



FABI

Forestry and Agricultural Biotechnology Institute



**Biennial Report
2002/2003**

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
Central Timber Co-operative (CTC)
China/South African Governments Agreement
CIRAD
Citrus Growers Association
CNRS/South African Government Agreement
CSIR
Department of Water Affairs and Forestry (DWAF)
Department of Trade and Industry through THRIP initiative
Department of Arts, Science and Technology through the Innovation Fund
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
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South African Avocado Growers Association
South African Litchi Growers Association
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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

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 of South Africans in the fields of forestry and agriculture

The association of FABI with the University of Pretoria, the largest residential university in South Africa, provides access to 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, Plant Production and Postgraduate School for Agriculture and Rural Development 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.

FABI is not a new emerging venture, but rather 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 April 1998, although it was only officially inaugurated on 13 March 1999. This third FABI biennial report covers the period from September 2001 to May 2003.

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Compilation, layout and design by TA Coutinho

Cover photograph: Cross section through the base of the South African native tree, *Celtis africana*, suffering from a root disease and showing discolouration and fungal zone lines (photograph taken by R Heath).



Forestry and Agricultural
Biotechnology Institute
"FUTURE FORESTS and FOOD"

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Director's Report



As I write this note, the fifth anniversary of the birth of FABI has just passed. It is truly amazing to look back and to realise that in this relatively short space of time, the Institute has achieved so much. FABI is now well recognised nationally as a leader in the broad field of plant biotechnology. The Institute has also rapidly gained international stature and there are few organisations with activities linked to ours that do not already know us well. This biennial report is the third in the history of the Institute and it is a great pleasure for me to share this brief summary of our activities during the last two years with you.

The FABI family has continued to grow and this has happened more rapidly than we might have expected. If you have followed our development, you will know that the founder FABIANS were approximately 50 in number. The group now represents about 130 mainly MSc, PhD students and post-doctoral fellows. This team is led by 14 academic staff that make up the core of the FABI management committee. We continue to function very much as a family, with all the privileges and responsibilities that go with being part of a family.

FABI's rapid growth has inevitably placed substantial pressure on our facilities. Space for students to work reasonably comfortably has become a contested resource. Armed with the statistics related to this problem and the fact that it was becoming necessary to limit the growth of some programmes, we have motivated for new research facilities. Of course here the business catch phrase "Return on investment" rapidly became dominant in discussions. However, the growth and great accomplishments of FABI have made it relatively easy to persuade the University of the need for additional facilities. The new three storey building extension to FABI has risen rapidly and we look forward to occupying this fantastic new facility during July 2003. Some illustrations of the growth of FABI during the latter part of 2002 and the early part of this year, are presented in this report to give you an idea of the changing face of our Institute.

The new FABI facilities will considerably enhance the scope of the research that we are able to undertake. It is impossible to provide details of the new building in this brief report but I must mention a few features. These include laboratory space and seating for 30 additional students. We will now have access to an auditorium that is able to easily seat all FABIANS and in this respect seminars and research meetings will be considerably more comfortable. A suite of world class quarantine greenhouses and a top quality facility for tissue culture and transgenic research plants also form part of the new building. To add to this, a third floor, not strictly limited to, but closely allied to the interests of FABI, has been added to the building to accommodate a bioinformatics centre and new laboratories for our growing DNA sequencing enterprise.

Biotechnology in the broadest sense of the word has continued to grow rapidly in South Africa. Obviously this has many positive repercussions for FABI. The group has access to new funding opportunities and our students, the prime *raison de etre* of FABI, have access to even better employment opportunities. I intentionally use the word "even better" because the post graduate students emerging from FABI have been in high demand both nationally and abroad. There is no question in my mind that biotechnology will continue to grow in South Africa, with many new businesses emerging. FABI will surely grow in concert and will continue to be an important source of trained scientists to participate in these exciting new ventures.

I wanted to include in this brief introduction, some news of new research groups that have joined FABI during the course of the last two years. This is difficult to do without absorbing more space than I have been promised. However, I must make mention of the outstanding new plant biotechnology research programmes of Prof Dave Berger and Dr Zander Myburg. We have also continued to build on our strong links with the CSIR and about two years ago, initiated a joint appointment for Dr Gert Marais to build on our mutual

interests in food products and industrial mycology. Our activities in forest entomology and insect pests on banana have also been substantially expanded. To add to this, research and services linked to food safety are also growing rapidly.

One always needs to be cautious in focussing on the achievements of a large number of outstanding people working in a group such as FABI. The danger here is that one inevitably forgets to mention someone. I must thus generalise but yet make the point that a great number of research leaders and graduate students have been recipients of special awards and honours. These have come from within the University of Pretoria, from organisations linked to science in South Africa and also from international groups. It is a great pleasure to see these accomplishments and to realise what a fabulous group the FABIANS represent.

It has always been my firm belief that diversity builds strength. In this sense, living and working in South Africa provides great opportunities. A country with 11 official languages can hardly fail to provide an educational institution with an incredible source of diversity. FABI has in the past achieved the status of having at least one student that speaks every one of South Africa's official languages. The Institute has also become a home to students from many countries, in every hemisphere and every continent. A small census towards the end of last year showed that 28 languages were spoken by FABIANS at that time and some details are presented in this report. I believe that this number has grown, although it changes from month to month. The point is merely that FABI has become a truly international institute.

Support for research in FABI comes from a wide range of organisations and private enterprises. The majority of our funding comes from outside the University of Pretoria and includes large industries, small companies, farmers groups, international aid organisations, overseas business ventures, government and state funds. We are most fortunate to have a very sound base of funding that is nurtured enthusiastically by the research enterprise of the University of Pretoria. On behalf of all FABIANS, I take this opportunity to thank our research sponsors for their support.

Research linked to education represents the core business of FABI. While we have close alliances with research providers that are more "product" oriented, all our work is in some way linked to student training. This is consistent with our existence in a university environment. Our main research focus remains linked to the broader field of plant sciences and as a research team, we are unified by some interest in plants, plant biotechnology and DNA-based interests. The establishment of FABI has had a profound impact on these fields at the University of Pretoria as well as in South Africa as a whole. There is every reason to expect that FABI is entering a growth phase in both size and quality.

One can only feel excited and enthusiastic when looking to the future of FABI. Alliances with international funding organisations have brought a much broader focus to our work than I had expected five years ago. Plant, insect and microbial molecular genetics projects were relatively well-established when FABI was founded. New areas of interest and focus have, however, emerged as important, and these are growing rapidly. They include projects linked to the impact of genetically modified organisms on the environment, a major thrust in plant and microbial biodiversity, and rapidly emerging projects in the area of industrial plant and microbial biotechnology. The establishment of powerful new facilities in bioinformatics, and the emergence of the first research products in this field clearly herald a major new area of growth for FABI.

I feel hugely privileged to lead this special group. On behalf of all of the FABI family, I again thank the many organisations and industries in South Africa and in other parts of the world that support our research. We are also most grateful to our many colleagues and friends that have supported us in reviewing publications, examining theses and many other elements of the education and academic enterprise.

Michael J Wingfield

Mondi Professor of Forest Pathology and Director of FABI and the TPCP

FABI Team



- Front row:** Carlos Rodas, Almuth Hammerbacher, Juan Vorster, Brenda Wingfield, Marlien van der Merwe, Susan Groenewald, René van Zyl, Elsie de Meyer, Maria-Noël Cortinas, Renate Zipfel, Sonja de Beer and Sanushka Reddy
- 2nd row:** Ntsane Moleleki, Anita Steyn, Dilzara Aghayeva, Claudia Rodas, Terry Aveling, Andrew Kiggundu, Mike Wingfield, Awelani Muthsembele, Thuto Matsioloko, Charline Kamburono, Brenda Buthelezi, Maria Onanena, Joyce Jakavula, Busisewe Tshabalala, Solomon Gebeyehu and Annabé Louw-Gaume
- 3rd row:** Therese Lotter, Retha Slabbert, Vivienne Clarence, Teresa Coutinho, Valentina Nkosi, Veloshinie Govender, Marinda Visser, Bongani Maseko, Lawrie Wright, Karl Kunert, Elizabeth Kola, Anna-Maria Oberholster, Mariette Truter, Marieka Gryzenhout, Rosemarie Visser, Leylani Grobler, Deshni Pillay, Thierry Regnier, Grace Nakabonge, Helen Doman, Izette Greyling, Barbara Nel, Martie van Zyl, and Irene Barnes
- 4th row:** Prem Govender, XuDong Zhou, Lancelot Maphosa, Alemu Gezaghne, Gavin Hunter, Jolanda Roux, Marelize van Wyk, Eduard Venter, Riana Jacobs, Cassi Myburg, Legesse Beyene, Sabine Lezar, Elizabeth Attinger, Eva Müller, Noëlani van der Berg, Joanne Weich and Danie Theron
- Back row:** Wilhelm de Beer, Dirk Swanevelder, Altus Viljoen, Lorenzo Lombard, Gert Marais, Bernard Slippers, Albé van der Merwe, Brett Hurley, Hardus Hatting, Ronald Heath, Dave Berger, Zander Myburg, Quinton Kritzing, Gina Swart, Juanita de Wet, Christelle van der Vyver, Draginja Pavlic, Schalk van Heerden and Paal Krokene

FABI growing – signs of a success story in plant biotechnology

FABI, the Forestry and Agricultural Biotechnology Institute of the University of Pretoria was formally established in April 1998. The Institute has grown rapidly and in just five years has become one of South Africa's premier plant biotechnology research groups. FABI has also rapidly gained international recognition for outstanding student training and research productivity. At the time of its establishment, FABI included some 55 full time scientists, MSc and PhD students and post doctoral fellows. The group making up the so-called FABI Family is now in excess of 130 mainly scientists, with a small core of Technical and administrative support staff.

Based on outstanding research and training outputs, the University of Pretoria agreed, late in 2000, to extend the research facilities of FABI. Expansion will include a three-story building connected to what is known as the "downstairs FABI" building. The new building will add new seminar facilities, four research laboratories, a large plant propagation and tissue culture facility, a state of the art suite of eight quarantine greenhouses, walk in growth rooms and refrigeration, a new DNA sequencing facility, a large bioinformatics centre, offices and much more. In all, the new facilities will more than double the floor space available used by FABI in the past.

Construction of the new FABI facilities began in October 2002 on a site adjacent to the main FABI facility. The building site, previously two old houses, utilised by the administration department of the Faculty of Biological and Agricultural Sciences. Thus, prior to commencing with building, these houses needed to be de-commissioned and demolished. In addition, the site was found to have a relatively high water table and a specialised foundation with reinforced concrete piles able to carry up to seven floors (planned for the longer term), needed to be constructed. Completion of the building project is planned for June 2003 and FABIANS look forward to populating the building shortly thereafter.

The following photographs illustrate phases in the growth of the new FABI facilities. The first picture is an artist's impression of the building which will be completed by the time this report is published.



An artists impression of the completed building. Who is the lady in the foreground we ask?



October 2002, prior to the demolition of the two faculty "houses"



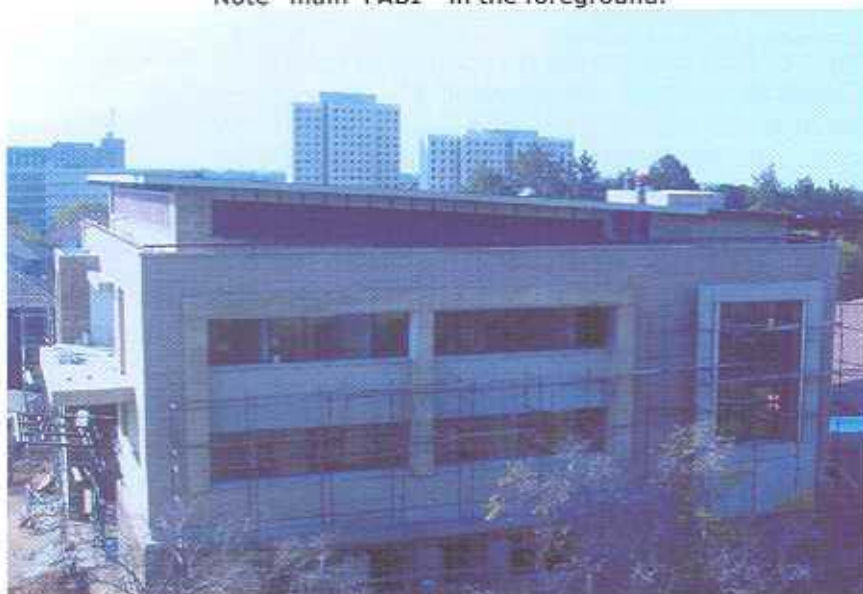
October 2002, the demolition begins!



October 2002, clearing the site



January 2003, second floor up and the start of the third floor.
Note "main' FBI" in the foreground.



June 2003, building is almost completed

RESEARCH REPORTS

Forest Protection

Research leader: Prof Mike Wingfield

Research team: Prof Teresa Coutinho
Prof Brenda Wingfield
Dr Jolanda Roux
Dr Prem Govender
Dr Oliver Preisig
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 so that they may be more effectively controlled
- Study and evaluate novel strategies for disease and pest control

Highlights of research 2002/2003:

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 highlights and important findings.

Cryphonectria canker

Cryphonectria canker of *Eucalyptus* caused by *Cryphonectria cubensis* remains one of the most important threats to plantation forestry in South Africa. One of our major initiatives has been to conduct screening trials on hybrid clones in areas where the disease has been most severe. These trials have brought a great deal of knowledge to forestry companies supporting the programme. Disease tolerant planting stock

has, in many cases, been identified in advance of planting. A limitation of this work has, however, been that the screening process is slow and results often emerge at a time when decisions have already been made regarding desirable material to plant. The research team will continue with screening inoculations in the future, but these trials will be much more specifically planned than in the past. They will also be geared to answering very specific questions such as whether different isolates of the pathogen respond similarly on different clones, or not.

The sudden appearance of *Cryphonectria* canker in South Africa and the known absence of the disease in the late 1970s has led us to believe that the causal agent was

introduced into the country. Knowledge pertaining to origin is highly important in terms of identifying appropriate planting stock and understanding the disease situation in South Africa. One of the problems in this regard is that the taxonomy of the genus *Cryphonectria* is poorly understood. In order to rectify this situation, we have undertaken a series of studies relating to the identification of *Cryphonectria* spp. Here we have begun to understand that species such as *C. cubensis* is very distantly related to, for example, the devastating causal agent of Chestnut blight, *C. parasitica*, in North America.



Sexual fruiting body of *C. cubensis*

An enigma regarding *Cryphonectria* canker in South Africa is that symptoms of the disease are somewhat different to those found elsewhere in the world. In addition, cankers in South East Asia and South and Central America, tend to be covered with sexual fruiting structures, whereas those in South Africa are covered by asexual fruiting structures. Early DNA sequencing studies in our laboratories showed no clear difference between isolates from these various areas of the world. However, more recently, we have undertaken studies where we have applied DNA sequence comparisons for multiple genes. Interestingly, the multiple gene genealogies show that the South African fungus is a distinct species. Data also indicate that the fungus in South America and that in South East Asia probably represent distinct species. These findings have significant implications for the management of *Cryphonectria* canker in South Africa and they will also impact strongly on quarantine measures. The latter actions must now be geared to ensuring that

C. cubensis in its South American or South East Asian manifestation does not reach South Africa. Similarly, we have to warn countries in these areas of the world against the introduction of the South African fungus into their plantations.



Asexual fruiting bodies of *C. cubensis*

A second *Cryphonectria* sp., *Cryphonectria eucalypti*, occurs commonly on *Eucalyptus* in South Africa. This fungus was previously known as *Endothia gyrosa* but intensive DNA-based studies in our laboratories have previously shown that this fungus is a species of *Cryphonectria* and that it probably originates on *Eucalyptus* in Australia. Knowing that a second species of *Cryphonectria* occurs in South Africa has led us to consider the pathogenicity of the fungus. There have been some suggestions that the fungus is only weakly pathogenic and a more accurate answer to this question has been desired. We have thus undertaken intensive inoculation studies on a wide range of *Eucalyptus* clones, using a large set of isolates of the fungus. Most interesting results have been obtained showing that isolates of the fungus differ in pathogenicity and that clones differ in susceptibility to this fungus. Results also suggest that the response of clones to inoculation is variable and is probably influenced by the time of inoculation. This would be consistent with our knowledge that the fungus tends to occur on the bark of somewhat stressed trees.

Eucalyptus snout beetle

The Eucalyptus Snout Beetle, *Gonipterus scutellatus*, was first recorded in South Africa in 1916, at Newlands, Cape Town. *Gonipterus scutellatus* was observed to

defoliate eucalypt trees, resulting in stunted growth and in severe cases, death. Native to Australia, *G. scutellatus* was without natural enemies and spread unhindered throughout South Africa, causing extensive damage to eucalypt plantations. The parasitic wasp, *Anaphes nitens*, a biological control agent from Australia, was introduced into South Africa. *Anaphes nitens* was very effective at most sites that have continued to experience high infestations of *G. scutellatus*. These infestations are especially prevalent at high altitude sites, where susceptible species, such as *Eucalyptus dunnii* and *E. smithii*, are planted.

In 2000, the research team undertook a study to investigate the influence of various abiotic factors (altitude, temperature and rainfall) over time on parasitism of *G. scutellatus* by *A. nitens*. Five study sites of varying altitude and rainfall were selected. All sites were compartments of *E. dunnii*. The percentage parasitism was measured at these sites.

Significant differences in parasitism between sites and over time were found. Parasitism at the highest altitude site was significantly lower than that at the lowest altitude site, confirming the reduced efficacy of *A. nitens* at high altitude sites. High altitude sites experienced an extended period of lower parasitism in winter. In both high and low altitude sites, parasitism dropped significantly after spring and increased again in summer.

Results of this research indicated that *G. scutellatus* infestations at high altitude sites are not only due to the presence of susceptible *Eucalyptus* species at these sites, but also due to the poor performance of *A. nitens*. This poor performance was attributed to the colder temperatures at high altitude sites. Cold temperatures can affect *A. nitens* indirectly, by reducing the number of hosts, and directly. The decrease in parasitism after spring was attributed to biotic factors, such as parasitoid – host interactions and superparasitism. Thus, the efficacy of *A. nitens* may be reduced by abiotic factors, such as temperature at high altitude sites and by biotic factors at high and low altitude sites.

Armillaria root rot

Armillaria root rot continues to be present in pine plantations in parts of KwaZulu/Natal and Mpumalanga. The disease is no longer considered serious but losses are found, particularly in damp river valley areas where the fungus is most active and aggressive. Much confusion has surrounded the identification of *Armillaria* spp. in South Africa and elsewhere. For many years, the fungus has been known as *Armillaria mellea*, but this name was clearly applied arbitrarily. There is particularly little knowledge available for the fungus in the southern hemisphere and we have maintained a small but active programme to identify species from the southern hemisphere. This has been linked to collaborations with colleagues in Australia and New Zealand as well as in collaboration with a colleague in Zimbabwe. These studies based on intensive DNA sequencing have shown fascinating relationships between Australasian species. They have confirmed the hypothesis that an undescribed species occurs in New Zealand. Furthermore, we have also shown that there are three species in Zimbabwe and that only one of these currently occurs in South Africa.

Mycosphaerella leaf blotch

Mycosphaerella leaf blotch was one of the first diseases to be recognised in South Africa as a threat to plantation forests. During the last two years, studies have been undertaken to determine which *Mycosphaerella* spp. occur, particularly on *E. nitens* in South Africa. This has important implications for disease control and for quarantine. In addition to understanding the composition of *Mycosphaerella* spp. associated with the disease, we have attempted to determine the relative importance of these species. Results have been extremely interesting and have indicated that the view that *M. juvenis* is the most important pathogen, is incorrect. At least for the areas surveyed, *M. nubilosa* is by far the most important cause of *Mycosphaerella* leaf blotch in South Africa. It is, however, possible that species composition changes with time and that different species are more important in some years, than in others. This question will have to be considered in the future. Because of the difficulty of identifying

Mycosphaerella spp., such surveys will require rapid identification protocols. Studies to develop these tools are currently under way and should be completed in the next year.

Dothistroma needle blight

In South Africa, *Dothistroma* needle blight is an enigma. The disease caused by *Dothistroma septospora* is one of the most important in the world and has caused huge losses to, mainly *P. radiata* plantations in countries such as Australia, New Zealand and Chile. What is interesting is that the disease is known to have been present in South Africa since the early 1960s, but it has remained on *P. radiata* and also in very limited areas of the eastern Cape. During the last year, the disease was also found associated with severe defoliation of *P. radiata* in the Limpopo Province.

Lack of understanding of *Dothistroma* needle blight in South Africa and concern that it could adapt to infect other hosts and perhaps hybrid pines, has prompted the research team to initiate a study of the pathogen. This study commenced in mid-2002 and will continue for three years. The key objectives will be to determine whether the pathogen is the same as that found elsewhere in the world. This question is important because of the enigma surrounding the distribution of the disease in South Africa. Another key objective will be to gain an understanding of the genetic diversity of the pathogen in the country and thus to consider the possibility of new disease outbreaks in the future.



Typical symptoms of *Dothistroma septospora* infection on a pine needle

Insect-related pathogens

Bark beetles are amongst the most important pests of forest trees in the world. There are literally hundreds of species and many are important. On pines, there are many examples of insects and these may pose a threat to South African plantations. At present, only three pine infesting bark beetles are known in South Africa and all three have been accidentally introduced into this country from Europe. The fact that bark beetles have already been introduced into pine-growing areas in this country suggests that others are likely to appear in the future. This could have a very negative impact on forestry in South Africa.

A fascinating and important aspect of bark beetles and particularly those infesting conifers, is that they live in close association with very specific fungi. These fungi, commonly known as the Ophiostomatoid fungi, include serious pathogens and agents of blue stain. The importance and role of many others is unknown. Researchers in the programme have a long track record of investigations into bark beetle biology and particularly concerning their association with fungi. Studies have elucidated important new relationships between these fungi and also between their bark beetle hosts. New species belonging to this group have been discovered including one on *Eucalyptus*. Although these studies have significant practical implications, they are largely also of a fundamental nature. The basic science surrounding these studies has enabled us to make strong connections with scientists in other parts of the world and thus to gain funding from initiatives such as the South Africa/Norway and South Africa/China bilateral government agreements. These in turn are helping substantially to expand the experience of students and staff of the group, particularly in the area of forest entomology.

Fungus gnats

Fungus gnats in the families Sciaridae and Mycetophilidae are suspected to transmit the pitch canker fungus, *Fusarium circinatum*, to pine seedlings in South African forestry nurseries. Our objectives have been to confirm the presence of and

to identify fungus gnats in these nurseries. Furthermore, to determine whether these fungus gnats assist in the transmission of *F. circinatum*. Four of the major pine growing nurseries in South Africa were used for our investigations.



Adult fungus gnat

Only one species of fungus gnat, *Bradysia difformis* (Sciaridae), was detected in the nurseries. This is the first record of *B. difformis* in South Africa. *Bradysia difformis* is a common commercial pest in Europe and *Bradysia* spp. have been recorded to transmit fungal pathogens in various overseas nurseries. However, *F. circinatum* was not isolated from the samples of *B. difformis* or any of the other flies collected in the nurseries. Studies are in progress to investigate whether any association exists between *F. circinatum* and the larvae of *B. difformis*.

Ceratocystis wilt

Ceratocystis species includes some of the world's most serious pathogens of trees. Notable examples are oak wilt in North America, sap streak disease and plane wilt in North America and Europe and *mal de machette* on a wide range of hosts in South America. In South Africa, wattle wilt caused by the newly discovered species and pathogen *Ceratocystis albobundus* has increased in importance each year since its discovery approximately a decade ago.

Studies on *Ceratocystis* spp. are leading us to conclude that these fungi are increasing in importance in forestry. We base this view on the discovery of new species such as *C. albobundus* and the growing evidence that this fungus is of African origin. Studies

conducted during the last year, for example, have shown clearly that *C. albobundus* occurs naturally on *Protea* spp. and that the fungus does not only occur in South Africa but also in central Africa (Uganda). Population biology studies using microsatellite markers that we have developed and including isolates from Uganda and South Africa have added substantial substance to our belief that *C. albobundus* is an African pathogen. In this regard, it poses a substantial threat to some Australian *Acacia* spp. in their native range.

Until relatively recently, *Ceratocystis* spp. were not considered as serious pathogens of *Eucalyptus* spp. However, discoveries made by members of the TPCP team have shown that the well-known wilt pathogen *C. fimbriata* can lead to the rapid death of *Eucalyptus* spp. in eastern Africa and in Brazil. This work has now been expanded to include studies on isolates from Uruguay, which have shown that *C. fimbriata* can infect and rapidly kill *Eucalyptus* spp. after pruning. Of greater concern is that the fungus has very recently been discovered on *Eucalyptus* in various parts of South Africa. This could be linked to commonly occurring but unexplained deaths of *Eucalyptus* trees, in plantations.

Studies by researchers in our group have begun to show that there are various pathogenic *Ceratocystis* spp. that have yet to be identified. An important question that has been asked in the past is whether *C. albobundus* might be present in Australia where *A. mearnsii* is native. As part of a sabbatical study, we were able to show that the fungus is probably not in that country, supporting our contention that it is native to South Africa. However, the study included *Eucalyptus* spp. and led to the discovery of a new and apparently pathogenic species of *Ceratocystis*. This fungus will be known as *Ceratocystis pirilliformis*. There is some evidence, although very preliminarily, to show that the fungus occurs in South Africa and that it is an aggressive pathogen. Studies are currently under way to test this hypothesis through robust experimentation.



Ostiolar hyphae at the apex of *C. pirilliformis*
prov. nom ascoma

Pitch canker

The pitch canker fungus *F. circinatum* has, in recent years, become one of the most serious impediments to pine propagation in South Africa. The pathogen was first observed in a single nursery in Mpumalanga in the early 1990s. Since that time, it has spread to nurseries in all parts of the country. In these nurseries, it has resulted in very substantial losses and continues to do so. *Fusarium circinatum* has now also been found associated with failed or poor pine establishment and this situation appears to be deteriorating. The fungus has also been found associated with the bark beetle *Hylastes angustatus*. Although the disease known as pitch canker, which is typified by resinous cankers on the stems of established trees, has not appeared in South Africa, it is widely believed that "full-blown" pitch canker will ultimately appear in this country. Strategies to ensure that pine propagation is not destroyed are thus being considered and management tools developed.

The research team has undertaken intensive studies on the pitch canker fungus in South Africa, and is internationally recognised as being a leader in this field. One of the recent questions that has been posed and that concerns the impact of the fungus on pines in South Africa, relates to the relationship between the pitch canker fungus and the very similar fungus that causes a disease known as mango malformation. We have thus obtained isolates of the mango malformation fungus and compared these with those from pines in South Africa. This

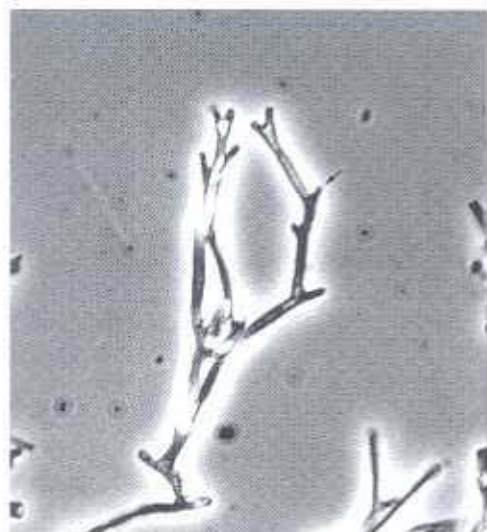
study has led to the discovery that there are two species of *Fusarium* causing mango malformation and that one is a species new to science. It is also now clear that there is no relationship between the pitch canker fungus and the causal agents of mango malformation. Consequently, there is also no risk of cross infection between the respective tree crops.

Many important questions pertaining to the pitch canker fungus in South Africa, remain to be answered. In our view, the most important of these are the following:

- Determine the role of the fungus in killing trees in nurseries. Intensive studies of potential associated insects such as fungus gnats will have to be included.
- Determine the importance of the pitch canker fungus in establishment failures and the role of *Hylastes angustatus* in this manifestation of the pathogen.
- Continue to evaluate the likelihood that "full-blown" pitch canker will appear in South Africa. These studies include some mentioned above pertaining to the pine weevils.
- Evaluate the changes in the population diversity of *F. circinatum* in South Africa and apply these data to impact assessment studies.
- Consider the relative susceptibility of South African pine planting stock and especially hybrids, to infection by the pitch canker fungus. These studies using artificial inoculation have already been established and will continue during the course of the next three to five years.

In order to undertake many of the above-mentioned investigations, the research team requires robust and rapid methods to identify isolates of the pathogen. Various studies have been completed linked to the identification of the pitch canker fungus and the question of differences between it and other related fungi. We are now using the data from these studies to produce a Real Time PCR technique for rapid identifications. This DNA-based technique will substantially increase the ease with which we work with the pitch canker fungus. Another tool that will be crucial in

our future studies of the pitch canker fungus, is one that will enable us to effectively identify individual genotypes of *F. circinatum*. To this end we have developed a robust set of microsatellite markers for *F. circinatum*. These will now be applied to evaluate and better understand the changes in the population structure of the pathogen in South Africa.



Polyphialides of *Fusarium circinatum*, the pitch canker fungus

Sirex wood wasp

One of the most important pests currently affecting pine plantations in South Africa is the wood wasp, *Sirex noctilio*. This pest was discovered for the first time, established in plantations of the western Cape, little more than a decade ago. Various biological control strategies were implemented with the support of SAFCOL who had primary interests in those plantations. Predictions were that the wasp would spread northwards at a rate of about 50km per annum. In 2001, the TPCP assumed responsibility for insect pest monitoring and the wasp was found near Knysna, considerably further north than was expected. During 2002, surveys showed for the first time that the wasp was established in the Umtata area. It has subsequently been found in Weza, close to Durban and in plantations in Stutterheim. Currently, we are not certain of the exact distribution of the pest. Surveys in 2003 will attempt to determine the northern most limit of *Sirex* in South Africa. For the present, we expect that it is considerably more widespread than anticipated and a

concerted effort will clearly be needed to reduce potential losses.



Sirex exit holes on a pine stem

Other than surveys, an important task in 2002 has been to undertake field extension visits to plantations where *Sirex* is present, and to inform foresters of its biology and presence. In addition, a *Sirex* Action Plan has been formulated and relevant committees have been established to deal with the pest. During 2003, the following actions are planned:

- Appointment of an entomologist to assume responsibility for the *Sirex* Action Campaign and assist foresters with surveys and implementation of biological control.
- Negotiations with the CSIRO in Australia for permission to utilise the biological control nematode, *Deladenus siricidicola*, throughout South African forestry areas. This nematode was previously introduced into South Africa but use was restricted to south of 32 degrees latitude. In addition, to negotiating for the rights to utilise the nematode, it will also be necessary to acquire permission to re-introduce this organism.

The coming few years will mark a time of considerable effort to evaluate the threat of the *Sirex* wood wasp in South Africa and to begin to reduce its impact.

Botryosphaeria canker

Botryosphaeria canker on *Eucalyptus* spp. is a relatively newly recognised disease in South African forestry. The disease is somewhat similar to *Diplodia pinea* shoot blight and die-back, in that both diseases occur on trees subjected to stress due to factors such as hail storms, drought and

many others. During the course of the last decade, we have learnt a great deal about *Botryosphaeria* canker on *Eucalyptus* and much progress has been made in testing and selecting clones resistant to infection. There are, however, many questions that remain unanswered. Perhaps most complicated of these is linked to the fact that we now know that there are at least two species of *Botryosphaeria* infecting *Eucalyptus* in South Africa. This substantially changes our understanding of the pathogen and will demand re-evaluation of susceptibility of clones in the future.

Botryosphaeria is a complex genus and this significantly complicates research on diseases caused by species in this genus. During the last few years, we have thus expanded substantial effort to establish a taxonomic and phylogenetic foundation that will allow us to identify isolates accurately. This will enable us to use these fungi appropriately in our pathogenicity and disease sensitivity trials. Although the work is not entirely complete, much progress has been made and these studies should be completed in the next two years. Ecological studies and pathogenicity tests are continuing concurrently with our phylogenetic studies.

Diplodia shoot blight and die-back

Shoot blight and die-back caused by the fungus known as either *Sphaeropsis sapinea* or *Diplodia pinea* is one of the best known and most important diseases of pine in South Africa. It was the first plantation tree disease to be described in this country and much has been written regarding its biology and control. Due to its importance, the research team has maintained a strong focus on this pathogen, and will continue to do so in the future. Previous reports have provided summaries of research accomplished and this report is intended to provide a brief update on results of recent investigations.

Amongst the intriguing concerns relating to *S. sapinea* is the fact that the fungus has been reported to exist in different forms that, in mycological terms, are known as morphotypes. We have reported extensively on this topic in the past and indeed described a new morphotype for the fungus.

Four morphotypes have thus been described for *S. sapinea* and these include the A, B, C and I types. Previous studies by members of our research group have shown that it is the A type that occurs in South Africa and that some of the others might present a threat to pine growing in this country.



Symptom development following artificial inoculation with *Diplodia sapinea*

Our recent research has led to the development of microsatellite markers for the A and C morphotypes of *S. sapinea*. This has provided the group with a powerful tool to track *S. sapinea* infections between plantations and between countries. In addition, we have been able to show that the A morphotype is the only one to occur in South Africa, Australia and New Zealand. Furthermore, the I morphotype appears not to be related to *S. sapinea* and is the same as *Botryosphaeria obtusa*. Further studies are now under way to consider the identity of the B morphotype and to determine whether it poses a threat to South African forestry.

Coniothyrium canker

Coniothyrium canker is now considered to be one of the most important diseases of *Eucalyptus*. The disease caused by *Coniothyrium zuluense* was first found in Zululand in 1990 and has spread rapidly throughout the country. It has now also been found in various parts of South America and South East Asia where it is causing serious damage to trees.

In South Africa, we have made considerable progress in reducing the impact of *C. zuluense*. This has mainly been through the selection of disease tolerant clones and

clonal hybrids. However, the disease still occurs and in some plantations is very damaging. There is also evidence that the fungus is adapting to infect new host genotypes. The implication is that this disease will continue to impart serious damage in the future and that research must continue to broaden our understanding of the pathogen and means to reduce its impact.

Apparent adaptation to previously selected resistant *Eucalyptus* genotypes has prompted the research team to consider variation in the population of *C. zuluense*. This has been done based on morphology as well as using the DNA-based AFLP technique. Results have shown a great deal of diversity in isolates of *C. zuluense* which is somewhat unusual as we have hypothesised that this is a fungus that was introduced into South Africa. But the results also help to explain great variation in the pathogenicity of isolates of *C. zuluense*. This work is clearly far from complete and further studies on the population diversity of the pathogen and its apparent synergistic relationship with two bacteria species, one of which is *Pantoea ananatis*, requires further study.

Pine weevils

The pine weevil, also known as the Deodar weevil *Pissodes nemorensis* is a well-known pest in South Africa. Damage by this pest has mainly been on *P. radiata* in the Southern Cape, although it occurs widespread in the country. Recent evidence has emerged to suggest that the pest is increasing in importance. In addition, there is considerable concern that *P. nemorensis* might emerge as an important vector or associate of the pitch canker fungus. The latter concern is based on the fact that *P. nemorensis* is associated with pitch canker in the south-eastern United States. TPCP team members have thus established a project to study *P. nemorensis*, and this study will include a specific focus on the potential for

the insect to act as a vector of *Fusarium circinatum*.

There are a great number of weevils (Curculionidae) that are serious pests of conifers. Amongst these is *Pissodes validirostris*, which is also known as the pine cone weevil. This insect is native to Europe and also has the potential to cause substantial damage to pines. Of particular concern to South African forestry is the fact that this insect is being considered as a biological control agent for so-called "weed pines". The insect has now been introduced into South Africa, for experiments in quarantine, in Stellenbosch. Of great concern to the TPCP and to the forestry industry is that this insect could also become an important associate of the pitch canker fungus. This situation represents a great risk to forestry in South Africa and therefore requires very careful attention.

Eucalyptus rust

Eucalyptus rust caused by *Puccinia psidii* is one of the most serious diseases of *Eucalyptus*. The pathogen is restricted to South and Central America where it occurs on a wide range of species of Myrtaceae. Eucalyptus rust seriously threatens plantation eucalypts outside its current area of occurrence and it is also seen as one of the greatest threats to native Myrtaceae in Australia. For this reason, the research team is participating in an international project funded by the Australian government (ACIAR) to evaluate the risks to Myrtaceae, posed by rust. This project has been active for three years and will be concluded during the coming year. Much progress has been made and results present a substantial contribution to our understanding of the threats linked to *P. psidii*.

For more information on the activities of the Tree Protection Co-operative Programme (TPCP), please visit our web site at: <http://www.up.ac.za/academic/fabi/tpcp>

Forest biotechnology: Propagation of pine species

Research leader: Prof Anna-Maria Oberholster (née Botha)

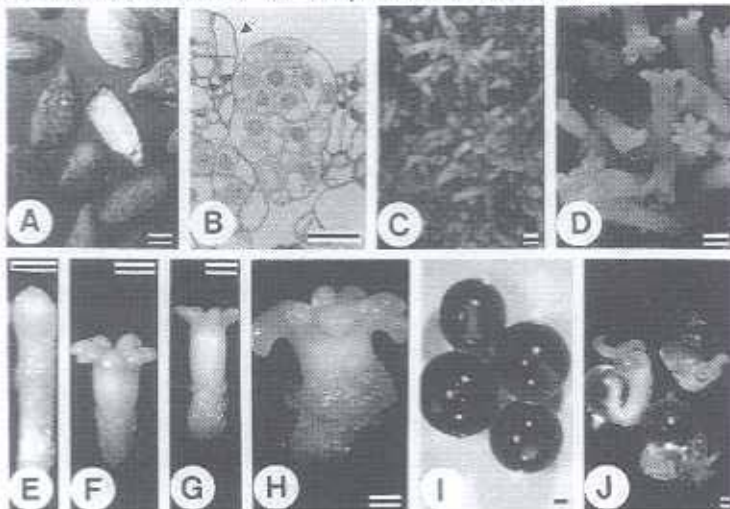
Objectives of the research programme:

- 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 understanding of the development of somatic embryos through a comparative study of somatic and zygotic embryos. This 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 2002/2003:

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. carabaea*. The programme that runs in collaboration with Professor Chris H Bornman (Denmark), 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 commercialisation. In a recent study, we compared aspects of the physiology/biochemistry (eg germination percentage, water, protein, lipid and carbohydrate content) of zygotic seed and its somatic counterpart. Conclusions drawn from this study include the fact that coniferous somatic embryos lack a suitable artificial megagametophyte, and that the somatic embryo, if it behaves like the isolated zygotic embryo cultured *in vitro*, does so with greatly reduced physiological vigour.



Different aspects of zygotic and somatic embryogenesis development . (A) Seed with embryo embedded in the megagametophyte, (B) Globular stage embryo surrounded by embryogenic tissue and callus (arrow); (C, D) Somatic embryos in torpedo stage, cotyledons visible; (E) Isolated zygotic embryo; (F-H) Mature (F), mature and partially desiccated (G) and teratogenic (H) somatic embryos; (I, J) Somatic embryos embedded in alginate (Bornman *et al.*, SA J of Bot. 2003).

Characterization of tree pathogenic and related fungi at the molecular level using DNA-based methodologies

Research leader: Prof Brenda Wingfield

Research team: Prof Mike Wingfield
Prof Teresa Coutinho
Prof Pedro Crous
Prof Wally Marasas
Dr Jolanda Roux
Dr Oliver Preisig

Objectives of the research programme:

- Develop molecular phylogenies for tree pathogenic and related fungi
- Develop DNA-based identification tools for fungi
- Investigate the population genetics of economically important fungal species using DNA-based polymorphic markers
- Characterise the viruses that occur in tree pathogens with a view to using these viruses as biological control agents.

Highlights of research 2002/2003:

The aim of this programme is to investigate the molecular phylogenetic relationships between economically important fungal pathogens. These relationships are studied both at the population as well as at the species level. Accurate molecular phylogenies and population genetics of fungal pathogens form the basis of disease management programmes. They are also the essence of biosecurity issues and are fundamental to South Africa's success in maintaining free trade and protection of our environment.

The last decade has seen huge opportunities emerge linked to advances in the use of molecular tools that enable a better understanding of phylogenetic relationships and population biology of micro-organisms. This research group has been at the forefront of producing some of the first molecular phylogenies for plant pathogens. These have proved invaluable in establishing effective and relevant pathogen management programmes, in South Africa as well as internationally.

This work has highlighted the tremendous need for such studies for all plant pathogens. In the last two years, the first phylogeny for southern hemisphere *Armillaria* species was developed. This has resulted in better understanding of this genus worldwide and results have led us to suggest that this genus probably has a Gondwanan origin and that it originated in Africa.

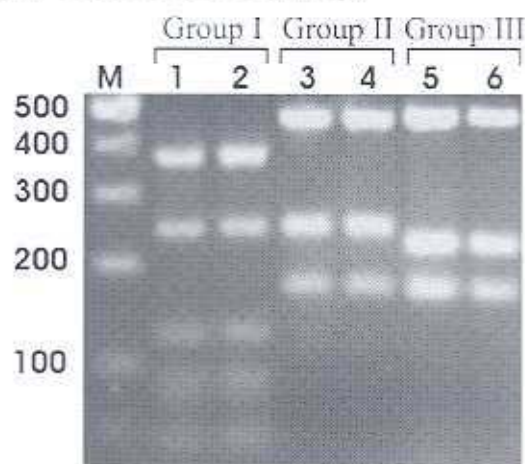
Our studies on the pitch canker pathogen, *Fusarium circinatum*, and related species have resulted in the discovery of a number of previously undescribed species related to this globally important pine pathogen. In addition, we have reported on the delineation of a cryptic species related to the maize pathogen *Fusarium subglutinans*. This cryptic species is particularly interesting as it apparently occupies the same host and geographic region as *F. subglutinans*. This suggests that it occupies that same niche but is no longer recombining with *F. subglutinans* in nature, although the two fungi are sexually compatible in the laboratory.

Cryphonectria cubensis is a tree pathogen well-known in many *Eucalyptus* planting areas of the world. When it was first discovered in South Africa in the late 1980s, it was assumed that the fungus had been introduced into the country. Molecular phylogenetic studies of this fungus have shown otherwise. Furthermore, gene genealogies produced for *C. cubensis* isolates collected globally, suggest that this is not a single species but a species complex. The groups within this complex are species in their own right and appear to have originated in different geographic regions of the world. What is puzzling is that these fungi are so closely related yet are apparently native to the areas where they are found. Phylogenetic studies suggest a very recent divergence of these species, but that they have their apparent origins many thousands of miles apart on different continents. The DNA sequence data suggests that these fungi diverged a long time after continental drift but before humans started migrating around the world.

Molecular phylogenies are only the starting point for fungal identification. Many fungi are capable of asexual reproduction and, therefore, may be spread clonally. Understanding the genetic diversity of a fungal population is an essential component of evaluating its potential to change and adapt in the field. It is only recently that robust molecular techniques have become available to easily evaluate genetics and diversity of fungal populations. In the last two years, we have developed molecular markers for more than 10 fungal genera. In the last two years, these have included markers for *Fusarium circinatum*, *Ophiostoma ips*, *Ceratocystis laricola* and *Botryosphaeria* spp. These markers have also been found to be useful for population studies on closely related species.

The polymorphic markers that we developed for *Sphaeropsis sapinea*/*Diplodia pinea* have allowed us to undertake an extensive study of the population diversity of this species from many countries of the world. This has allowed us to better understand the

disease that this pathogen causes in pine plantations. I have also made it possible for us to examine the value of quarantine strategies associated with the movement of pine seed to and between South Africa, New Zealand and Australia.



A 3% agarose gel showing *AluI* restriction fragments for *Armillaria* isolates from Zimbabwe. Groups I, II and III probably represent new species. Lane labelled with M indicates a 100 bp molecular marker with band sizes in base pairs

Our studies on fungal viruses continue to lead to the discovery of novel viruses. We have recently discovered our first mitochondrial viruses in *Cryphonectria cubensis*. There are probably a great number of viruses waiting to be discovered in the fungal pathogens that we study. Many of them are unrelated although they infect similar hosts. What is interesting is that a small number seem to be present in more than one species. This suggests co-evolution of these viruses with their hosts.

The molecular tools that we employ to study fungi are becoming increasingly sophisticated. A number of fungal genomes have now been sequenced. This has provided a framework for a better understanding of the relationships between different orders not merely at the level of genes but now also at the genomic level. Establishing the degree of synteny between species of closely related fungi will allow us better comprehension of species relationships, as well as enabling a better understanding of species evolution and pathogen differentiation.

Genes for superior fibre

Research Leader: Prof Brenda Wingfield

Research Team: Prof Mike Wingfield
Dr Jolanda Roux

Objectives of the research programme:

- Support of goal-directed research and promote a source of expertise for molecular biology of Forest tree species. Discovery of new genes, which are important in resistance of trees to fungal pathogens
- Provide genetic fingerprinting tools to the forestry companies. Produce microsatellite markers for the forestry industry and investigate the potential of DNA micro-arrays for this purpose.
- Provide marker-aided selection/molecular screening and develop molecular markers to screen specific pedigrees for DNA-based markers linked to disease resistance
- Facilitate accelerated breeding techniques through the production of molecular tools. Through close working relationships with tree breeders, develop the DNA tools for commercial use in tree breeding
- Promote education and capacity building in the field of plant molecular genetics. Provide forestry companies with a ready source of well-trained postgraduate students and assist them in maintaining a high level of competency in this field.

Highlights of research 2002/2003:

This research programme has already provided the forestry industry with access to the latest molecular based technology. A number of the forestry companies are already using these techniques in their own research and development programmes. In the area of molecular biology the technology is changing very rapidly. It is, therefore, essential that this programme be involved in continuous technology development and implementation initiatives.

A number of microsatellite markers have been developed in this programme for *Eucalyptus grandis*. These markers, as well as others developed elsewhere, have been used successfully to fingerprint *Eucalyptus* trees in the forestry industry during 2001 and 2002. The technique that was developed to obtain the *Eucalyptus* microsatellite markers has also been adapted successfully for use on a number of fungal pathogens including *Cryphonectria cubensis*. Understanding the fungal population diversity is an essential component of developing disease resistant *Eucalyptus* clones. Using this technology, we are now confident that the fungus in South Africa is a species different to the one which is found in other parts of the world. This has significant implications when selecting for disease resistant trees for commercial plantations.

The first DNA array using *Eucalyptus* DNA was produced in 2001. This DNA chip has been tested to determine its usefulness in fingerprinting and identification of *Eucalyptus* clones in South Africa. Our preliminary results suggest that the DNA chip that we have produced will be useful for fingerprinting *E. grandis* trees.

Cereal genomics

Research leader: Prof Anna-Maria Oberholster (née Botha)

Objectives of the research programme:

In this programme we study the super family of resistance (R) and defence related (DR) gene sequences applicable to insect resistance in wheat. To achieve this we address the following issues:

- Isolation and characterization of Resistance Gene Analogs (RGAs) from cDNA in bread wheat utilising modern technologies and bioinformatic tools (eg PCR using degenerate primers based on conserved motifs obtained from the *Arabidopsis* and rice genome projects; 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 of differential expression of Expressed Sequence Tags (ESTs) and R/DR genes upon RWA infestation using DNA microarray technology. 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 wheat $F_{2/3}$ populations

Highlights of research 2002/2003:

In a recent study on feeding behaviour of *Diuraphis noxia* (Russian wheat aphid, RWA) on wheat (*Triticum aestivum* L.), we confirmed that the RWA probed more and feed less on resistant cultivars, resulting in the formation of more lesions in the resistant cultivars. We investigated the possible reasons for this feeding behaviour by studying the leaf morphology and ultrastructure, and transcriptome and proteome expression in RWA resistant and susceptible cultivars.

In our study on the morphology and ultrastructure of wheat leaves, we compared the leaf epicuticular wax ultrastructure and leaf trichomes of three bread wheat cultivars (two susceptible and one RWA resistant cultivar). There were no significant differences in the lengths of the trichomes in the three wheat cultivars examined. However, the resistant cultivar ('Tugela DN') had a significantly greater trichome density than the susceptible cultivars. Examination of the position of the trichomes revealed that there were differences for the adaxial and abaxial surfaces. Trichomes on all three wheat

cultivars were found to occur mostly on the leaf veins of the adaxial surfaces, and on the leaf veins as well as between them on the abaxial surfaces. Leaf trichome density and position may act as a physical obstacle to Russian wheat aphid feeding as the aphid feeds on leaf veins of the adaxial leaf surfaces. The high trichome density on the leaf veins found in the resistant 'Tugela DN' cultivar could prevent the Russian wheat aphid from finding a suitable feeding site. Comparison of the scanning electron micrographs showed that the epicuticular wax structure was found to be similar for both the adaxial and abaxial surfaces amongst the three wheat cultivars. The wax structure was similar in the Russian wheat aphid resistant and susceptible cultivars and does not seem to affect Russian wheat aphid feeding.

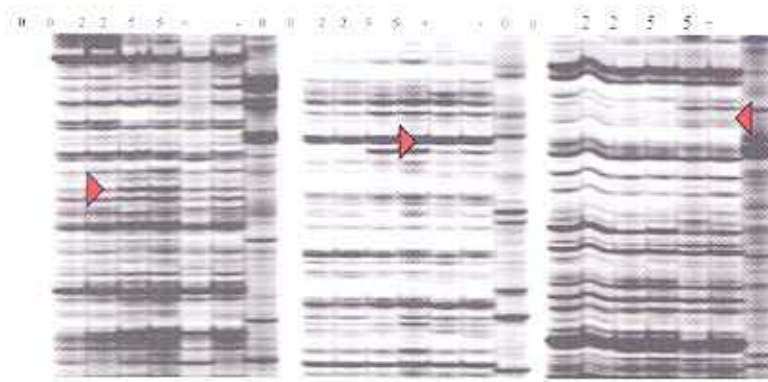
In studies conducted on the intercellular washing fluid of wheat resistant to the Russian wheat aphid ('Tugela DN'), we found that proteins were induced within six days of infestation. These induced proteins were visible as five bands using

SDS-PAGE analysis. Two proteins disappeared from the protein profile of 'Tugela DN' when the Russian wheat aphid was allowed to feed. Analysis of these proteins using two-dimensional gel electrophoresis showed that the five induced protein bands corresponded to seven proteins. Subsequently, the > 20 kDa band observed with SDS-PAGE analysis, was shown to represent three proteins using two-dimensional gel electrophoresis with pI values of 5.0, 5.2 and 5.8. Unfortunately, the induced proteins could not be sequenced because of their low concentrations in relation to the other proteins. Overexpression and down regulation of proteins were also visible after Russian wheat aphid infestation. Two of the induced proteins (> 36 and 26 kDa) are possibly β -1,3-glucanases and chitinases, as both the pI and molecular mass corresponds with results obtained previously by the group. What was also evident from these results was that both HR and SAR are involved in the host resistance response against the RWA. In a study on the localization of the PR proteins, a intercellular β -1,3-glucanase was purified and polyclonal antibodies were raised against it. This was then used to localize the β -1,3-glucanase *in planta* with the immunogold labelling technique. The antibodies recognized both the inter- and intracellular isoforms of β -1,3-glucanase. In contrast to dense gold labelling in infested resistant plants, very little labelling was found in infested susceptible plants. Labelling was observed in the cell walls of different cells, but was denser in the vascular bundle cells. Gold labelling was also detected in the chloroplasts and thus, it seems that gold accumulated in tissues that were affected most by the feeding aphids.

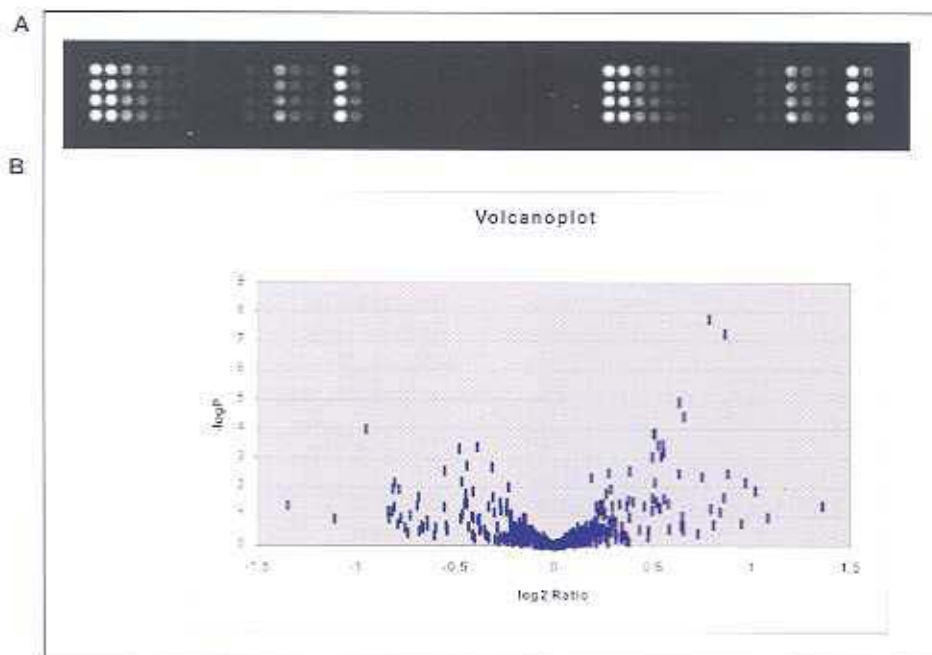
Recently, cloning of multiple R genes from various plant species has revealed conserved domains at the amino acid level. The most notable being the

presence of nucleotide binding sites (NBS) and leucine repeat regions (LRR). The presence of a NBS and a LRR is consistent with the protein products playing a significant role in signal transduction and having a putative role in protein-protein interactions. Recently, much of the research effort in this research group has been focused on gene sequences containing NBS-LRR motifs. In studies using RWA infested material containing the RWA resistance genes *Dn1*, *Dn2* and *Dn5*, and utilizing degenerate primers sets designed from the consensus NBS motif from other genome studies (eg *Arabidopsis* and rice), subtraction suppression hybridization (SSH), RACE-PCR, and cDNA libraries, several NBS-LRR sequences related to the RWA were obtained. Linkage to the RWA resistance response was verified using cDNA microarray expression profiling, Real-time PCR, Northern and Southern Blot analysis. In a comparative functional analysis, we tested the feasibility of using a degenerate PCR-based approach to obtain sequences of interest. It was found that 18% of all the obtained ESTs showed significant homology to resistance genes from other plants on amino acid level (E value < 10⁻⁵) rendering the approach highly feasible. Results obtained in our microarray analysis indicate that wheat homologs to *RGA-2* and *WIR* pathogen R genes were also induced in the RWA resistance response.

Besides understanding the underlying genetic mechanisms involved in host resistance, the rationale for the programme is also driven by the need for a high through-put marker system (DNA microchip and SSR/AFLP-based), which can be utilized by breeders to select for superior disease resistant material. We are currently testing all the cDNA clones that we identified from our microarray profiling analysis for linkage to RWA resistance using segregating F_{2/3} populations.



A small portion of the first cDNA-AFLP gel recently produced at UP on a LI-COR DNA fragment analyser. Lanes 1-2, 3-4, and 5-6 of each panel are cDNA-AFLP profiles of a wheat line infested with Russian wheat aphids after 0,2 and five days respectively. Lane 7 of each panel contains a tomato positive control cDNA-AFLP profile. Each panel was produced with a different selective primer combination. Arrows indicate differentially expressed cDNA fragments



Results obtained after cDNA clones were spotted on a slide, and then hybridised with Cy3-and Cy5-labelled cDNA. The labelled cDNA was synthesized from mRNA isolated from leaf tissue 2 days post Russian wheat aphid infestation (a). Volcano plot obtained after analysis of the resulting data, which illustrate the significance of data points (b)

Diseases and pests of bananas

Research leader: Dr Altus Viljoen

Research team: Prof Teresa Coutinho
Prof Mike Wingfield
Prof Karl Kunert
Prof Dave Berger
Prof Brenda Wingfield
Dr Prem Govender
Mr Johan van der Waals

Objective 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 pests and diseases of banana.
- To isolate, identify and manipulate genes in banana with resistance to pests and diseases

Highlights of the research 2002/2003:

The South African banana industry is one of the largest agricultural industries in the country, with an annual turnover of approximately R600 million. The industry not only provides employment for thousands of South Africans, but also provides millions of South Africans with a cheap and very nutritious food source. Although the banana industry is well-developed in South Africa, using high yielding cultivars under optimised horticultural conditions, the local industry has been devastated by the soil-borne disease known as Fusarium wilt (caused by *Fusarium oxysporum* f.sp. *cubense* [Foc]), also known as Panama disease. This disease has already caused losses of up to 40% of fields in two of the five banana growing areas of South Africa. It is now again threatening to halt banana production on additional farms in these areas.

Research on Fusarium wilt of banana has been conducted for more than 100 years in almost every banana growing country where the disease occurs. The only effective and semi-permanent solution has been the replacement of Gros Michel, a banana cultivar susceptible to Foc race 1 in the tropics, with Cavendish type cultivars.

However, Cavendish cultivars are highly susceptible to race 4 of Foc in the subtropics, and now also in some tropical countries where a new variant of the pathogen has been discovered. There is no control measure to prevent bananas from being killed by Fusarium wilt once Foc has been introduced into fields. The only possible control measure is to find or develop plants with resistance to the pathogen, or to develop a novel control strategy to destroy the pathogen. In our research program on Fusarium wilt of banana, we address both of these strategies. In addition to research on Fusarium wilt, studies are also undertaken on banana leaf diseases and the banana weevil borer.

Studies on the epidemiology and management of Foc subtropical race 4

In surveys undertaken in Kiepersol and KwaZulu/Natal, it was found that the introduction of Fusarium wilt into new fields is often associated with infected but symptomless planting material, or machinery and other field equipment that have previously been used in affected fields. Fusarium wilt has recently been discovered in two banana-growing areas that were previously free of the disease. In

both cases, strict quarantine measures assured the isolation of the disease.



Cleaning of field machinery with a disinfectant

Foc has not been found on alternative hosts such as *Strelitzia* spp. *Fusarium oxysporum* has been isolated from irrigation water, and whether these isolates belong to *Foc* is now being determined. Several management strategies have been implemented on new *Fusarium* wilt-affected fields. Where the disease is already well-established, little can be done other than eventually replacing Cavendish banana plants with other crops. Temperature plays a major role in the development of *Fusarium* wilt on banana, as has been shown in both field and greenhouse experiments. Several fungicides and sterilants have been tested for effectiveness against *Foc* *in vitro*. As some of these products are more effective than those used previously, environmentally acceptable and non-corrosive, they have been recommended to farmers for use in future.

Enhancing *Fusarium* wilt tolerance by means of biological control, plant nutrition and SAR treatment

In order to understand the factors that might contribute to enhanced plant tolerance to *Fusarium* wilt in Cavendish bananas, disease suppressive and conducive soils were compared. Several species of *Trichoderma*, *Fusarium oxysporum* and bacteria were collected for biological control purposes. The effect of nitrogen and pH on Panama disease development was determined in greenhouse studies. Six different chemicals that induce systemically acquired

resistance in plants were evaluated in the greenhouse, and four in the field.

Selection and testing of bananas with resistance to *Foc*

Cavendish plants can be improved for tolerance or resistance in two ways: random mutations and transformation. Random mutation in micropropagated plants is known as somaclonal variation that occurs naturally or is induced in the laboratory. Field selection is probably the fastest and least expensive means to find somaclonal variants with both tolerance/resistance to Panama disease, and acceptable agronomic qualities. Field trials to evaluate Cavendish selections and FHIA hybrids and selections for Panama disease resistance are planted in several locations in South Africa. Preliminary data have shown good resistance in all the FHIA hybrids and selections, and good tolerance in DRS1 (a selection of DuRoi laboratories). Further work is being done to understand and to increase disease tolerance in this Cavendish selection.



Evaluation of banana varieties for resistance to *Fusarium* wilt

Isolation and identification of resistance genes in Cavendish bananas

In South Africa, only Cavendish banana cultivars are planted, and they are all susceptible to *Foc*. However, tolerance available in DRS1 offers the opportunity to understand the nature of resistance in Cavendish bananas. This knowledge can be used to screen Cavendish selections or somaclonal variants for disease tolerance/resistance, and can eventually be introduced into susceptible plants. In order to understand why tolerance/resistance is expressed in DRS1, Suppression

Subtractive Hybridisation (SSH) was used to isolate differentially expressed genes. mRNA was extracted from Williams (susceptible) and DRS1 (tolerant) plants, and cDNA developed from RNA templates. The cDNA from the tolerant and susceptible plants has been used in the identification of resistance genes that are expressed after infection with *Foc*. Southern blots were performed to validate the efficiency of each subtraction. Subtracted genes were cloned into a vector and two different libraries (each containing 600 clones) were generated for each of the subtractions. Fragments of interest were sequenced and approximately 20 fragments have thus far shown high homology to defence related genes that are available on international databases. Some of the more interesting genes are currently being subjected to additional experimentation to show that they are up-regulated.

Genotypic and phenotypic analysis of *Foc*

The population structure of *Foc* has been extensively investigated in South Africa. Vegetative compatibility group (VCG) analysis on 160 South African isolates revealed that only VCG 0120, representing subtropical race 4 of *Foc*, occurs in this country. A mating type study showed that *Foc* in South Africa contains only *mat*-2 genes, making it impossible for the fungus to reproduce sexually and diversify in a manner other than by natural mutation. Crosses between different isolates were attempted on Carrot agar, but only pseudothecial structures without ascospores (sexual spores) were produced.

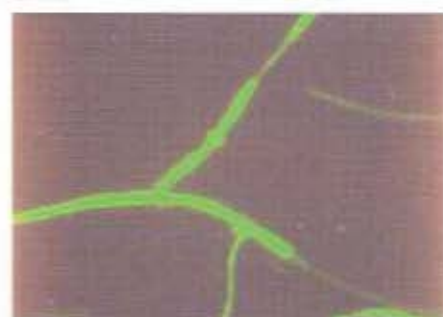
To further determine the phylogenetic relationships of South African isolates of *Foc*, the β -tubulin gene, α -elongation factor, and the mitochondrial region 5 and 7 were compared to isolates of *Foc* from around the world. *Foc* is known to include five independent clonal lineages based on molecular phylogeny. Clonal lineages 2 and 4 fall within clade 1, and clonal lineages 1, 3, and 5 within clade 2. The β -tubulin sequence could not resolve the phylogeny among isolates of *Foc*, because of poor resolution. The other independent loci, however, placed the South African

population within clonal lineage 2 and 4, thus clade 1.

The phenotypic diversity of the genotypically homogenous population of *Foc* was investigated. A large diversity in colony appearance and virulence was found. This prompted the group to further investigate pathogenicity factors involved in *Foc* virulence in order to design counter defence strategies to the pathogen armoury.

Transformation of *Foc* with the GFP

Foc isolates from South Africa and other parts of the world were genetically modified using a protein marker called the green fluorescent protein (GFP). This is an excellent tool to study host pathogen interactions inside the vascular tissue of a diseased plant, and it is the first time that *Foc* has been transformed with the GFP protein. This could further be applied in the search for resistance responses that are activated upon host-pathogen recognition. Transformed isolates appear bright green under ultra violet (UV) or blue light. Transformed isolates have not lost pathogenicity and no variation in cultural morphology was observed. The foreign protein was thus shown to have been taken up and it is very stable in the fungal genome.



Foc transformed with the green fluorescent protein

Development of molecular markers to rapidly identify *Foc* race 4

Microsatellite markers for *Foc* have been developed. Sequence analyses and genome walking of the fungal DNA was performed and 12 specific primers to *Foc* were identified through the polymerase chain reaction (PCR). From these a total of eight different polymorphic microsatellite markers have been selected that

differentiates among isolates of *Foc* from different geographic regions. These are currently being used to further describe the population genetics of *Foc*. Microsatellite markers specific for *Foc* will be further used for the analysis of the population genetic structure, to determine the reproductive mode, genetic isolation and gene flow between geographically separated populations.

Training in tissue culture and transformation techniques

A banana tissue culture facility has been established for research purposes. Techniques established include micropropagation through meristem tip culture, multiplication, rooting and hardening-off of Ladyfinger and Cavendish bananas. Some of these bananas have already been planted in the field. Additionally, experiments to produce callus from immature male flowers have been initiated. From the callus, cell suspensions that can be used in a banana transformation system will be produced. A temporary immersion system for the increased production and rooting of micropropagated plants was developed along with procedures for the long-term storage of shoot tip cultures.

Leaf diseases of banana

To determine the present status of leaf diseases in South Africa, a survey was conducted in the five banana growing regions of the country. Yellow Sigatoka, caused by *Mycosphaerella musicola* and *Mycosphaerella speckle*, caused by *M. musae*, were the dominant diseases. Cordana leaf spot, caused by *Cordana musae* was also found in all regions, while Cladosporium speckle (caused by *Cladosporium musae*) was reported for the first time in the country, and only occurs in the Levubu area. Various other saprophytic and endophytic fungi were also associated with banana leaves in the country, many of them for the first time. Apart from identifications based on morphology, analysis based on sequences of the ITS region of the ribosomal DNA operon for *Mycosphaerella* spp., and pathogenicity tests were done. In this way, it was confirmed that black Sigatoka (caused by

M. fijiensis) does not occur in the country, and that *Mycosphaerella speckle* is caused by two species, *M. musae* and another closely related to *M. colombiensis*.



Fruiting body of *Pseudocercospora musicola* (teleomorph: *Mycosphaerella musicola*), causal agent of yellow Sigatoka

Studies on the banana weevil borer

The banana weevil borer, *Cosmopolitus sordidus*, is the most important pest of banana in South Africa and in the world. Since it is most active under moist and warm conditions, its behaviour in the subtropics differ from that known in the tropics. Studies to determine its population dynamics and genetic structure in South Africa, is currently being conducted. This information will be used to design an integrated pest management programme that will consist of cultural, chemical and biological control. In addition, studies are undertaken on the identification and manipulation of genes related to resistance in banana to the weevil (see report by Kunert), and the use of fungal endophytes as biological control agents against the weevil.



Damage caused to the banana pseudostem by the banana weevil

Molecular plant-pathogen interactions

Research leader:

Prof Dave Berger

Objectives of the research programme:

- Describe mechanisms whereby plants defend themselves against viral, bacterial and fungal 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 2002/2003:

The ACGT (African Centre for Gene Technologies) Microarray Facility is situated in the laboratory of Professor Dave Berger of the Botany Department, Forestry and Agricultural Biotechnology Institute (FABI) housed in the Agricultural Sciences building of the Faculty of Natural and Agricultural Sciences. This facility represents a joint venture between the CSIR and UP, which came to fruition in 2002 with the launch of a microarray service in mid-2002 for both internal and external users. A website with pricing and sample preparation instructions has been posted at <http://fabinet.up.ac.za/microarray>. To date, internal users from the Department of Genetics, Department of Microbiology and Plant Pathology, Department of Botany, FABI and CSIR-Bio/Chemtek have made use of the service. Laboratory protocols for the preparation of microarrays and the multi-step process required to measure gene expression differences in control and experimental plant samples have been optimized in the laboratory of Prof Berger using the model plant *Arabidopsis thaliana*.



Arabidopsis plant

A collaborative project with Dr Y Marco at CNRS-INRA Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes in Toulouse, France is under way. The topic concerns the interaction of *Arabidopsis* with the bacterial wilt pathogen, *Ralstonia solanacearum*, particularly isolates from *Eucalyptus* trees. Through exchange visits with the French lab, expertise is being built based on this pathosystem. The aim will be to use microarray analysis to understand the interaction. A PCR-RFLP method has been used for rapid identification of *Ralstonia solanacearum*, which will be particularly useful for diagnosis during outbreaks of this disease in *Eucalyptus* plantations.

A laboratory workshop aimed at developing molecular expertise relevant to Microarray technology was held in April 2002. The workshop was entitled "Differential gene expression profiling and bacterial genome subtraction using Suppression Subtractive Hybridisation (SSH)" and was facilitated by visiting scientists from the Scottish Crops Research Institute. The workshop was held in the Microarray Laboratory at the University of Pretoria and was jointly organized with FABI at UP (Prof MJ Wingfield) and the University of Durban-Westville (Prof G Pillay). Fifteen participants from throughout South Africa attended (workshop report posted at <http://fabinet.up.ac.za/ssh>).



Arabidopsis defence gene microarray

The SSH technique has been applied in two different research projects in the lab, resulting in the generation of gene libraries enriched for fungal resistance genes from banana and millet. The libraries are currently being characterized using DNA microarrays.

The improvement of maize by genetic engineering has focused mainly on yellow maize varieties, which supply the animal feed industry. Yellow maize makes up 98% of the 700 million tons produced world wide annually. However, in certain regions of the world such as southern Africa, white maize varieties are produced in larger quantities due to the requirements of the local market. White

maize used for human consumption makes up 65% of the 10 million tonnes harvested annually in South Africa. Since it is time-consuming to introduce transgenes from yellow maize into local white maize varieties by backcross breeding, an alternative approach for maize improvement in developing countries concerns the identification and isolation of agronomically-useful genes and subsequent introduction of these directly into the genome of selected elite white maize lines.

In collaborative research between the University of Pretoria and CSIR-Bio/Chemtek, a method was developed for genetic engineering of local elite white maize in which stable integration of marker genes was shown. This research was carried out in line with the regulations of the Genetically Modified Organisms Act (#15 of 1997). This success led to a research grant being awarded by the European Union in which both conventional and genetic engineering approaches are being used to improve maize for fungal resistance (for more information, see <http://www.up.ac.za/academic/botany/safemaiz.html>). This project, co-ordinated by Prof Berger of the Botany Department, Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, involves co-operation between UP, CSIR-Bio/Chemtek and ARC-Roodeplaat laboratories in Pretoria, and serves as an example for the newly formed African Centre for Gene Technologies (<http://www.acgt.co.za/>). International partners include Plant Research International in The Netherlands, University of Rome and University of Zambia.

Molecular stress physiology

Research leader: Prof Karl Kunert

Research team: Dr Christell van der Vyver
Ms Louw Gaume

Objectives of the research programme:

The goal of this programme is to evaluate labile DNA regions present in plant genomes, which vary in response to environmental stress. Research questions concern (1) the identification and isolation of labile DNA regions in plant genomes, which might change to a stressful event and (2) the evaluation of cysteine proteinase inhibitors as protectants against abiotic and biotic stresses in plants. Research on stress-induced changes has focused on tobacco, date palm and an inland wild oat grass.

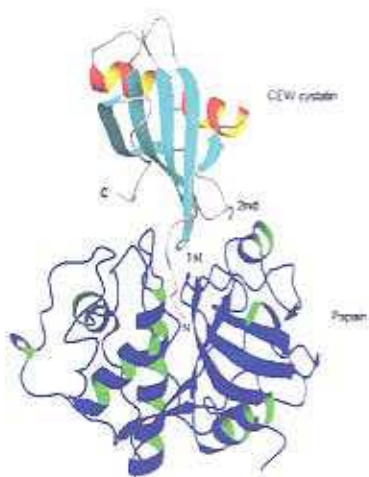
Highlights of research 2002/2003:

Cysteine proteinase inhibitors

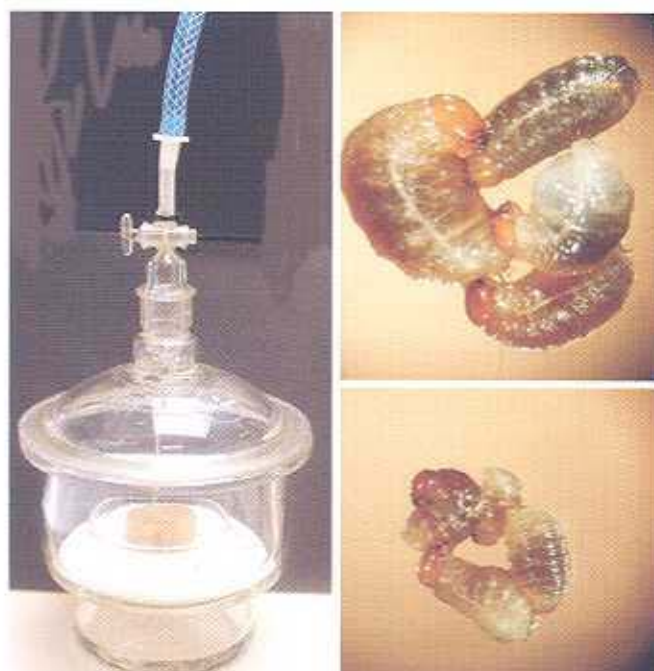
A first research project on cysteine proteinase inhibitors has focused on the evaluation of tobacco plants, expressing an exogenous rice cysteine proteinase inhibitor transgene (OCI) for abiotic stress resistance. Expression of the transgene in genetically modified tobacco, which is stably expressed under severe stress conditions, without any degradation, resulted in improved acclimation to chilling and increased biomass in OCI expressing plants. Inhibition of elongation expressed under low light intensity is currently being investigated in more detail by genome analysis. An interesting phenotype, was further discovered in these plants.

A second research project has focused on the evaluation of a strategy to use cysteine proteinase inhibitors of plant origin as a target gene for transformation of banana to improve resistance/tolerance to the banana weevil stem borer, *Cosmopolites sordidus* (Germar, 1824; Coleoptera: Curculionidae). Through the use of evolutionary guided protein engineering involving protein

sequence/structural analysis and site-directed mutagenesis. The project also seeks to optimise the inhibition activity of plant cysteine proteinase inhibitors to obtain novel inhibitors with increased activity against cysteine proteinases in the gut of banana weevils, and to acquire intellectual property rights. Native plant-derived and mutated inhibitors currently under intensive investigation are being produced in large purified amounts by cloning, expression in *E. coli* and purification by affinity chromatography. Inhibitors are tested with both *in-vitro* and *in-vivo* assay systems. A system for the isolation of proteinase enzyme containing gut extracts of the banana weevil and subsequent analysis of activity/inhibition *in-vitro* has recently been developed. Using this system, cysteine proteinase activity was confirmed in both adult and larval banana weevils as has been reported for many other coleopteran insects. In addition, a purified inhibitor recently infiltrated into banana tissue using vacuum infiltration, showed strong inhibition of banana weevil larval growth and development.



[LEFT] A three-dimensional structural plot of the complex between a typical proteinase enzyme (papain; blue and green) and a typical cystatin (chicken egg white cystatin; CEW) coloured light blue, red and yellow (PDB accession No 1STF). The complex is presented in front view to show the V-shaped active site of papain and how the N-terminal region of CEW covers it thus making it unavailable for protein hydrolysis



[LEFT] Set up of the apparatus used for vacuum infiltration of banana stems (A) with either cystatin solution or distilled water (control). Ten day old banana weevil larvae after developing in banana stem infiltrated with distilled water (B) and with papaya cystatin solution (C)

Stress-induced genomic changes in plants

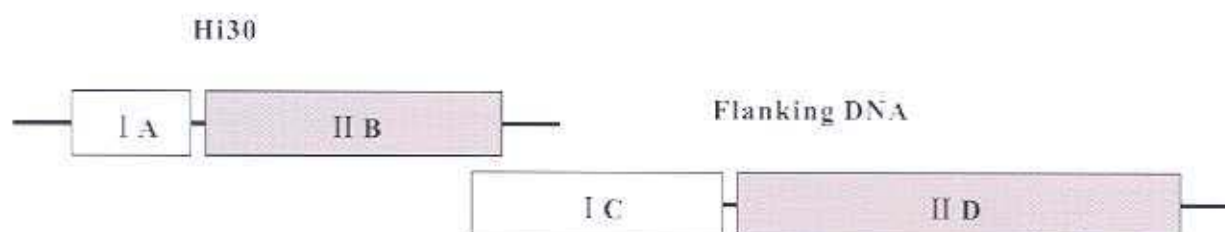
Several labile DNA sequences have been isolated from these plants representing a group of labile DNA sequences in the plant genome that easily change under stressful natural or artificial environmental conditions. Their potential of being applied as molecular markers for stress tolerance is currently being evaluated. Research in this programme represents a joint effort

between the Case Western Reserve University, Ohio, USA and the University of Pretoria. It specifically focuses on plasticity present in plant genomes when exposed to a stressful event. By applying the technique of "Representational difference analysis" or RDA we were able to successfully isolate both highly (date palm, tobacco) and low repetitive DNA (tobacco, wild oats) with DNA point mutations, deletions and insertions. Detailed information about such

susceptible DNA regions has been obtained in the last two years in tobacco derived from a stressful plant tissue. These subtraction products represented possible DNA sequence differences between wild-type non-transformed plants and plants derived from a plant tissue culture/transformation process. Using bioinformatics tools, two methylation-sensitive subtraction products, Hp12 and Hp14, were identified to be similar to part of the tobacco chloroplast genome and the tobacco 18S rRNA gene, respectively. A third non-methylation sensitive DNA subtraction product, Hi30, had no significant homology to any reported DNA sequences.

As a further result, this study allowed for the successful identification of adjacent sequence fragments of these variable DNA sequences in the plant genome. Screening a genomic library derived from a transformed plant confirmed the plastid origin of Hp14 and Hp12, while the Hi30 sequence could be localized in regions with

culture/transformation process. Three different DNA sequences from transformed tobacco were isolated and characterized, homology to known repetitive DNA sequence families. By applying Tail PCR it was further determined that the flanking sequence of Hi30 was partially homologous to sequences of a *Shewanella* 16S RNA gene, DNA sequences of different cloning vectors as well as to *Arabidopsis thaliana* mRNA for ATP synthase. These DNA sequences likely indicate DNA insertions at a labile DNA region into the tobacco genome. Recently, we were also able to identify a similar process of bacterial DNA insertion into a variable region of wild oats. In future research, a variety of both wild-type and plant tissue culture/transformation-derived tobacco plants will be screened in this project by PCR for sequence variation in identified labile DNA regions. The aim is to evaluate the potential of these regions to serve as DNA-based stress markers.



Schematic diagram of subtraction product, Hi30 and its adjacent flanking DNA region. A and B represent a variable (30 bp; A) and conserved (125 bp; B) DNA region present within the Hi30 subtraction product whereas C and D represent a variable (90 bp; C) and conserved (186 bp; D) region present within the flanking sequence. Region D showed, in part, significant homology to a *Shewanella* 16S RNA gene, DNA sequences of different cloning vectors, as well as to *Arabidopsis thaliana* mRNA for ATP synthase

Indigenous food crop pathology

Research leader: Prof Terry Aveling

Research team: Prof Lise Korsten
Dr Nico Labuschagne
Prof Teresa Coutinho

Objectives of the research programme:

- Survey of plant pathogens occurring on indigenous food plants
- Collect, isolate and identify plant pathogens reducing yields of these plants
- Establish a reference collection of plant pathogens on these plants
- Study the biology and seed pathology of these pathogens
- Train and educate students with relevant expertise to undertake extension work in disadvantaged communities
- Establish a centre of excellence of diseases of indigenous food plants important for small-scale farmers

Highlights of research 2002/2003:

Prof Aveling and five postgraduate students visited resource-poor on-farm cowpea and bambara groundnut farmers field trials in Mpumalanga during February 2001 and 2002. Diseased plant samples were collected for further laboratory studies. A web page was established. Information on cowpeas, their importance, cultivation, fungal diseases and diagnosis is available at:

http://www.up.ac.za/academic/microbio/plant/pr_cowpea.html



Students at a field trip to Maputo, Mozambique, to look at cowpea and bambara groundnut diseases

A collaborative project together with the International Institute of Tropical

Agriculture (IITA), Eastern and Southern Africa Regional Center (ESARC), Kampala, Uganda on the viruses of cassava was established. Mr Joseph Ndunguru from Tanzania commenced his PhD conducting research on these viruses on cassava in Tanzania and Uganda. Biological control of *Sclerotium* on bambara groundnut was investigated. A *Trichoderma* formulation provided good control, greatly reducing pre- and post-emergence damping-off. The production of fumonisins in cowpea by *Fusarium proliferatum* was reported for the first time. This is also the first report of this fungus producing fumonisins in legumes. This is an important discovery as these mycotoxins cause adverse health effects in animals and humans.



Different subsistence legume crops

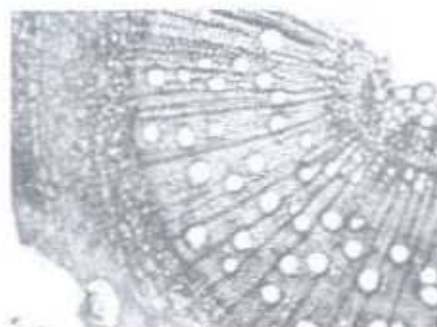
Forest molecular genetics programme: Designer genes for designer fibre

Research leader: Dr Alexander Myburg

Objectives of the research programme:

The Forest Molecular Genetics Programme is a new industry-supported research programme within FABI and the Department of Genetics at UP. The first phase of this programme (2003 – 2005) has four main objectives:

- *To discover and characterise genes involved in wood formation.* The programme will focus on genes and regulatory sequences that affect lignin biosynthesis, cellulose biosynthesis and vascular development in trees. High-throughput molecular genetic techniques such as microarray analysis and AFLP transcript profiling are being developed to identify genes involved in these biological processes.
- *To establish a model system for functional analysis of tree genes.* The model plant *Arabidopsis thaliana* can be induced to form secondary xylem and can therefore be used as a model system for wood and fibre development. The new FABI building will contain excellent plant growth facilities for *Arabidopsis*, and bioinformatics facilities to harness the wealth of genomic information available for this model system.
- *To characterise allelic (nucleotide) diversity in wood and fibre genes in forest tree breeding populations.* Very little is known about allelic diversity in forest trees (and in plants in general). This information will be used to develop gene and allele-based markers for association genetic studies in forest tree populations.
- *To develop molecular tools for biotechnology-assisted tree improvement.* Gene and allele information acquired under the first three objectives will be used to develop tools for marker-assisted breeding or direct genetic engineering of forest trees. The programme will also focus on the development of general molecular breeding tools for applications such as fingerprinting, paternity analysis, pollen competition studies, and estimation of inbreeding/outcrossing rates.



Cross section through a young *Eucalyptus* stem

Highlights of research 2002/2003:

Dr Myburg joined the University of Pretoria at the end of 2001 after completing a PhD degree programme in Forestry and Genetics at North Carolina State University. He established the Forest Molecular Genetics Laboratory in the Department of Genetics and FABI in 2002. During this year, much needed capacity

was established for high-throughput molecular genetic analysis of forest trees. Two automated DNA fragment analysis systems were purchased from LI-COR Biosciences (Lincoln, Nebraska). These two instruments, purchased with partial funding from Sappi Forests, allow a capacity of more than 100 000 molecular

marker determinations per week (enough to completely map most plant genomes in little more than a month). The LI-COR instruments will serve as a technology platform for much of the research performed in the Forest Molecular Genetics programme and other Plant Biotechnology programmes in FABI.

Four BSc Hons student projects were completed in the Forest Molecular Genetics programme in 2002. One of these projects focused on the development of a DNA microarray chip for marker analysis of interspecific hybrids of *Eucalyptus*. This powerful new technology holds the promise of genotyping thousands of markers in a single assay for genomic mapping or fingerprinting of tree genomes. A prototype chip of 384 DNA fragments was produced in this project and will be tested in a follow-up study in 2003.



LI-COR AFLP gel image

Microsatellite or simple sequence repeat (SSR) sequences provide highly informative molecular markers for genetic mapping and fingerprinting of plant genomes. A genome-wide set of microsatellite markers for *Eucalyptus* was recently developed by researchers in Brazil. The Forest Molecular Genetics programme is in the process of

transferring these markers (kindly provided by Dr Dario Grattapaglia) to UP. Two BSc Hons student projects applied a set of these markers for comparative genomic mapping and SSR analysis of pollen competition in polymix crosses of *Eucalyptus* in 2002.



Dr Myburg collecting xylem from a *Eucalyptus* tree

The complete genome sequences of *Arabidopsis* and rice, and the soon to be completed sequence of the poplar tree genome, offers a tremendous resource for gene discovery and comparative analysis in other plant species. These resources can be accessed through on-line bioinformatics tools and local analysis packages. The University of Pretoria will host a major node of the National Bioinformatics Network. This facility in the new FABI building will offer valuable support for comparative analysis of wood and fibre genes in *Eucalyptus* and *Arabidopsis*. To position the Forest Molecular Genetics programme for comparative research in *Arabidopsis* and *Eucalyptus*, a local database of wood and fibre development genes in these two species was established in 2002 (focus of a fourth BSc Hons student project). This database is being updated in 2003 by two Bioinformatics research assistants.

Three new MSc projects and two BSc Hons projects were initiated in 2003. These projects are part of a new Wood and Fibre Molecular Genetics programme supported by Sappi Forests and Mondi Forests and THRIP.

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 molecularly 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
- Train and educate students in an important field of plant pathology
- Establish a centre of excellence in phytobacteriology

Highlights of research 2002/2003:

Bacterial blight and die-back is a relatively newly discovered disease of *Eucalyptus* in South Africa. During the course of the last three years the disease has increased in occurrence, importance and distribution. Isolates of the causal agent have been identified and characterized and have been shown to be *Pantoea ananatis*, which is a known pathogen of other crop plants but has never previously been found on *Eucalyptus*. The appearance of *P. ananatis* on different *Eucalyptus* species, hybrids and clones in a range of environmental habitats is of concern. In order to understand the genotypic relatedness between strains from different species, clones and hybrids of *Eucalyptus* and from different environments, a genetic diversity study is being conducted. This study involves the use of a number of techniques such as sequencing of the 16S gene and interspacer region as well as Amplified Fragment Length Polymorphisms (AFLPs). The study does not focus only on isolates from *Eucalyptus* but *P. ananatis* isolates from different hosts (pineapple, onions, cantaloupe fruit, sudan grass, rice, sugarcane and honeydew melons) are included in order to determine their relatedness. This study will provide information on the possible origin and the current distribution of the bacterium will be determined.

Pantoea ananatis is seedborne in onion. There is evidence to suggest that the introduction of the disease into new environments has been via the use of contaminated seed. We are currently investigating methods of rapidly identifying this pathogen using a differential media and molecular-based techniques. A related species, *P. agglomerans*, also occurs on seed and its exact role, if any, in causing a disease in onion is also being investigated.

Preliminary results have shown that *P. ananatis* together with an undescribed *Pantoea* sp. are associated with *Coniothyrium zuluense* fungal canker pathogen of *Eucalyptus*. There appears to be a synergistic relationship between the *Pantoea* spp. and this fungus. Investigations are currently under way to determine the identity of the second *Pantoea* sp. as well as their role in disease expression of what is known as *Coniothyrium* canker.

Bacterial wilt of enset is an economically important disease in Ethiopia and Uganda. In both countries, large scale losses are being experienced. There is only one published paper on the disease, which described the causal agent as *Xanthomonas campestris* pv. *musacearum*. Studies are currently under way to verify this identification and characterize isolates from both Ethiopia and Uganda.

Industrial mycology

Research leader: Dr Gert Marais

Research team: Mr Lucky Madzivandila
Ms Annelie Lübben

Objectives of the research programme:

- Building collaboration between FABI and CSIR Bio/Chemtek
- Expanding mycological research at FABI
- Finding industrial applications for the FABI fungal culture collection
- Screening local ophiostomatoid fungi for the ability to produce flavours and fragrances
- Promoting mycology as a research discipline through education

Highlights of research 2002/2003:

Through the closer relations between the University of Pretoria and the CSIR, collaboration between FABI and Bio/Chemtek was promoted through the secondment of Gert Marais to FABI in June 2001. One of the identified joint initiatives is the utilisation of the FABI fungal culture collection in the search for metabolites or active compounds applicable in markets such as agriculture, food and pharmaceutical industries.

During two decades of plant pathological research, FABI has obtained a very unique and extensive collection of more than 12000 fungal isolates, including approximately 2000 cultures from South African plants, forests and other environments. Many of these fungi are associated with specific insect vectors and have developed various specialised mechanisms to attract their vectors. This includes the production of pheromones, flavours, fragrances and the development of mutualistic relationships, some of which are so interwoven that the survival of the one is dependant on the other.

Access to this collection, and the expertise at CSIR Bio/Chemtek in the industrial development and commercialisation of microbial products, was seen as an ideal opportunity to join forces. This has led to an agreement that was signed by the two

institutions in December 2002, to launch a screening process for active compounds that might be used to combat cancer, bacteria, fungi and insects. In addition, the screening also includes new and interesting flavours and fragrances for the food industry.

Flavours and fragrances

A joint project between FABI, CSIR Bio/Chemtek and an industrial partner in the flavour and fragrance industry was submitted and awarded by the BioPAD BRIC in March 2003. The project includes the commercialisation of the production of blue cheese flavour compounds, a peach flavour, and two other flavours. Production is based on the utilisation of fungi from FABI and CSIR fungal culture collections. The ultimate goal is to establish a venture business that is based on using fermentation processes in the production of bio-flavours.

During a period of four months since the signing of the agreement between FABI and CSIR Bio/Chemtek, 350 ophiostomatoid fungi were screened for their ability to produce aromatic compounds. Of these, approximately 16% were found to produce desirable flavours and were earmarked for further investigation.

Fungi in thatched roofs

The role that fungi play in the deterioration of thatched roofs and their contribution to health risks in South Africa have largely been neglected. Thatched roofs are aesthetically important, especially in the tourist industry, but maintenance costs, insurance and possible health risks for inhabitants make them very costly. A project was launched in collaboration with the Pretoria Technikon to study the role of fungi in the quality and health of thatched roofs. This is part of an ongoing project and covers 38 case studies in 12 weather zones in South Africa. The study has included the determination of the level of fungal infestation in thatched roofs and compares these with the fungal aerosols inside and outside the dwellings.



Fungal growth on the inside of a thatched roof in Durban

Results have shown that thatched roofs in South Africa are infested with plant pathogenic fungi and opportunistic saprophytes. In most of the case studies, environmental conditions seem to contribute significantly to the prevalence of fungi inside the dwellings. *Cladosporium cladosporioides*, *Alternaria alternata*, *Phoma sorghina*, *Epicoccum nigrum* and various *Penicillium* species seem to be predominant in most of the fungal aerosols. *Aspergillus versicolor* seems to occur rather in the environmental aerosols in Mpumalanga and not in the rest of the country. Similar fungi were isolated from the thatch, although *Eurotium* species occurred in significant numbers in the thatch in certain cases.



Fungal aerosol samples showing mainly *Cladosporium* species

It was also shown that the Kruger National Park and the coastal areas of KwaZulu/Natal and the Eastern Cape Province are high-risk areas for fungal bio-aerosols and damage to thatched roofs. In many cases, the surrounding environment contributes significantly to the levels of fungal loads inside the dwellings. However, there were cases where the likely source of fungi could be associated with the degradation of the thatch.

Mycotoxigenic fungi

In March 2003, a project was awarded to FABI, CSIR Bio/Chemtek and the ARC by the Maize Trust. The project covers the evaluation of the most dominant maize cultivars in South Africa to withstand infestation of mycotoxigenic fungi. The project aims to identify molecular markers closely linked to genes responsible for resistance to specifically mycotoxigenic fungi and to lay a platform for developing resistant cultivars in future.

A wide variety of fungi are associated with the staple food of South Africa such as maize. These include both field and storage fungi of which some are known producers of mycotoxins, implicated in the cause of various disorders and cancers in humans and domestic animals. The best known and studied mycotoxin in South African maize is fumonisin B₁. It is associated with oesophageal cancer in humans and the cause of leucoencephalomalacia (LEM) in horses, mules and donkeys. It is also responsible for liver damage in rats and lung disorders

in swine. *Stenocarpella maydis* is also one of the more prominent fungi in South African maize. However, in this case, the mycotoxin is unknown although the fungus is associated with diplodiosis in cattle and sheep, as well as dehydration, growth inhibition and mortality in poultry. The presence of these fungi is mostly associated with the maize kernels due to high humidity and moisture during harvesting and storage. Field fungi are metabolically inactive at water activities lower than 0.85 (moisture content lower than 20%) while storage fungi thrive between 0.65 and 0.8 (moisture contents between 13% and 19%).



Maize kernels infected with *Stenocarpella maydis*

Field fungi such as *Fusarium*, *Alternaria*, and *Stenocarpella* spp. are regularly associated with South African maize kernels and can cause, besides the production of mycotoxins, physical damage that can influence the quality and milling properties. Storage fungi such as *Eurotium*, *Aspergillus* and *Penicillium* species also contribute to this and due to the colour of their spores can cause milled products to be aesthetically unacceptable. The presence of these fungi also results in other problems such as off flavours and

odours, lack of germination, mouldy kernels and shortened shelf life of products.

It was found that, during a study for the Department of Health in 2000, mycotoxins such as aflatoxin B₁ and aflatoxin M₁, beauvericin, citreoviridin, deoxynivalenol, diplodiatxin, diplosporin, fumonisins, fusaproliferin, fusarin C, fusarenon X, moniliformin, secalonic acid D, trichothecin and zearalenone were positively associated with fungi isolated from maize in South Africa. This information, however, only includes reports and manuscripts published in the scientific and public domains. Many fungi are associated with maize, especially after the milling process. However, not much is published on the presence of fungi during the storage or milling processes of maize. There is also little information on the levels of fungi in the products destined for human and animal consumption. Future work will be based on addressing these questions.

Diagnostic mycology

In April 2002, a mycological diagnostic clinic was initiated at FABI that addressed the mycological needs of the food and feed industries in South Africa. The services provided by the clinic mainly include the identification of fungi to species level, risk assessments, fungal enumeration, fungal aerosols, environmental impact studies, and various others. More than 150 samples were handled by the clinic in the first 12 months of its existence. Samples were received from the food, feed, milling, industrial processes and agricultural industries in South Africa and neighbouring countries.

Citrus rootstock resistance

Research leader: Dr Nico Labuschagne

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

Objectives of the research programme:

- Identify rootstocks with resistance/tolerance to the main fungal root pathogens of citrus
- Develop techniques for rapid screening of rootstocks for resistance
- Elucidation of the mechanisms involved in rootstock resistance

Highlights of research 2002/2003:

Phytophthora species cause severe diseases of citrus worldwide. Tolerance of citrus rootstocks to *Phytophthora* has been amply demonstrated and it offers an excellent means of reducing losses due to *Phytophthora* root and collar rot. Resistance responses are usually characterized by the early accumulation of secondary phenolic compounds that effectively isolates the pathogen at the point of infection. The production of antifungal phenolic compounds has been demonstrated in citrus fruits, peels, leaves and in citrus roots.

The fungicide fosetyl-aluminium (aluminium tris-[o-ethyl phosphonate]) is known to be effective for control of some diseases caused by the Peronosporales. Fosetyl-Al exhibits a complex mode of action acting both directly on the pathogen and indirectly by inducing host defence responses. Despite numerous studies on the fungicide the exact mode of action of fosetyl-Al is not fully understood.

The levels of total soluble phenolics were determined in citrus rootstocks tolerant and susceptible to *Phytophthora nicotianae* following inoculation with the pathogen. The effect of fosetyl-Al treatment on total phenolic concentrations in the various rootstocks was also studied. Phenolic concentrations were higher in the roots of *Phytophthora*-tolerant swingle, macrophylla, troyer and sour orange rootstocks 21 days after inoculation,

whereas only minor increases occurred in susceptible rough lemon and volkamer lemon rootstocks. The highest phenolic concentrations were recorded after 21 days in troyer, rendering a maximum of 58.2 mg gallic acid equivalents/gram dry root material, in comparison to 48.1 mg/g in rough lemon. Total phenolic concentrations correlated positively with tolerance to *P. nicotianae*. Treatment with the systemic fungicide fosetyl-Al further elevated phenolic levels in the roots. Total phenolic concentrations were two to three times greater in inoculated rootstocks treated with fosetyl-Al than in uninoculated, untreated rootstocks.

Results of the present study provide strong evidence that the increase in phenolic concentrations in citrus roots plays a key role in resistance of citrus against *P. nicotianae* root rot. The ELISA plate Folin-Ciocalteu method used in this study has potential as a high throughput method for screening citrus rootstocks for resistance. Coinciding with its ability to protect rootstocks against *P. nicotianae*, fosetyl-Al increased total soluble phenolic concentrations even further following inoculation with the pathogen. This provides evidence that elevation of phenolic levels is involved in the mechanism of action of fosetyl-Al in the control of *Phytophthora* root rot, therefore supporting an indirect antifungal mode of action.

Citrus and subtropical fruit research

Research leader: Prof Lise Korsten

Research team: Dr Gina Swart
Dr Thierry Regnier
Dr Petra Labuschagne

Objectives of the research programme:

Citrus

- Determine differences between virulent and avirulent *G. citricarpa* isolates and prove non-infective status of black spot lesions on fruit particularly for export consignments
- Epidemiology of citrus black spot to respond to the European Union's comments on the Risk Assessment study
- To develop an effective, environmentally friendly disease control option for *Guignardia citricarpa*, the causal agent of citrus black spot
- To develop an effective post-harvest disease control strategy using antagonists in a biological control programme and identify and develop new, innovative biological control strategies
- To develop an effective *Alternaria* disease control strategy using bees to disseminate natural antagonists for control of navel-end rot

Mango

- Develop new innovative disease control strategies, such as the use of plastic caps impregnated with copper based fungicides or biocontrol agents, to control fruit disease.
- Epidemiological studies on soft brown rot.

Avocado

- Biological control of avocado fruit diseases using bees to disseminate antagonists to flowers to control stem end rot
- Development of a biological control predictive forecasting program

Highlights of research 2002/2003:

South Africa has 47 422 ha under citrus cultivation, of which Mpumalanga and Limpopo province are the most important production areas (14 138 and 11 997 ha). The South African citrus industry has more than 20 million trees, which produce more than 930 000 tons of fruit annually, with more than 40 million cartons being exported to more than 60 countries around the world. The industry is expected to export more than 60 million cartons of citrus worth R2.5 billion in the year 2003. The South African citrus industry is regarded as one of the largest agricultural industries in the country and plays a significant role in the national

economy being worth R1.7 billion annually. Several post-harvest diseases such as green and blue mold contribute significantly to post-harvest losses, but citrus black spot is one of the most important pathogens affecting the citrus industry.

Mangoes and avocados are economically important crops cultivated in South Africa for both local consumption, processing and export. These South African industries have developed expertise over the years that enabled the industry to successfully export the bulk of its fruit mainly to Europe. Due to stringent quality

assurance standards, fruits with any blemishes are rejected for export. Control of pre-harvest fruit diseases is thus important in ensuring high quality fruit for export. One of the most important mango pre-harvest diseases is bacterial black spot (BBS) caused by *Xanthomonas campestris* pv *mangiferae*indicae. In general, it is extremely difficult to control bacterial plant diseases and current practices focus on timely copper sprays. However, new more effective disease control options are required to ensure consistent high levels of quality fruit. For avocado, *Cercospora* spot caused by *Pseudocercospora purpurea* is a major pre-harvest disease, which can be controlled with timely copper or *Bacillus subtilis* (Avogreen) sprays. The commercial biocontrol agent Avogreen developed in our laboratories, is currently used as a substitute for copper sprays for particular organic farming or integrated with reduced copper sprays in conventional farming. Post-harvest losses on the export market have been significant for both avocado and mango and have resulted in inferior quality fruit and reduced profits. The most important post-harvest diseases of mango are anthracnose caused by *Colletotrichum gloeosporioides*, and soft brown rot (SBR) and stem-end rot (SER) both caused by *Botryosphaeria* spp. With the shift in international trade in terms of food safety standards, more stringent quality and safety of fruit are expected for export. New innovative alternative disease control strategies are thus currently evaluated and developed for the South African mango industry.

The most significant highlights for 2002 and 2003 include the successful use of biocontrol agents to control bacterial black spot in mangoes, particularly the use of the innovative plastic cap with woolly inner linings, which give a slow release effect of both biocontrol agents and copper fungicides. In addition, other application methods including the use of bees to disseminate biocontrol agents to flowers to control stem end diseases in fruit, have shown great promise, both for citrus and avocado. Biocontrol agents and

certain plant extracts have also shown promise in controlling citrus black spot and post-harvest diseases.

Several breakthroughs have been made in terms of elucidation of citrus black spot epidemiology and taxonomy, which could provide critical information for the EU response in the risk mitigation arguments, thereby protecting several sensitive export markets for the South African citrus industry. These include the development of PCR primers to detect and distinguish between the pathogenic (*Guignardia citricarpa*) and non-pathogenic (*Guignardia mangiferae*) species. This is of great importance for the citrus industry, both in terms of export and prevention of inadvertent disease spread through infected nursery trees.

Citrus

Determine differences between virulent and avirulent *Guignardia citricarpa* isolates and prove non-infective status of black spot lesions on fruit particularly for export consignments

It is of quarantine importance to distinguish between the citrus black spot pathogen, *Guignardia citricarpa* and the omnipresent endophyte, *G. mangiferae*, if South Africa exporters wish to retain their competitive edge in the European and access the United States of America, markets. Species-specific primers were designed that are able to detect and distinguish between these two species, since *G. mangiferae* is not a sanitary and phytosanitary risk. Application of the primer set Citric 1 and Camel 2 in conjunction with the ITS 4 primer yielded 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 repeated tests verified accuracy. 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 black spot lesions. We are therefore able to distinguish *G. citricarpa*

and *G. mangiferae* unequivocally using a PCR-based technique, thereby shortening the time needed to test fruit for *G. citricarpa* in export consignments.

With the development of this technique, we are now able to accurately and quickly identify *Guignardia* species from both fruit lesions and leaves. This has enormous implications for the citrus industry. The first application of this test is that rejected or suspect fruit may be tested from as little as a single lesion, irrespective of what type of symptom. This implies that growers may test their fruit before consignments are packed for export if they have any doubts about their CBS status, or if fruit have been rejected at the packhouse or harbour with confirmations obtained within eight hours. A second important application is the use of the primers for epidemiological studies and confirmation of the CBS status of production areas. This is due to the optimisation of the test so that *Guignardia* species may be detected in any type of plant material, including degraded leaf litter. To date, nursery material suspected of being infected with CBS has not yet been tested using this technique. Since this material would most probably be asymptomatic, a protocol is now being developed/adapted for this purpose. Furthermore, the identity of ascospores counted in spore traps should also be confirmed, since current prediction models are based on their presence. Testing of trapped ascospores should also be optimised, although the removal from the spore trap represents a challenge. It is clear that there are several applications for this test and they should all be optimised in order to provide a maximum benefit for the citrus industry.

Investigate epidemiology of citrus black spot and explore other control measures

Ascospores are considered the most efficient means of dissemination of CBS in areas where the disease is established. It has been reported that the spread of CBS through conidia could be as important as the role of ascospores in establishing the disease. As age-related resistance in plant

hosts to fungal pathogens is a commonly recognised phenomenon, the influence of Valencia leaf age and susceptibility to conidia infection was investigated. Conidia harvested from cultures and prepared as spore suspensions were used to infect the leaves at 30-day intervals. The pathogen could be re-isolated from the leaves consistently up to four months in the preliminary trial and to date the second and currently ongoing trial shows that the spores could be re-isolated from six-month-old leaves. The cultures were identified by the PCR technique. The amount of soluble phenol and sugars do not differ from the control and phytoalexins were not detected.

To date, the effect of leaf age on infection by the black spot pathogen has not been evident. From the two experiments, it seems as if the physiological and chemical barrier found in older leaves is not sufficiently developed in up to six-month-old leaves. This experiment will continue for three more months after which a final conclusion will be made in terms of leaf age and susceptibility. If the pathogen is still recovered from the leaves at the end of this experiment, it will then demonstrate that age does not play a role in protecting the leaf from infection and that spores could survive on the surface of the leaf for a longer time than previously suggested. As leaves can remain on the trees for up to three years, a follow-up experiment needs to be conducted over a period of up to three years.

Determination of optimal environmental conditions for the germination of black spot conidia is the first step in understanding disease development and improving strategies for disease management. This information is also critical for development of accurate disease forecasting models. Since ascospores remain the major source of inoculum, they are often used to test the effect of different parameters on the fungus. The aim of this study was to investigate the influence of temperature, pH and light on the germination of *Phyllosticta citricarpa*. Most germ tubes (GT) and appressoria (AP) developed at mild temperatures between 20°C and 25°C and

lower pH values. It was observed that at 10°C, and 20°C most appressoria were sessile with short germination tubes. At higher temperatures, they were attached to longer germination tubes.

Differences in germination rates were observed in the experiments indicating that light, ambient temperature (25°C) and lower pH values (pH 4.5) enhanced the development of GT and AP. No germination was observed at 4°C while very long GT and a few AP developed at 37°C compared to that observed at 20°C. At 10°C and 20°C the AP tended to be sessile and at 25°C it was attached to a longer germ tube. Germination was lower in distilled water and leaf extract, compared to 2% orange juice. After four days, when using the orange juice, the percentage of GT and AP was $\pm$ 15.83% and 62.37% respectively. At 2% dilution of the stock solution, the presence of several minerals such as calcium, magnesium, or ascorbic acid without any inhibiting compounds (phenolic compounds) could provide a good nutrient supply to the spores.

The *in vitro* toxicity of commercial formulations of preventive and systemic products to pathogens is not always well-known. It was postulated that citrus black spot was more severe in cultivars where low levels of antifungal compounds like limonene and citrus oil were found in the rind tissue. All chemicals and natural products tested inhibited fungal germination at commercially recommended concentrations. The minimum concentration required varied according to the product. Scoparone and scopoletin, phytoalexins produced in fruit with black spot symptoms strongly inhibited the germination of the pathogen.

Removal or immobilisation of overwintering inoculum of *Guignardia citricarpa* was investigated in two adjacent 30-year-old Valencia on Rough Lemon orchards near Burgersfort, one 1.36 ha in size and the other 1.03 ha. In both orchards, all leaves on the orchard floor were manually removed and burned. Four blocks of 16 trees each were demarcated

in the 1.36 ha orchard and three blocks in the 1.03 ha orchard. The surface under the trees in the blocks in the 1.36 ha orchard was mulched with a layer of wheat straw, whereas leaves were again removed from the 1.03 ha orchard and the unmulched area in the 1.36 ha orchard. Orchards were sprayed with fungicides except for the trees in the demarcated blocks. No ascospores of *G. citricarpa* could be trapped with a Quest spore trap in the 1.03 ha orchard during December 2001 and January 2002. Evaluation of the orchards indicated a CBS incidence of 2.3% in the mulched blocks and 13.2%, almost six times higher, in the unmulched blocks. None of the infected fruit in the mulched blocks had more than five CBS lesions, 26% with 6-50 lesions, and 9% with extensive infection in the unmulched blocks. Virtually no CBS was evident on chemically sprayed fruit.

The decomposition of infected citrus leaf litter was also evaluated on a small scale in the greenhouse. Mature infected leaves were picked on three different occasions and placed in plastic containers in contact with organic material. Treatments included: (1) different organic material: sterilised sand as control, old mango compost, new mango compost, citrus compost, citrus compost mixed with bark, chicken, cattle and sheep manure; (2) placement of leaves: on top of material and underneath material; (3) watering: no water and watering twice a week. Leaves were evaluated weekly for two months. The amount and frequency of watering had the greatest effect on rate of decomposition, followed by duration of leaf wetness and area of contact between the leaves and organic material. There was also a positive correlation between the level of microbial activity of the organic compound and decomposition rate of the leaves. The experiment will be repeated and different organic material will be included.

Development of an effective, environmentally friendly disease control option for *Guignardia citricarpa*, the causal agent of citrus black spot

Garlic (*Allium sativum*), clove and *Coprosma repens* extracts were evaluated for their biological activity against *Penicillium digitatum*, *P. italicum*, and *Guignardia citricarpa* both *in vitro* and on artificially inoculated (in the case of *P. digitatum* and *P. italicum*) and naturally infected (in respect of *G. citricarpa*) Valencia oranges stored at $8\pm1^{\circ}\text{C}$ and 90%-95% relative humidity (RH) for four weeks. Garlic was evaluated alone or in a mixture with vegetable cooking oil (0.1% v/v). Both garlic and *Coprosma* exhibited varying degrees of antifungal activity against all pathogens, and all concentrations of extracts were significantly effective when compared with the control in checking disease incidence, but were not as effective as the commercial fungicide, which gave complete control of both *P. digitatum* and *P. italicum*. Mixing garlic extracts with oil remarkably improved its activity. As a result, the treatment comprising garlic extracts (1 000 ppm) mixed with oil was as effective (100% control) as the fungicide treatment in the control of both *P. digitatum* and *P. italicum*. *Coprosma repens* extract on its own was not as effective and the percentage control achieved varied between 67% and 81%. *In vitro* studies indicate that the mode of action of extracts is inhibition of spore germination and germ-tube development.

Development of an effective post-harvest disease control strategy using antagonists in a biological control programme and identify and develop new, innovative biological control strategies

Three *Bacillus subtilis* isolates (F1, L2 and L2-5) were evaluated along with other commercial biocontrol products *Bacillus subtilis* (Avogreen powder and Avogreen liquid), and *Candida saitoana* (Biocure and Biocoat) for their antifungal activity against *Penicillium digitatum*, the cause of citrus green mold, under simulated export

conditions in 2000, 2001 and 2002. The *B. subtilis* isolates were evaluated either alone or in combination with sodium bicarbonate (SB) at 1% (w/v). The efficacy of treatment was negatively affected by time of treatment application. Treatments were generally more effective when applied at the beginning of the season than when used later in the season when fruits have started 'ageing'. Neither the *B. subtilis* isolates on their own, nor the formulated products were as effective as the commercial fungicide treatment, which gave complete control of the disease throughout the season. Combining *B. subtilis* isolates with SB resulted in a remarkable improvement in the biocontrol activities of all isolates. Isolate F1 combined with SB was as effective as the fungicide treatment in some instances.



Oranges heavily infested with *P. digitatum* (left) and the control (right)

Development of an effective *Alternaria* disease control strategy using bees to disseminate natural antagonists for control of navel-end rot

The commercial *Bacillus subtilis*, previously screened successfully *in vitro* for antagonism against *Alternaria alternata*, was dusted on citrus flowers at the concentration of 10^7 colony forming units (cfu) per ml with a paint brush. This powder formulation was also placed in pollen inserts in the entrance of a beehive to test the ability of foraging bees (*Apis mellifera*) to disseminate this antagonist to citrus flowers for control of *Alternaria* rot of citrus. Mean recovery of *Bacillus subtilis* commercial powder sprayed on

flowers was 10^3 cfu per stamen. Based on electron microscopy, this organism multiplied preferentially on the stylar end of the flower. Bees emerging from the hive acquired 10^4 cfu per bee of commercial *Bacillus subtilis* powder. When observed under the scanning electron microscope, the powder was observed on the legs and thorax of the bees. The mean population of *Bacillus subtilis* recovered from flowers visited by the bees was 10^4 cfu per stamen. Trees that were visited were maintained in an enclosure. Electron microscopy studies showed that the organism attached on the stylar end of the flowers. This study showed that *Bacillus subtilis* colonised citrus flowers effectively and that bees can be efficient vectors of this antagonist for control of *Alternaria alternata*.

Mango

Disease control strategy ie plastic caps impregnated with copper-based fungicides or biocontrol agents

In South Africa, mangoes are an important sub-tropical export commodity. Most of these are exported by sea and long shipment times are conducive to the formation of post-harvest diseases like anthracnose, stem-end rot and soft brown rot. These post-harvest diseases result in significant financial losses due to rejection on export markets. Limited control has been achieved by the use of existing fungicides. However, pathogen resistance has been reported to certain fungicides, and many are no longer allowed on fruit destined for export due to new EU pesticide regulations. Pre-harvest application of biocontrol agents (*Bacillus licheniformis*) on their own or integrated with copper fungicides were evaluated as alternative strategy to control mango fruit diseases. The use of plastic woolly caps impregnated with the biocontrol agents or copper fungicides were evaluated as alternative method of application to provide sustained disease control throughout the rainy season by means of a slow release effect. The woolly plastic caps provided more effective sun protection to fruit than commercial plastic caps. Although the antagonist could

effectively attach, colonize and survive on the plastic caps it could not provide more effective control of mango fruit diseases compared to the field spray applications.

The aim of the current study was to evaluate new and "softer" chemicals for the control of fruit diseases. Trials were performed under semi-commercial conditions. New disinfectants were tested in packhouses, where they replaced existing disinfectants or wash media or were used in combination with chemicals or biocontrol agents. Fruit from the trials were stored in conditions simulating local and export market conditions. Preliminary results showed that a combination of prochloraz with strobilurins added to wax provided the highest levels of control. The use of disinfectants on its own did not produce significant results, but the use of Prasin with prochloraz was relatively effective when compared to the commercial control. Further studies will be conducted to understand the mode of action and optimise the use of these new chemicals.

Several disease management strategies have been applied both pre- and post-harvestly in order to control mango fruit diseases. To date, chemical products have provided some control of these diseases. However, growing concern over food safety issues, build-up of pathogen resistance and the high cost of commercializing new chemicals have resulted in a move towards safer, more environmentally friendly control measures. Biological control has been used successfully in packhouses to control several post-harvest diseases of several food commodities. In this study, *Bacillus licheniformis*, previously tested with success in preliminary trials, was used individually or in combination with standard packhouse treatments, as well as integrated with other chemical treatments. Two packhouses were chosen from different geographical regions using similar packline systems. After treatments, fruits were packed into boxes and one half of the total treatments stored at room temperature and the other half in cold storage, to simulate local and export

market conditions. Results show that the antagonist combined with standard packhouse treatments was more effective than the untreated control. The degree of effectiveness differed between the two packhouses. These results combined with previous trials will give a better indication of the feasibility of incorporating antagonists in commercial packhouse operations. Fruit treated with biocontrol agents have an additional benefit in terms of being acceptable for the organic market.

Epidemiological studies on soft brown rot

Mangoes were introduced from tropical areas into South Africa. South Africa does not have a tropical climate and the tree had to adapt to these climatic conditions. This environment also places the tree under stress making it more susceptible to a wide spectrum of pathogens. One of these used by *Botryosphaeria* cause die-back and can lead to a reduction in yield, since infected inflorescence die and young fruitlets are aborted during the early development stages. In order to investigate die-back, five orchards were chosen at a large mango estate in Mpumalanga. One of these orchards was salt stressed, two orchards had typical and atypical die-back symptoms respectively, and two control orchards were included. Tree samples which consisted of twigs, leaves, bark and fruit were evaluated for the occurrence of *Botryosphaeria* spp. and other known endophytes. *Botryosphaeria* spp. were isolated from all orchards with varying degrees of incidence. The highest incidence was found in the orchard under salt stress conditions, closely followed by the orchard with typical die-back symptoms. This indicates that *Botryosphaeria* spp. were more prevalent than was originally thought. It also confirms that *Botryosphaeria* spp. are opportunistic, endophytic pathogens, which attack trees under stress. A disease cycle is currently being constructed to illustrate the relationship between the host and the pathogen, as well as the spread between and within trees and primary and secondary inoculum resources. The information gained can be important to

better manage die-back in mango orchards.

In order to investigate the role nurseries play in the dissemination of the pathogen through infected trees, all commercially important mango nurseries and associated mother blocks were surveyed in the Mpumalanga area. The associated orchards are those from which mother material and rootstock scion material are obtained. In each nursery, two blocks of ten-week-old Kent trees were analysed as well as Kent mother block orchards. In addition a Sabre orchard from which rootstock scion material were used was also included in the study. In all nurseries *Botryosphaeria* spp. were isolated from almost every tree sampled, irrespective of age. *Fusarium* was also isolated from these trees although at a lower frequency. In the Kent and Sabre orchards, *Botryosphaeria* spp. were also isolated but at a higher frequency. This study clearly showed the impact of *Botryosphaeria* spp in mother block orchards and that it could be transferred from infected mother material to new trees. In future, nurseries should give more careful consideration to nursery practices and grafting material should be screened before use.

Avocado

Biological control of avocado fruit diseases

Post-harvest fungicide treatments against latent infections cannot give complete control unless the fungicides have the ability to penetrate through the fruit cuticle and reach the infection site. Besides, there is increasing public pressure against the use of fungicides on harvested commodities. This, together with the increasing appearance of pesticide resistance in plant pathogens, is shifting the focus of plant disease control towards alternative control with a reduced risk to the consumer and the environment. One such alternative is biological control. Although various researchers have reported successful control of post-harvest fruit diseases little has been reported on pre-harvest approaches to control post-harvest fruit diseases. One of the difficult

aspects in pre-harvest biocontrol is the successful delivery of the antagonist to the infection court at the critical stage of infection. It has been shown that an important infection route for fruit pathogens is through flowers. Hence, combating stem-end rot (SER) pathogens at the flowering stage can prove to be effective in suppressing the incidence of the disease. The biocontrol agent must however be able to attach, survive and colonise the flowers so that the pathogens will be prevented from establishing on the flowers. Avogreen (*Bacillus subtilis*) has been successfully tested pre-harvestly against avocado and is currently commercially used for *Cercospora* control.



Bacteria on the surface of an avocado flower

To determine effective attachment and colonisation of antagonist to avocado flowers, Avogreen was used to "inoculate" flowers and samples were prepared for Scanning Electron Microscopy (SEM) immediately and after 1, 2, 4, 6, 12, 24, and 48 hours. Dilution series were also done from inoculated flowers at the same time intervals to determine the survival of antagonist on flowers over time. Results showed that the bacterium can effectively colonise and survive on avocado flowers.

Previously, the potential of bees in disseminating antagonists to citrus flowers were successfully shown. In this study the effective dissemination to avocado flowers was assessed by mixing Avogreen powder formulation with fluorescent powder and monitoring bee dissemination during the evening using portable UV torches. Five beehives on a block of Fuerte were used for the experiment. Dispensers were refilled three times a day i.e. at 9:00, 11:00, and 13:00. Trees around the hive

were selected and marked. Apart from bee dispersal field sprays with Avogreen liquid, spray formulation and copper were used for comparative purposes.

To see the interaction between the antagonist and the pathogens, spores of pathogens (*Dothiorella*, *Phomopsis*, *Pestalotiopsis* and *Colletotrichum*) and antagonistic bacteria were inoculated on flowers in either one of the following patterns: antagonist first followed by the pathogen, pathogen followed by antagonists, antagonist and pathogens at the same time. The flowers were prepared for SEM observation. Excellent results were obtained for *Dothiorella* and *Phomopsis* showing interaction between the pathogen and antagonists.

Due to the increased economic and environmental pressure, research on several aspects of biological control has increased substantially. Integration of disease forecasting models with a biocontrol predictive system may provide growers with a disease control program that adheres to the requirements of organic programmes and EurepGap. This model will also focus on eliminating variable parameters that may influence product performance. These parameters include climatic variables and agricultural practices. During a preliminary study, the effect of certain agricultural practices on the survival of *Bacillus subtilis* (Avogreen) was evaluated. It was found that mist blower and handgun application methods were equally effective in delivering the antagonist to the phylloplane, but that spray volumes could affect performance. Repeated pre-harvest applications resulted in a higher build-up of bacterial numbers on the leaves and increased the success of post-harvest disease control. Results showed that spreader and sticker agents should be avoided to ensure survival of the bacteria. The compilation of a predictive system for *B. subtilis* will consist of a combination of climatological information and antagonist behavior as influenced by agricultural practices.

SERVICES

Forest health extension

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

Activities 2002/2003:

Extension activities form an important component of the Forest Protection Research Programme. These activities are divided into a number of components. They include all activities linked to monitoring of diseases and pests in forest plantations. Monitoring is further sub-divided into efforts to detect new pathogens and pests in a timely fashion and evaluation of the change in status of pathogens and pests that 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 for the South African forestry industry.

During 2001/2002 the Diagnostic clinic dealt with a significantly increased number of samples compared to previous years. Although this placed pressure on the group maintaining the clinic, the results justified the increasing efforts. Thus, data emerging from diagnoses were not only important to those requesting advice, but also contribute significantly to disease monitoring and the establishment of research priorities.

From January to December 2002, the clinic received a total of 461 samples. This is considerably more than the 271 samples received in 2001. The majority of samples were received in July, November and December. Most samples received were from pine (72%) followed by eucalypts (19%). Fewer samples wattle samples were received (4%). A number of water, growth media, seed samples and samples from indigenous trees (5%) were also tested for the presence of pathogens. Insect samples (5%) are included in this category.

Field extension and monitoring are a crucially important focus of the team leading the Tree Protection Co-operative Programme. Members of the group undertook field studies in all forestry areas of South Africa and spent a total of 636 person days in the field during 2002. This is a record as can be seen in contrast to the 483 person days spent on field work during 2001. An important component of extension and field activities included presentations on forest pests and diseases and training of foresters and farmers at field days.

"Tree Protection News", the newsletter of the research programme, continued to appear twice each year and remains an important means of distributing new information to members of the Programme. This newsletter now firmly incorporates both entomology and pathology activities of the Programme. Our experience has been that the publication of this newsletter results in significant feedback to the programme and that it is generally well received and appreciated by members. "Tree Protection News" continues to be distributed by the Institute for Commercial Forestry Research (ICFR) together with ICFR News, a system that has

functioned effectively during the course of the past three years. In addition to "Tree Protection News", the programme places shorter news items in two other issues of ICFR News. Similarly, news items are provided to various other members at their request for inclusion in their company newsletters and articles are provided for the two major forestry magazines in the country.

FIELD EXTENSION ACTIVITIES:



TPCP students examining fruiting bodies on an *Eucalyptus* tree



The two photographs above illustrate participants from the major forestry companies at a field day learning about tree diseases and methods used to evaluate them

Microarray service

Facility manager: Prof Dave Berger

Technical assistant: 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.

The Facility is equipped with data capture software and a data analysis pipeline is being established. In future, with completion of the new building adjacent to FABI, which will house a Bioinformatics facility on the third floor, the Microarray Facility aims to provide microarray bioinformatics support in addition to the current wet-lab service.

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



Microarray spotter

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These lists include only publications that had appeared by the end of May 2003. Manuscripts in press and submitted for publication are not included.

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- SITHOLE H., GOVENDER P. & VILJOEN A. 2001. Integrated pest management of the banana weevil, *Cosmopolites sordidus*. Congress of the Entomological Society of Southern Africa, Pietermaritzburg. July 2001.
- SLIPPERS B., COUTINHO T.A., WINGFIELD B.D., CROUS P.W. & WINGFIELD M.J. 2001. Taxonomy and phylogeny of *Botryosphaeria* species. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, Nelspruit. 21-24 January 2001.
- STEYN T.L., KRUGER K. & GOVENDER P. 2001. Complex sensory stimuli influence decisiveness in the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Congress of the Entomological Society of Southern Africa, Pietermaritzburg. July 2001.
- STRONKHORST L.D., VILJOEN A., CLAASSENS A.S., NEL B. & VAN DER WAALS J.H. 2003. The effect of N-fertilization and pH on the incidence of Fusarium Wilt (Panama disease) of banana in greenhouse trials. Golden Jubilee Congress (Soil Science Society of South Africa, South African Society of Crop Production, Southern African Society for Horticultural Sciences), Stellenbosch. 20-23 January 2003.
- SURRIDGE A.K.J., VILJOEN A. & WEHNER, F. 2001. Distribution of banana leaf spot pathogens in South Africa in 2000. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, Nelspruit. 21-24 January 2001.
- SURRIDGE A.K.J., VILJOEN A. & WEHNER, F. 2001. Fungi associated with banana leaves in South Africa. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, Nelspruit. 21-24 January 2001.
- SWANEVELDER Z.H., VAN DER MERWE M., VAN WYK A.E. & BOTHA A-M. 2001. Development of microsatellite markers for application in *Clivia miniata* (Amaryllidaceae). SAAB, Grahamstown, 8-11 January 2003.
- SWANEVELDER Z.H., VAN DER MERWE M., VAN WYK A.E. & BOTHA A-M. 2003. Chloroplast DNA variation in *Clivia miniata* (Amaryllidaceae). SAAB, Pretoria, 7-11 January 2003.
- SWART G. & KORSTEN L. 2001. An investigation into chemical resistance on mango - is it a problem? South African Mango Research Symposium, Tzaneen. 21 June 2001.
- SWART G. & KORSTEN L. 2001. Anthracnose on mango - an overview. South African Mango Research Symposium, Tzaneen. 21 June 2001.
- SWART G.M. & KORSTEN L. 2001. Detection of citrus greening disease from different sources and distinguishing between leaf symptoms using PCR. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, White River. 21-24 January 2001.
- SWART G.M. & KORSTEN L. 2002. A comparative study of *Colletotrichum gloeosporioides* from avocado and mango. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 21-24 January 2002.
- SWART G.M., MEYER L. & KORSTEN L. 2002. Practical application of species-specific primers in the citrus industry. The 2nd Southern African Citrus Research Symposium, Infrutec, Stellenbosch. 23-24 July 2002.
- SWART G.M., MEYER L. & KORSTEN L. 2002. Application of species specific primers in the South African citrus industry. Biotechnology in Africa Conference, University of Pretoria.
- SWART S.H., KORSTEN L., SERFONTEIN J.J. & KALINOWSKI J. 2002. Post-harvest control of CBS symptoms. The 2nd Southern African Citrus Research Symposium, Infrutec, Stellenbosch. 23-24 July 2002.
- TSHABALALA B., COUTINHO T.A., DE BEER Z.W., BURGESS T. & WINGFIELD M.J. 2002. Phenotypic diversity in a South African population of *Lasiodiplodia theobromae* from Pinus. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 19-23 January 2002.
- VAN BROEKHUIZEN W., KORSTEN L. & HAMMES P.S. 2002. Development of an alternative method for the detection of *Ralstonia solanacearum* in naturally infested soil. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 21-24 January 2002.
- VAN DEN BERG N. & AVELING T.A.S. 2001. Evaluation of six fungicides for controlling *Alternaria cassiae* on cowpea seeds. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, Nelspruit. 21-24 January 2001.
- VAN DEN BERG N., BIRCH P., VILJOEN A., WINGFIELD M.J. & BERGER, D.K. 2003. The identification of genes associated with tolerance/resistance to *Fusarium oxysporum* f.sp. *cubense* in Cavendish bananas. SAAB National Conference, Pretoria, South Africa, 7-11 January 2003.
- VAN DER MERWE M., VAN WYK A.E. & BOTHA A.E. 2003. Molecular variation within the southern African Myrtaceae. Joint International Conference of the South African Association of Botanists & International Society for Ethnopharmacology Pretoria, 8-11 January 2003.
- VAN DER MERWE M., VAN WYK A.E. & BOTHA A-M. 2003. A phylogenetic study of *Eugenia* (Myrtaceae). Joint International Conference of the South African

Association of Botanists & International Society for Ethnopharmacology Pretoria, 8-11 January 2003.

VAN DER MERWE N.A., WINGFIELD B.D. & WINGFIELD M.J. 2002. Characterisation of *Cryphonectria cubensis* populations using polymorphic DNA markers. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

VAN DER MERWE N.A., WINGFIELD B.D. & WINGFIELD M.J. 2001. Genetic diversity in the South African *Cryphonectria cubensis* population determined using DNA markers. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, White River. 21-24 January.

VAN DER WAALS J.E. & KORSTEN L. 2002. Completing the pyramid. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 21-24 January 2002.

VAN NIEKERK J.M., CROUS P.W., GROENWALD J.Z., VERKLEY G. & WINGFIELD M.J. 2002. Systematic appraisal of the genus *Coniella*, with specific reference to species occurring in South Africa. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

VAN WYK M., BARNES I., CHHETRI D.B., KRISITS T., ROUX T., WINGFIELD B.D. & WINGFIELD M.J. 2003. A new species of *Ceratocystis* from Bhutan. The 41st Annual Congress of the Southern African Society for Plant Pathology, Baine's Game lodge, Bloemfontein. 19-22 January 2003.

VENTER E., JACOBS A., WINGFIELD B.D. & BOTHA A-M. 2002. What drives *Fusarium circinatum*? Analysis of pathogenicity at the molecular level. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

VENTER E., WINGFIELD B.D., WINGFIELD M.J. & BOTHA A-M. 2002. *Fusarium circinatum*-induced mRNA sequences in *Pinus patula*. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

VENTER M., MYBURG H., WINGFIELD M.J. & WINGFIELD B.D. 2002. Reconsideration of the conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

VILJOEN A., VAN DER WAALS J., NEL B. & WIESE L. 2002. The potential application of disease suppressive soils in the management of *Fusarium* wilt of banana. The 13th Annual Soil-borne Disease Symposium, Stellenbosch.

VISMER H.F., MARASAS W.F.O. & COUTINHO T.A. 2001. The effects of fenpropathrin and other compounds on the growth and presence of mites in fungal cultures. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, Nelspruit. 21-24 January 2001.

VORSTER B.J. & KUNERT K.J. 2001. Identification of Genetic "Hotspots" in the Genome of Plants. The 27th

Annual Conference of the South African Association of Botanists. Johannesburg, South Africa. 14-18 January 2001.

WEICH J.P., GAUME A., VAN DER MERWE M., VAN WYK A.E. & BERGER D.K. 2003. Thale cress (*Arabidopsis thaliana*): is it native, introduced or naturalized in South Africa? SAAB (International conference of the South African Association of Botanists and the International Society of Ethnopharmacology South African Association of Botany), Pretoria. 7-11 January 2003.

WEICH J.P., POUSSIER S., TRIGALET-DEMERY D., TRIGALET A., NAKABONGE G., BERGER D.K. & COUTINHO T.A. 2003. Rapid identification of African strains of *Ralstonia solanacearum*. The 41st Annual Congress of the Southern African Society for Plant Pathology, Bloemfontein. 19-22 January 2003.

ZHOU X.D., BURGESS T., DE BEER Z.W., WINGFIELD B.D. & WINGFIELD M.J. 2002. Development of polymorphic microsatellite markers for the tree pathogen and sapstain agent, *Ophiostoma ips*. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

ZHOU X.D., DE BEER Z.W., AHUMADA R., CIBRIAN D., WINGFIELD B.D. & WINGFIELD M.J. 2002. Molecular and morphological identification of *Ophiostoma* spp. associated with bark beetles in Mexico and Chile. The 40th Annual Congress of the Southern African Society for Plant Pathology, Dikhololo. 20-23 January 2002.

ZHOU X.D., DE BEER Z.W., WINGFIELD M.J. & WINGFIELD B.D. 2001. Pathogenicity of *Ophiostoma ips*, *Leptographium serpens* and *L. lundbergii* to pines in South Africa. The 39th Annual Congress of the Southern African Society for Plant Pathology, Greenway Woods, White River. 21-24 January.

Seminar presentations

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

Special seminars

Dr Francois Lieutier February 2001	Conifer defences and bark beetle attack strategies
Dr Hugh Glen June 2001	The "nut" parade
Prof Dr Michael Göttfert July 2001	Molecular insights into the <i>Bradyrhizobium japonicum</i> /soybean symbiosis – an analysis of the bacterial partner
Prof Michael Wingfield August 2001	Thunder dragon land: a forest pathologist's view of Bhutan
Dr Eddie Mwenje February 2002	Characterization of <i>Armillaria</i> in Zimbabwe
Dr Gert Marais July 2002	Fungi and mycotoxins in South African foods and feeds
Prof John Leslie September 2002	Genetic mapping – an example from <i>Fusarium</i>
Dr Christiaan Steinberg September 2002	Soil suppression to soilborne diseases: interactions between biotic and abiotic antagonistic modes of action
Dr Marcus Conradt October 2002	Expression profiling tools at Memorec: SAGE (Serial Analysis of Gene Expression) and cDNA microarrays
Prof Pedro Crous January 2003	Life in the fast lane – living with my thumb in the dyke
Dr Paul Krokene January 2003	Bark beetles, blue-stain fungi and conifer defence mechanisms
Dr D Aanen January 2003	The evolution of fungus-growing termites and their mutualistic fungal symbionts
Prof H Davies February 2003	Targeted analysis or profiling approaches to detect unintended effects on GMOs
Dr Karin Jacobs March 2003	Hitch-hikers and parasites: adventures in taxonomy

Prestige seminars

Emma Steenkamp (PhD)

March 2001

Molecular taxonomic studies of selected species in the *Gibberella fujikuroi* complex

Noëlani van den Berg (MSc cum laude)

April 2001

A new disease of cowpea caused by *Alternaria cassiae*

Adriana Jacobs (MSc)

June 2001

The genus *Phialocephala* – a taxonomic study

Percy Chimwamurombe (PhD)

September 2001

Molecular plant pathogen interactions with special reference to *Eucalyptus grandis* PGIPs, which attack fungal polygalacturonases

Irene Barnes (MSc cum laude)

October 2002

Taxonomy, phylogeny and population biology of *Ceratocystis* species with particular reference to *Ceratocystis fimbriata*

Gavin Hunter (MSc cum laude)

November 2002

Mycosphaerella species causing leaf blotch on *Eucalyptus* species in South Africa

Karen Surridge (MSc)

April 2003

Banana leaf diseases in South Africa

Grace Nakabonge (MSc cum laude)

April 2003

Diseases of *Eucalyptus* in Uganda

FABI TEAM 2002/2003

Full time academic & research Staff

Prof Lise Korsten
 Prof Karl Kunert
 Prof Brenda D. Wingfield
 Prof Michael J. Wingfield
 Assoc Prof Terry Aveling
 Assoc Prof Dave Berger
 Assoc Prof Teresa Coutinho
 Assoc Prof Anna-Maria Oberholster
 Dr Prem Govender
 Dr Nico Labuschagne
 Dr Petra Labuschagne
 Dr Gert Marais
 Dr Zander Myburg
 Dr Thierry Regnier
 Dr Jolanda Roux
 Dr Gina Swart
 Dr Christel van der Vyver
 Dr Altus Viljoen
 Mr Brett Hurley

Technical Staff

Ms Trish Beart
 Ms Sonja de Beer
 Ms Gerda Fourie
 Mr Hardus Hatting
 Ms Joyce Jakavula
 Ms Pritty Khumalo
 Ms Amelita Lombard
 Ms Eva Müller
 Ms Karin Muller
 Ms Valentina Nkosi
 Ms Amanda Redmond
 Ms Anita Steyn
 Mr Danie Theron
 Ms Lydia Twala
 Ms Kerien van Dyk
 Ms Martie van Zyl
 Ms R. van Zyl

Administrative staff

Ms Elizabeth Attinger
 Ms Vivienne Clarence

Ms Helen Doman
 Ms Judy Hasset (June – December 2002)
 Ms Daleen Muller
 Ms Liana Viljoen
 Ms Rose Visser

Computer support

Mr Chris Visagie

Information specialist

Ms Marie Theron

Honorary professors/lecturers

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

Sabbatical visitor

Dr Paäl Krokene (2003)

Postdoctoral fellows

Dr Dilzara Agayeva

Insect/fungal pathogen associations

Dr Marcus Conradt

Scientific consultant: plant gene discovery using SAGE (Serial analysis of gene expression)

Dr Alain Gaume

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

Dr Solomon Gebeyene

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 Ntsane Moleleki

Pathogenic determinants of *Fusarium circinatum*

Dr Ana Slaughter

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

Dr Retha Slabbert

The effect of cold stress on *Fusarium* wilt development in banana

Dr Marlien van der Merwe

A phylogenetic study of the *Eugenia* (Myrtaceae)

Previous postdoctoral fellows

Dr Oliver Preisig (1997-1999)

Dr Dennis Willson (1998-1999)

Dr Hong Li (1998-1999)

Dr Jingua Chen (1998-1999)

Dr Anupama Gaur (1998-1999)

Dr Treena Burgess (2000-2001)

Dr Gwen Koning (2000-2001)

Dr Thierry Regnier (2000-2002)

Dr Linda Meyer (2000-2001)

Dr Percy Chimwamurombe (2001-2002)

Current postgraduate students**PhD students****Appolinaire Adandonon**

Damping-off of cowpea in Benin

Advisors: TAS Aveling & W Hammond

Shahasi Athman

Biological control of the banana nematode *Radopholus similis* with fungal endophytes

Advisors: N Labuschagne & A Viljoen

Hugues Baimey

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

Advisors: N Labuschagne & D Coyne

Getu Beyene

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

Advisor: K Kunert

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 & T Afriyie

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

Bridget Campbell

Elucidation of disease resistance in monocotyledonous plants through DNA microarray analysis

Advisor: D Berger

Martin Coetzee

Molecular characterization of *Armillaria* (Basidiomycetous Agaricales Tricholomycetaceae)

Advisors: BD Wingfield, MJ Wingfield & P Bloomer

Chris Cooper

Studies on the bioremediation of oil-based contaminants

Advisors: VS Brözel & TA Coutinho

Maria-Noël Cortinas

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

Advisors: BD Wingfield & MJ Wingfield

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 Wehnier

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

Food safety for fruit crops

Advisors: L Korsten

Alemu Gezaghne

Diseases of plantation forest trees in Ethiopia

Advisors: J Roux, MJ Wingfield & BD Wingfield

Teresa Goszczynska

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

Advisors: TA Coutinho & SN Venter

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, BD Wingfield & P Govender

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 & J Jaffa

Gavin Hunter

Mycosphaerella leaf blotch of *Eucalyptus* in South Africa

Advisors: MJ Wingfield, BD Wingfield & PW Crous

Riana Jacobs

Interaction between *Pinus radiata* and the fungal pathogens *Fusarium circinatum* and *Sphaeropsis sapinea*

Advisors: TA Coutinho, I Dubery & MJ Wingfield

Sinnia Kappindu

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

Advisors: A Viljoen, B Niere, D Coyne & C Gold

Andrew Kiggundu

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

Advisors: K Kunert, 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, AA Myburg & F Wehner

Quenton Kritzing

Mycotoxins and medicinal properties of cowpea

Advisors: TAS Aveling & N Lall

Sabine Lezar

Microarray analysis of disease resistance in *Eucalyptus grandis*

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

Michael Luttig

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

Advisors: BD Wingfield & B Manicom

Mauricio Marin

Molecular taxonomy of *Ceratocystis polonica sensu lato*

Advisors: MJ Wingfield, O Preisig & BD Wingfield

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

Cylindrocladum spp. in Venezuela

Advisors: MJ Wingfield, TA Coutinho & BD Wingfield

Josephine Mukiibi

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

Advisors: K Kunert, A Viljoen & M Pillay

Cassi Myburg

Molecular studies on *Cryphonectria* canker of eucalypts

Advisors: BD Wingfield & MJ Wingfield

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

Joseph Obagwu

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

Advisors: L Korsten & T Regnier

Dean Oelofse

Molecular approaches towards anthranose resistance in lupins

Advisors: I Dubery & D Berger

Yolisa Pakela

Interaction between cowpea and *Collectotrichum dematium*

Advisors: TAS Aveling & TA Coutinho

Ida Paul

Mapping and distribution of citrus greening in South Africa

Advisors: A van Jaarsveld & L Korsten

Shadrack Phophi

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

Advisor: L Korsten

Sanuska Reddy

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

Advisors: D Berger & K Denby

Bernard Slippers

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

Advisors: MJ Wingfield, TA Coutinho, BD Wingfield & PW Crous

Ezanne Swanepoel

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

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

Noëlani van den Berg

Resistance mechanisms of Cavendish bananas against *Fusarium oxysporum f.sp. cubense*

Advisors: A Viljoen, D Berger & MJ Wingfield

Albé van der Merwe

Population genetics of *Cryphonectria cubensis*

Advisors: BD Wingfield & MJ Wingfield

Jacque van der Waals

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

Advisors: L Korsten, TAS Aveling & T Regnier

Madel van Eeden

Monitoring biocontrol systems under commercial conditions

Advisors: L Korsten & F Wehner

Lynelle van Emmenes

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

Advisor: A-M Oberholster

Schalk van Heerden

Studies on *Cryphonectria cubensis* in South Africa

Advisors: MJ Wingfield, O Preisig & BD Wingfield

Chantel van Niekerk

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

Advisors: A-M Oberholster & K Kunert

Eduard Venter

Host resistance in South African *Pinus* spp.

Advisors: A-M Oberholster, BD Wingfield & MJ Wingfield

Marinda Visser

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

Advisors: A Viljoen, MJ Wingfield, BD Wingfield & T Gordon

Gezachew Weidemichael Assefa

Xanthomonas campestris pv. *musacearum* associated with onset 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 water sanitizers

Advisors: N Labuschagne & FC Wehner

Tessa Bandounas

Infection studies on white rust of sunflower

Advisors: A Viljoen & TAS Aveling

Raksha Bhoora

Genetic transformation of *Eucalyptus* clones

Advisors: BD Wingfield, MJ Wingfield, D. Berger & P. Chimwamurombe

Jane Boshoff

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

Advisors: N Labuschagne & L Korsten

Alicia Bouwer

Development of a food safety assurance system for the fruit packing industry

Advisor: L Korsten

Carrie Brady

Molecular typing of *Pantoea ananatis* from different hosts

Advisors: SN Venter & TA Coutinho

Brenda Buthelezi

Population study on a *Cylindrocadium* sp. associated with *Eucalyptus* in South Africa

Advisors: TA Coutinho, O Preisig, MJ Wingfield & PW Crous

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

Oliver Dickens

Somatic embryogenesis in the *P. elliotii* x *P. caribae* hybrid

Advisors: A-M Oberholster & C Bornman

Mapula Julia Domola

Survey, indexing and serological detection of sweet potato viruses in South Africa

Advisors: TAS Aveling & G Thompson

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

Anton Fourie

Mechanisms of resistance in citrus rootstocks against fungal pathogens

Advisors: N Labuschagne, Z Apostolides & D Berger

Nonnie Geldenhuys

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

Advisors: MJ Wingfield & J Roux

Veloshinie Govender

Evaluating biological control systems for mango postharvest disease control

Advisor: L Korsten & T Regnier

Izette Greyling

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

Advisors: TA Coutinho, SN Venter & MJ Wingfield

Edzard Grimbeek

Management strategies for panama wilt disease of banana in South Africa

Advisors: A Viljoen, TA Coutinho & MJ 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

Advisors: L Korsten

Ronald Heath

Disease of Myrtaceous and Melastomataceous hosts

Advisors: MJ Wingfield, J Roux & BD Wingfield

Zhou Honghai

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

Advisor: AA Myburg

Stephan Honibal

Biocontrol of False Codling Moth, *Cryptophlebia leucotreta* (Meyr.) (Lepidoptera: Tortricidae)

Advisors: P Govender & A Schoeman

Brett Hurley

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

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

Charline Kamburona

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

Advisors: A-M Oberholster & A Cilliers

Elizabeth Kola

Seed pathology of bambara groundnuts

Advisors: TAS Aveling & T Regnier

Begashaw Leulseged

The potential of rhizosphere microflora in the biocontrol of root rot and growth enhancement of lettuce (*Laetuca sativa*)

Advisors: L Korsten, F Wehner & N Labuschagne

Natalie Levendall

Delivery of lysozyme to plant cell surfaces using the bean polygalacturonase inhibitor protein (PGIP)

Advisor: D Berger

Lorenzo Lombard

Pathogens associated with hydroponically grown eucalypts in South Africa

Advisors: TA Coutinho, MJ Wingfield & B Janse

Therese Lotter

Characterisation of a polygalacturonase gene encoding a possible virulence factor from the lupin antracnose fungus *Colletotrichum acutatum*

Advisor: D Berger

Lara Mansfield

The bioeconomics and control of scarabaeid pests attacking inland sugarcane and forestry in KwaZulu/Natal

Advisor: P Govender

Lance Maphosa

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

Advisors: BD Wingfield, MJ Wingfield & E Mwenje

Celia Martinze

Stability of Bt toxin in transformed tobacco under drought

Advisor: K Kunert

Fhumalani Mashau

Risk assessment of fire blight on pears

Advisors: L Korsten & C Pistorius

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

Advisor: A-M Oberholster

Patrick Mphahlele

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

Advisors: L Korsten & RM Crewe

Awelani Mutshembele

Development of a DNA-based identification technique for *Fusarium circinatum*

Advisors: BD Wingfield & MJ Wingfield

Barbara Nel

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

Advisors: A Viljoen, C Steinberg & PS van Wyk

Marie Onanena

Isolation of labile regions from wild oat

Advisors: K Kunert & C van der Vyver

Draginja Pavlic

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

Advisors: TA Coutinho, MJ Wingfield & B Slippers

Anneke Prins

The protective role of oryzacystatin-I during abiotic stress

Advisors: K Kunert & A-M Oberholster

Nditsheni Rabambi

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

Advisors: N Labuschagne, Z Apostolides & G Thompson

Koreen Ramessar

Transformation of maize with antifungal genes towards engineering resistance to *Fusarium verticillioides*

Advisors: D Berger & M O'Kennedy

Martin Ranik

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

Advisor: AA Myburg

Wayne Rathbone

Development and assessment of biotechnology curriculum for high school scholars

Advisors: BD Wingfield & MJ Wingfield

Carlos Rodas

Diseases of plantation forest trees in Colombia

Advisors: MJ Wingfield, J Roux & TA Coutinho

Mashudu Silimela

Alternative methods for preventing pre- and post-harvest diseases and sunburn on mango fruits

Advisor: L Korsten

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

Dirk Swanevelder

A population study on the genus *Clivia* using microsatellite markers

Advisor: A-M Oberholster, BE van Wyk & M vd Merwe

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

Busi Tshabalala

Population and pathogenicity studies of *Lasiodiplodia theobromae* in South Africa

Advisors: TA Coutinho, MJ Wingfield & W de Beer

Itani Tshivhandekano

Water quality in Gauteng contributing to food safety risk in agriculture

Advisor: L Korsten & W du Plooy

Americo Uaciquete

Epidemiology, control and germplasm screening for powdery mildew, *Oidium anacardii*, of cashew in Mozambique

Advisors: L. Korsten & TAS Aveling

Marilize van Wyk

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

Advisors: MJ Wingfield & BD Wingfield

René van Zyl

Transformation of Cavendish bananas for Fusarium wilt resistance

Advisors: A Viljoen & J-V Escalant

Fanie Verwey

Control of *Pythium* root disease of hydroponically grown lettuce

Advisors: N Labuschagne & FC Wehner

Juan Vorster

The application of representational difference analysis and plant differentiation

Advisors: K Kunert & A-M Oberholster

Joanne Weich

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

Advisors: D Berger & TA Coutinho

Anita Willis

Status of *Guignardia mangiferae* in South African mango orchards

Advisors: L. Korsten & P. Labuschagne

4th year and honours students

Kobus de Wet (2001)

Andrew Gallagher (2001)

Leylani Grobler (2001)

Susan Groenewald (2001)

Thuto Maria Matshololo (2001)

Lorenzo Lombard (2001)

Barbara Nel (2001)

Deshni Pillay (2001)

Wimpy Prozesky (2001)

Gladys Ramotshodi (2001)

Jenny Smith (2001)

Besrat Tesfagiorgis (2001)

Gerda Vermeulen (2001)

Kevin Barry (2002)

Carrie Brady (2002)

Elsie de Meyer (2002)

Louise Killen (2002)

Karen Muller (2002)

Ronnie Nelson (2002)

Madelien Wessels (2002)

Aneen Belgrove (2003)

Nicky Creux (2003)

Rosita Endah (2003)

Frank Maleka (2003)

Zelda Pieterse (2003)

Innocentia Phuto (2003)

Bernice Porter (2003)

Robert Walters (2003)

Student assistants

Jake Darby (2001)

Marius Bisset (2002)

Erika Boshoff (2002)

Nicky Creux (2002; 2003)

Robert Walters (2002)

Muhammed Ebrahim (2003)

Vincent Kekana (2003)

Duncan Newman (2003)

Luke Solomon (2003)

Recent graduates**PhD****Percy Chimwamurombe (2001)**

Molecular plant-pathogen interactions with special reference to *Eucalyptus grandis* polygalacturonase inhibiting proteins and fungal polygalacturonases

Advisor: BD Wingfield

Co-advisors: MJ Wingfield & A-M Oberholster

Henriette Britz-van Heerden (2002)

Taxonomic, genetic and molecular studies on *Fusarium* spp. within the *Gibberella fujikuroi* complex

Advisor: MJ Wingfield

Co-advisors: TA Coutinho, BD Wingfield and WFO Marasas

Ntsane Moleleki (2002)

Hypovirulence in the pine pathogen, *Sphaeropsis sapinea*

Advisor: MJ Wingfield

Co-advisors: O Preisig and BD Wingfield

Esme van Jaarsveld (2002)

Studies on *Phytophthora nicotianae*, the cause of black shank of tobacco

Advisor: MJ Wingfield

Co-advisor: BD Wingfield

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

Christell van der Vyver (2003)

Stress induced genomic changes in plants

Advisor: K Kunert

Co-advisor: C Cullis

Prem Govender (2003)

Impact assessment of soil pests affecting the establishment of wattle, eucalypts and pine seedlings in South Africa

Advisor: C Scholtz

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-advisors: MJ Wingfield

MSc

Adriana Jacobs (2001)

The genus *Phialocephala*: a taxonomic study

Advisor: MJ Wingfield

Co-advisors: K Jacobs & BD Wingfield

Jo-Marie Lottering (2001)

Identification and characterization of markers linked to the leaf rust resistance gene *Lr41*.

Advisor: A-M Oberholster

Co-advisor: FR Kloppers

Christiaan Troskie (2001)

Identification and characterization of markers linked to the leaf rust resistance gene *Lr37*.

Advisor: A-M Oberholster

Co-advisor: FR Kloppers

Jackie Doyle (2001)

Determining gene flow, linkage and parental contribution in *Pinus elliottii* x *P. caribaea* hybrids

Advisor: A-M Oberholster

Co-advisor: BD Wingfield

E Botes (2001)

Molecular characterization of toxin-producing and non-toxin producing strains of *Microcystis aeruginosa*

Advisor: JU Grobbelaar

Co-advisor: A-M Oberholster

Lieschen Bahlmann (2002)

Russian wheat aphid (*Diuraphis noxia*) induced gene expression

Advisor: A-M Oberholster

Co-advisor: P Govender

Wilhelm de Beer (2002)

The occurrence of Ophiostomatoid fungi on wood and wood products in South Africa

Advisor: MJ Wingfield

Co-advisor: BD Wingfield

Irene Barnes (2002)

Population, taxonomic and phylogenetic studies on *Ceratocystis fimbriata*

Advisor: MJ Wingfield

Co-advisor: J Roux and BD Wingfield

Gavin Hunter (2002)

Mycosphaerella leaf blotch of Eucalyptus in South Africa

Advisor: MJ Wingfield

Co-advisors: J Roux, TA Coutinho, PW Crous & BD Wingfield

René Jacobs (2002)

Characterisation and identification of *Botryosphaeria* spp. from mango in South Africa

Advisor: L Korsten

Co-advisors: MJ Wingfield and B Slippers

Karen Surridge (2003)

Fungi associated with banana leaf diseases in South Africa

Advisor: A Viljoen

Co-advisor: PW Crous

Rodrigo Ahumada (2003)

Diseases of commercial *Eucalyptus* plantations in Chile

Advisor: MJ Wingfield

Co-advisors: BD Wingfield and G Hunter

Grace Nakabonge (2003)

Diseases associated with plantation forestry in Uganda

Advisor: J Roux

Co-advisors: TA Coutinho and MJ Wingfield

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

Anton Jordaan (2003)

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

Advisor: A-M Oberholster

MSc (Agric)/MinstAgrar

Wilma van Broekhuizen (2002)

Detection and suppression of *Ralstonia solanacearum*, causal agent of potato bacterial wilt, in naturally infested soil

Advisor: L Korsten

Co-advisor: P Hammes

Margareth Schoeman (2002)

Comparative studies on *Dothiorella* on avocado

Advisor: L Korsten

Co-advisor: G Swart

Hendrik Sithole (2002)

Integrated pest management of the banana weevil, *Cosmopolites sordidus* in South Africa: a critical review of the literature

Advisor: P Govender

Co-advisor: A Viljoen

Karin Louw (2002)

Antimicrobial activity of indigenous bulbous plant extracts to control selected pathogens

Advisor: L Korsten

Co-advisor: T Regnier

Prestigious NRF bursary holders

Martin Coetzee

Eduard Venter

Lynelle van Emmenes (née Lacock)

Marinda Visser

Marieka Gryzenhout

Noëlani van den Berg

Irene Barnes

Gavin Hunter

Almuth Hammerbacher

Dirk Swanevelder

Ezanne Swanepoel

Johan De Graaf

Shadrack Phophi

Franco du Preez

Aaron Klug scholarship

Juanita de Wet

Mellon Foundation grants

Ntsane Moleleki

Bongani Maseko

Martin Coetzee

Bernard Slippers

Jacque van der Waals

Eduard Venter

Christell van der Vyver

Albé van der Merwe

Lynelle van Emmenes (née Lacock)

Lieschen de Vos

Sanuska Reddy

Gavin Hunter

Irene Barnes

Noëlani van den Berg

NRF scarce skills scholarships

Carrie Brady (2002; 2003-2004)

Elsie de Meyer (2002)

Rene van Zyl (2002; 2003-2004)

Izette Greyling (2003-2004)

Other scholarships

Andrew Kiggundu (Rockefeller Foundation)

Sinnia Kappindu (IITA)

Josephine Muklibi (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)

Appolinaire Adandonon (IITA, Nigeria)

Joseph Ndunguru (IITA, Nigeria)

Ntsane Moleleki (Claude Harris Foundation)

Karin Jacobs (University of Pretoria; L'Oreal Award)

MANAGEMENT

Management committee

Professor MJ Wingfield (Chairman)
Professor BD Wingfield
Professor K Kunert
Professor L Korsten
Assoc Professor D Berger
Assoc Professor A-M Oberholster
Assoc Professor TAS Aveling
Assoc Professor TA Coutinho
Dr A Viljoen
Dr Z Myburg
Dr G Marais
Dr N Labuschagne
Dr J Roux
Dr P Govender
Mr B Hurley
Eduard Venter (Postgraduate student representative 2001)
Schalk van Heerden (Postgraduate student representative 2002)
Gavin Hunter (Postgraduate student representative 2003)

Advisory committee

Professor R Crewe, 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
Assoc 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 C Machethe, Head of the Postgraduate School for Agriculture & Rural Development
Professor M Wingfield, Chairman

Board of Control

Dr C Seele, Chairman of NCT, Vice-President of the South African Wattle Growers Union, Director of CTC
Mr M Edwards, Executive Director, Forest Owners Association
Prof J Malherbe, Vice-Rector (Research)
Prof R Crewe, Dean of the Faculty of Natural and Agricultural Sciences
Prof MJ Wingfield, Director of FABI

Some social highlights in FABI 2002/2003

Annual SPOOF* meeting

*Society for the Publication of Outrageous Findings
Theme: Vikings



Sonja de Beer, Jana and Bernard Slippers



Rodrigo Ahumada, Sonja and Wilhelm de Beer and
Rose Visser (front)



Irene Barnes, Rodrigo Ahumada, Rene Jacobs and
Dilzara Agayehu

Year end function 2002



Carlos and Claudia Rodas, Maria-Noel Cortinas and Michael Cunningham



Stéfan Fouché and Joanne Weich, Sanusha and Tyrell Naidoo and Danie Theron



Back row: Irene and Kevin Barnes, Bongani Maseko, Gavin de Vos, Ronald Heath

Front row: Jolanda Roux, Gavin Hunter, Barbara Nel, Lorenzo Lombard and Leon Labuschagne

Is FBI becoming "FABYLON"?

A stranger walking through the passages of the Forestry and Agricultural Biotechnology Institute (FABI) on the main campus of the University of Pretoria, might wonder if he/she has not perhaps rediscovered the Babylon of old? This impression would arise due to the large number of foreign Post-doctoral fellows, Masters and PhD students that populate FABI. Of course, the many South Africans speaking a wide array of local languages contribute to this, not unpleasant, cacophony. In September this year, an informal survey was conducted on the FABITEAM listserver to determine exactly how many languages are currently spoken in the Institute. Of the 66 people who responded to the questionnaire, all but one FABIANS, speaks two or more languages. And it is known that the one person who recorded that she speaks only English, understands Afrikaans perfectly well! The person who speaks the most languages in FABI, is South African PhD student Bongani Maseko, who speaks nine languages. Then there are seven FABIANS who each speak six languages, five speak two languages, four speak four languages, twelve speak three, and the remaining 34 speak two languages. The languages spoken (numbers of people in brackets) included: from South Africa: Afrikaans (30), Zulu (5), Xhosa (4), S-Sotho (4), Tswana (4), N-Sotho (3), Swazi (2), Ndebele (1), Hindi (1). The number of Afrikaans speaking people seems high, but this is mainly because almost all the African language speakers also speak Afrikaans as a second or third language, while very few of the Afrikaans speaking students speaks any of the native African languages. FABIANS from the remainder of Africa speak the following languages: Amharic (3), Oromigna (2), Swahili (1), Luganda (1), Shona (1), Tonga (1), Chewa (1), Ndebele (1), Herero (1). Those from Europe and Asia: German (7), French (3), Azerbaijanian (1), Russian (1), Turkish (1), Serbian (1), Portuguese (1), Italian (1), Chinese (1). FABIANS from South America: Spanish (6). All of the respondents speak English, which is also the operational language in FABI. In celebration of the international flavour in FABI, the FABI Christmas card for the 2002 festive season include the season's greetings in 22 of the 28 languages spoken in this very multicultural institute.



FABI Christmas Card 2002

