries to the key academic staff and support for the physical facilities and some of the equipment. Research projects and student support comes from a multiplicity of private, public and statutory organizations, which are far too numerous to list here. They do, however, include both South African and other governments in many parts of the world, local and international funding bodies as well as private industry, locally and internationally. Some of these are listed in this report and we take the opportunity to thank all the sponsors of FABI research for their support during the past two years. Without this support, it would clearly not have been possible to achieve the remarkable outputs of the Institute. Perhaps most importantly, the impact that this support has had on human resource development through postgraduate education will be felt for decades to come.

We seek to publish a FABI Biennial Report in June of every second year. This is sent to colleagues and friends around the world and the contents is also placed on the FABI Web Site. Just in the first part of this year, our web site has had some 800 000 hits, which illustrates the interest that FABI is drawing and it also highlights the power of electronic communication in a rapidly shrinking world. We are well on our way to another FABULOUS two years before the publication of our next formal report. But in the interim, you will certainly be hearing from us through the many conduits of communication that typify the modern world. Thank you for your part in supporting FABI and we look forward to working with you in the coming year.

Mike Wingfield Ph.D. (Minnesota), FRSSA, ASSAf
Mondi Professor of Forest Pathology
Director of FABI, the Tree Protection Co-operative Programme (TPCP) &
The DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB)

FABI TEAM



In alphabetical order:

Mohammed Abdo, Aneen Belgrove, Dave Berger, Legesse Beyene, Mesfin Bogale, Jane Boshoff, Carrie Brady, Vivienne Clarence, Martin Coetzee, Maria-Noel Cortinas, Teresa Coutinho, Elna Cowley, Nicky Creux, Pranitha Dawlal, Sonja de Beer, Wilhelm de Beer, Pieter de Maayer, Elsie de Meyer, Lieschen de Vos, Juanita de Wet, Helen Doman, Wilhelm Dreyer, Derian Escheverri, Joanne Fouché, Magda Fouché, Gerda Fourie, Solomon Gebeyehu, Tanja Gevers, Christian Giesel, Dina Gomez, Veloshinie Govender, Izette Greyling, Joha Grobbelaar, Susan Groenewald, Marieka Gryzenhout, Jenny Hale, Almuth Hammerbacher, Hardus Hatting, Ronald Heath, Ursula Heiniger, Gavin Hunter, Brett Hurley, Willeke Keesenberg, John Kemp, Pritty Khumalo, Andrew Kiggundu, Barnabas Kiula, Lise Korsten, Karl Kunert, Marija Kvas, Nico Lasbuschagne, Adrene Laubscher, Seonju Lee, Sabine Lezar, Amelita Lombard, Lorenzo Lombard, Evelyn Madoroba, Grieta Mahlangu, Martha Mahlangu, Frank Maleka, Gert Marais, Wolfgang Maier, Babalwa Mbebe, James Mehl, Calvin Molepe, Buyi Mthlane, Eva Muller, Karin Muller, Claire Munro, Zander Myburg, Sanuschka Naidoo, Grace Nakabonge, Barbara Nel, Valentina Nkosi, Gilbert Nkuekan, Draginja Pavlic, Zelda Pieterse, Bernice Porter, Martin Ranik, Thierry Regnier, Carl Roux, Jolanda Roux, Thea Schultz, Gladys Shabangu, Diana Six, Ana Slaughter, Bernard Slippers, Lisel Solms, Luke Solomon, Anita Steyn, Danie Theron, Marié Theron, Jacob Thobatsi, Lydia Twala, Albé van der Merwe, Leon van Eck, Marelize van Wyk, Martie van Zyl, Rosie van Zyl, Altus Viljoen, Chris Visagie, Rosemarie Visser, Liesl Vorster, Brenda Wingfield, Mike Wingfield, Lawrie Wright, XuDong Zhou, Karin Zeeman, Renate Zipfel

RESEARCH REPORTS

Forest Protection

Research leader: Prof Mike Wingfield

Research team: Prof Teresa Coutinho
Prof Brenda Wingfield
Prof Jolanda Roux
Mr Brett Hurley

Objectives of the research programme:

- Development of field monitoring techniques to recognize the appearance of new pests and diseases as well as to monitor the spread and impact of those already established in South Africa.
- Identify new and important tree pests and pathogens and evaluate their genetic structure so that they can be more effectively controlled.
- Develop methods to screen trees for tolerance to the most important diseases present in the country.
- Establish and evaluate contemporary breeding strategies in order to produce disease and pest tolerant species, clones and hybrids.
- Establish an understanding of the biology of tree pests and pathogens to promote their better management.
- Study and evaluate novel strategies for disease and pest control.

Highlights of research 2003/2004:

In this report, a brief summary of the various research activities of the team members and postgraduate students of the Tree Protection Co-operative Programme (TPCP) is provided. This is a condensed review with the focus on selected highlights and important findings. The research team represents approximately 55 people, mostly M.Sc. and Ph.D. students as well as post doctoral fellows and research visitors. Thus a great number of pest and disease problems are handled and this report can only touch on a very select few of these. Publication and project lists, as well as information on the FABI website (<http://fabinet.up.ac.za>) provide a more comprehensive view.

During the course of the past two years, the South African Forestry Industry has continued to be severely challenged by pest and disease problems. The prediction that forest health problems will be increasingly important to the industry has become a clear reality. New disease and insect problems have emerged

virtually every year and some of those that are already established have increased in importance.

One of the more fascinating but worrying discoveries in recent years is that many new disease and pest problems are being caused by native insects and microbes. The unexpected appearance of a serious wood-boring cossid moth on *Eucalyptus* during 2004 illustrates this worrying trend. Damage due to this pest has been severe on this important forestry species. Larvae of the insect were found and these were reared to the adult stage. This work led to the conclusion that the insect is the native cossid moth *Coryphoderma tristis*, also known as the Goat Moth, due to the unpleasant odour associated with infestations. The recent damage caused by *Coryphoderma tristis* on *E. nitens* is sufficiently significant to the forestry industry to have necessitated an intensive study. Current activities include a search for the cause of the sudden host shift as well as to consider the moth's natural enemies. This will

make it possible to formulate an effective pest management program in the context of South African forestry practices.



Tunnels and larvae of the cossid moth

Pest and pathogens native to other countries also continue to threaten South African forestry. Two of the most serious of these are the *Sirex* wood wasp and the pitch canker pathogen. The *Sirex* wood wasp, *Sirex noctilio*, together with its fungal symbiont, *Amylostereum areolatum*, is now undoubtedly one of South Africa's most threatening forest pests. A major focus of the research on *Sirex* during 2004 has been on the deployment of the parasitic nematode, *Beddingia siricidicola*, for biological control. Techniques for rearing, deployment and storing the nematodes have been tested and perfected. This led to the first major deployment of nematodes in all areas where *Sirex noctilio* is active. There is a significant lack of knowledge concerning the biology of *Sirex noctilio*, its fungal symbiont and its nematode parasite in South Africa. For this reason, a collaborative effort has been established with colleagues in Sweden who have many years of experience studying this system.



Adult female *Sirex* wasp

Fusarium circinatum, also known as the pitch canker fungus, has become one of South Africa's most important forest tree pathogens. The fungus is highly aggressive and has caused devastating losses to pine nurseries in the country. One of the most important aspects of the research on this pathogen is linked to reducing losses in nurseries. One of the greatest threats to the South African forestry industry lies in the question of whether the pitch canker fungus will emerge to infect established trees, as is the case elsewhere in the world. There are arguments both indicating that this will happen in time and others that suggest that factors such as insects and climate in South Africa are not suitable for the development of "full blown" pitch canker. Research in this programme is considering various of these factors and one of particular interest is the relationship between the fungus and the eastern pine weevil *Pissodes nemorensis*. This insect is known to be associated with the pitch canker fungus in the South Eastern United States and the same might emerge to be true in South Africa in the future.



Pine seedlings infected with *F. circinatum*

Cryphonectria canker is one of the most serious diseases of *Eucalyptus* in South Africa. The disease has been known in the country for approximately 15 years and it is reasonably clear that it was not a problem prior to this time. Cryphonectria canker also occurs almost predominantly in warmer, sub-tropical regions of South Africa. Recent research conducted in this programme has focused on the distribution of the disease. Predictions have also been made concerning global warming and the likely extension of the range of this disease if temperatures in the country were to rise.

Intriguing and important results have continued to emerge concerning the origin of the *Cryphonectria* canker pathogen. This disease agent has been known in South Africa as *Cryphonectria cubensis* and the assumption has been that it was introduced into the country, either from Latin America or from South East Asia. During the last few years, research in this programme has accumulated increasing evidence that the pathogen is native to South Africa. This has led to a change of name for the pathogen that is now been split into two species. The fungus in South Africa has been named *Chrysosporthe austroafricana*, while *C. cubensis* from other parts of the world is now known as *Chrysosporthe cubensis*. *Chr. austroafricana* threatens *Eucalyptus* forestry in other parts of the world, including native *Eucalyptus* and potentially other Myrtaceae in countries such as Australia. There is also a great danger of *Chr. cubensis* being introduced into South Africa, as all breeding and selection work done up until now was against a pathogen native to the country and will probably not apply to *Chr. cubensis*.



Conidiomata of *Chrysosporthe austroafricana*

Discovering that *Chr. cubensis* (formerly *C. cubensis*) does not occur in South Africa has raised the question of the origin of this fungus. This is important to South Africa because such knowledge will increase our capacity to exclude the fungus from the country. Some early evidence has suggested that the origin of the pathogen might be South America, and that the fungus of the same name in South East Asia, might represent a different species. As a result of research

with a student from Colombia, we have gained added information supporting the view that *Chr. cubensis* is probably native to South America. This work is not complete and is being continued through additional collections and intensive DNA sequence comparisons.



Cryphonectria canker

Another new development in the South African forest health system has been the recent discovery of Quambalaria leaf and shoot blight on plantation trees. Quambalaria leaf and shoot blight was first identified in South Africa in the late 1980's from a single nursery on the sub-tropical Zululand coast. Infection resulted in leaf spots, leaf and shoot blight and seriously impacted on cutting production. The disease in South Africa is caused by *Quambalaria eucalypti* (syn. *Sporothrix eucalypti*) and was not considered a major restraint to commercial forestry as it was so limited in distribution and was not seen for many years after its initial discovery. However, around 2000 the disease re-appeared in a second nursery in the Zululand area, affecting a wider range of clones and sporadically impacting on cutting production. More recently, the disease has been found on one-year-old *E. nitens* plants in the Mpumalanga Highveld. This is of concern as it is the first report of the fungus from this part of the country, the pathogen appears to be adapting to colder climates and it was also found to cause extensive lesions on *E. nitens*. Research is currently underway to determine whether the fungus in Mpumalanga is the same as that previously found in Zululand and to better understand the host range of the pathogen.

Cereal Genomics

Molecular genetic mechanisms involved in host resistance to pests

Research Leader: Prof AM Botha-Oberholster

Objectives of the research programme:

In the programme we assess the super family of resistance (*R*) and defence related (*DR*) gene sequences applicable to insect resistance in wheat, by addressing the following issues:

- Isolation and characterization of Resistance Gene Analogs (RGAs) from cDNA in bread wheat utilising modern technologies and bioinformatic tools; Suppression Subtractive Hybridisation (SSH) using driver (induced) and tester (non-induced) plant material, and cDNA-Amplified Fragment Length Polymorphisms (cDNA-AFLPs).
- The in depth study into the differential expression of Expressed Sequence Tags (ESTs) and *R/DR* genes upon Russian Wheat Aphid (RWA) infestation using the DNA micro-array technology. As well as the characterization and mapping of *DR* genes containing the nucleotide-binding site-leucine rich repeat (NBS-LRR).
- Development of a marker system for mass screening of breeding material using microarray technology.
- Map the relevant sequences using segregating populations.

Highlights of research 2003/2004:

The molecular basis of plant-aphid interactions remains poorly understood, despite the fact that aphids are the largest group of phloem-feeding insects. A common feature among the reported studies is the activation of genes known to be involved in defenses against bacterial and fungal pathogens. Such responses are generally not associated with chewing insects. As with plant-pathogen interactions, the defense against RWA is accompanied by the production of reactive oxygen species (ROS), including H₂O₂. ROS induce the accumulation of cellular salicylic acids (SA) concentrations and trigger the expression of pathogenesis related (PR) proteins. Several defense-related products have been shown to accumulate in the apoplast of resistant wheat cultivars, including pathogenesis related (PR) proteins, i.e. β -1,3-glucanases, chitinases and peroxidases. The analogy in plant responses stimulated by phloem-feeding aphids and pathogens could be due to the similar effects that the aphid's stylets and fungal hyphae have on the host during insect feeding and fungal infestation. The path of the stylet during

feeding is mainly intercellular or intramural and such feeding limits the damage that is distinctive from chewing insects, and it is thus not surprising that a novel defense-regulation could be elicited by phloem-feeding insects.

In the most simplistic scenario, the recognition of invading pathogens is facilitated through resistance (*R*)-genes products and the activation of responses results in the impediment of pathogen growth. *R*-gene mediated response is dependent on the expression of a complementary avirulence (*Avr*) gene, and if a host *R*-gene product interacts either directly or indirectly with a corresponding, pathogen-derived *Avr*-gene product, recognition occurs and an incompatible interaction follows. Incompatibility often results in a rapid signalling cascade, leading to an active defense response. In the absence of either the *R*-gene or the corresponding *Avr*-gene, a compatible interaction occurs, and the pathogen is able to proliferate and cause disease.

This genetic relationship between the hosts and pathogens, termed gene-for-gene interaction is involved in a wide range of pathogen types including fungi, bacteria, viruses, nematodes and more recently it was suggested for the RWA-wheat interaction. Resistance genes that function in a gene-for-gene manner generally belong to one of four general classes based on their amino-acid motifs that are found within the encoded protein sequence. Members of the largest class encode for cytoplasmic proteins with nucleotide-binding site (NBS) and several leucine-rich repeats (LRRs). Although the proteins encoded by the NBS-LRR of resistance genes can be further subdivided in either coiled-coil (CC) or Toll-interleukin-1 receptors (TIR) homology domain at the amino terminus, only the CC domains occur in wheat, where they may have a function in directing certain protein-protein interactions. *R*-genes belong to a large gene family where arrays of similar sequences allow for recombination events that can lead to the evolution of the gene for novel recognition specificities. Despite selection of divergence, many of these race-specific resistance genes retain the requirement for similar downstream events. Considering the function of NBS-LRRs in plant defense, and taking into account that several copies of the same ESTs, that encode for proteins with significant homology to NBS-LRRs (i.e. wheat homologs to RGA-2, leucine rich-like protein, a LZ-LRR-NBS, and *Mla* locus — also a CC-NBS-LRR) were isolated from RWA induced libraries, the significance of these clones during RWA defense was investigated. Upon infestation by RWA, all the ESTs were significantly regulated. Linkage to *Dn1* gene has previously been confirmed for NBS-LRR and RGA-2 using a segregating F₃ 'Tugela' x 'Tugela Fast Grow' population, further provide supporting evidence for the role as receptor and/or signalling agent. RGA-2 is also an NBS-LRR-like protein with a putative receptor-like function, and thus may be involved in signal transduction and/or invader recognition. Moreover, even though these RGAs map to the appropriate *Dn1* harboring region in wheat, they may not encode the specific, functional *Dn1* R-gene product especially

since *R*-genes are typically found clustered in genomes. Importantly, this data strongly suggest that *Dn1* might be an RGA in the truest sense and further characterization of the *Dn1* containing genomic region might identify additional gene candidates for downstream functional analysis (e.g. overexpression and phenotype assessment in transgenics etc.).

The downstream events after infestation by RWA have been studied extensively for the NILs 'Tugela' and 'TugelaDN', and include a general and specific defense response. Using cDNA-AFLP transcript profiling, it was shown that the response against RWA is rapid, and the first transcript changes happen within 1-2 hours of RWA feeding. Recently, it was further demonstrated, that NILs of 'Tugela' containing different *Dn*-genes expressed similar as well as novel transcripts after infestation with RWA providing supporting evidence for a gene-for-gene type of interaction. Another exciting observation made by the group indicates that RWA feeding damages the photosynthetic machinery (i.e. PSI reaction centre) and in resistant cultivars ATP synthase facilitate the "recovery" of the reaction centre which enable resistant plants to cope with the stress, while their susceptible counterparts dies.



Feeding Russian wheat aphids on resistant varieties result in little visual damage. The recent development of several new RWA biotypes is a matter of great concern to researchers and farmers alike.

Cereal Genomics

Quantifying diversity and heterosis in maize germplasm

Research Leaders: Prof AM Botha-Oberholster
Dr AA Myburg

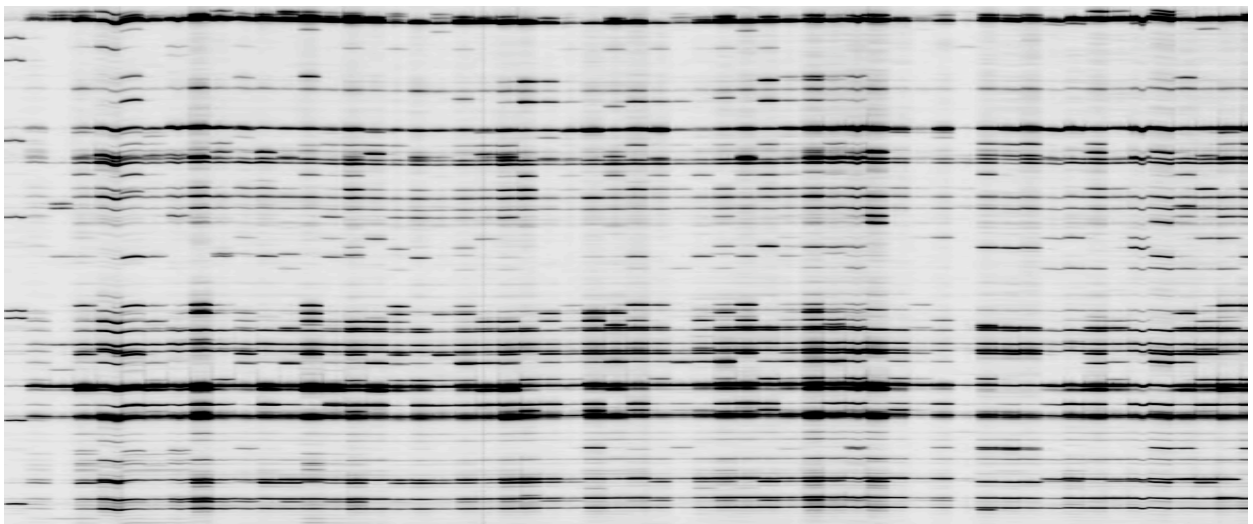
Objectives of the research programme:

- To establish the diversity and heterosis of selected maize varieties of interest to Africa using molecular tools.
- Identify and develop markers for Gray leaf spot resistance in maize.
- Develop markers to enable maize breeders from different African countries to optimise their breeding efforts.

Highlights in the programme 2003/2004:

Research efforts in this programme at FABI involve the identification of genetically diverse maize inbred lines for highland maize regions in Ethiopia. Highland inbred lines have been characterized for their divergence and heterotic groups have been suggested for the later use as genetically divergent source materials. A representation of this work using AFLP is

indicated below. The research efforts at FABI using molecular markers (AFLP and SSR) form part of the efforts underlined to identify heterotically divergent inbred lines. The programme further identified maize hybrids with good potential application to Gray leaf spot “hot spot” regions in the Tanzanian maize producing areas.



A representation of the AFLP profile generated for 56 highland and mid-altitude adapted maize inbred lines with *EcoRI* -ACA/ *MseI* -CAC primer combination

Forest Biotechnology

Propagation of Pine Species

Research Leader: Prof AM Botha-Oberholster

Research Team: Mrs Anita Steyn

Objectives of the research programme:

- Supply Komatiland Forest Research with a protocol for somatic embryogenesis using female gametophytes/immature embryos as explants.
- Evaluate changes at the genomic level in cell lines in culture/stored for extended periods.
- Increase the understanding of the development of somatic embryos through a comparative study of somatic and zygotic embryos. The research has focused on similarities and differences between somatic and zygotic embryos in terms of morphology, histology, biochemical and metabolic pathways.
- Determine the role of late embryogenic associated proteins in the development of somatic embryos.

Highlights of research 2003/2004:

Research in this programme represents a joint effort between Komatiland Forest Research and the University of Pretoria. It especially focuses on the propagation of several pine species of commercial importance, namely *Pinus patula* and the hybrid *P. elliotti* x *P. caribaea*. The programme runs in collaboration with Professor Chris H Bornman (Denmark) and Dr. Krystyna Klimaszewska from Canadian Forestry has produced somatic embryos in *P. patula*, *P. radiata* and *Picea abies* (model system), while embryogenic structures have been obtained for the hybrid species. The study not only focused on protocol development, but also on understanding the difficulties as to why the successes with the process is low in terms of commercialization. Conclusions drawn from this study include the fact that some of the coniferous somatic embryos seem to 'behave' like recalcitrant seed. Somatic embryos of *Picea abies* behave like isolated zygotic embryos *in vitro* but with greatly reduced physiological vigour.



Germinating somatic embryo of
Pinus patula

Molecular Plant-Pathogen Interactions

Research Leader: Prof Dave Berger

Objectives of the research programme:

- Describe mechanisms whereby plants defend themselves against pathogens.
- Study plant anti-fungal polygalacturonase-inhibiting proteins (PGIPs).
- Produce genetically modified (GM) plants as a tool to study plant resistance mechanisms.
- Use DNA Microarrays as a tool in understanding plant function.

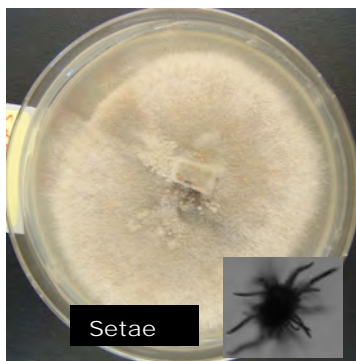
Highlights of research 2003/2004:

Lupin anthracnose in South Africa
The Stock Feed manufacturing industry in South Africa requires high quality grain to provide the nutritional requirements for chicken, pig and other livestock producers in the country. Much of this grain is provided by legumes such as soybean and lupin. Lupin grain has the highest level of protein (20-45%), and can be processed without heat treatment or an oilpress, an advantage over soybean. The species *Lupinus albus* is the most desirable, since it has the highest protein levels (34-45%) compared to *Lupinus angustifolius*. However, farmers have been forced to halt production of *L. albus* in South Africa since it is highly susceptible to the anthracnose fungus.

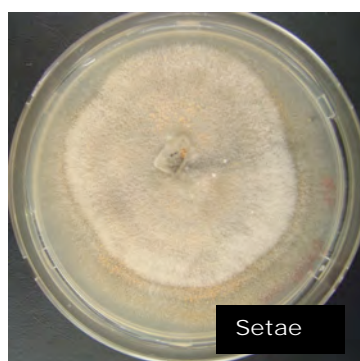
Much controversy has surrounded the identity of this anthracnose fungus, which

is highly specific to lupins. This controversy was solved in a project supported by the Protein Research Foundation, which aims to make the South African Feed manufacturing industry less dependent upon grain (protein) imports from overseas. The lupin anthracnose fungus had previously been named *Colletotrichum gloeosporioides* or *Colletotrichum acutatum*. Characterization of lupin anthracnose isolates from different regions in South Africa was carried out using morphological and molecular tests (ribosomal DNA and β -tubulin gene sequencing), and it was concluded that the causal agent is *Colletotrichum lupini* var. *setosum* (see figure below). The results have broad implications for other lupin producing regions of the world, such as Australia.

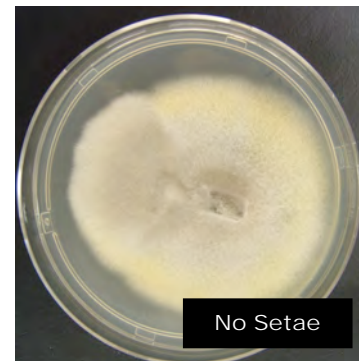
Colletotrichum (from lupins in South Africa)



Colletotrichum lupini var. *setosum* (ex-type)



Colletotrichum lupini var. *lupini* (ex-type)



Lupin anthracnose fungus in South Africa identified as *Colletotrichum lupini* var. *setosum*

Gene discovery using Microarrays

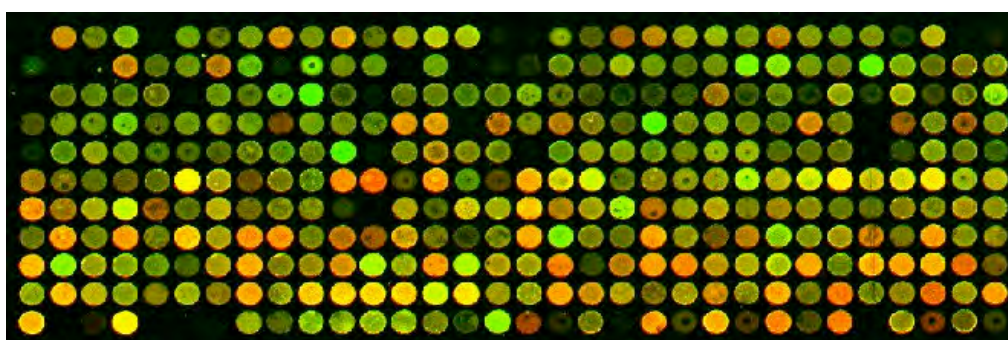
Plants respond to pathogen attack by the activation of biochemical defence responses. We are interested in gene expression underlying these responses with the aim to discover novel genes that can be used for crop improvement. We developed a strategy for gene expression profiling on glass slide microarrays that can be generalised for any non-model organism, an important consideration in aligning research with national priorities.

The method was jointly applied to the identification of pathogen defence genes in the African crop pearl millet (with ACGT and CSIR-Bio/Chemtek) and genes involved in resistance to Fusarium-wilt (Panama disease) in banana in co-operation with the Scottish Crops Research Institute (see report of Banana research group). cDNA libraries enriched for several thousand transcripts that were differentially expressed in response to pathogen elicitors were constructed using the suppression subtractive hybridisation (SSH) technique.

A quantitative method was developed to screen the SSH libraries on microarrays, and this indicated that the libraries were of high quality. For example, 77% of the clones from the pearl millet forward library represented up-regulated transcripts, and only 5% of the clones were derived from rRNA (see Figure below). Up-regulation of selected clones was confirmed by inverse Northern analysis, a microarray-independent technique. Sequence analysis revealed that the libraries contain potentially novel defence genes. Expression profiling of the pearl millet defence response is currently underway and the information will be used to design strategies for crop improvement.

This project illustrates the synergy facilitated by the FABI environment, which resulted in a novel strategy published in an international journal that was developed in co-operation between the Molecular-Plant Pathogen Interactions research group, the Banana research group, ACGT, CSIR-Bio/Chemtek and the Scottish Crops Research Institute.

Microarray Screening of millet cDNA library



Note: Only 1/12 of Microarray image is shown.
Green spots represent genes induced by pathogen elicitors.
Orange/red spots represent genes repressed by elicitors.

Bioinformatics

The Molecular-Plant Pathogens research group has been closely involved in the establishment of Bioinformatics research in the new FABI Square Bioinformatics building. Open source database and software platforms have been implemented for Microarray data analysis

(see section on Microarray service). In addition, as part of ongoing biochemical studies of the anti-fungal polygalacturonase inhibiting proteins (PGIPs), a bioinformatics approach was used to identify PGIP genes of *Arabidopsis thaliana* prior to release of the annotated whole genome sequence.

Centre for Applied Mycological Studies (CAMS)

Research Leader: Dr Gert Marais

Research Team: Ms Annelie Lübben (CSIR)

Objectives of the research programme:

- Building collaboration between FABI and CSIR Bio/Chemtek.
- Promoting mycological research in South Africa.
- Exploiting the fungal culture collections of FABI and CSIR for value added products in the agricultural, food, medical and industrial fields.
- Promoting mycology as a research discipline through education.

Highlights of research 2003/2004:

Establishment of CAMS

The Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria and CSIR Bio/Chemtek have joined forces to establish the Centre for Applied Mycological Studies (CAMS). The aim of CAMS is to build capacity in the study of fungi (mycology) in various fields and to utilise South Africa's fungal resources as a means of creating value within the country. The aim is also to ensure the protection and maintenance of our fungal biodiversity. CAMS aims to create an environment where international collaborators can liaise with a local institution such as CAMS at FABI to do research and development on South African fungi, to ensure that benefits flow back to the country.

Flavours and fragrances

CAMS is currently involved in a BioPAD BRIC project to commercialize the production of blue cheese flavour compounds. An industrial process was developed in conjunction with the CSIR and a technology package was finalized recently.

The project also includes research on the production of a peach flavour. For this purpose, more than 1000 fungal isolates from the fungal culture collections of CSIR, FABI and CAMS have been screened. Promising results were obtained and a hit rate of approximately 22% was achieved during the screening process. The CSIR, in conjunction with CAMS, is now looking into the economic

viability and optimization of the fermentation process.

In a separate study, 155 fungal isolates from CSIR, FABI and CAMS were screened for their ability to produce flavours such as pyrazines. Results showed that a significant number of fungi produce aromas resembling caramel, meaty, nutty, chocolate, coffee and green flavours.

Fungi associated with thatched roofs

The thatch roof industry in South Africa is relatively informal and normally thatching grass is used that grows in the vicinity where the roofs are constructed. Thatch is a good source of food for microorganisms and these can contribute significantly to the degradation during ideal weather conditions. Thatched roofs are aesthetically important in the tourist industry, but could be expensive when considering maintenance or insurance of the roof. In addition, the health risks based on allergens can present important problems.

An ongoing study is being conducted by CAMS and the Tshwane University of Technology to compare the level of fungal infestation from different thatch roof samples taken from all 12 weather zones in South Africa. Air samples have also been taken inside and outside the dwellings. The aim has been to determine the extent that fungal spores, originating from the thatched roofs, contribute to the health of inhabitants.

Results showed that the structure and the method of thatching influences the lifespan of the roof and contribute to the

tempo of degradation by fungi. Parameters such as the thickness and density of the thatch, the pitch and damage due to handling, sunlight and rain can significantly affect the quality of the roof. Insect infestation also indicates a high incidence of fungal contamination. Based on the mycological data, areas such as the Kruger National Park and the coastal areas of Kwazulu-Natal and the Eastern Cape Province are high-risk areas for fungal degradation and aerosols in thatch roof dwellings.

First report of Ochratoxin A in RSA

Mycotoxins in South African commodities are of great concern, especially in seasons where significant rains are experienced just before or during harvesting. It is known that staple foods like maize can contain mycotoxins such as fumonisin B₁, DON and zearalenone. There are also fungi on maize, such as *Stenocarpella maydis* in which case the mycotoxins are not known.

Recently, it was discovered by researchers in CAMS that ochratoxin A, a mycotoxin that causes kidney damage and cancer in humans and animals, is found in considerable amounts in vine fruit and dried products. During the past few years this toxin has received serious consideration at the Codex Committee on Food Additives and Contaminants (CCFAC). International levels are recommended and this mycotoxin is becoming an issue when South Africa exports agricultural crops to elsewhere in the world.

Maize resistance to mycotoxigenic fungi

CAMS is currently conducting a project in conjunction with The Maize Trust and THRIP to identify resistant commercial maize cultivars against mycotoxigenic fungi during storage. The rationale behind the project is that considerable work has been done on resistance of maize against plant pathogens in the field. However, humans consume only processed maize that is sometimes stored for a considerable time. During the storage process mycotoxigenic fungi are introduced and change the fungal population and thus mycotoxins that could be detrimental to the health of humans and animals. The project also aims to identify resistant genes, which

will assist us in the future development of resistant maize cultivars.

CAMS is involved in the SAFEFOODS project between the CSIR, UP, ARC and the European Commission in the 6th framework. The project aims to compare genetically modified maize and potatoes (GMO's) to conventional crops and determine whether differences exist based on nutrition, health, and various other characteristics. The role of CAMS is to compare the fungal populations associated with genetically modified maize and non-GMO's.

Mycotoxigenic fungi in animal feed

Mycotoxigenic fungi and their mycotoxins are of great concern to the feed industry. In cases where food crops are not fit for human consumption it is earmarked for animal feed. The reason for the poor quality is mainly because of fungal spoilage that contributes to the presence of mycotoxins. CAMS recently conducted a survey on pet food in the Pretoria area that included most of the pet food brands available in supermarkets. Results showed that a wide variety of fungi are associated with dog and cat food. These include *Aspergillus flavus*, *A. versicolor*, *Penicillium*, *Eurotium*, *Mucor* and *Trichoderma* species. Indications are that mycotoxins such as aflatoxins, sterigmatocystin, and various other known and unknown mycotoxins could be present. In addition, a project is also currently conducted to look at the quality of feed destined for livestock.

Fungal biodiversity in indigenous trees

As part of the CTHB, a study to investigate and isolate fungi that are associated with indigenous trees in the Northern Cape Province is currently conducted. The aim of the project is to determine the diversity of fungi associated with the environment and the trees. Due to the lack of knowledge pertaining to the associated mycoflora of these trees, it is envisaged that new and interesting fungal species will be found. Although the Northern Cape Province is known as an arid climatic zone, many fungi were isolated to date and efforts are underway to identify these fungi to species level.

Molecular stress physiology

Research leader: Prof Karl Kunert

Objectives of the research programme:

The overall objective of this research is to understand the mechanism of stress protection in plants. A particular first major research concern in 2003/4 has been the investigation of the cysteine proteinase/cysteine proteinase inhibitor system and its involvement in biotic stress protection and stress-induced plant senescence. A second research concern has been to evaluate labile DNA regions present in plant genomes, which vary in response to environmental stress.

Highlights of research 2003/2004:

Cysteine proteinase inhibitors (phytocystatins)

Research work carried out by A. Kiggundu has focused on optimising phytocystatin action for insect control (black maize beetle and banana weevil) for use in a plant genetic engineering approach. Phytocystatins, when ingested by phytophagous feeding, prevent the digestion of dietary proteins by binding to the active sites of proteinase enzymes present mainly in the mid-gut of insects (Fig. 1). However, there is considerable evidence that native cystatins provide only limited protection against insects. In our approach the assumption has been made that the protein to be engineered has not attained its optimum state of function through evolution.

Cystatin optimisation is currently done via site-directed mutagenesis and engineered mutated cystatin coding sequences are expressed, purified and used either in enzymatic assays with isolated insect gut extracts, or in a newly developed explant infiltration technique with insects. So far, several phytocystatins with significantly improved activity for coleopteran insects have been produced, which are currently being tested in transgenic maize plants for improved insect resistance as part of the involvement in a SA Innovation Fund project.

Together with the group of Prof. Michaud at Laval University in Canada we have also analyzed phytocystatin sequences for directed evolution at amino acid sites. Using site directed-mutagenesis we could engineer based on this information improved papaya cystatins with increased

activity papain and insect gut cysteine proteinases (Kiggundu *et al.*, Adaptive evolution among plant cystatins, and modulation of their anti-papain inhibitory activity by single mutations at a positively selected amino acid site. Submitted to Plant Journal).

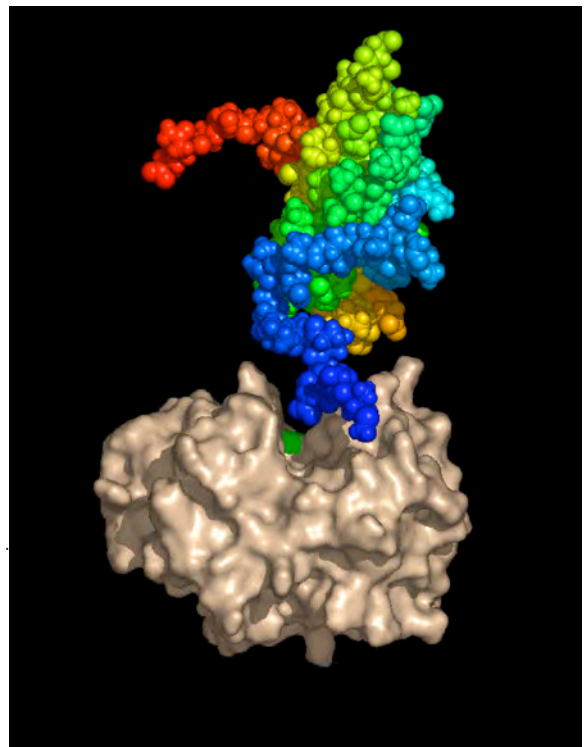


Fig. 1. Model how phytocystatin (blue part of upper molecule) binds to a pocket in the cysteine proteinase

Research carried out by Anneke Prins also focused on the analysis of the leaf proteome to explore exogenous cystatin action under abiotic stress conditions. In particular, transcript profiles of nuclear-encoded proteins under different abiotic stresses have been studied in

transformed tobacco expressing an exogenous rice cystatin. This included Rubisco activase and a chlorophyll binding protein for which transcript profiles under non-stressed and heat stress were measured. In general, plants expressing an exogenous phytocystatin had a lower transcript level for the light-harvesting chlorophyll binding protein. This indicates a possible involvement of the cysteine proteinase/cystatin system in the chlorophyll binding protein turnover. However, no clear protection of Rubisco activase, glutathione synthetase, ascorbate peroxidase, or D1 protein could be observed.

Cysteine proteinases

Through application of the RACE technique, Getu Beyene was able to isolate and characterize two novel cysteine proteinase genes from tobacco named NTCP1 (nucleotide GenBank accession number AY881011) and NTCP2 (nucleotide GenBank accession number AY881010) [Beyene *et al.* Isolation of two cysteine proteinases, NTCP1 and NTCP2 genes from tobacco (*Nicotiana tabacum* L.) leaves. Submitted to Plant MolecularBiology]. Expression studies revealed that NTCP1 is expressed only in senescing leaves (Fig. 2). This proteinase can therefore be used as a specific marker for leaf senescence. In contrast, NTCP2 has been found to be the first KDEL tailed cysteine proteinase cloned from tobacco being expressed in green mature leaves and down-regulated during drought and heat stress. Cysteine proteinase homologs of NTCP2 with KDEL motifs are known to be involved in programmed cell death in other plants like in germinating castor bean seeds and senescing flowers.

Labile genome regions

Research carried out by Christel van der Vyver and Juan Vorster focused on the isolation of variable DNA sequences from irradiated cowpea plants that show resistance to drought. Through the application of the technique of Representational Difference Analysis rapidly changing DNA sequences have been isolated and characterized. These rapid sequence changes might provide an adaptive mechanism to respond to stressful environments. Changes include base deletions and substitutions including changes from C→A, G→T, G→C C→T and G→A. Such base pair changes represent some of the most commonly observed point mutations in plants under stress and these mutations are therefore very likely a consequence of gamma radiation treatment. This DNA region is currently being characterized in greater detail by screening for DNA sequence variability and possible recovery from mutation in a large number of cowpea mutant lines using PCR. Detailed characterization might ultimately enable the identification of irradiation-associated variations that might further differ from those associated with naturally occurring mutations.

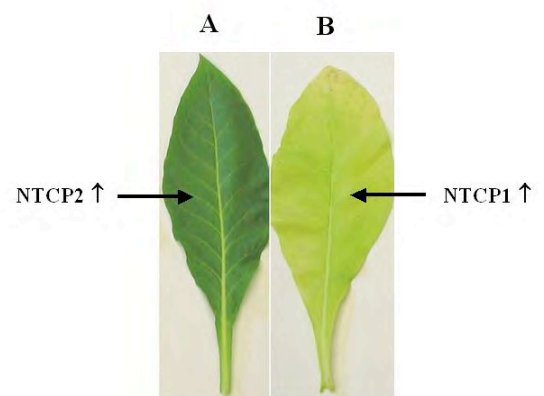


Fig. 2. Leaf development showing green (A) and senescing leaves (B) with increased expression (↑) of NTCP2 and NTCP1, respectively.

Resistance of Citrus Rootstocks to Root Pathogens

Research leader: Prof Nico Labuschagne

Research team: Dr Zeno Apostolides (Dept of Biochemistry)
Dr Thierry Regnier

Objectives of the research programme:

- Establishing which mechanisms are involved in resistance / tolerance of citrus rootstocks against fungal pathogens.
- Identifying specific biochemical compounds (phytoalexins) that are involved in disease resistance of citrus rootstocks.

Highlights of the research 2003/2004:

The viability of the citrus industry is to a great extent dependant on the rootstocks in use. Rootstocks must, therefore, have a high degree of resistance or tolerance to root and collar rots. In the search for alternative rootstocks, it is thus necessary to develop screening techniques that are rapid and reliable. In our research it has been shown that the determination of total soluble phenolics from citrus roots correlated well with tolerance or susceptibility towards *P. nicotianae*. Consequently, it can be used as a good pre-screening method for citrus rootstock resistance against *Phytophthora* root rot. Accumulation of the phytoalexin scoparone was examined in the roots of citrus rootstocks, to determine whether scoparone, which was shown to be correlated with resistance to *P. citrophthora* collar rot, also plays a role in resistance against *P. nicotianae* root rot. No correlation between the accumulation of scoparone and citrus root rot

resistance could however be demonstrated.

Thin layer chromatography (TLC) analysis of citrus root extracts revealed a number of other unidentified fluorescent compounds. A distinct yellow fluorescent compound, provisionally labelled U82, was detected in tolerant Swingle, Sour orange, Troyer and Macrophylla rootstocks (Fig. 1). This compound was absent in susceptible Rough lemon and Volckameriana rootstocks. Unknown compound U82 and other phenolic compounds were further characterized using TLC and HPLC and their antifungal activity determined by means of a bioassay with *Cladosporium* as an indicator fungus. Several compounds, including the yellow fluorescing compound, displayed antifungal activity during the *Cladosporium* bioassay (Fig. 2).

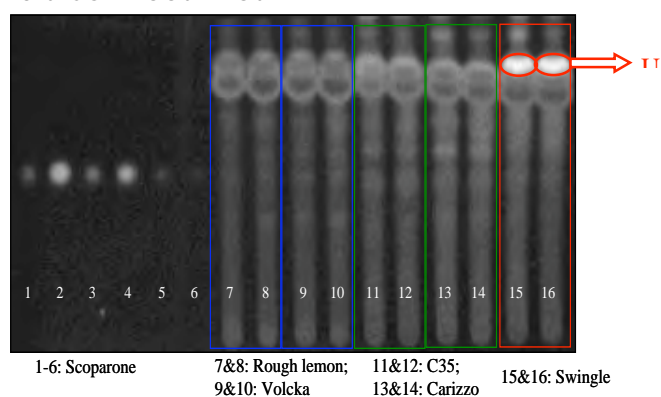


Fig. 1. TLC analysis of crude phenolic extracts from the roots of different citrus rootstocks, following inoculation by *P. nicotianae*. Chromatograms were developed with Toluene: Ethyl acetate (1:1) and fluorescence observed under UV light (360 nm). Lane 1 - 6, scoparone standards 10-200 µg/mL and

Lane 7 - 16, induced citrus root extracts. Arrow indicates unknown yellow fluorescent compound (U82) with Rf value of 0.82.

Unknown compound U82 was separated and purified from other citrus root phenolics by means of preparative TLC (Fig. 3). This procedure did work to some extent, but it was rather tedious and yielded low quantities of U82, which was not completely pure. A solid phase extraction (SPE) method was implemented to resolve this problem. Strata-X SPE columns (Phenomenex) was used to bind citrus root phenolics that allowed for selective subsequent elution. Strata-X SPE columns produced considerable pure amounts of the compound U82. These, therefore, represent a high throughput method, allowing large volume cleanup or purification. During the structure determination and identification of U82, ultraviolet spectroscopy revealed a maximum absorbance at 280 nm, which

is characteristic of some flavanoid type of compounds. Nuclear magnetic resonance (NMR) revealed a complex hydrogen composition or framework, indicating that U82 is a reasonably large compound compared to that of other phenolic compounds or phytoalexins. Although the SPE extraction method produced pure and considerable amounts of U82, it proved to be not sufficient for a proper NMR analysis of the carbon-framework. Current efforts are now focussed on obtaining sufficiently large quantities pure compound for adequate analysis. Such a unique compound that is constitutively produced does not need induction and that is only associated with tolerant rootstocks could potentially be used in developing a high throughput screening technique for citrus rootstock resistance.

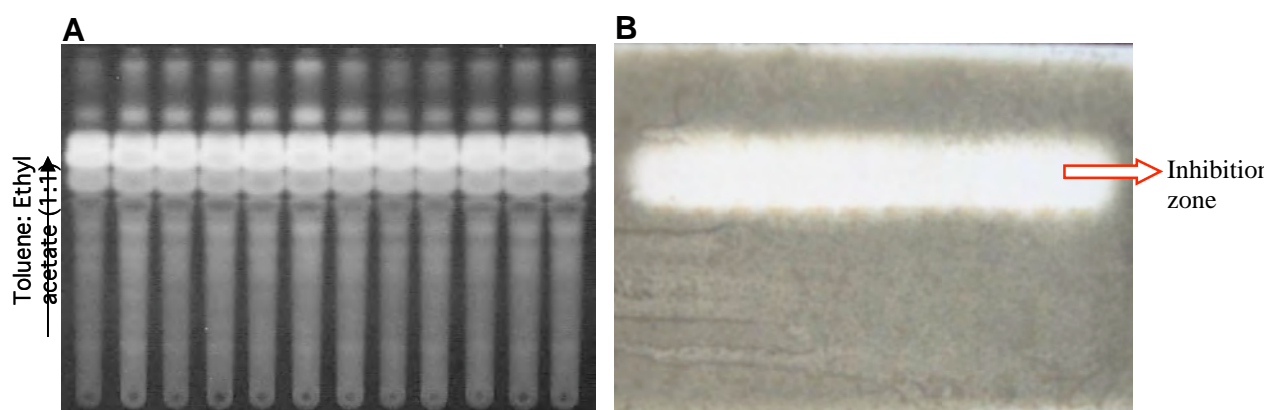


Fig. 2. TLC analysis of crude phenolic extracts from the roots of Swingle rootstock (A) and bioassay with *Cladosporium cladosporioides* (B).

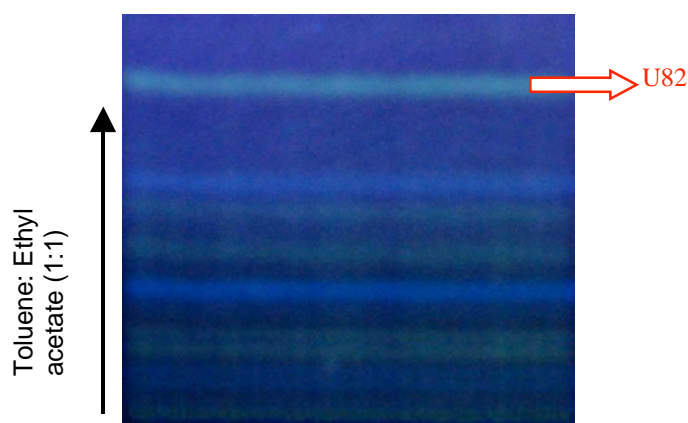


Fig. 3. Preparative TLC analysis of crude phenolic extracts from the roots of Swingle rootstock following inoculation by *P. nicotianae*. Arrow indicates unknown yellow fluorescent compound (U82) with Rf value of 0.82.

Newly Emerging Plant Pathogenic Bacteria

Research leader: Prof Teresa Coutinho

Research team: Dr Fanus Venter (Dept of Microbiology & Plant Pathology)
Prof Mike Wingfield
Dr Altus Viljoen

Objectives of the research programme:

- Develop rapid, reliable methods of accurately identifying plant pathogenic bacteria.
- Characterise and type isolates of pathogenic bacteria responsible for economically important diseases of *Eucalyptus*, bananas and other agricultural crops.
- Study the epidemiology and biology of selected emerging plant pathogenic bacteria.
- Determine the association of pathogenic bacteria with fungal pathogens of plants.
- Identify virulence factors of selected plant pathogenic bacteria.
- Train and educate students in phytobacteriology, a neglected field of plant pathology.
- Establish a centre of excellence in phytobacteriology.

Highlights of research 2003/2004:

The research during this period focused primarily on two bacterial pathogens of plants, viz. *Pantoea ananatis* and an, as yet, only partially characterised pathovar of *Xanthomonas campestris* known as *musacearum*.

Genus *Pantoea*

The genus *Pantoea* belongs to the Enterobacteriaceae. There are seven species and two sub-species with the majority associated with plants causing a wide range of symptoms on a number of hosts. In South Africa, *P. ananatis* has been found to cause bacterial blight and die-back of eucalypts, central rot of onions and a stalk rot disease of maize. Since the first description of *P. ananatis*, in 1998, causing bacterial blight and dieback in different *Eucalyptus* species, hybrids and clones in South Africa, there have been numerous reports of this disease emerging in other parts of Africa, South America and Asia. *P. agglomerans* has also been found to cause blight of onion. None of the other plant associated *Pantoea* spp. have been reported in South Africa. However, due to the complexity in identifying *Pantoea* spp., they may well occur but remain undetected.

One of our first objectives was to develop a rapid, molecular-based method of identifying *Pantoea* spp. Various methods were evaluated including 16S-23S ITS-PCR. AFLP analysis, however, proved to be the most reliable and reproducible method of typing the species and sub-species in the genus. The species cluster in distinct groups with their type strains. This method is now used routinely to identify isolates of *Pantoea* from different hosts and countries world-wide.



Bacterial blight symptoms on eucalypt leaves

We collaborate closely with the Laboratory voor Microbiologie at the University of Gent, Belgium. In

November 2004, our research on *Pantoea* was presented at a 1-day workshop in Gent. The collaboration has been extended by a further 2 years and is funded by the Flemish/South African Governments agreement.



From left to right: Dr Marc Vancanneyt, Carrie Brady, Dr Fanus Venter, Prof Jean Swings, Prof Teresa Coutinho, Teresa Goszczynska and Ilse Cleenwerck

***Xanthomonas campestris* pv. *musacearum* (Xcm)**

Xanthomonas campestris pv. *musacearum* causes a bacterial wilt disease of ensete (*Ensete ventricosum*), a banana-like plant that serves as a staple food crop for about 20 million people in Ethiopia. This pathogen also attacks banana plants in Ethiopia and Uganda. Large scale losses of these crops due to bacterial wilt could lead to malnutrition and starvation in central and southern Africa, where an estimated 100 million people rely on them as a staple diet.

Xanthomonas campestris pv. *musacearum* isolates have been collected from both ensete and banana in Ethiopia and from banana in Uganda and the Central Republic of the Congo. Isolates were phenotypically identified as *Xanthomonas campestris*. The 16S rRNA gene from various isolates was sequenced to verify the identity of a number of isolates. The AFLP technique is currently being used to determine the genetic relatedness among the three populations. The results will clarify

questions related to the epidemiology and spread of this pathogen in central Africa.



Male flower infected with Xcm



Young banana plant infected with Xcm

Diseases of Indigenous Food Crops Grown by Smallholder Farmers

Research leader: Prof TAS Aveling

Research team: Prof N Labuschagne
Dr N Lall (Dept of Botany)

Objectives of the research programme:

To identify and study the plant pathogens, storage fungi and associated mycotoxins of cowpea and other indigenous food crops

Highlights of the research 2003/2004:

Initially the "diseases of indigenous food crops" research programme of Prof. Aveling focused on the diseases of cowpeas but the programme has expanded to include Bambara groundnut, cassava and sweet potato. Some research is also being done on maize grown by small-holder farmers. Numerous excursions and field evaluations by the programme leader and her postgraduate students have been conducted in Maputo in Mozambique, Makatini Flats in Kwa-Zulu Natal and Kanyamazane/Kangwane in Mpumalanga.



Students recording results of a greenhouse fungicide trial

Other cowpea research focused on mycotoxins and the effect of essential plant oils on storage fungi of cowpeas. Cowpea leaf extracts were investigated for antimicrobial properties and the results showed that the extracts did indeed exhibit antifungal activity at 2.5 and 5 mg/ml. This work has been

published in the South African Journal of Botany and also represents the first report of cowpea extracts exhibiting antimicrobial activity. A world first report of the presence of fumonisins and the causal fungus on cowpea was made and published in the Journal of Agricultural and Food Chemistry.

A survey amongst small-scale farmers of cowpea and maize in Mpumalanga was started towards the end of 2003. This entailed visiting the farmers in the rural communities and having them completing a questionnaire. The questionnaire included questions on storage practices, the uses of cowpea and maize and the importance of the crop to the farmer's livelihood. The survey involved 71 farmers from rural communities in Mpumalanga.



Cowpeas intercropped with maize in Mozambique

The importance of the cowpea research was emphasised by Prof. Aveling being elected as Chairperson of the Organising Committee for the 1st International Edible Legume Congress in conjunction with the 4th World Cowpea Congress held at the International Convention Centre in Durban from 17-21 April 2005 (www.up.ac.za/conferences/ielc). The conference was a huge success attracting delegates from 20 countries of which 15 were African.

Physiological and biochemical characterisation of four South African varieties of Bambara groundnut was done in collaboration with the Swedish Biodiversity Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden. Germination potential, emergence percentage, plant height, plant weight, leaf number/plant and total phenolics were quantified, to supplement the known phenotypic descriptions of the cultivars. In another study, the seed-borne mycoflora present on the seed coat of six Bambara groundnut cultivars, differing in seed coat colour, was determined and related to surface topography and/or the amount and localisation of polyphenolic compounds.



Germinating cowpea seed infected with a *Fusarium* sp.

Research on viruses of sweet potato in collaboration with the Agricultural Research Institute, Roodeplaat, was part of a project funded by the EU. A collaborative project on the viruses of cassava was established with the

International Institute of Tropical Agriculture (IITA), Eastern and Southern Africa Regional Center (ESARC), Kampala, Uganda. Mr Joseph Ndunguru from Tanzania started his PhD in 2003 at the University of Pretoria researching these viruses on cassava in Tanzania and Uganda. The thesis has been passed by all the examiners and four manuscripts in accredited journals have been accepted for publication, two in the prestigious journal, *Virology*.

Prof. Aveling's research speciality is seed pathology and she has been nominated as member of the Scientific Programme Advisory Committee (SPAC) for the 28th International Seed Testing Association Symposium to be held in Brazil in 2007. Prof. Aveling was also nominated and appointed Vice-Chairperson of the Storage Committee of the International Seed Testing Association and also made a Member of the Plant Disease Committee of the International Seed Testing Association for the period 2001-2005 at the 26th Congress of the International Seed Testing Association held in Angers, France from 14 - 22 June 2001. Both positions have been renewed to 2007 at the 27th International Seed Testing Association Triennial Congress, held in Budapest, Hungary from 17 - 20 May 2004.



Cowpea seed stored under poor conditions in Mpumalanga

Fusarium Diversity in South Africa

Research leader: Dr E Steenkamp

Research team: Prof BD Wingfield
Prof MJ Wingfield
Prof WFO Marasas

Objectives of the research programme:

The objective of this research programme is to obtain a global view of the evolution of species in the fungal genus *Fusarium*. The research focuses strongly on relationships within and among species, but also on deeper taxonomic relationships among sections and/or species complexes. Accordingly, our major goals are to:

- Characterise *Fusarium* species that are of ecological, agricultural, commercial and medical importance using morphology- and DNA-based approaches.
- Analyse the population biology of these important species using cultural and molecular characters.
- Build an overall picture of the relationships among *Fusarium* groups, sections and species complexes using molecular phylogenetic approaches.

Highlights of the research 2004/2005:

Our major research highlight for 2005 was the initiation of the waterberry floral malformation project. Floral malformation in waterberry (*Syzygium cordatum*) is characterized by abnormal and diseased flowers that do not bear fruit (Fig. 1). This disease closely resembles the inflorescence malformation disease of mango, where the causal agent of the disease was shown to be a *Fusarium* species (*F. mangiferae*) in the *Gibberella fujikuroi* complex. Our preliminary results suggest that *Fusarium* may also play a significant role in waterberry malformation, because this fungus was isolated from all the diseased waterberry samples we have examined thus far. Interestingly, all the samples also harboured large numbers of mites (*Eriophyes afroensis*, Fig. 2), which is analogous to the situation in mango malformation where the disease seem to be associated with the mite *Eriophyes mangiferae*. In the year ahead we hope to establish the role, if any, of *Fusarium* and *Eriophyes* in the malformation disease of waterberry flowers.



Fig. 1. Malformed (left) and healthy (right) inflorescence of waterberry (*Syzygium cordatum*)

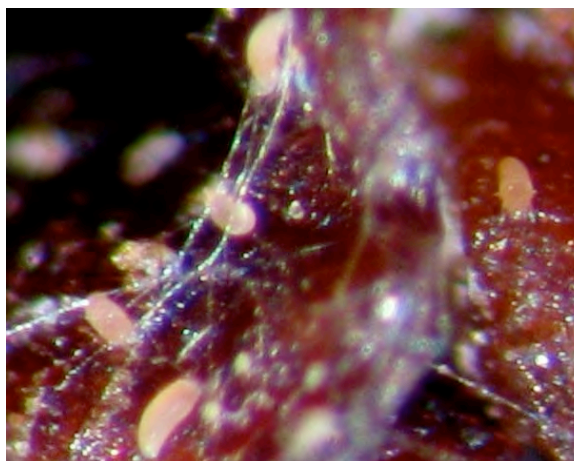


Fig. 2. Mites (*Eriophyes afroensis*) on malformed waterberry floral tissue

Rhizobial Diversity Associated with Indigenous Legumes

Research leader: Dr E Steenkamp

Research team: Dr I Law (ARC-PPRI)

Objectives of the research programme:

Our overall objective is to characterise the diversity of rhizobia associated with Southern African legumes, especially those that are of agricultural, commercial and medicinal value. To accomplish this, we isolate and identify the rhizobial bacteria from the root nodules of legumes from various regions in Southern Africa. Such identifications are generally achieved with a combination of traditionally used morphology and biochemical tests, as well as DNA-based approaches. These bacteria are also evaluated for their ability to nodulate (Fig. 1) and to fix atmospheric nitrogen. Ultimately these data provide an indication of the distribution of rhizobial species and their ecological significance for indigenous legumes.

Highlights of the research 2004/2005:

We currently have two ongoing projects in this programme. The major aim of the one project is to determine the evolution and population biology of *Bradyrhizobium* species associated with cowpea in Southern Africa. For this purpose we are employing a phylogenetic approach using the combined sequence information from multiple nuclear and symbiotic genes. The second project aims to identify the rhizobial bacteria associated with the root nodules of uniquely South African *Hypocalyptus* species. In the year ahead, we also hope to start studying the diversity and significance of rhizobia

associated with the root nodules of kiat (*Pterocarpus angolensis*).

Pterocarpus angolensis is a native South African leguminous tree. Parts of the tree are commonly used for medicinal purposes and carving. This project will form an integral part of the Centre of Excellence in Tree Health Biotechnology (CTHB) housed in FABI.

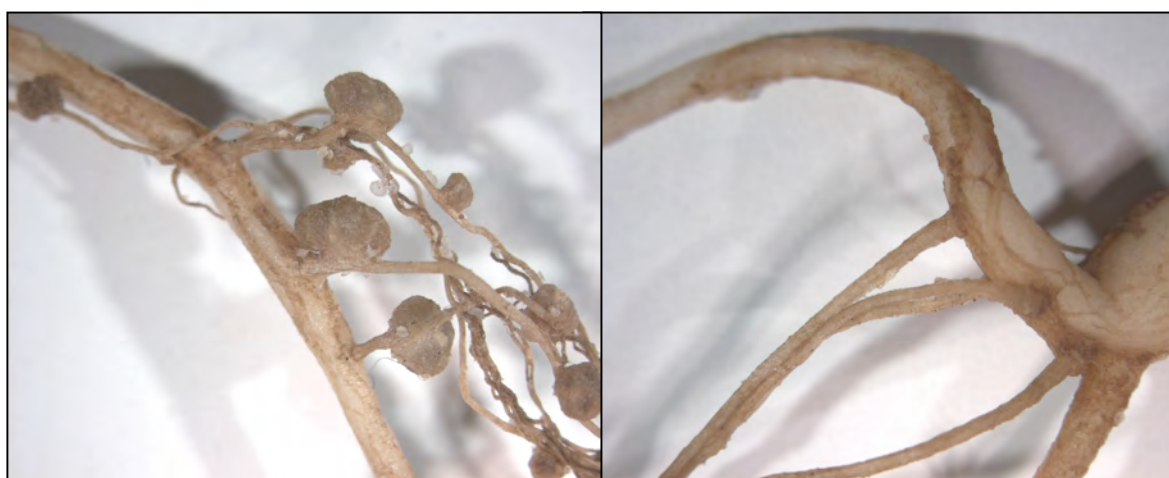


Fig. 1. Nodulation assay on siratro (*Macropitilium atropurpureum*) roots, where bacterial isolates either generate numerous root nodules (right) or are unable to successfully infect and colonize the roots (left).

Protist Origins of Animals and Fungi

Research leader: Dr E Steenkamp

Research team: Dr F Lang (University of Montreal)

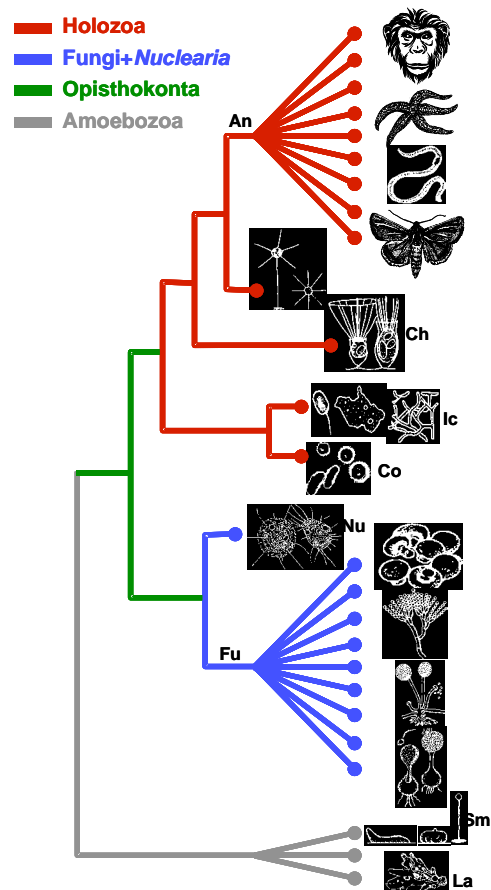
Objectives of the research programme:

The objective of this research programme is to study the early evolution of fungi and their protistan allies. For this purpose we employ a variety of phylogenetic approaches to infer evolutionary relationships from concatenated sequence data. Our main research questions are:

- What was the nature of the ancestral fungus?
- Which protists represent sistergroups of fungi?
- How are lower fungi related to their protistan sister taxa?
- How are protistan sistergroups of fungi related to other eukaryotes?

Highlights of the research 2004/2005:

Our major research highlight for 2005 was the initiation of the *Nuclearia* EST and mitochondrial genome sequencing projects. *Nuclearia* is an amoeboid protist that, together with fungi and animals (Metazoa) represents the super-kingdom 'Opisthokonta'. Within this grouping, several additional protists are specifically associated with Animals, together designated as 'Holozoa'. The known holozoan protists are *Ministeria* spp. (stalked or free-living amoebae with symmetrically-distributed, radiating pseudopodia), Choanoflagellata (aquatic uniflagellates with collars of tentacles that resemble the choanocytes of sponges), Ichthyosporea (fungus-like, amoeboid, and uniflagellate animal parasites), and *Corallochytrium* spp. (simple free-living, non-flagellated saprophytes). However, among the known opisthokont protists, only *Nuclearia* is specifically associated with fungi. Preliminary results of our genomic analyses of this unique protist suggest that it would add considerable resolution to the base of fungal phylogenetic trees. In the year ahead we hope to generate strongly supported and well-resolved phylogenies of the early evolution of fungi. We also plan to explore South African freshwater environments for the presence of additional protists that branch close to the origin of fungi.



Phylogeny of Opisthokonta. Within the opisthokont clade, the holozoan protists are indicated as follows: Mi - *Ministeria* spp.; Ch - Choanoflagellata; Ic - Ichthyosporea; and Co - *Corallochytrium* spp. Within the Fungi + *Nuclearia* clade, *Nuclearia* spp. are indicated with 'Nu'. The tree is rooted with the amoebozoan taxa slime moulds (Sm) and lobose amoebae (La). Branches leading to animals and fungi are indicated with 'An' and 'Fu', respectively.

The Forest Molecular Genetics (FMG) Programme: Biotechnology for Superior Fibre

Research leader: Dr Zander Myburg

Objectives of the research programme:

The Forest Molecular Genetics Programme is hosted within FABI and the Department of Genetics at UP. The main focus of the programme is to learn more about the molecular genetics of fibre development and to develop biotechnology tools for the improvement of wood and fibre quality in plantation tree species. High-throughput molecular technologies are used for:

- Gene discovery research (e.g. gene expression profiling in developing wood).
- Functional genetics research (e.g. testing of tree genes in model systems such as *Arabidopsis thaliana*).
- Allele discovery research (characterization of allelic diversity in candidate wood formation genes).
- The development of molecular breeding tools (e.g. AFLP, SSR, SNP markers) for the genetic improvement of plantation forest tree (*Eucalyptus* and *Pinus*) species.

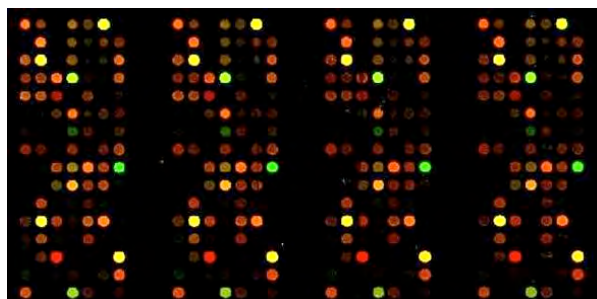
Highlights of the research 2003/2004:

Most of the current research activities in this programme are funded under the Wood and Fibre Molecular Genetics Programme, which is a joint research and development venture of the University of Pretoria, Sappi Forests and Mondi Business Paper South Africa. Additional financial and student support is provided by the Technology and Human Resources for Industry Programme (THRIP) and the National Research Foundation (NRF) of South Africa.

During the past two years we have made tremendous progress in the high-throughput profiling of gene expression in differentiating woody tissues of *Eucalyptus* trees. More than 6000 cDNA-AFLP gene fragments were profiled in mature xylem, immature xylem, phloem and cork collected from fast-growing *Eucalyptus* trees. This allowed the identification of more than 1000 fragments representing genes that are differentially regulated during wood formation in *Eucalyptus*. We have also used a 2600-gene wood formation gene chip (kindly provided by Prof. Ron Sederoff at NC State University) to profile gene expression in different tissues collected from the same and from different *Eucalyptus* trees (clones). These

studies are allowing us to obtain a detailed overview of gene expression variation within and between genotypes of *Eucalyptus*.

Candidate genes isolated in forest trees can (in some cases) be tested in model genetic systems such as *Arabidopsis thaliana*. During the past year we have focused on the use of vascular and biochemical mutants of *Arabidopsis* to study the function of genes isolated in *Eucalyptus*.



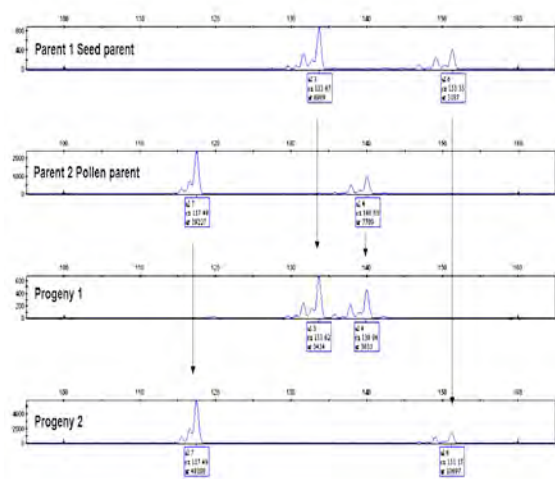
Microarray profile of gene expression in xylem vs. phloem tissues of *Eucalyptus*



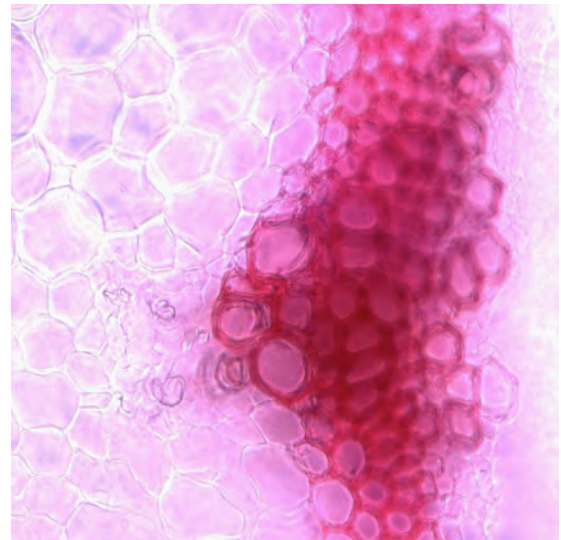
Taking a peek at fibre formation

Three M.Sc. research projects in the programme are aimed at investigating allelic diversity in wood and fibre genes of various *Eucalyptus* and pine species. This work allows us to achieve a better understanding of molecular evolution in wood and fibre genes, and provides a better understanding of the structure of genetic diversity in tree breeding populations. Single nucleotide polymorphism (SNP) markers have been identified for several candidate lignin and cellulose biosynthesis genes and are currently being used to assay allelic diversity in these genes.

Simple sequence repeat (SSR) markers are powerful tools that can be used to



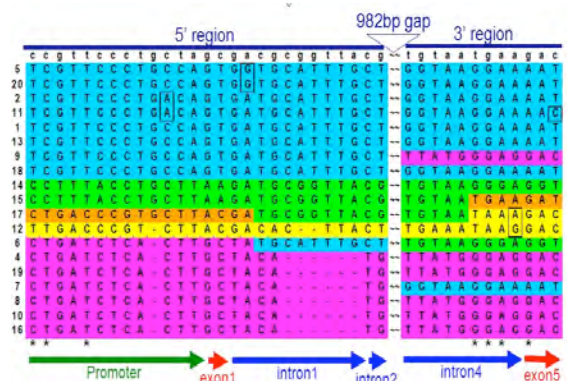
Parentage analysis in *Eucalyptus* using SSR markers



Lignin staining of xylem tissue in *Arabidopsis*

fingerprint closely related trees and support routine tree breeding activities. In the past two years, we have used SSR markers to successfully determine the level of pollen contamination in controlled crosses and the extent of pollen competition in polymix crosses of *Eucalyptus* trees.

In Nov 2005, we will have the privilege of co-hosting the IUFRO Tree Biotechnology 2005 meeting, which will bring world leaders in tree molecular biology and tree genomics to South Africa.



Allelic diversity in a lignin gene of *Eucalyptus*

Diseases and Pests of Banana

Research leader: Dr A Viljoen

Research team: Prof K Kunert
Dr R Chikwamba

Objectives of the research programme:

- To develop integrated disease and pest management strategies for the South African banana industry.
- To develop biotechnological tools for research on diseases and pests of banana.
- To isolate, identify and manipulate genes in banana with resistance to diseases and pests of banana.
- To develop a transformation strategy for the unconventional improvement of banana plants.

Highlights of the research 2003/2004:

Integrated disease and pest management

Biological control: Non-pathogenic isolates of *Fusarium oxysporum* are used for the biological control of pathogens, such as *F. oxysporum* f.sp. *cubense* (*Foc*), and pests, such as *Cosmopolitus sordidus* and *Radopholus similis*, of banana. The mode of action and colonisation patterns of non-pathogenic isolates of *F. oxysporum* is also investigated. Non-pathogenic isolates of *F. oxysporum*, other microbial biological control agents and commercial products that reduced the incidence of Fusarium wilt of banana in the greenhouse will, in future, be included in field trials for biological control of Fusarium wilt. Research on biological control of the banana weevil and nematodes is conducted in collaboration with the International Institute for Tropical Agriculture (IITA) in Uganda.

Chemical control: Two commercial products that are environmentally friendly and non-corrosive, Sporekill and Prazin, were effective as sterilants against *Foc*. Both products are now recommended for surface disinfection of machinery and field equipment in order to prevent the spread of Fusarium wilt to uninfected banana fields in South Africa. These products have replaced the less effective and environmentally unfriendly sterilants previously used.

Several fungicides inhibited the mycelial development of *Foc in vitro*, with the DMI fungicides and Benomyl found to be most effective. The same fungicides also reduced severity of Fusarium wilt in the greenhouse, but have not been evaluated in the field. Certain pesticides have proved to be very effective in reducing banana weevil damage when injected into pseudostems in the field, and could be recommended to local banana producers.

Chemical activators provide one of the more attractive options to combat Fusarium wilt of banana, as they stimulate the plants' own defence responses. Greenhouse and field evaluation showed that some activators reduced Fusarium wilt significantly, both in the greenhouse and field, and should be considered for inclusion in an integrated disease management strategy.

Cultural control: The effect of plant nutrition, soil pH and planting date on Fusarium wilt development have been investigated both under greenhouse and field conditions. Preliminary data suggest that the form and amount of nitrogen, and the pH, influence plant vigour, but did not reduce the incidence of Fusarium wilt. Deleafing practices at the onset of spring have almost entirely eliminated yellow Sigatoka from banana fields in South Africa. Pheromone trapping could be applied effectively and economically for the control of the banana weevil. The effect of field sanitation and bunch

harvesting practices on weevil damage has also been investigated.

Plant resistance: In collaboration with the South African banana producers, banana tissue culture companies and international banana improvement programmes, new banana hybrids, somaclonal variants and field selections are continuously tested for tolerance and resistance to *Fusarium* wilt under the subtropical environmental conditions in South Africa.



Embryogenic callus with somatic embryos from immature male flower of Cavendish bananas cv Williams (AAA)

Development and application of molecular and biotechnological tools for banana research

Comparative genomics: DNA-based technologies such as AFLP analysis, PCR-RFLPs, DNA sequencing and microsatellite marker analysis have been used for characterization and population studies involving several pathogens and non-pathogens of banana. These include *F. oxysporum* f.sp. *cubense*, *Mycosphaerella musicola*, *M. fijiensis*, *M. musae*, *M. colombiense*, *Cladosporium musae* and *Xanthomonas campestris* pv. *musacearum*. AFLP analysis has also been used to study the population structure of the banana weevil borer, *C. sordidus*.

Molecular markers are developed for the rapid detection of vegetative compatibility groups in *F. oxysporum* f.sp. *cubense*, and for *Mycosphaerella* spp. associated with banana leaves.

Suppressive subtraction hybridisation is used for the isolation and identification of genes associated with resistance in banana to *Fusarium* wilt (see D. Berger), and representational difference analysis for genes associated with resistance to the banana weevil (report by K. Kunert).

Functional genomics: Expression profiles of banana genes associated with resistance to *F. oxysporum* f.sp. *cubense* were analysed using microarray analysis and rtPCR (report by D. Berger). In order to enhance its activity levels against the banana weevil, a papaya systatin gene was modified by means of site-directed mutagenesis (report by K. Kunert). The presence of genes associated with virulence in fungi, such as *fmk1*, *pg1* and *xy13*, were established in *F. oxysporum* f.sp. *cubense* and other *formae speciales* of *F. oxysporum*, and phylogenetically compared. To better understand virulence in *F. oxysporum* f.sp. *cubense*, selected genes will be disrupted in collaboration with scientists at Cornell University by means of site-directed mutagenesis.

Establishment of a tissue culture and transformation facility for banana
A tissue culture facility was established at FABI for the micropropagation of banana varieties required for research purposes. Embryogenic cell suspensions, produced from immature male flowers of high-yielding Cavendish bananas, have been genetically modified by means of *Agrobacterium*-mediated transformation with a plasmid containing the GUS and rice systatin genes. Expression levels are currently being investigated.



Banana plant infected with *Foc*

Mushroom Research

Research leaders: Prof L Korsten
Prof TAS Aveling

Research team: Dr P Labuschagne
Dr T Regnier
Dr L Meyer

Objectives of the research programme:

In the commercial cultivation of white button mushrooms (*Agaricus bisporus*), compost colonized with mushroom mycelium is covered with a casing layer, to initiate development of mushrooms. In this layer the switch from vegetative (mycelium) to reproductive (mushroom) growth takes place. Physical, chemical and microbiological factors determine the suitability of a casing material. Peat neutralized with calcitic lime is the most widely used casing material for mushroom cultivation throughout the world. Its water holding capacity and structural properties are widely accepted as ideal for the purpose of casing. However, problems associated with its use, especially regarding its availability which is directly linked to the depletion of our natural reserves and the alteration of ecosystems, have led to the search for alternative materials. New legislation is further under consideration concerning the utilization of South African peat lands. The local reed-sedge peat is currently used as casing material in button mushroom cultivation in South Africa and due to the questionable sustainability of peat supplies to the local mushroom industry, research needs to be done to evaluate agricultural wastes as alternatives that can fulfil the functions currently performed by peat.

Standard physical, chemical and biological methods have been defined and used as parameters in the evaluation of different agricultural waste products (i.e. citrus, sugarcane and grape byproducts) as potential casing materials. Physical parameters have been found essential in defining substrates for casing materials, because the main role of such substrates is to provide the proper physical conditions for mushroom development. The most important parameters are: pH, electrical conductivity, bulk density, porosity and water holding capacity. These parameters have been optimised and are currently used to predict whether a particular substrate has the potential to be a good casing material. Furthermore, this information is currently being used to alter a particular casing material to improve on its cropping potential. In order to have performance characteristics at least equal to peat, a competitive cost, stable quality, continuity of supply, absence of pests and diseases and ease in handling are some of the characteristics that can be used to screen by-products. The primary focus of our research program is the development and optimization of alternative casing material for *A. bisporus* cultivation. A temperature and humidity controlled growing facility was therefore developed on the main campus and is currently used for small-scale experiments to evaluate different agricultural wastes that could be used as an alternative for peat as a mushroom casing layer for button mushroom cultivation. The casing project was expanded in 2004 to include postgraduate students Ms Z Pieterse and Mr H du Plooy investigating one of the mushroom pathogens, *Mycogone perniciosa* (wet bubble) of the button mushroom under supervision of Prof Terry Aveling.

The most characteristic symptom of wet bubble disease is the development of distorted masses of mushroom tissue, which are initially white and fluffy which become brown as they age and decay. It is the wet decay and the shape of affected mushroom tissue that gives the disease its common name (Fletcher et al., 1994). One of the most important means of control is the elimination of primary sources of the pathogen. General sanitation, which includes sound hygiene practices, is key to the control of the disease. As with all diseases, early detection of the pathogen is important for effective control.

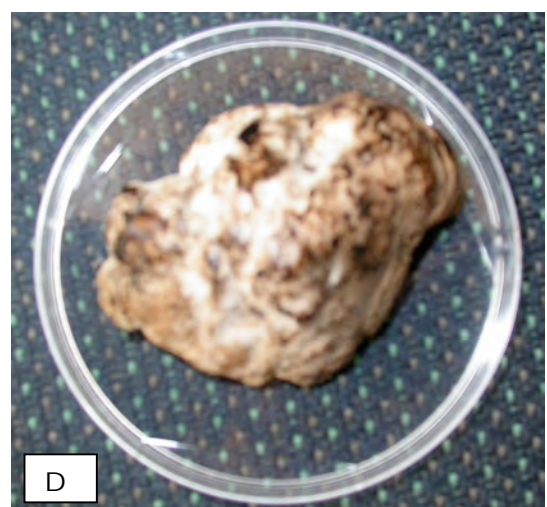
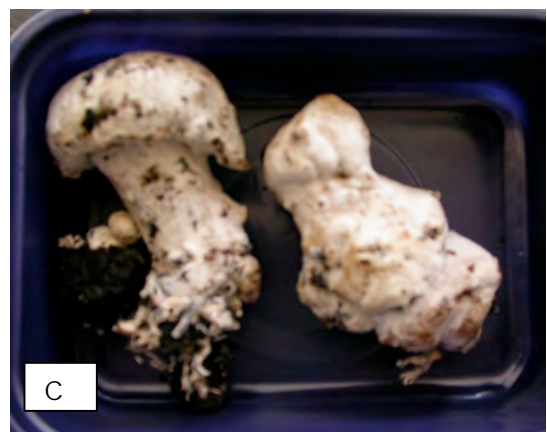
Currently, our project is aimed at designing a rapid, easy to apply PCR-based protocol that is able to detect and quantify the pathogen in various types of samples, with emphasis on casing materials.

Highlights of the research 2003/2004:

A temperature and humidity controlled growing facility was constructed on the main campus of the University of Pretoria, with funding granted by the South African Mushroom Farmers' Association and THRIP. The facility is currently used for small-scale experiments to evaluate different agricultural waste products that could be used as an alternative for peat as a mushroom casing layer. Six different casing trials have been conducted. The first trial effectively resulted in reduction of casing mixes that proved impractical and low yielding. Different mixes of wattle bark, bagasse, filter cake, coir and Topterra casing were extensively evaluated. Apart from yield, the different casing mixes were also compared in terms of their water absorption abilities. The trials did highlight some potential materials that will be used in future trials. Extensive cinnamon brown mould contamination during some of the trials was found to be due to excessive pasteurisation of the wattle bark.



White button mushroom experimental cultivation (A) and the presence of cinnamon brown mold on wattle bark casing material (B)



Wet bubble disease (C and D)

Citrus Black Spot Research

Research leaders: Prof L Korsten
Prof TAS Aveling

Research team: Dr T Regnier
Dr L Meyer
Dr J van der Waals

Objectives of the research programme:

The South African citrus industry is the 12th largest citrus producer in the world, and the third biggest exporter of fresh fruit. Currently, 60% of all citrus produced in South Africa is exported fresh to mainly European countries. Currently, the industry is negotiating market access into the USA and China, which will cause significant growth in this sector. However, with increased free trade there will also be new restrictions in the form of phytosanitary barriers to trade.

Citrus Black Spot caused by *Guignardia citricarpa* Kiely is one of the most important fruit diseases on citrus in South Africa and other mainly southern hemisphere countries. The disease does not occur in European citrus producing countries and has therefore become a major barrier to trade. This is despite the more than 80 years of unrestricted exports from South Africa to the EU. In order to remain competitive on the European markets and to access new markets, a risk assessment (RA) study had to be done to ensure continued access into the EU. Comments and concerns from the EU on the RA document from South Africa have been addressed within this research project.

Highlights of the research 2003/2004:

Rapid detection of citrus black spot infected fruit

Application of the primer set Citric 1 and Camel 2 in conjunction with the ITS 4 primer yields PCR amplicons of approximately 580 bp and 430 bp for *G. citricarpa* and *G. mangiferae* respectively. Results obtained with these primers are in accordance with sequence data and tests were repeated five times with the same results. A BLAST search revealed no matches other than *G. citricarpa* and *G. mangiferae*, and no positive PCR results were obtained with *Colletotrichum gloeosporioides*, the most common contaminant in blackspot lesions. We are therefore able to distinguish *G. citricarpa* and *G. mangiferae* unequivocally using a PCR based technique. This significantly shortens the time needed to test export consignments for positive CBS black spot infection.

Monitoring *Guignardia* spp. in soils

The SoilMaster DNA kit is being used in conjunction with the FastDNA Spin kit and FastPrep Instrument to extract DNA of the *Guignardia* citrus pathogen and

endophyte from soil. The PCR technique is currently being used further to detect the pathogen on asymptomatic fruit. The sensitivity of the procedure must still be determined. PCR reactions with the designed primers for *Guignardia citricarpa* and *G. mangiferae* are being used with the addition of 300 ng/μl BSA to the reaction mix to alleviate possible inhibitory substances, such as humic and phenolic acids in soil samples. Negative results can now be screened for inhibition before final confirmation of the pathogen in environmental samples.

Seasonal availability of ascospore inoculum

Leaf litter has been collected on a monthly basis from a lemon orchard near the town of Brits. The leaf litter was processed without further incubation and spores captured with the Kotzé Inoculum Monitor. Ascospores of *Guignardia* spp. were captured from the leaf litter collected in May, with the highest capturing of ascospores from litter collected during March.

Litchi Research Project

Research leaders: Prof L Korsten

Research team: Dr T Regnier

Objectives of the research programme:

South African litchi fruit are mainly exported to lucrative European markets, which require extended storage life, an attractive red colour and high fruit quality. Pericarp browning, desiccation and post harvest decay are major problems restricting expansion of the South African industry. Currently, litchi fruit is commercially fumigated with sulphur dioxide (SO₂) to overcome these problems. However, SO₂ fumigation leaves undesirable residues, alters the fruit taste and may potentially represent a health hazard for sulphur sensitive consumers and pack house workers. Furthermore, strict new pesticide regulations and maximum residue levels (MRL) enforced by the European community has resulted in a reduced allowable maximum sulphur residue level of 10 µg g⁻¹ in the edible portion of the fruit. This has necessitated the development of an alternative postharvest treatment to maintain overall quality during storage and transportation. The South African litchi growers experience a high percentage of losses at the export end of the fruit chain, which prevent them from attaining premium prices on the export markets.

Understanding the factors that contribute to postharvest decay and managing it more effectively to ensure quality remains a critical aspect for the industry if future growth is to be ensured. *Penicillium* spp. play an important role in postharvest decay and are often associated with saprophytic growth on the fruit surface rendering the product unmarketable. In order to effectively detect and trace the source of origin of the pathogen it is essential to have a thorough understanding of population variation, type and range of species found in different fruit handling environments and on fruit. Furthermore, it is essential to develop a rapid direct *in situ* (on fruit) detection method that eliminates lengthy isolation and identification methods, which can take several months to complete. A PCR method can be developed that can detect the pathogen directly on the fruit within 6 hrs, providing a very helpful tool in legal disputes. The PCR method can be used to further detect the source of origin and thereby providing a helpful tool that can be used in the eradication or better management of the postharvest disease problem currently experienced by the industry.

Understanding the microbial composition, interaction and buffering capacity on plant surfaces has become critical to more effective control of fruit diseases and ensure improved product quality. Recent studies indicate that different chemical treatments adversely affect the microbial buffering capacity on fruit surfaces, often leading to increased disease caused by epiphytes/ pathogens previously regarded as unimportant. In this project the microbial dynamics of litchi fruit surfaces from flowering up until marketing are being studied. In view of this, a study was initiated to monitor the microbial composition of litchi fruit from the early flower bud stage till the end point at the export market.

Micro cracking is a serious concern for litchi growers since it ultimately affects the postharvest quality of the fruit. In order to better understand the processes that lead to micro cracking, a preharvest study was initiated to monitor cuticle development. In addition, recent international requirements to ensure product safety have forced the industry to become Good Agricultural Practices (GAP) compliant and adopt food safety systems. Although producers remain responsible for the product until it is sold at the retail end of the supply chain, they do not control the product once it leaves the farm gate. Reliance on exporters, importers, distributors and retailers therefore becomes critical. If handlers of the fruit further down the chain do not similarly comply with basic

hygiene standards and careful handling practices, product quality can be severely compromised. Food safety and quality is a shared responsibility and require a holistic approach to achieve the ultimate goal of top quality fruit. Further, a critical need exists to find a replacement for SO₂ fumigation for litchis since the EU and the USA have become more restrictive in terms of sulphur applications on fresh produce. This places pressure on the local industry to urgently find an effective alternative which is part of this project's main objectives. The litchi research project covers several research focus areas which include microbial ecological and wax studies of the fructoplane, population genetic studies of *Penicillium* spp. and the development of alternative disease control options and food safety of exported products.

Highlights of the research 2003/2004:

Alternatives to Sulphur dioxide treatments

Modified Atmosphere Packaging (MAP) was used in combination with hot water treatments to retain the postharvest quality of litchi fruit during long-term storage. Quality assessment indicated that hot water dips at high temperatures affected the colour retention of the fruit. The light intensity, *L*, *chroma* *C*, colour coordinates *a* and *b* showed a decline, while the hue angle *H*⁰ showed an increase. Exposure to higher temperatures affected the fruit firmness and higher weight loss was observed. Soluble solids concentration (Brix) was low and titratable acidity was high in fruit exposed to higher temperatures. Although hot water dip treatments combined with MAP minimized the incidence of fungal decay, higher decay incidence was observed at 50°C. It was evident from this study that fruit stored in MAP alone without any hot water dip treatment retained quality the most effectively (Fig.1).

Determining genetic diversity of *Penicillium* spp.

Penicillium species are among the most common fungal species affecting litchi fruit. These species have the ability to colonise almost any surface from fruit to packhouse walls and air conditioning systems. The presence of these spores in storage and transport facilities therefore holds great potential for postharvest spoilage of fruit and may result in significant economic losses to the exporting fruit industry. In this study, representative *Penicillium* spp. in the litchi export chain from South Africa to the European markets have been isolated. Fourteen different *Penicillium* spp. were identified throughout the



Fig. 1. Litchi fruit at harvest (A) and after 40 days postharvest storage in the modified atmosphere packaging treatment (B)

export chain. The species include *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. citreonigrum*, *P. citrinum*, *P. corylophilum*, *P. decumbens*, *P. expansum*, *P. fellutanum*, *P. glabrum*, *P. janthinellum*, *P. rugalosum*, *P. solitum* and *P. viridicatum*. In comparison to previous studies, many of these species identified do not naturally infect and colonise litchi fruit. The wide diversity of

species identified in the export chain is an indication of potential cross-contamination of fruit that can occur within and between crops. Currently the project focuses on population genetic studies on these fungi.

Litchi fruit surface studies

Micro cracking was observed 20 days after initial fruit set (Fig. 2). Cuticle accumulated on the pericarp surface during fruit development (Fig. 3). In Mauritius cultivar fruit, cuticle accumulated over the entire surface of the epidermal layer from an early stage of fruit development. The cuticle lines were parallel to each other in the early stages, and became more wrinkled, compact and protruded as the fruit matured. This study will be extended to include other important litchi cultivars and will in future focus on the chemical composition of the wax.

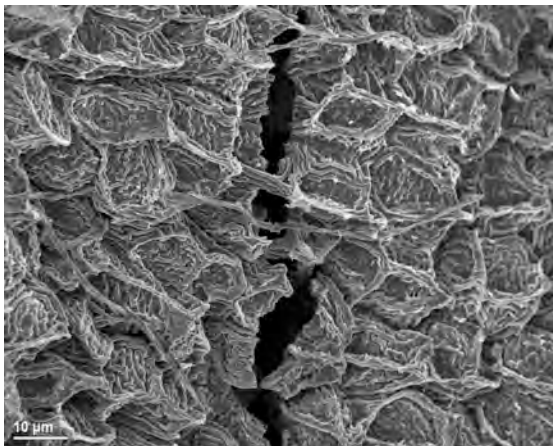


Fig. 2. Micro cracking at fruit set (upper surface)

Food safety within the litchi export chain

Foodborne pathogens may be transmitted to fresh produce during handling, washing of fruit, irrigation or field spraying practices. In addition unhygienic conditions in the packhouse and coldrooms or along the export chain may contribute to product contamination. In this study, litchi product safety was investigated throughout the export chain, starting in the field all the way through to the consumer. No major foodborne pathogens could be isolated from litchi fruit surfaces.



Fig. 3. Parallel cuticle layers developing on litchi fruit

DST/NRF CENTRE OF EXCELLENCE IN TREE HEALTH BIOTECHNOLOGY

The South African National Research and Development Strategy has identified the need to create “centres and networks of excellence” in science and technology, including the social sciences, as a key component of the human capital and transformation dimensions of government policy. It was envisaged that such centres would stimulate sustained distinction in research while simultaneously generating highly qualified human resource capacity to impact meaningfully on key national and global areas of knowledge.

Centres of Excellence (CoE) are physical or virtual centres of research which concentrate existing capacity and resources to enable researchers to collaborate across disciplines and institutions, on long-term projects that are locally relevant and internationally competitive in order to enhance the pursuit of research excellence and capacity development. In 2003, over 70 applications were submitted to the NRF for peer review and consideration for funding as Centres of Excellence. Of these, six applications were successful and were nominated for funding in 2004. One of these was the DST/NRF Centre of Excellence in Tree Health Biotechnology at FABI (CTHB), under the Directorship of Prof Mike Wingfield and hosted by the University of Pretoria.

The official general launch of all the Centres of Excellence took place in 2004 and this was a glittering occasion. Each individual Centre was responsible for organizing its own launch and inviting its stakeholders. The official launch of the CTHB took place on Monday, 31 January 2005 in the Sanlam Auditorium at the University of Pretoria and was attended by approximately 100 people. The guests and students were welcomed by the Principal and Vice-Chancellor, Prof Calie Pistorius. Other speakers included the Vice-Principal: Research, Prof Robin Crewe, who spoke about the Centres of Excellence and Research at the University. Dr Rob Drennan of the NRF

congratulated Prof Mike Wingfield, Director of the new Centre of Excellence and his team, on the honour of being one of the six groups to be charged by the DST and NRF with taking research to new heights. Prof Braam van Wyk, presented a fascinating and stimulating talk entitled “Southern African trees: outstanding features”.

Prof Wingfield’s team at FABI, are well experienced in researching diseases of commercial forest plantation trees through the long-standing and very successful Tree Protection Cooperative Programme (TPCP). The solid research base and expertise established through the TPCP will assist in developing the new research foci of the Centre of Excellence (CoE). Thus the objectives of the new CoE do not pose a threat to the work of the TPCP. The research efforts and foci of the two programmes should complement each other and provide new challenges. The Centre of Excellence will allow for research on trees such as Acacias, Watter Berry, Kiaat, Proteas and Baobabs, which are native to South Africa and form an important part of the country’s biodiversity. Studies on these native species have previously been neglected, and there is no question that they are also threatened by little known pests and diseases.



Back row: Prof Mike Wingfield, Prof Coert Geldenhuys, Dr Rob Drennan, Prof Anton Ströh
Front row: Dr Eddie Mwenje, Prof Robin Crewe, Prof Urmilla Bob, Prof Brenda Wingfield, Mr Mike Edwards, Ms Jenny Hale

The DST/NRF Centre of Excellence in Tree Health Biotechnology (at FABI) held its first Board meeting, followed by a dinner, at FABI, University of Pretoria on 3 November 2004. The CTHB Board is constituted of nine members and one co-opted member. Prof Wingfield was given the authority by the Board to invite another person with scientific expertise in the field of tree health of "native/indigenous" trees, to serve on the Board in future.

The vision for the Centre of Excellence in Tree Health Biotechnology includes a strong mentoring and outreach programme. Such a system of mentorship will make a significant impact with regards to training future academics for South Africa. In the "Education and Training" section of the CoE Business Plan that was presented to the CoE Board in November 2004, the new "mentoring programme" for offering undergraduate assistantships was outlined. In 2005 1st and 3rd year students have been targeted, but in subsequent years the idea will be to expand the mentoring programme to include 1st, 2nd and 3rd year students.

The inauguration of this new Centre of Excellence in FABI at the University of Pretoria reflects the recognition and achievement of FABI. The Centre has already started to have a significant impact in FABI and the next two years will enable it to grow to being another significant programme in FABI.



Back row:
Prof Teresa Coutinho, Dr Gert Marais, Mr Brett Hurley, Dr Bernard Slippers, Prof Brenda Wingfield
Front row:
Prof Jolanda Roux, Prof Mike Wingfield, Ms Jenny Hale, Ms Lisel Solms (Absent Prof Wally Marasas and Prof Pedro Crous)

The Members of the CTHB Board and their role on the Board/areas of expertise:

Name	Institution	Role/Area of expertise
Prof Urmilla Bob	University of Kwazulu-Natal	Humanities advisor
Ms Anati Canca	Department of Science and Technology	DST representative
Prof Robin Crewe	University of Pretoria	Chairperson of the Board
Dr Rob Drennan	National Research Foundation	NRF representative and Director of the CoE Programme
Mr Mike Edwards	Forestry South Africa	Industrial advisor
Prof Coert Geldenhuys	Associate Professor, University of Stellenbosch and Consultant	Technical advisor and forest ecologist
Dr Eddie Mwenje	University of Bulawayo	Provincial/technical advisor
Prof Anton Ströh	University of Pretoria	Dean: Faculty of Natural and Agricultural Sciences
Prof Brenda Wingfield	University of Pretoria	Programme leader and co-opted member of the Board
Prof Mike Wingfield	University of Pretoria	Director

Core Research leaders of the COE:

Name	Institution	Position in the Centre
Prof MJ Wingfield (Mike)	FABI, UP	Director
Prof BJ Wingfield (Brenda)	FABI, UP	Programme leader
Prof TA Coutinho (Teresa)	FABI, UP	Research leader
Dr PW Crous (Pedro)	CBS, Utrecht, Netherlands	Research leader
Mr BP Hurley (Brett)	FABI, UP	Research leader
Dr GJ Marais (Gert)	FABI, UP/CSIR	Research leader
Prof WFO Marasas (Wally)	MRC, Tygerberg	Research leader
Prof J Roux (Jolanda)	FABI, UP	Research leader and newly appointed Associate Professor to the CTHB
Dr B Slippers (Bernard)	FABI, UP	Research leader and newly appointed Senior Lecturer to the CTHB
Dr E Steenkamp (Emma)	FABI, UP	Research leader
Prof J Zwolinski, (Janusz)	University of Natal, PMB	Research leader

SABBATICAL VISITS

Prof. Teresa Coutinho

Host: Prof. Jean Swings

Location: Laboratorium voor Microbiologie, University of Gent, Belgium

Duration: 1 September – 30 November 2003

Objective: The objective of my research was two-fold. Firstly, I attempted to identify and characterise *Pantoea* spp. infecting eucalypts locally and in countries where symptoms similar to those observed in South Africa had been reported. Preliminary results suggested that more than one member of the Enterobacteriaceae are involved in this disease. Secondly, I attempted to identify and characterise two species of *Pantoea* associated with Coniothyrium canker of eucalypts in South Africa. These two bacterial species are believed to have a synergistic interaction with the fungal pathogen, *Coniothyrium zuluense*.

During the course of the past two years, we have obtained authentic *Pantoea ananatis* strains from a number of hosts including rice, onions, pineapple, sudan grass, melons and a clinical strain. These strains were included in this study together with strains isolated from eucalypts showing typical symptoms of bacterial blight and die-back from various countries in South America and Uganda. Preliminary results obtained in South Africa indicated that we were dealing with *P. ananatis* and one or more unknown *Pantoea* spp. After examining the results obtained in South Africa, it was decided that my first research objective in Gent would be to try and group the strains using another molecular method, namely, REP-PCR. Included in this study, were the eight *Pantoea* type species. It was also decided to include two authentic strains of each of these species to determine the variation in profiles within a species. A computer programme, Bionumerics, was used to analyse the results obtained. It was decided to only consider the results obtained from the ERIC profiles because they were more distinct than those obtained using BOX primers. All 68 strains used in this study, including the eight found associated with *Coniothyrium zuluense*, grouped into 5

clades. The type strain of *P. ananatis* grouped with the largest clade. The type strain of *P. agglomerans* also grouped with one of the clades. All other types strains were distinct and grouped separately. These results were then compared to those obtained in South Africa and the strains grouped accordingly.

A following step in this research was to conduct DNA:DNA hybridisations between selected strains from the various sub-clades and the type strains of *Pantoea*. This is a complex process which requires DNA of a very high quality and quantity. The first task was to grow up sufficient quantities of bacteria for DNA extraction (i.e. at least 1 gram). Because of time constraints, it was decided that three strains from Uganda, eight strains from South America and eight strains associated with *C. zuluense* would be hybridised with the type strain of *P. ananatis*. Hybridisation with the other type strains would proceed once we ascertained which strains represented *P. ananatis*.

The hybridisation method, which is very sensitive and requires very careful preparation, is based on fluorometric measurements. Micro-dilution well plates are used so each hybridisation is repeated four times and results averaged and analysed statistically. None of the strains obtained from Uganda and South America were found to be *P. ananatis* (DNA binding values range from 4 – 56%). Three of the eight strains associated with *C. zuluense* represented *P. ananatis* (DNA binding values range from 74 – 94%; others had a values between 39 – 56%). Based on these results, DNA from two strains from Uganda and two strains associated with *C. zuluense* but not *P. ananatis* were hybridised with DNA from the types of *P. agglomerans* and *P. stewartii* subsp. *indologenes* (based on 16S rRNA gene sequences these two species are closely related to *P. ananatis*). None of these strains were either *P. agglomerans* or *P. stewartii* subsp. *indologenes* and are thus probably undescribed *Pantoea* spp.

Dr. Emma Steenkamp

Host: Dr B. Franz Lang (Organelle Genome Sequencing and Protist EST Programmes)

Location: Department of Biochemistry, University of Montreal, Montreal, Canada

Duration: 15 March 2005 – 25 April 2005

Objective: To generate cDNA and mitochondrial DNA libraries for use in the *Nuclearia* EST and organelle genome sequencing projects.

Currently various genome sequencing programs of diverse eukaryotes are underway or completed (for a comprehensive list see the Genomes OnLine Database, <http://www.genomesonline.org/>). Most of these projects focus on organisms of great economic, medical or veterinary importance, since it is financially not viable to sequence the genomes of organisms of 'lesser importance'. However, the Protist EST Program (PEP), as well as some others, has succeeded in securing funding for sequencing the genomes of organisms that would traditionally not form the bases of expensive research endeavors. PEP is also unique in that they use a phylogenetically-broad approach, where protistan taxa from all the major eukaryotic lineages are explored. This is important since protists comprise more evolutionary, ecological and biochemical diversity than the multicellular lineages combined.

Protists in the genus *Nuclearia* are typically filose amoebae with extremely fine pseudopodia that lack microtubules. Recently this amoeba has been the focus of much attention since molecular phylogenetic studies suggest that it represents the only known unicellular relative of the eukaryotic kingdom Fungi. Morphologically, fungi share no obvious characters with *Nuclearia*. Nucleariids are multi- or uninucleate phagotrophic amoebae with fine pseudopodia that feed on algae and bacteria, while fungi are absorptive heterotrophs that usually produce networks of apically extending branched multinucleate tubes (hyphae) in which they live. A detailed examination of nucleariid traits such as cell-wall chemistry, modes of reproduction,

ultrastructure, etc. may reveal characters that unite these apparently divergent taxa. Such analyses and further molecular dissection may also shed considerable light on the origin of fungi and evolution of this important group of eukaryotes.



University of Montreal

During my 6-week visit to the University of Montreal, normalized cDNA libraries for two *Nuclearia* species were established. The mitochondrial genome for one of these were also purified, randomly fragmented and cloned. Subsequently, thousands of ESTs and hundreds of mitochondrial clones have been sequenced. As with other mitochondrial genome and EST sequencing projects, the information gathered during the *Nuclearia* sequencing projects will greatly enhance our understanding of the classification and evolution of this protist. Preliminary analyses of the sequences generated thus far have already produced the strongest support yet for the *Nuclearia*-Fungi sister relationship. Currently, various other relationships between *Nuclearia* and the most basal fungi (i.e. chytrids and certain zygomycetes) are also being tested. Ultimately, these results will provide important insights, not only into the overall evolution of fungi, but also into the nature and origin of the ancestral fungal cell.

SERVICES

Tree Health Extension

Responsible researchers: Prof Jolanda Roux (Extension and Monitoring)
Prof Teresa Coutinho (Diagnostic Clinic)
Mr Brett Hurley (Pest Monitoring and Extension)
Prof Mike Wingfield

Activities 2003/2004:

Extension activities form an important component of the Tree Protection Co-operative Programme (TPCP) and Centre of Excellence in Tree Health Biotechnology (CTHB). These activities are divided into a number of components. They include all activities linked to monitoring of diseases and pests in forest plantations. Monitoring includes efforts to detect new pathogens and pests in a timely fashion and evaluation of the change in status of pathogens and pests, which have been present for many years. One of the key components of the monitoring programme is the Diagnostic Clinic that provides one means of rapid detection of new diseases and pests. Data from the clinic and field extension/monitoring activities also form part of a longer term historical record of pests and diseases for the South African forestry industry.

During 2003/2004 the Diagnostic clinic dealt with a significant number of samples. Most samples were received in the first half of the year (Jan-July). Of the samples sent, the majority were from pine (81%). Only 14% of the samples received were from eucalypts and 4% were those from other sources. These included insect, seed, growth media and water samples. Less than 1% of the samples received were from black wattle (*Acacia mearnsii*).

Field extension and monitoring are a crucially important focus of the team leading the TPCP. Members of the group undertook field studies in all forestry areas of South Africa and for example, spent a total of 466 and 356 person days in the field during 2003 and 2004, respectively. Four survey visits were also undertaken to other countries in Southern and Eastern Africa to obtain a better idea of the pests and diseases in these countries. Several presentations and

field days were held during these visits. An important component of extension and field activities included presentations on forest pests and diseases and training of foresters and farmers at field days and several field days were also attended in South Africa.

From the beginning of 2005, the extension activities of the TPCP have been extended to cover CTHB activities. This has already included presentations on tree diseases at a symposium in Kruger National Park and networking with botanists and conservationists.

The newsletter of the TPCP, the "Tree Protection News" continues to be an important means of distributing new information to members. Tree Protection News now firmly incorporates both entomology and pathology activities of the TPCP. Two issues of the newsletter have been distributed each year and these are dispatched by the Institute for Commercial Forestry Research (ICFR) together with ICFR News. In addition, short reports have been dispatched together with the ICFR News in the two quarters when a full newsletter was not produced. In addition to our own newsletter, articles were regularly produced for magazines or newspapers to inform foresters and the public of our activities. Various news items have been posted on the TPCP web site (<http://fabinet.up.ac.za>). The TPCP is also an active participant of the newly re-constituted Forest Invasive Species Network for Africa (FISNA) and has published new disease outbreaks and a list of our research activities on their webpage (<http://www.fao.org/forestry/foris/webview/fisna/index.jsp?siteid=6381&sitetreeld=26951&langld=1&geold=0>). This network aims to improve communication between foresters and researchers on invasive species on the African continent.

FIELD ACTIVITIES OF THE TPCP



Derien Escheverri and Solomon Gebeyehu searching for the Eucalyptus snout beetle *Gonipterus scutellatus*



TPCP students learning to identify Eucalypt diseases



Wilhelm Dreyer and Buyi Mthlane evaluating *Eucalyptus* diseases



Coniothyrium inoculations in Zululand



Diagnostic clinic members visiting a nursery during a training session



Solomon Gebeyehu and Dina Gomez collecting wood chip samples

Pine Pitch Canker Screening Facility

Facility management team:

Prof Teresa Coutinho
Prof Jolanda Roux
Prof Mike Wingfield

Technical manager:

Mr Kgosi Mangwaketsi

Technical committee:

Dr A Kanzler (Sappi Forests)
Mr E Kietzka (Mondi Business Paper)
Mr C Wentzel (Komatiland Forestry)

One of the major initiatives of the Tree Protection Co-operative Programme (TPCP) relating to the pitch canker fungus in South Africa is to ensure that *Fusarium circinatum* tolerant planting stock is available for planting. During 2004, a great deal of research was conducted on this question, particularly regarding techniques and standardisation. Results clearly showed that the system of inoculation for disease

screening can be used in South Africa and that it produces results consistent with those from similar programmes elsewhere in the world. The programme now has a large scale, high throughput screening system established. This is being utilised by various South African Forestry companies and various organisations elsewhere in the world have expressed interest in using it.



Screening facility on University of Pretoria Experimental farm



A drop of inoculum is added to the cut surface of a pine tree



After 6 weeks die-back caused by *F. circinatum* is measured



Measurements are recorded and analysed giving an indication of disease tolerance

Sirex Wood Wasp Control Programme

Responsible researchers: Mr Brett Hurley
Dr Bernard Slippers
Prof Mike Wingfield

Objectives of the control programme:

- Develop an Integrated Pest Management system for the control of the Sirex woodwasp, *Sirex noctilio*.
- Investigate and apply mass-rearing and release techniques of the nematode *Beddingia siricidicola*, primary biological control agent against *S. noctilio*.
- Establish a better understanding the biology of *S. noctilio*, its symbiotic fungus *Amylostereum areolatum*, and its parasites.

Highlights of research 2003 / 2004:

The licence to use the parasitic nematode, *B. siricidicola* for the control of *Sirex noctilio*, was purchased in 2003. This allowed us to mass-rear the nematode at FABI. Existing rearing techniques were adapted to decrease losses from contaminations. In 2004, the nematode was released in the Eastern Cape and KwaZulu-Natal for the first time and re-released in the Western Cape. In total about 180 million nematodes were released. Parasitism success was assessed and research areas identified which will increase parasitism in future releases.

Nematode releases will now occur on an annual basis. There will also be significant increases in the numbers of nematodes released as the rearing and release techniques are further perfected. Two of the main research questions currently being considered are the life-cycle of *S. noctilio* in the KZN area and factors affecting establishment of *B. siricidicola* in *Pinus patula* plantations. Although ongoing, these studies have already provided crucial information for the success of the biological control programme against *S. noctilio*.



Flasks used for mass-rearing of the nematode *B. siricidicola*



Mycetophagous form of the nematode *B. siricidicola*

Microarray service

Facility manager: Prof Dave Berger

Microarray scientific officer: Mr Danie Theron

The ACGT (African Centre for Gene Technologies) Microarray Facility provides a service of arraying (spotting) DNA samples on glass slides at a density up to 4900 genes/slide on 36 replicate slides in a single spotting run. Arraying is done by a GEN III Array Spotter (Molecular Dynamics, Sunnyvale, California, USA) housed in a controlled-environment room. Arrayed slides are then made available to users who carry out the required experimental procedures in their own laboratories. After this, users return their slides for the Scanning service, in which the hybridization signals across the glass slide are measured and quantified using a GenePix 4000B Scanner (Axon Instruments, Foster City, California, USA). The results in the form of images and data sheets are then provided to the user electronically.

Recent developments at the ACGT

Microarray Facility include:

- Upgrade of the Array Spotter software to spot DNA samples at higher density.
- Upgrade of the Genepix Scanner software.
- Array Designer Software (Premier Biosoft, USA) for design of gene-specific long oligonucleotides for arraying.
- Upgrade of Vector NT software for analysis of cDNA sequence information.
- Introduction of a Microarray "starter kit":

New users are able to carry out all steps of a microarray experiment over three days under the guidance of Danie Theron. Control reagents and microarray slides are provided at cost. This has proven to be a very effective way to embark on this technology and has been attended by researchers from FABI, Citrus Research International, National Laser Centre, CSIR, and several Departments in the Faculty of Natural and Agricultural Sciences, and Faculty of Health Sciences at UP.

- Establishment of Open Source software:
Microarray Database in B.A.S.E format (BioArray Software Environment).
Microarray Data analysis (TM4 software suite from The Institute for Genome Research (TIGR), USA (<http://www.tigr.org/software/tm4>);
Gene Expression Pattern Analysis Suite GEPAS from the Spanish Bioinformatics Institute (<http://gepas.bioinfo.cnio.es>) and Bioconductor, USA (www.bioconductor.org).

For more information, please consult <http://microarray.up.ac.za/>

Several FABI research groups have used the ACGT Microarray Facility to produce research outputs that have been published internationally. These include the development of a cDNA library screening method (see reports of Molecular Plant-Pathogen Interactions and Banana research groups) and development of a Microarray-based fingerprinting method for *Eucalyptus* trees.

WORKSHOPS & CONFERENCES

International *Fusarium* Workshop

Each year a hands-on international *Fusarium* laboratory workshop is held in a different location in the world. Six workshops have thus far taken place, mainly in the USA. The instructors are internationally recognised experts in the field of *Fusarium* taxonomy. The scope of the workshop includes morphological and molecular characterisation of many species in this important fungal genus. *Fusarium* species are not only pathogens of agricultural and forestry crops, but are also important in the medical field. Some species produce mycotoxins and when ingested by humans and animals can cause certain forms of cancer as well as other disease symptoms. Resource poor farmers are particularly badly affected as their grain is usually stored under less than optimum conditions. There have been many documented reports of mycotoxin-associated oesophageal and other cancers from people living in rural areas of South Africa, for example.

In 2004 the Forestry and Agricultural Biotechnology Institute (FABI) hosted and Professor Teresa Coutinho organised the International *Fusarium* workshop. It was held from the 26th September to the 2nd of October. This was the first time that the workshop has been held in Africa. Due to a limitation in space and resources, the number of participants was limited to 42. The registered delegates came from seven African countries, Sweden, the Netherlands, Switzerland, Venezuela, Chile, Brazil, Venezuela, Argentina, USA, Canada and New Zealand.

The instructors included the following five eminent scientists:

David Geiser – Assistant Professor in the Department of Plant Pathology and Director of the *Fusarium* Research Center, Pennsylvania State University, USA. Experience with molecular

evolution of fungi and fungal population genetics.

John F. Leslie – Professor in the Department of Plant Pathology at Kansas State University, USA. Over 20 years experience with *Fusarium* genetics and population analysis.

Walter F.O. Marasas – Director of the PROMEC Unit of the South African Medical Research Council. Has more than 30 years experience in the taxonomy of toxigenic *Fusarium* species, the mycotoxins produced by them, and their effects on human and animal health.

Brett Summerell – Senior Research Scientist at the Royal Botanic Gardens (Sydney, Australia). Has 15 years of experience in the taxonomy, identification and description of new species of *Fusarium* and has conducted research on *Fusarium* diseases of palms, ornamental plants, maize, wheat and vegetables, and co-authored two laboratory manuals on *Fusarium* identification.

Brenda Wingfield – Professor in the Department of Genetics and the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Has 15 years experience in molecular taxonomy and phylogenetics of *Fusarium* species particularly those occurring in the *Gibberella fujikuroi* complex.



Back row:

Brett Summerell, Brenda Wingfield, Wally, Marasas, David Geiser, John Leslie and Teresa Coutinho with the Marimba Band



The International Edible Legume Conference

The University of Pretoria hosted the IV World Cowpea Congress in conjunction with the 1st International Edible Legume Conference (IELC) 2005 at the International Convention Centre (ICC), Durban in South Africa from 17 to 21 April 2005. Prof Theresa Aveling was chairperson of the organizing committee and Dr Quenton Kritzing (Department of Botany) was secretary. Other members of the organizing committee included Dr Andre Cilliers (Plaaskem), Dr Eugenia Barros (CSIR), Mr Claus Coetzee and Mrs Engela van Eyssen (Drybean Producers Organization), and Dr Dean Oelofse (ARC-Roodeplaat). The major sponsors were the Department of Agriculture, IITA and Syngenta SA.



1st International Edible Legume Conference
4th WORLD COWPEA CONGRESS
I.C.C. Durban, South Africa 17 - 21 April 2005

The aim of the joint conference/congress was to include all involved in the international pulse/edible legume industry and research institutions worldwide in a forum at which the many facets

of the industry - ranging from research to production to processing to marketing - could be placed in perspective to the mutual benefit of all participants.

The conference succeeded in bringing together researchers from 20 countries, working on all aspects of edible legumes, and provided opportunities for African researchers from 15 countries to interact with colleagues from the rest of the world.



The Opening address was given by Mr Kosy Dongo (Head of Research, Department of Agriculture: Mpumalanga) and was titled "Legume production in Southern Africa: Its role in agricultural development and food security". There were two plenary lectures by Dr B.B. Singh (Nigeria) and Prof Irv Widders (USA). The socials included a welcoming cocktail reception, a traditional South African braai at the Point Yacht Club and a gala dinner at the ICC with Zulu dancers for entertainment.

A Southern Africa Grain Legume Working Group was established. The aim of this group will be to establish collaboration between all the researchers working on bambara groundnut, cowpea, pigeonpea and all other dry beans in the southern African region.

The 2nd International Edible Legume Conference combined with the V World Cowpea Congress will be held in Kenya in 2010.

GCP workshop on Plant Genetic Diversity and Molecular Marker Assisted Breeding

The Generation Challenge Program (GCP) held a training workshop at FABI for 10 days in May 2005. The workshop was run by international and local resource scientists and included Theresa Fulton and Sharon Mitchell from the Institute of Genomic Diversity at Cornell University (Ithaca/USA), Chris Cullis from Case Western Reserve University (Cleveland/USA), Carmenn de Vicente from IPGRI (Cali/Columbia) Zhikang Li from CAAS (Beijing/China), Fred van der Post from PANNAR (Greytown/South Africa), Jane Morris from ACGT (Pretoria South Africa) Zander Myburg, Dave Berger and Karl Kunert from the University of Pretoria/FABI. Fourteen participants representing 10 African countries (Nigeria, Ghana, Sudan, Eritrea, Ethiopia, Uganda, Kenya, Tanzania, Namibia and South Africa), were selected from 63 applicants. Local resource people also included a FABI research scientist and FABI post-graduate students responsible for assisting in laboratory exercises as well as assisting in general administration in the course (Gerda Fourie, Juan Vorster, Andrew Kiggundu, Christell van der Vyver, Rosita Endah).

The lectures, discussions and practicals were all held in the FABI facilities including the ACGT computer laboratory. The goal of this workshop was to provide both conceptual and hands-on training in characterizing plant genetic diversity and to use DNA molecular marker assisted breeding. Emphasis was on practical applied usage and improving the links between plant breeding, germplasm management and utilization, and molecular biology methods, with a particular focus on the use of microsatellite markers. Sessions included topics such as: DNA-based markers, PCR-based markers, comparing molecular marker techniques, AFLP markers, Linkage mapping and QTL discovery and analysis, use and analysis of microsatellite markers, marker-assisted breeding strategies, and practical applications. Laboratory practicals included DNA extractions, PCR, electrophoresis, the use of microsatellites and basic informatics and computer

searches for SSR markers on public databases.

During the workshop Mike Wingfield also gave a presentation about the history, purpose and future of FABI and a postgraduate FABI student (Maria-Noel Cortinas) described the methodology for isolating SSR markers. Throughout the course there was active participation from the workshop attendees in the discussions.

A field trip to a South African breeding company (PANNAR) connected the basic science with practical applications was undertaken. Also commercial South African commercial breeders from PANNAR (Christiaan Troskie and Fred van der Post) and SAKATA VEGENETICS (Schalk van Heerden) contributed to the discussions on the commercial potential of molecular markers in plant breeding. A second field trip to Inqaba Biotech offered the opportunity to participants to set up connections for supply and technical services in Africa. A session devoted to research proposal development provided the culmination of the course. Due to the available travel schedules some of the participants were able to spend a longer time at FABI and were able to take advantage of extended discussions with scientists at the host institution. The workshop provided opportunities for the participants to interact and form collaborative networks. Subsequent encouragement of the participants to formalize these interactions specifically with FABI and generate research proposals together with FABI is likely to be an ongoing activity.



Group photo of the presenters and attendees

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These lists include only publications that had appeared by the end of May 2005. Manuscripts in press and submitted for publication are not included.

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SEMINAR PRESENTATIONS

All postgraduate students linked to FABI present two seminars each year on a Thursday morning. Special seminars, presented by invited speakers, are also regularly held. Once a postgraduate student has been awarded a degree, he/she is invited to present a prestige seminar.

Special seminars

Prof Pedro Crous
CBS, The Netherlands
January 2003
Life in the fast lane – living with my thumb in the dyke

Dr Paul Krokene
Norwegian Forest Research Institute (SKOGFORSK)
January 2003
Bark beetles, blue-stain fungi and conifer defence mechanisms

Dr D Aanen
University of Copenhagen, Denmark
January 2003
The evolution of fungus-growing termites and their mutualistic fungal symbionts

Prof H Davies
Scottish Research Institute, UK
February 2003
Targeted analysis or profiling approaches to detect unintended effects on GMOs

Dr Karin Jacobs
Postdoctoral report, Agriculture Canada, Canada
March 2003
Hitch-hikers and parasites: adventures in taxonomy

Dr. Jean-Luc Hofs
CIRAD, France
May 2003
Bt cotton in South Africa

Dr. Hugh Glen
National Botanical Institute, Durban
June 2003
Computer-aided ID – some of the options (and computer-aided keys and descriptions)

Prof. Yaojian Xie
Deputy Director General, China Eucalypt Research Centre, China
September 2003
Eucalypt research in China

Prof. R.A. Blanchette
Dept of Plant Pathology, University of Minnesota, USA
October 2003
Microbes at the extreme

Prof. H. Hirt
Institute of Microbiology and Genetics, Vienna Biocenter, Vienna, Austria
October 2003
How do plants think? Lessons from studying stress signaling MARK pathways

Dr. Paul Abrahams
CEO of eGoli BIO
October 2003
Business of biotechnology

Dr. D. Aghaveva
Institute of Botany, Azerbaijan
November 2003
Taxonomy of the Ophiostomatoid fungi occurring on different woody hosts in Azerbaijan and China

Prof. M. Wagner
North Arizona University
November 2003
Genetic resistance and companion planting as pest management strategies for a West African tree species

Colleen Aldous
University of Natal, Pietermaritzburg
November 2003
Investigating genetics student competencies with some practices of Science

Jan Perold
Affiliated with AFROSEARCH, South Africa
July 2004
Public perceptions to science, scientists and
GMOs

Prof Hugh Robertson
Dept of Entomology and Dept of Cell and
Structural Biology, University of Illinois,
USA
July 2004
The honey bee genome project:
sequencing, initial assemblies and
preliminary analysis

Prof Braam van Wyk
Dept of Botany, University of Pretoria
September 2004
Plants, people and cultures: perspectives
from Southern Africa

Dr. Gert Kema
Plant Research International, Wageningen,
The Netherlands
September 2004
Mycosphaerella and *Fusarium* research at
Plant Research International, the
Netherlands: Screens and genes

Dr. Celeste Linde
ETHZ, Zurich, Switzerland
September 2004
Population genetics of *Phynchosporium*
secalis

Dr. Brett Summerell
Royal Botanical Gardens, Sydney, Australia
October 2004
Phytophthora root rot of indigenous plants
in Australia

Prof. Christine Foyer
Rothamsted Research, UK
November 2004
Roles of mitochondria in the orchestration
of photosynthetic C/N metabolism and
redox signalling

Dr. Diana Six
College of Forestry and Conservation,
University of Montana, USA
November 2004
Bark beetles – fungus symbiosis: teasing
apart interaction types

Prof. Yong-Suk Jang & Dr. Moon-Sik
Yang
Chonbuk University, Korea
December 2004
Plant-based oral vaccine development and
plant cell culture-based useful protein
production

Prof. Volker Brözel
Dept of Biology and Microbiology, South
Dakota State University, USA
January 2005
Multicellular behaviour of *Bacillus cereus*

Dr. Walter Gams
CBS, The Netherlands
Fungi in the field and in culture
February 2005

Prof. Jens Kossmann
Institute for Plant Biotechnology, University
of Stellenbosch
February 2005
Understanding and influencing starch
biochemistry

Dr. Matthias Stoll
Universität Tübingen, Lehrstuhl für
Spezielle Botanik und Mykologie
February 2005
Coevolution in smut fungi and their hosts

Prof. Dale Bergdahl
Dept of Forestry, University of Vermont,
USA
February 2005
Concepts in forest pathology in the face of
exotic pathogens: a focus on butternut
canker disease

Dr. Diana Six
College of Forestry and Conservation,
University of Montana, USA
March 2005
Temporal variation in bark beetle fungal
symbionts

Allison Hansen
College of Forestry and Conservation,
University of Montana, USA
March 2005
Homogenization of ground beetle
assemblages mediated by an exotic forb in
Rocky Mountains savannas

Kjerstin Skov
College of Forestry and Conservation,
University of Montana, USA
March 2005
Fire effects on Douglas-fir beetle
reproduction in the first year after fire

Prestige seminars

Karen Surridge (MSc)
April 2003
Banana leaf diseases in South Africa

Grace Nakabonge (MSc cum laude)
April 2003
Diseases of *Eucalyptus* in Uganda

Alemu Gezahgne (PhD)
June 2003
Diseases of exotic plantation forestry trees
in Ethiopia

Cassie Myburg (PhD)
June 2003
Phylogenetic and morphological studies on
species of *Cryphonectria* and *Endothia*

Esme van Jaarsveld (PhD)
June 2003
Phytophthora nicotianae on tobacco and its
control in South Africa

XuDong Zhou (PhD)
July 2003
Taxonomy and biology of Ophiostomatoid
fungi associated with conifer-infesting bark
beetles

Mauricio Marin (PhD)
November 2003
Phylogenetic and molecular population
biology studies on *Ceratocystis* spp.
associated with conifer and coffee diseases

Dirk Swanevelder (MSc cum laude)
November 2003
Diversity and population structure of *Clivia
miniata* Lindl. (Amaryllidaceae): Evidence
from molecular genetics and ecology

Ronald Heath (MSc cum laude)
April 2004
Studies to consider the possible origins of
three canker pathogens of *Eucalyptus* in
South Africa

Marelize van Wyk (MSc cum laude)
March 2005
Taxonomy and population biology of
selected *Ceratocystis* spp. with hat-shaped
ascospores

Barbara Nel (MSc cum laude)
April 2005
Management of Fusarium wilt of banana by
means of biological and chemical control
and induced resistance

FABI TEAM

2003-2005

Full time academic & research staff

Prof Teresa Coutinho
Prof Lise Korsten
Prof Karl Kunert
Prof Anna-Maria Oberholster
Prof Brenda D. Wingfield
Prof Michael J. Wingfield
Assoc Prof Terry Aveling
Assoc Prof Dave Berger
Assoc. Prof Nico Labuschagne
Assoc. Prof Jolanda Roux
Dr Rachel Chikwamba
Dr Petra Labuschagne
Dr Gert Marais
Dr Zander Myburg
Dr Thierry Regnier
Dr Bernard Slippers
Dr Jacquie van der Waals
Dr Altus Viljoen
Mr Brett Hurley

Technical staff

Ms Trish Beart
Ms Elna Cowley
Ms Sonja de Beer
Mr Neil de Jager
Ms Gerda Fourie
Mr Hardus Hatting
Ms Joyce Jakavula
Ms Pritty Khumalo
Ms Amelita Lombard
Ms Grieta Mahlangu
Mr Kgosi Mangwaketsi
Ms Eva Müller
Ms Karin Muller
Ms Valentina Nkosi
Ms Judy Ratner
Ms Amanda Redmond
Ms T Schultz
Ms Anita Steyn
Mr Danie Theron
Ms Lydia Twala
Ms Kerien van Dyk
Ms Martie van Zyl

Administrative staff

Ms Vivienne Clarence
Ms Helen Doman
Ms Gerda Fourie
Ms Jenny Hale
Ms Adrene Laubsher
Ms Martha Mahlangu
Ms Daleen Muller
Ms Judy Ratner
Ms Lisel Solms
Ms Liana Viljoen
Ms Rose Visser

Computer support

Mr Chris Visagie

Information specialist

Ms Mari_ Theron

Honorary professors/lecturers

Prof PW Crous
Prof WFO Marasas
Prof JP van der Walt
Prof J Webster
Dr T Burgess
Dr B Eisenberg
Dr O Preisig

Sabbatical visitors

Dr Paäl Krokene (2003)
Dr Diane Six (2004/2005)
Dr Ursula Heiniger (2004/2005)
Dr Dale Bergdahl (2005)

Postdoctoral fellows

Dr Solomon Gebeyehu
Studies on *Pissodes nemerensis* and its possible role in vectoring *Fusarium circinatum*
Dr Karin Jacobs
Molecular taxonomic studies on *Ophiostoma* and *Ceratocystis*, particularly of species linked to forest biosecurity

Dr Ana Slaughter

Genetic improvement of maize to enhance food safety by introducing resistance to *Fusarium moniliforme*

Dr Adele McLoed

Genetic studies of resistance to the bacterial pathogen, *Ralstonia solanacearum*, in *Arabidopsis thaliana*

Dr Marlene van der Merwe

A phylogenetic study of *Eugenia* (Myrtaceae)

Dr Retha Slabbert

The effect of cold stress on the physiology of banana plants during infestation by *Fusarium oxysporum* f.sp. *cubense*

Dr Solomon Kebede

Development and use of microarray technology for genotyping and gene expression analysis in *Eucalyptus* trees

Dr Martin Coetzee

Population genetics of *Armillaria fuscipes* in South African pine plantations

Dr Seonju Lee

Fungi on Protea species

Dr Wolfgang Maier

Global phylogeny of rust fungi with specific focus on African species

Dr Jane Wright

The global population biology of *Fusarium circinatum*

Dr Dharini Sivakumar

Improving litchi quality and shelf life through the export chain focusing on all aspects from production to consumption

Dr XuDong Zhou

Fungi associated with bark beetles

Current postgraduate students

PhD students

Shahasi Athman

Biological control of the banana nematode *Radopholus similis* with fungal endophytes and the study of host-pest-endophyte interactions

Advisors: N Labuschagne, A Viljoen & T du Bois

Hugues Baimey

Scutellonema bradys as a pathogen of yam (*Dioscorea* spp.) in Benin

Advisors: N Labuschagne, D Coyne & A McDonald

Irene Barnes

Taxonomy, phylogeny and population biology of the red band needle blight fungus and related species

Advisors: MJ Wingfield & BD Wingfield

Getu Beyene

Expression and the stability of a rice oryzacystatin I in transgenic tobacco under abiotic stress

Advisors: K Kunert & CH Foyer

Lesesse Beyene

Genetic diversity in maize inbreds and its association with test cross performances, combining ability and heterosis

Advisors: A-M Oberholster, AA Myburg, K Pixely & TK Tumasi

Yoseph Beyene

Characterization of genetic diversity in Ethiopian highland maize (*Zea mays* L.) populations

Advisors: AA Myburg & A-M Oberholster

Mesfin Bogale

Fusarium spp. associated with teff production in Ethiopia

Advisors: BD Wingfield, MJ Wingfield & ET Steenkamp

Carrie Brady

Examining the global epidemiology of *Pantoea ananatis* using MLST

Advisors: SN Venter & TA Coutinho

Maria-Noël Cortinas

Population genetics of the stem canker pathogen, *Coniothyrium zuluense*

Advisors: BD Wingfield & MJ Wingfield

Bridget Crampton

Elucidation of disease resistance in monocotyledonous plants through DNA microarray analysis

Advisor: D Berger

Johan de Graaf

Integrated Pest Management of *Cosmopolitus sordidus* Germar (Coleoptera: Curculionidae) in South Africa

Advisors: P Govender, A Viljoen, A Schoeman & S Bruwer

Elizabeth de Jager

Post-harvest quality standards in the South African litchi industry

Advisors: L Korsten & F Wehner

Pieter de Maayer

Virulence factors associated with *Pantoea ananatis*

Advisors: TA Coutinho & SN Venter

Lieschenn de Vos

Characterization of the *Fusarium circinatum* genome

Advisors: BD Wingfield, MJ Wingfield & Z Myburg

Juanita de Wet

Molecular taxonomy and phylogeny of *Sphaeropsis sapinea* and its association with dsRNA elements

Advisors: MJ Wingfield, O Preisig & BD Wingfield

Wilma du Plooy

Mango (*Mangifera indica* L) fruit rind morphology and chemistry and their implications for post harvest quality

Advisor: L Korsten

Joanne Fouché

Population and infection biology studies on *Xanthomonas campestris* pv. *musacearum* on bananas in central Africa

Advisors: TA Coutinho & A Viljoen

Anton Fourie

Efficacy of rhizobacteria for growth promotion and biocontrol of soil-borne pathogens on selected vegetable crops

Advisors: N Labuschagne & L Korsten

Teresa Goszczynska

Pantoea ananatis and *P. agglomerans* associated with onion seed in South Africa

Advisors: TA Coutinho & SN Venter

Veloshinie Govender

Seed pathology and vigour of maize stored under subsistence farming conditions

Advisor: TAS Aveling

Andile Grootboom

Increasing the lysine content in maize by engineering proteinase inhibitor

Advisors: R Chikwamba & KJ Kunert

Marieka Gryzenhout

Revision of the taxonomy of the fungal genera *Endothia* and *Cryphonectria*

Advisors: MJ Wingfield & BD Wingfield

James Harrison

Complementary morphological and molecular approaches to plantation white grubs (Scarabaeidae) identification

Advisors: MJ Wingfield & C Scholz

Ahmed Hassen

Efficacy of *Rhizobacteria* for growth promotion and biocontrol of selected soilborne pathogens of sorghum in Ethiopia and South Africa

Advisors: N Labuschagne & L Korsten

Ronald Heath

Studies of wound infecting pathogens of plantation hardwood trees in Southern and Eastern Africa

Advisors: J Roux, MJ Wingfield & BD Wingfield

Gavin Hunter

Mycosphaerella leaf blotch of *Eucalyptus* in South Africa

Advisors: MJ Wingfield, BD Wingfield & PW Crous

Riana Jacobs

Studies on the *Fusarium* spp. in the *Gibberella fujikuroi* complex

Advisors: TA Coutinho, MJ Wingfield, BD Wingfield & WFO Marasas

Sinnia Kappindu

Mechanisms of banana weevil (*Cosmopolitus sordidus*) biocontrol using fungal endophytes

Advisors: A Viljoen, N Labuschagne & T du Bois

Andrew Kiggundu

Identification of candidate genes for resistance to banana weevil in East African Highland bananas

Advisors: K Kunert, D Michaud, A Viljoen, M Pillay & C Gold

Barnabas Kiula

Effect of gray spot of testcross performance, combining ability and heterosis of Tanzanian inbred and open-pollinated maize varieties

Advisors: A-M Oberholster & DE Lyimo

Sabine Lezar

Fingerprinting in *Eucalyptus* using microarrays

Advisors: BD Wingfield, AA Myburg, D Berger & MJ Wingfield

Lorenzo Lombard

Phylogeny and taxonomy of *Cyclindrocladium* spp. with obpyriform to ellipsoidal vesicles

Advisors: MJ Wingfield, BD Wingfield & PW Crous

Michael Luttig

Characterisation of a citrus tristeza closterovirus population which interferes with Huanglongbing infection

Advisors: BD Wingfield & B Manicom

Evelyn Madoroba

The use of molecular tools for quality control of starter cultures

Advisors: ET Steenkamp & TE Cloete

Bongani Maseko

Phytophthora root rot associated with cold tolerant eucalypts in South Africa

Advisors: TA Coutinho, MJ Wingfield, BD Wingfield & T Burgess

Sissay Mekbib

Identification of citrus (*Citrus sinensis* L.) postharvest pathogens from Ethiopia and its control

Advisor: L Korsten

Sari Mohali

Cylindrocladium spp. in Venezuela

Advisors: MJ Wingfield & BD Wingfield

Calvyn Molepo

Molecular phylogeny of the *Ravenelia* spp. in South Africa

Advisors: BD Wingfield, W. Maier & MJ Wingfield

Lorraine Moses

Fumonisin regulating genes in *Fusarium verticillioides* and other fumonisin producing fungi

Advisors: MJ Wingfield, BD Wingfield & WFO Marasas

Josephine Mukiibi

Studies of mechanisms of resistance to the banana weevil (*Cosmopolitus sordidus* Germar) within the *Musa* germplasm

Advisors: K Kunert, A Viljoen & Chikwamba R

Sanuska Naidoo

Genetic studies of resistance to the bacterial pathogen, *Ralstonia solanacearum*, in *Arabidopsis thaliana*.

Advisors: D Berger & K Denby

Grace Nakagonge

Studies on *Cryphonectria* spp. in Africa

Advisors: J Roux & MJ Wingfield

Joseph Ndunguru

Molecular characterization and dynamics of cassava mosaic geminiviruses in Tanzania

Advisors: TAS Aveling, G Thompson, J Legg & C Fauquest

Marie Onanema

Impact of cartegena protocol on Cameroon

Advisors: KJ Kunert & Chikwamba R

Pamela Paparu

Plant-endophyte interactions in East African Highland bananas (AEHB)

Advisors: A Viljoen & T du Bois

Ida Paul

Mapping and distribution of citrus greening in South Africa

Advisors: A van Jaarsveld & L Korsten

Draginja Pavlic

Population biology of *Botryosphaeria* spp. from native and introduced hosts in Southern Africa

Advisors: B Slippers, MJ Wingfield & TA Coutinho

Shadrack Phophi

International accepted best marketing practices based on minimum food safety and quality requirements

Advisor: L Korsten

Anneka Prins

Characterization of the cystatine gene family

Advisors: KJ Kunert & CH Foyer

Ezanne Swanepoel

Mapping *Diuraphis noxia* resistance loci in *Triticum aestivum*

Advisors: A-M Oberholster, AA Myburg & MT Labuschagne

Dirk Swanevelder

Signal transduction during RWA defense

Advisors: AM Oberholster & E Venter

Noëlani van den Berg

The identification of banana genes associated with tolerance against *Fusarium oxysporum f.sp. cubense* subtropical race 4

Advisors: A Viljoen, D Berger, P Birch & MJ Wingfield

Albé van der Merwe

Population genetics of *Cryphonectria cubensis*

Advisors: BD Wingfield & MJ Wingfield

Magriet van der Nest

Compatibility in *Amylostereum areolatum*

Advisors: MJ Wingfield, BD, Wingfield, B Slippers & J Stenlid

Madel van Eeden

Monitoring biocontrol systems under commercial conditions

Advisors: L Korsten & F Wehner

Chris van Ginkel

Environmental, pathogen and host related aspects in the quest for sustainable management practice of citrus black spot

Advisor: L Korsten

Marelize van Wyk

The genus *Ceratocystis*

Advisors: MJ Wingfield & BD Wingfield

Juan Vorster

Horizontal gene transfer in plants

Advisors: KJ Kunert & C Cullis

Gezachew Weidemichael

Xanthomonas campestris pv. *musacearum* associated with enset in Ethiopia and Uganda

Advisors: TA Coutinho & A Viljoen

Current MSc/MSc (Agric) students

Mohammed Abdo

Molecular markers for *Fusarium circinatum*

Advisors: BD Wingfield, TA Coutinho & MJ Wingfield

Roger Bagnall

Control of Pythium wilt and root rot of hydroponically grown lettuce by means of chemical treatment of the nutrient solution

Advisors: N Labuschagne & TAS Aveling

Aneen Belgrove

The application of non-pathogenic forms of *Fusarium oxysporum* for the biological control of Fusarium wilt of banana

Advisors: A Viljoen, C Steinberg & B Nel

Jane Boshoff

Biological control of Pythium wilt and root rot of hydroponically grown lettuce

Advisors: N Labuschagne, L Korsten & T Regnier

Gerda Britz

Water quality of citrus packhouse dip tanks and the role and importance of *Salmonella* spp. in food safety

Advisor: L Korsten

Nicky Creux

Characterization of tissue-specific promoters involved in wood formation in *Eucalyptus* trees

Advisors: AA Myburg, DK Berger & V van Staden

Minique de Castro

Nucleotide diversity in cellulose biosynthetic genes of *Eucalyptus*

Advisor: AA Myburg

Elsie de Meyer

Fungi associated with utility poles in South Africa

Advisors: MJ Wingfield & ZW de Beer

Besrat Demoz

Honey bee dispersal of antagonist to avocado flowers to control stem-end rot pathogens

Advisor: L Korsten

Pranitha Dawlal

Resistance of South African maize cultivars against mycotoxigenic fungi

Advisor: GJ Marais

Franco du Preez

Analysis and origin of the different classes of nucleotide binding site motifs present in bread wheat

Advisors: A-M Oberholster & AA Myburg

Rosita Endah

Characterization of NPR1 like genes in banana

Advisors: Chikwamba R & Kunert KJ

Derien Echeverri

Understanding and managing the *Eucalyptus* snout beetle (*Gonipterus scutellans*) (Coleoptera: Curculionidae) in South Africa

Advisors: S Gebeheye & MJ Wingfield

Gerda Fourie

The evolutionary biology of *Fusarium oxysporum* f.sp. *cubense*

Advisors: A Viljoen, E Steenkamp & T Gordon

Nonnie Geldenhuis

Studies on fungi associated with dying *Schizolobium parahybrum* in Ecuador

Advisors: MJ Wingfield & J Roux

Tanja Gevers

Identification and characterization of virulence factors in *Fusarium oxysporum* f.sp. *cubense*

Advisors: A Viljoen & A Churchill

Christian Giesel

Pyramiding of anti-fungal genes in transgenic plants

Advisors: DK Berger & B Crampton

Dina Gomez

Ophiostomatoid fungi from bark beetles in China with special reference to species with *Leptographium* and *Pesotum* anamorphs

Advisors: X-D Zhou, K Jacobs & MJ Wingfield

Izette Greyling

Studies on the *Pantoea* spp. associated with Coniothyrium canker in South Africa

Advisors: TA Coutinho, SN Venter & MJ Wingfield

Joha Grobbelaar

Molecular phylogeny and population genetics of *Ophiostoma quercus*

Advisors: MJ Wingfield & BD Wingfield

Leylani Grobler

Pathogens associated with mango die-back

Advisors: L Korsten & G Swart

Susan Groenewald

The biology and pathogenicity of *Fusarium oxysporum* f.sp. *cubensis*

Advisors: A Viljoen, N van der Berg & WFO Marasas

Almuth Hammerbacher

Epidemiology of the pitch canker fungus in South Africa

Advisors: TA Coutinho, MJ Wingfield & BD Wingfield

Wilma Havenga

Mode of action of *Bacillus subtilis* as biocontrol agent of postharvest diseases of avocado

Advisor: L Korsten

Zhou Honghai

Functional analysis of *Eucalyptus* wood formation genes in *Arabidopsis*

Advisor: AA Myburg

Brett Hurley

Species composition, pathogen interactions and management of fungus gnats in forestry nurseries

Advisors: P. Govender, MJ Wingfield, TA Coutinho & BD Wingfield

Bedel Kalonji

Biological and chemical control of seedling diseases of lettuce

Advisors: TAS Aveling, N Labuschagne & JE van der Waals

Charline Kamburona

Evaluating genetic diversity and performance of peanut (*Arachis hypogaea*) lines

Advisors: A-M Oberholster & A Cilliers

Gilbert Kamgan Nkuekam

A study of the Ceratocystis and Ophiostoma species infecting wounds on trees

Advisors: J Roux & MJ Wingfield

Delphin Kandolo

Effect of fungicide seed treatments on vigour and germination of various crops

Advisor: TAS Aveling

Willeke Keesenberg

Food safety and quality throughout the apple export chain

Advisor: L Korsten

John Kemp

Eco-TILLING of wood and fibre genes in forest trees

Advisors: AA Myburg, L van Rensburg & J Greef

Daniel Khumalo

Seed treatment of cowpea to control seedling diseases

Advisor: TAS Aveling

Marija Kvas

Fusarium spp. associated with *Syzigium cordatum* malformation

Advisors: ET Steenkamp, BD Wingfield & MJ Wingfield

Natalie Levendall

Development of the polygalacturonase inhibitor protein (PGIP) for delivery of foreign proteins to the surfaces of plant cells

Advisor: D Berger

Therese Lotter

Characterisation and expression of a polygalacturonase gene from the lupin antracnose fungus identified as *Colletotrichum lupine* var. *setosum*

Advisor: D Berger

Rebecca Makhado

Endophytic studies on *Pantoea* spp. associated with eucalypts in South Africa

Advisors: TA Coutinho & SN Venter

Frank Maleka

Comparative analysis of nucleotide diversity in a lignin and cellulose biosynthetic genes of *Eucalyptus* and *Arabidopsis*

Advisors: AA Myburg & P Bloomer

Happy Maleme

Botryopshaeria spp. on eucalypts in South Africa

Advisors: MJ Wingfield & BD Wingfield

Vonia Mampuru

Biodiversity of Opisthokont protists in South African freshwater

Advisor: ET Steenkamp

Lance Maphosa

Taxonomic and population biology on *Armillaria* spp. in Zimbabwe

Advisors: BD Wingfield, M Coetzee, MJ Wingfield & E Mwenje

Celia Martinze

Stability of Bt toxin in transformed tobacco under drought

Advisor: K Kunert

Lerato Matsaunyane

Isolation and characterization of the apple polygalacturonase inhibiting protein 2 gene (*pgip 2*) from apple and investigation into the proteins' antifungal activity

Advisor: D Berger & D Oelofse

Thuto Matsioloko

Using cDNA-AFLP and microarray analysis for rapid identification of *Diuraphis noxia* induced expressed genes

Advisors: A-M Oberholster & AA Myburg

Aisha Mahomed-Ali

Flavours and fragrances produced by South African fungi

Advisor: GJ Marais

Claire Munro

Molecular markers in bananas

Advisor: A Viljoen

Karin Muller

Mapping *Dn1* in a "Tugela DNA" and "Tugela Fast Grow" mapping population

Advisor: A-M Oberholster

Zelda Pieterse

Interaction between *Mycogone perniciosa* and *Agaricus bisporus*

Advisors: TAS Aveling & PM Labuschagne

Nditsheni Rabambi

Antimicrobial activity of tea (*Camellia sinensis*) extracts against plant pathogenic viruses on selected vegetable crops

Advisors: N Labuschagne, Z Apostolides & G Thompson

Martin Ranik

Gene discovery in differentiating xylem of *Eucalyptus* and *Arabidopsis*

Advisor: AA Myburg

Moses Ramusi

Biological and chemical control of seedling diseases of cowpea

Advisors: TAS Aveling, N Labuschagne & JE van der Waals

Luke Solomon

Identification of circadian rhythms in the expression patterns of wood-formation genes in *Eucalyptus*

Advisors: AA Myburg & DK Berger

Liesl Stronkhorst

The effect of pH and N-fertilization practices on the incidence of *Fusarium* wilt (Panama disease) of bananas

Advisors: J van der Waals & A Viljoen

René Sutherland

Transformation of Cavendish bananas for *Fusarium* wilt resistance

Advisors: A Viljoen, J-V Escalant & K Kunert

Annie Thomas

Impact of genetically modified plants on the South African flora

Advisors: K Kunert & AJ Buys

Mariette Truter

Epidemiology and control of black scurf and stem canker of potatoes

Advisor: F Wehner

T Tshifaro

Antimicrobial activity of tea (*Camellia sinensis*) extracts against plant viruses of selected vegetable crops

Advisors: N Labuschagne & Z Apostolides

Itani Tshivhandekano

Water quality in Gauteng contributing to food safety risk in agriculture

Advisors: L Korsten & W du Plooy

Leon van Eck

Transcript profiling in Tugela near isogenic lines in response to RWA feeding

Advisors: A-M Oberholster & N Lapitan

Rosie van Zyl

Identification of virulence factors secreted by the RWA during feeding

Advisor: A-M Oberholster

Fanie Verwey

Control of Pythium wilt and root rot in hydroponically grown lettuce

Advisors: N Labuschagne & FC Wehner

Michelle Victor

MiRNA profiling in differentiating woody tissues of *Eucalyptus*

Advisors: AA Myburg, H Huismans & J Theron

Robert Walters

Development of markers for identification of different aphid biotypes

Advisors: A-M Oberholster & V Tolmay

Anita Willis

Status of *Guignardia mangiferae* in South African mango orchards

Advisors: L Korsten & P Labuschagne

Michelle Wilmot

Antimicrobial activity of tea (*Camellia sinensis*) extracts against pathogenic fungi on selected vegetable crops

Advisors: N Labuschagne & Z Apostolides

Dewald Zaayman

Transcript profiling in Gamtoos *Dn7* a gene with bimodal functioning

Advisors: A-M Oberholster & N Lapitan

4th year and honours students

Aneen Belgrove (2003)

Nicky Creux (2003)

Jake Darby (2003)

Elsie de Meyer (2003)

Rosita Endah (2003)

Joha Grobbelaar (2003)

Frank Maleka (2003)

Zelda Pieterse (2003)

Innocentia Phuto (2003)

Bernice Porter (2003)

Robert Walters (2003)

Henrich du Plooy (2004)

Daniel Khumalo (2004)

James Mehl (2004)

Joylene Moulder (2004)

Tanja Gevers (2004)

Claire Munro (2004)

Luke Solomon (2004)

Steven van Niekerk (2004)

Chyreene Wingfield (2004)

Chrizzelle Beukes (2005)

Bianca Hinze (2005)

Murray Logan (2005)

Mpho Makinta (2005)

Clair Strange (2005)

Lorinda Swart (2005)

Ariska van der Nest (2005)

Francois van der Walt (2005)

Bernett van der Merwe (2005)

Nadine Varelas (2005)

Marinda Veenendaal (2005)

Markus Wilken (2005)

Student assistants

Nicky Creux (2003)

Muhammed Ebrahim (2003)

Hardus Hatting (2003)

Vincent Kekana (2003)

Duncan Newman (2003)

Luke Solomon (2003)

Wilhelm Dreyer (2005)

Buyi Mthlane (2005)

Recent graduates

PhD

Deidre Fourie (2003)

Bacterial diseases of dry beans in South Africa with special reference to common bacterial blight and its control

Advisor: PS van Wyk

Co-advisor: MJ Wingfield

Alemu Gezaghne (2003)

Diseases of plantation forest trees in Ethiopia

Advisor: J Roux

Co-advisors: MJ Wingfield & BD Wingfield

Mauricio Marin (2003)

Molecular taxonomy of *Ceratocystis polonica sensu lato*

Advisor: MJ Wingfield

Co-advisors: O Preisig & BD Wingfield

Cassi Myburg (2003)

Molecular studies on Cryphonectria canker of eucalypts

Advisor: BD Wingfield

Co-advisor: MJ Wingfield

Dean Oelofse (2003)

Molecular approaches towards antracnose resistance in lupins

Advisor: IA Dubery

Co-advisor: DK Berger

Christell van der Vyver (2003)

Stress induced genomic changes in plants

Advisor: K Kunert

Co-advisor: C Cullis

XuDong Zhou (2003)

Ophiostomatoid fungi with reference to those species associated with three bark beetles in South Africa

Advisor: MJ Wingfield

Co-advisor: BD Wingfield

Appolinaire Adandonon (2004)

Damping off and stem rot of cowpea in Benin caused by *Sclerotium rolfsii*

Advisor: TAS Aveling

Co-advisor: M Tamo

Martin Coetzee (2004)

Molecular characterization of *Armillaria* (Basidiomycetous Agaricales Tricholomycetaceae)

Advisor: BD Wingfield

Co-advisors: MJ Wingfield & P Bloomer

Joseph Obagwu (2004)

Developing biopesticides for control of citrus fruit pathogens of importance in global trade

Advisor: L Korsten

Co-advisor: T Regnier

Yolisa Pakela (2004)

Interaction between cowpea and *Colletotrichum dematium*

Advisor: TAS Aveling

Co-advisor: TA Coutinho

Bernard Slippers (2004)

The taxonomy, phylogeny and ecology of Botryosphaericeous fungi on selected woody hosts

Advisor: MJ Wingfield

Co-advisors: TA Coutinho, BD Wingfield & P Crous

Jacque van der Waals (2004)

Implementing a disease forecasting system for early blight for the South African potato industry

Advisor: L Korsten

Co-advisors: TAS Aveling & T Regnier

Lynelle van Emmenes (2004)

Gene expression profiling in *Triticum aestivum* line PI 137739 in response to *Diurapsis noxia* feeding

Advisor: A-M Oberholster

Schalk van Heerden (2004)

Studies on *Cryphonectria cubensis* in South Africa

Advisor: MJ Wingfield

Co-advisors: O Preisig & BD Wingfield

Chantel van Niekerk (2004)

Analysis of gene expression in *Triticum aestivum* cultivar "Tugela DN" after *Diurapsis noxia* infestation

Advisor: A-M Oberholster

Co-advisor: K Kunert

Eduard Venter (2004)

Host resistance in South African *Pinus* spp.

Advisor: A-M Oberholster

Co-advisors: BD Wingfield & MJ Wingfield

Marinda Visser (2004)

Population biology of the banana panama wilt pathogen, *Fusarium oxysporum* f.sp. *cubense*

Advisor: A Viljoen

Co-advisors: MJ Wingfield, BD Wingfield & T Gordon

Quenton Kritzing (2005)

Mycotoxins and medicinal properties of cowpea

Advisor: TAS Aveling

Co-advisor: N Lall

MSc

Rodrigo Ahumada (2003)

Diseases of commercial *Eucalyptus* plantations in Chile

Advisor: MJ Wingfield

Co-advisors: BD Wingfield and G Hunter

Anton Jordaan (2003)

Transformation of *Nicotiana tabacum* cv. Samsun with melanin and indigo genes.

Advisor: A-M Oberholster

Shilo Loots (2003)

Isolation and characterization of *Diuraphis noxia* induced sequences from wheat line PI 294994

Advisor: A-M Oberholster

Co-advisor: E Venter

Inge Maritz (2003)

Evaluation of polygalacturonase-inhibiting protein (PGIP)-mediated resistance against *Verticillium dahliae*, a fungal pathogen of potato

Advisor: D Berger

Co-advisor: D Oelofse

Grace Nakabonge (2003)

Diseases associated with plantation forestry in Uganda

Advisor: J Roux

Co-advisors: TA Coutinho and MJ Wingfield

Carlos Rodas (2003)

Diseases of plantation forest trees in Colombia

Advisor: MJ Wingfield

Co-advisors: J Roux & TA Coutinho

Karen Surridge (2003)

Fungi associated with banana leaf diseases in South Africa

Advisor: A Viljoen

Co-advisors: F Wehner & PW Crous

Dirk Swanevelder (2003)

Population diversity in *Clivia miniata*

Advisor: A-M Botha-Oberholster

Co-advisors: A van Wyk & M van der Merwe

Tessa Bandounas (2004)

Infection studies on white rust of sunflower

Advisor: A Viljoen

Co-advisor: TAS Aveling

Raksha Bhoora (2004)

Genetic transformation of *Eucalyptus* clones

Advisor: BD Wingfield

Co-advisors: MJ Wingfield, D Berger & P Chimwamurombe

Ronald Heath (2004)

Diseases of Myrtaceous and Melastomataceous hosts

Advisor: MJ Wingfield

Co-advisors: J Roux & BD Wingfield

Anton Fourie (2004)

Biochemical mechanisms for tolerance of citrus rootstocks against *Phytophthora nicotianae*

Advisor: N Labuschagne

Co-advisor: Z Apostolides

Lorenzo Lombard (2004)

Pathogens associated with hydroponically grown eucalypts in South Africa

Advisor: TA Coutinho

Co-advisors: MJ Wingfield & B Janse

Marie Onanena (2004)

Isolation of labile regions from wild oat

Advisor: K Kunert

Co-advisor: C van der Vyver

Anneke Prins (2004)

The protective role of oryzacystatin-I during abiotic stress

Advisor: K Kunert

Co-advisor: A-M Oberholster

Koreen Ramessar (2004)

Genetic modification of maize by introduction of anti-fungal genes to confer resistance to *Fusarium verticillioides*

Advisor: DK Berger

Co-advisor: M O'Kennedy

Americo Uaciquete (2004)

Epidemiology and control of powdery mildew (*Oidium anacardii* Noack.) on cashew nut (*Anacardium occidentale* L.) in Mozambique

Advisor: L Korsten

Co-advisor: TAS Aveling

Marelize van Wyk (2004)

Taxonomic and population biology of *Ceratocystis* spp. on *Eucalyptus* and other Myrtaceae

Advisor: MJ Wingfield

Co-advisors: BD Wingfield & J Roux

Juan Vorster (2004)

The application of representational difference analysis and plant differentiation

Advisor: K Kunert

Co-advisor: A-M Botha-Oberholster

Joanne Fouche (2005)

Pathogenicity of African isolates of bacterial wilt on *Arabidopsis thaliana*

Advisor: D Berger

Co-advisor: TA Coutinho

Veloshinie Govender (2005)

Evaluating biological control systems for mango postharvest disease control

Advisor: L Korsten

Co-advisor: T Regnier

Barbara Nel (2005)

Management of *Fusarium* wilt of banana by means of biological, chemical control and induced resistance

Advisor: A Viljoen

Co-advisors: C Steinberg & PS van Wyk

Draginja Pavlic (2005)

Botryosphaeria spp. endophytic in eucalypt and *Syzygium* in South Africa

Advisor: TA Coutinho

Co-advisors: MJ Wingfield & B Slippers

MSc (Agric)/MInstAgrar

Elizabeth Kola (2004)

Mycoflora and polyphenolics of various coloured seeds of Bambara groundnut (*Vigna subterranean* L. Verdc.)

Advisor: TAS Aveling

Co-advisor: N Lall

Julia Domola (2004)

Survey and characterization of sweet potato viruses in South Africa

Advisor: TAS Aveling

Co-advisor: G Thompson

Patrick Mphalele (2004)

Honey bee dissemination of *Bacillus subtilis* to citrus flowers for control of *Alternaria*

Advisor: L Korsten

Mashudu Silimela (2004)

Alternative methods to control pre- and postharvest diseases of mango and sunburn

Advisor: L Korsten

Leulseged Begashaw (2004)

Commercial exploitation of plant growth promoting microbes for biocontrol and growth enhancement of some vegetable crops

Advisor: L Korsten

Fhumalani Mashau (2005)

Risk assessment of fire blight on pears

Advisor: L Korsten

Prestigious NRF bursary holders

Irene Barnes

Johan de Graaf

Franco du Preez

Marieka Gryzenhout

Almuth Hammerbacher

Ronald Heath

Gavin Hunter

Luke Solomon

Ezanne Swanepoel

Dirk Swanevelder

Noëlani van den Berg

Magriet van der Nest

Marelize van Wyk

Michelle Victor

Aaron Klug scholarship

Juanita de Wet

Mellon Foundation grants

Irene Barnes

Lieschen de Vos

Gavin Hunter

Bongani Maseko

Sanushka Naidoo

Noëlani van den Berg

Albé van der Merwe

NRF scarce skills scholarships

Carrie Brady (2002; 2003-2004; 2005-2008)

Elsie de Meyer (2002)

Rene Sutherland (2002; 2003-2004)

Izette Greyling (2003-2004)

Aneen Belgrove (2005)

Other scholarships

Andrew Kiggundu (Rockefeller Foundation)
Sinnia Kappindu (IITA)
Josephine Mukiibi (Belgium Embassy & INIBAP)
Gizachew Weidemichael Assefa (Ethiopian Agricultural Institute)
Charline Kamburona (DAAD, TUCSAN Scholarship)
Lesesse Beyene (EARO, Ethiopia)
Yoseph Beyene (EARO, Ethiopia)
Mesfin Bogale (EARO, Ethiopia)
Joseph Ndunguru (IITA, Nigeria)
Pamela Paparu (IITA)
Shahasi Athman (IITA)
Rene Sutherland (NRF Mobility grant to travel to Orlando, Florida for "Plastid transformation of bananas and plaintains)
Michelle Victor (UP Postgraduate Research Visit Bursary to Prof V Chiang, NC State University, USA)
Grace Nakabonge (TWOS [Third World Organization for Women in Science])

MANAGEMENT

Management committee

Professor MJ Wingfield (Chairman)
Professor BD Wingfield
Professor K Kunert
Professor L Korsten
Professor TA Coutinho
Professor A-M Oberholster
Assoc Professor D Berger
Assoc Professor TAS Aveling
Assoc Professor J Roux
Assoc Professor N Labuschagne
Dr A Viljoen
Dr Z Myburg
Dr G Marais
Dr E Steenkamp
Dr B Slippers
Dr R Chikwamba
Mr B Hurley
Gavin Hunter (Postgraduate student representative 2003)
Dirk Swanevelder (Postgraduate student representative 2004)
Irene Barnes (Postgraduate student representative 2005)

Advisory committee

Professor A Ströh (Chairman), Dean of the Faculty of Natural and Agricultural Sciences
Professor H Huismans, Head of the Dept of Genetics
Professor TE Cloete, Head of the Dept of Microbiology & Plant Pathology
Professor J Verschoor, Head of the Dept of Biochemistry
Professor M Meyer, Head of the Dept of Botany
Professor C Reinhardt, Head of the Dept of Plant Production
Professor S Nicolson, Head of the Dept of Zoology & Entomology
Professor M Wingfield, Director of FABI

Some social highlights in FABI 2004

Annual SPOOF* meeting

*Society for the Publication of Outrageous Findings
Theme: Where in the world is FABI



Koos Eloff, Martin Coetzee and Lorenzo Lombard



Joha Grobbelaar and her sister



Hardus Hatting and his date



Karen Jacobs and her twin daughters

Year end function 2004



Left to right: Lorenzo Lombard, Marelize van Wyk and Ronald Heath



Aisha Mohamed-Ali, Marija Kvas and Rebecca Makhado

FABI Square Inauguration



Dr Zander Myburg explaining aspects of his research programme



Visitors examining nematodes, cultured for Sirex biological control



Dr Adi Patterson, Deputy Director of the Dept of Science and Technology planting a tree to acknowledge the contribution of the SA Government to research in FABI



Delegates representing the SA Forestry Industry including Bruce Hulett (Mondi Business Paper), Lolly Stuart (WOODFOR), Mike Edwards (FSA), Colin Dyer (ICFR) and Flic Blakeway (CSIR)

Sponsors of research

Many of these commercial companies or organisations fund more than one programme in FABI

ACIAR (Australia)
Agropolis Advanced Research Platform (France)
Banana Growers Association of South Africa
Belgium Embassy
BIOPAD
Central Timber Co-operative (CTC)
China/South African Governments Agreement
CIRAD
Citrus Growers Association
CNRS/South African Government Agreement
CSIR
DFG (Deutsche Forschungs-Gemeinschaft: German Research Foundation)
Department of Water Affairs and Forestry (DWAF)
Department of Trade and Industry through THRIP initiative
Department of Science and Technology through the Innovation Fund and CTHB
Department of Science and Technology through the NRF
Du Roi QMS
EARO, Ethiopia
ESKOM
European Union
Flemish/South African Governments Agreement
Forestry South Africa
Global Forestry Products
Hans Merensky Holdings
INIBAP
Innovation Fund
International Institute of Tropical Agriculture (IITA)
Italian/South African Governments Agreement
Mellon Foundation
Mondi Business paper and Mondi Shanduka
National Research Foundation
Norway/South African Governments Agreement
Protein Research Foundation
Rockefeller Foundation
SAFCOL/Komatiland Forestry
Sappi
SIDA/South African Government Agreement
South African Avocado Growers Association
South African Litchi Growers Association
South African Mushroom Farmers Association
South African Wattle Growers Union
Tanzanian Government
Technology and Human Resources and Industry Programme (THRIP)
Tuscan Namibia/DAAD (Germany)
Wattle Growers Union of South Africa (SAWGU)
Wheat Cereals Trust

