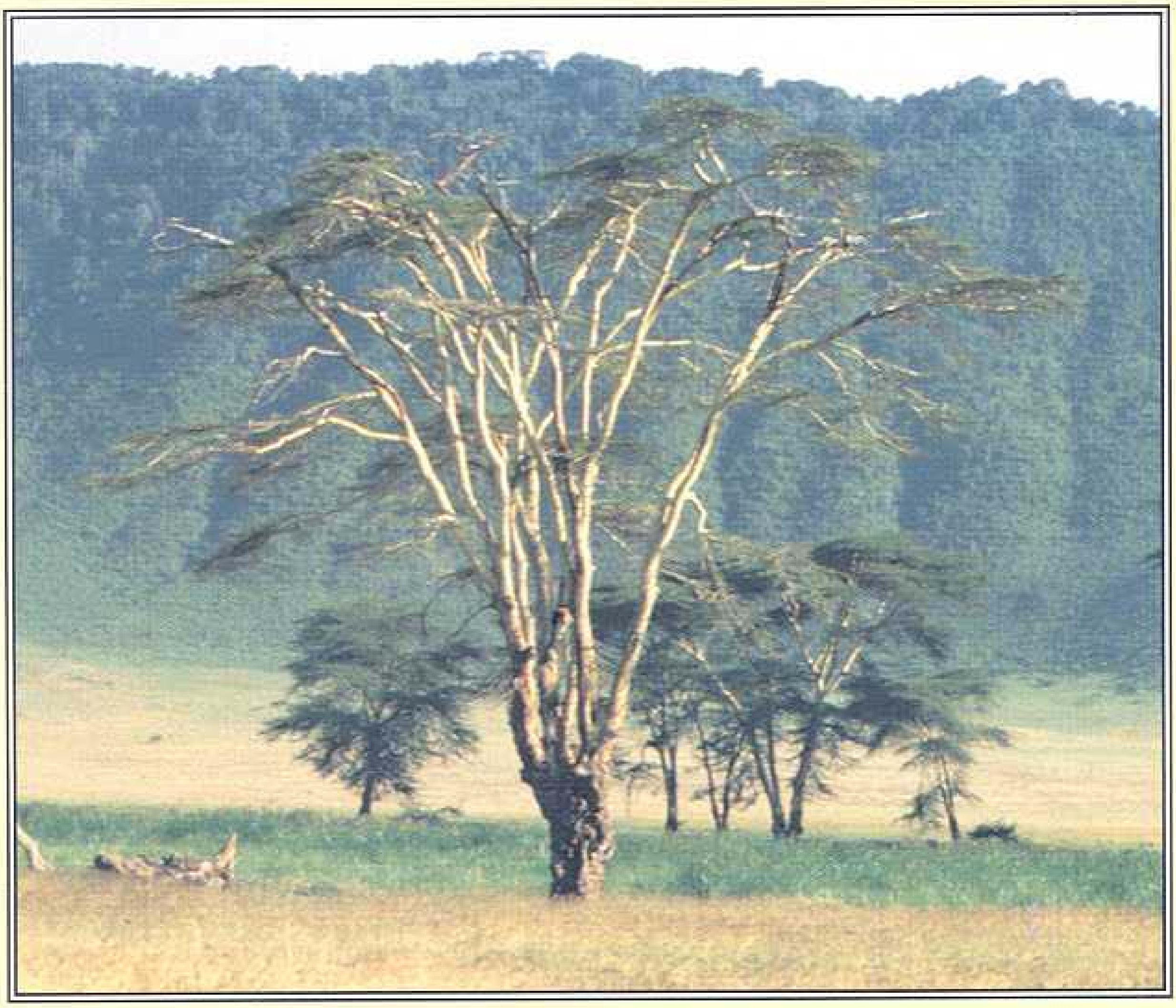
FABI

Forestry and Agricultural Biotechnology Institute



Biennial Report 1998 / 1999

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 calibre, 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
- Promote the future development of FABI and the University of Pretoria

The association of FABI with the largest University in South Africa provides access to enormous 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 the 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 the 13th of March 1999. This first FABI biennial report thus covers the period from April 1998 to August 1999. Future reports will cover a two year period from September 1999 to September 2001.

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FABI

Forestry and Agricultural Biotechnology Institute
FUTURE FORESTS and FOOD

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Director's Report

It gives me tremendous pleasure to provide this introductory statement to accompany this first biennial report for FABI. This is particularly true because FABI has had such an exciting birth and early life. Indeed, it would be fair to say that this process has totally exceeded all expectations.

The idea to establish FABI emerged in early 1997 after discussions amongst interested role players at the University of Pretoria, partners in the South African forestry industry and other industries linked to the broader field of plant sciences. In a remarkably short period of time, plans to establish a significant physical infrastructure were completed, the building process was begun and the necessary human resources to undertake relevant research projects were acquired. By the end of 1998, the first research staff and students attached to FABI were already becoming settled and scientifically productive.

There are some conflicting views as to when FABI actually began. I think it is well recognised and commonly known that a major research programme on which FABI was established is the Tree Pathology Co-operative Programme (TPCP). Thus, we might consider the arrival of the business office of the TPCP in Pretoria in April 1998 as a formal birth date for this exciting new Institute. Having said this others might prefer to look to the formal inauguration of FABI in March 1999 as an appropriate starting point. Whatever time we might choose as the birth date for FABI, the fact remains that the Institute has become one of the most significant ventures in plant biotechnology in South Africa.

Establishing an entirely new research Institute is a process filled with excitement and challenge. Certainly, we have very rapidly discovered that, while facilities and equipment are essential considerations, establishing a firm atmosphere of learning and culture of scientific investigation is most important. What was required was the establishment of regular events such as a seminar series, journal clubs and similar events. Doing so in FABI has been a truly successful exercise that is already paying great scientific dividends.

Establishment of FABI has required much more than entrenching a culture of learning. It has been necessary to find an appropriate management system for the Institute. As director of FABI, I have been fortunate to have the support of a fantastic group of colleagues that now constitute the Management Committee of the Institute. The Management Committee of FABI has the support of the Advisory Committee that is led by Professor Robin Crewe, Dean of Science, and the Heads of the Departments of Microbiology and Plant Pathology, Zoology and Entomology, Genetics, Botany, Biochemistry, Plant Production as well as the Director of the Institute for Rural Development.

The research programmes of FABI have grown beyond our wildest expectations. While the TPCP and the Forest Molecular Biology Programme (FMBC) were the foundation research programmes, numerous other programmes were rapidly added to FABI in a short period of time. It is impossible in this short introduction to treat all of these. I do wish to briefly mention some exciting **new** initiatives that have emerged subsequent to the establishment of FABI:

- AECI PLANT BIOTECHNOLOGY
  Shortly after the establishment of FABI, AECI made a decision to terminate its plant biotechnology programme. After some negotiation, this programme was moved to FABI.

- PANAMA WILT DISEASE PROJECT
  Late in 1998, FABI entered into a partnership with the Banana Growers Association (BAGASA), to reduce the impact of the devastating panama wilt disease. This project has already grown substantially and now also includes a focus on Sigatoka disease.

- FOREST ENTOMOLOGY
  Early in 1999, negotiations with major players in the forestry industry to expand FABI's tree protection activities to include entomology were undertaken. The team of the TPCP has already included some entomological work and this expansion marks a logical augmentation of current activities. Significant opportunity also exists to gain significant synergy between forest pathology and forest entomology activities of the TPCP.

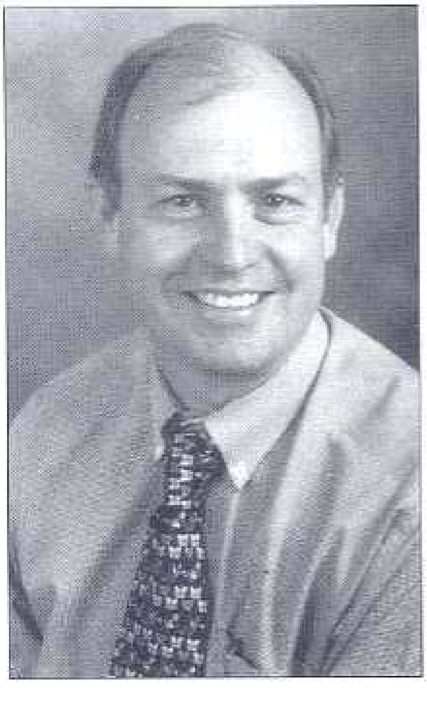
- ADVANTA BIOTECHNOLOGY
  Starting in 2000, FABI will begin a significant research collaboration with the International seed company ADVANTA. This programme will focus on the biotechnology needs of ADVANTA and will aim to produce new products and to train students.

- POSTHARVEST TECHNOLOGY
  During 1999, a new postharvest technology forum was established focusing on postharvest research, development and training through the CSIR-UP convergence.

Being based in a University environment means that FABI must have a distinct focus on education. Thus, while all research projects have clear practical objectives, we also maintain a distinct long term view and a commitment to high quality basic science. In this way, FABI is also able to provide outstanding training for postgraduate students in the broad field of plant sciences. Thus, FABI boasts a significant group of postgraduate students including 20 M.Sc., 26 Ph.D. and 6 post-doctoral fellows.

I am convinced that FABI will grow from strength to strength. Future issues of this report will track the growth and development of FABI.

Michael J. Wingfield
Mondi Professor of Forest Pathology and Director of FABI and the TPCP



RESEARCH REPORTS

Tree Pathology Co-operative Programme (TPCP)

Michael Wingfield, Teresa Coutinho and Jolanda Roux

The Tree Pathology Co-operative Programme (TPCP) is a collaborative venture between the South African Forestry Industry and the University of Pretoria. The TPCP was established in 1990 and has a proven track record in resolving forest tree disease problems. The services offered to the members of the programme include the maintenance of an active and reliable diagnostic clinic, a research programme focused on diseases of priority, monitoring of diseases to gain a perspective of their relative importance, an extension service through which foresters are informed on strategies to reduce the impact of diseases, and education at the undergraduate and postgraduate level. Successes achieved over the past year can be summarised as follows:

Diagnostic Clinic

The clinic provides a reliable, responsible and reasonably rapid diagnoses of disease problems of forest trees. Thus, samples that come to the clinic - and numbers of these have increased steadily over the years - are subjected to a range of reasonably complex tests prior to a diagnosis being provided. Diagnostic services function through foresters submitting samples to the Programme for analysis. Isolations for pathogens are conducted from plant material, soils and water, using state of the art techniques.

From January to the end of December 1998 the clinic received a record number of 168 samples. This is substantially more than the 126 samples received in 1997. In April alone, the month that we moved into the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, we received a total of 30 samples. This year, the majority of samples were from pine (43.5%) followed by eucalypts (32.2%). In previous years we had tended to receive an equal proportion of pine and eucalypt samples. We also conducted analyses of a number of water, growth media and seed samples for the presence of pathogens.

Culture Collection

The culture collection of the TPCP is one of the most precious reference sources for the South African Forestry Industry. It is also the largest, single collection of fungi associated with disease from woody hosts in the world. In years to come, cultures - many that have originated from routine diagnostic work - will be used for comparisons and to derive information on changes that might have occurred in populations of pathogens.

Extension Services

Field studies, monitoring of disease problems and general extension services make up a key component of the TPCP. Maintaining an effective disease-monitoring programme is beset with difficulties. A decision some years back was that the most effective means of doing so would be through the regular inspection of permanent sampling plots. These would also need to be distributed throughout plantations in such a way that they represent the diversity of species, clones and age classes of trees. Monitoring should be conducted by company staff and the TPCP together with the MMRC (Mensuration and Monitoring Research Consortium). The MMRC would provide support in the form of data analysis, verification of field observations and disease monitoring. In order to assist foresters with the monitoring function, the TPCP has produced a series of guidelines and pamphlets outlining desired disease monitoring techniques. In addition, the MMRC, with the help of the TPCP, has compiled a field-size booklet to assist foresters. In addition the TPCP has made contributions to various newsletters (ICFR, NCT etc) and also produces two regular issues of Tree Pathology News, which is the Newsletter of the TPCP.

Field trips to plantations to conduct experimental work, as well as to present extension lectures, were undertaken regularly during the year. There were a slightly lower number of person days spent in plantations (in excess of 400), this was not due to a reduced number of field trips. Rather, it is linked to the fact that a greater number, but shorter trips, including fewer staff, were undertaken. The fact that fewer people undertook field trips is directly linked to the time that had to be spent establishing the new FABI facilities.

Research

The research component of the TPCP has remained multi-faceted. Thus, research of an immediate problem-solving nature is an important component of the Programme. In addition, medium and longer term research is being conducted that will ensure that, in terms of forest protection and biotechnology, the South African Forestry Industry will remain at the forefront of the field. Invitations to provide reviews on various aspects of tree health such as a recent review for a book on pine diseases by the American Phytopathological Society reflects the status of the Programme. In this report, a brief summary of the status of important disease problems was provided. This should also serve to illustrate the breadth and depth of research programmes linked to the TPCP.

The following section provides a condensed review of various research activities. The focus is clearly on highlights and most important findings.

Cryphonectria canker of *Eucalyptus*

Cryphonectria canker, caused by *Cryphonectria cubensis*, remains one of the most serious diseases of eucalypts in South Africa. The disease occurs in all sub-tropical forestry areas although it is much less common, now that disease tolerant clones have been extensively planted in these areas. Screening trials conducted by the TPCP have contributed greatly to selection of disease tolerant clones and thus the reduction of impact of the disease. The causal agent of the disease remains one of the most virulent pathogens known to forestry and ongoing efforts to reduce its impact must continue.

Cracked tree root collar typical of infection by *C. cubensis* on older trees

The TPCP has now conducted research on *C. cubensis* for ten years and the group is internationally recognised for contributions leading to a more comprehensive understanding of the pathogen. One of the focus areas for our research lies in developing biological control agents for Cryphonectria canker. In this regard we have a sincere interest in exploiting opportunities linked to dsRNA-mediated hypovirulence. Some of the advances that have been made in our efforts to reduce losses due to Cryphonectria canker are summarised in a review from an invited presentation at a recent IUFRO symposium on Tree Phytophage interactions in France.

Die-back caused by *Sphaeropsis sapinea*

Die-back caused by *Sphaeropsis sapinea* is the most serious disease of pines in South Africa. This is ironical when one considers that the causal agent is an opportunistic fungus which is less aggressive than pathogens such as *C. cubensis* or the pitch canker fungus *Fusarium subglutinans* f.sp. *pini* (= *F. circinatum*). As has been stated in previous reports, *S. sapinea* owes its notoriety in South African to the combination between susceptible trees (*P. patula* and *P. radiata*) and an environment (hail storms in summer) that is conducive to disease.

One of the most fascinating discoveries emerging from TPCP studies in recent years is that *S. sapinea* is represented by a highly diverse population in South Africa. This is completely contrary to the expectation that introduced (exotic) pathogens, and particularly those such as *S. sapinea* that only reproduce asexually, would have very limited genetic diversity. This is attributed to the fact that the pathogen has probably been introduced into South Africa a number of times from a wide variety of sources. These repeated introductions probably reflect the seed borne nature of the fungus and the fact that great volumes of pine seed have been introduced into the country during the course of the last Century.

Symptoms associated with infection of lumber with *S. sapinea*

One of the most exciting focuses of the TPCP is linked to attempts to reduce the impact of *S. sapinea* through hypovirulence. During the course of the last five years, students in the group have discovered the presence of dsRNA viruses in this fungus. These viruses have the potential to infect *S. sapinea* and to reduce its pathogenicity. During the course of the past year, we have completed a first phase of this project to fully characterise the virus infecting *S. sapinea* at the molecular level. It was consequently discovered that what was thought to be a single virus is actually two viruses, which we now know as SSRV1 and SSRV2. Studies are currently underway to screen a large population of the fungus to determine the distribution of the viruses. Another aim is to develop techniques to transfect healthy isolates of the fungus for later use in biological control trials.

Amongst the most useful strategies to reduce losses due to *S. sapinea* infection is through the planting of disease-tolerant stock. The TPCP is currently involved in a long-term programme to identify disease tolerant individuals among 200 selected progeny of *P. patula* and *P. radiata* from in two highly desirable families that belong to SAFCOL. A further aim of this project is to develop rapid screening techniques such as marker aided selection that will be useful to the entire forestry industry or future selections.

Sirex Wood Wasp

Subsequent to the appearance of the Sirex wood wasp, *Sirex noctilio,* in South Africa, the TPCP engaged in an intensive study on its fungal symbiont, *Amylostereum areolatum*. A comprehensive review of the literature has been conducted and this should be valuable to foresters. Some of the most important questions in this project have been to:

***Sirex noctilio*, wood wasp**

- Confirm at the molecular level that the fungus is the same as that found elsewhere in the world.
- Determine the genetic diversity of the fungus in South Africa and thus evaluate how many introductions of the wasp might have occurred here.

- Attempt to determine the origin of the wasp in South Africa and thus contribute to our understanding of threats and likely sources of pathogens in the future.

Data from these studies have emerged rapidly and indicate that the South African *S. noctilio* population probably had its origin in South America. The fungal symbiont is clonal in South Africa and a single or very limited introduction is certain to have occurred. Futher details will be presented in the next report of the TPCP.

Pitch Canker of Pines

Pitch canker of pines, caused by *Fusarium subglutinans f.sp. pini* (= *Fusarium circinatum*), is one of the most serious pathogens these trees where they are grown as exotics in plantations. Some of the most fundamental recent information pertaining to the disease have emerged from studies conducted in South Africa. This is ironic because the disease, in the sense of cankers on mature trees, does not occur in this country. Thus far, the pathogen has remained restricted to nurseries where it can cause severe damage.

Die-back symptoms caused by the pitch canker fungus

One of the most interesting aspects of the pitch canker fungus in South Africa is the fact that isolates have been shown to readily undergo sexual recombination in the laboratory. We have recently shown that this is due to the fact that the local population has a high degree of female fertility. A practical consequence of this discovery is the fact that the fungus population is likely to be genetically diverse in South Africa and that management strategies will be hampered by this. Furthermore, these and other findings suggest strongly that the pathogen was introduced into South Africa from Central America and not the United States. This presumably occurred on imported seed.

Armillaria Root Rot

Armillaria root rot is one of the better-known diseases of pines (to a lesser extent eucalypts) in South Africa. Losses due to the disease are not great, but they continue to occur and the TPCP is regularly called upon to deal with the disease. From an international perspective, Armillaria root rot is one of the best known and most important diseases of woody plants. Thus, peers elsewhere in the world also expect members of TPCP to focus some attention on the problem.

Intensive research has been conducted on Armillaria root rot in recent years and it has been shown that, in the Northern Hemisphere, the disease is caused by more than ten species. The species responsible for the pine disease in South Africa has remained obscure. Our recent research has shown that, contrary to popular belief, the pathogen is neither *A. mellea* nor *A. heimii*. It is most likely related to *A. fuscipes*.

Coniothyrium canker of *Eucalyptus*

Coniothyrium canker is an unusual disease that has caused tremendous damage to eucalypt plantings in Natal and northern parts of the forestry region. The disease, caused by *Coniothyrium zuluense*, has resulted in losses of some of the most sought after eucalypt clones. Some extensive plantings have virtually had to be abandoned.

One of the most difficult matters to deal with when working with *Coniothyrium zuluense* is the fact that it grows slowly and it is extremely difficult to identify. The disease has been known only from

South Africa and there is thus no reference literature on which to rely. During the course of the last year, there have, however, been reports of the disease from Thailand, Argentina and Brazil, which imply that the disease is either spreading from South Africa or from some other unknown source. We have confirmed the fact that the fungus occurring in Thailand is the same as that in South Africa. The South American reports remain to be confirmed.

The TPCP, together with members such as Mondi that have experienced serious losses due to Coniothyrium canker, has made tremendous progress in reducing the impact of the disease. Considerable efforts have been focussed on disease monitoring and also on selecting clones that are not susceptible to infection by *C. zuluense*. The current focus of the TPCP is to understand more about the biology of the pathogen, its genetic diversity and factors that affect susceptibility in trees. This disease remains a priority in South Africa and it is necessary to retain a significant focus on it. Breeding trials and programmes of the FMBC to develop marker-aided selection will also include Coniothyrium canker.

Botryosphaeria Canker of *Eucalyptus*

Botryosphaeria canker of eucalypts was unknown in South Africa until 1990. It was, however, most probably present, but not recognised, long before that time. Currently, studies on Botryosphaeria canker are focussed on determining whether *Botryosphaeria dothidea* is the only species associated with the disease. This question arose due to equivocal results that emerged from disease screening trials. We now believe that there are at least two species of *Botryosphaeria* infecting eucalypts and, subsequently to characterisation, these will need to be incorporated into screening trials.

Eucalyptus Leaf Diseases

Eucalyptus leaf and shoot diseases result in occasional losses which, other than in the case of *Mycosphaerella juvenis* on *E. nitens*, are of relatively minor importance. Nevertheless, samples are submitted to the diagnostic laboratory and it is important to have a knowledge of these fungi. For this reason, the TPCP has maintained an active programme of collecting these pathogens from infected eucalypt leaves and shoots to provide a reliable and accurate diagnoses when they do occur. Amongst the key pathogens of eucalypts are *Cylindrocladium* spp. Research on these, in collaboration with Prof. P. Crous (University of Stellenbosch) has continued during the course of the last year. These studies, significantly increase our knowledge of diseases that are likely to become more important in the future. These data are also valuable in terms of international trade and related quarantine issues.

Blotches caused by a *Mycosphaerella* sp. on *Eucalyptus* leaves

Eucalyptus Rust

Eucalyptus rust, caused by *Puccinia psidii*, is one of the most feared diseases of eucalypts worldwide. The pathogen is native to South and Central America and has its origin on native Myrtaceae in that area. It has adapted to be able to infect certain *Eucalyptus* spp., of which *E. grandis* and *E. saligna* are most susceptible.

The importance of Eucalyptus rust has led to a realisation that an international effort is needed to reduce the potential impact of *P. psidii*. For this reason, the TPCP has entered into discussions with colleagues in Australia who share our interest and concern about the disease. During the course of the last three years, we have developed various proposals and reviews to gain funding to work on the disease. At the end of 1998, ACIAR (Australian Centre for International Agricultural Research) approved a phase one research effort. This research will be conducted as a collaborative effort between the CSIRO Division of Forestry, the TPCP and the Federal University of Viscosa, Brazil. Each party will cover costs pertaining to work in areas of particular interest to them. It is hoped that this collaborative approach will lead to a maximum output of knowledge at the lowest possible cost.

Insect Associated Diseases

The interaction between insects and forest pathogens is underestimated and overlooked. Researchers linked to the TPCP have an international reputation for work in this field. Such research sustains significant status and thus funding from groups such as the NRF. This work has continued with a small number of students committed to projects to understand the importance and identity of fungi carried by conifer infesting bark beetles. The work has also been sustained by considerable collaboration with colleagues outside South Africa and with funding from groups such as the USDA Forest Service.

Phytophthora Root Rot

Phytophthora root rot, caused primarily by *P. cinnamomi*, is a serious pathogen on some *Eucalyptus* spp. in South Africa. The TPCP has thus maintained capacity to study this disease. A particular question, which relates to the likely durability of disease tolerance, is whether the pathogen is native or introduced in South Africa. This is a controversial question and is also one of considerable international debate. Through the use of intensive isozyme studies, we have shown that the fungus has a relatively limited genetic base in South Africa. Consequently, we have reached a preliminary conclusion that the fungus was introduced into the country. We are currently considering this question using a more robust RFLP approach and also have conducted extensive pathogenicity tests on eucalypts using a large number of isolates.

Typical Phytophthora root rot symptoms in a plantation

Guava Wilt

Guava (*Psidium guajava*) is a close relative of eucalypts and a host for eucalypt pathogens such as rust. In South Africa, wilt is the most serious disease of Guava. In TPCP studies, it has been shown that the guava wilt pathogen can infect and kill eucalypts. There is concern that field outbreaks of the disease on eucalypts might occur. Very little is known about the guava wilt pathogen and its identity is quite obscure. In order to increase our understanding and our capacity to recognise the fungus, the TPCP has undertaken a study of its taxonomy. Preliminary conclusion are that the pathogen is possibly *Penicillium vermoesenii* (not a true *Penicillium*), which also has the ability to infect members of the Palmaceae. Further studies to determine its natural occurrence on eucalypts are planned.

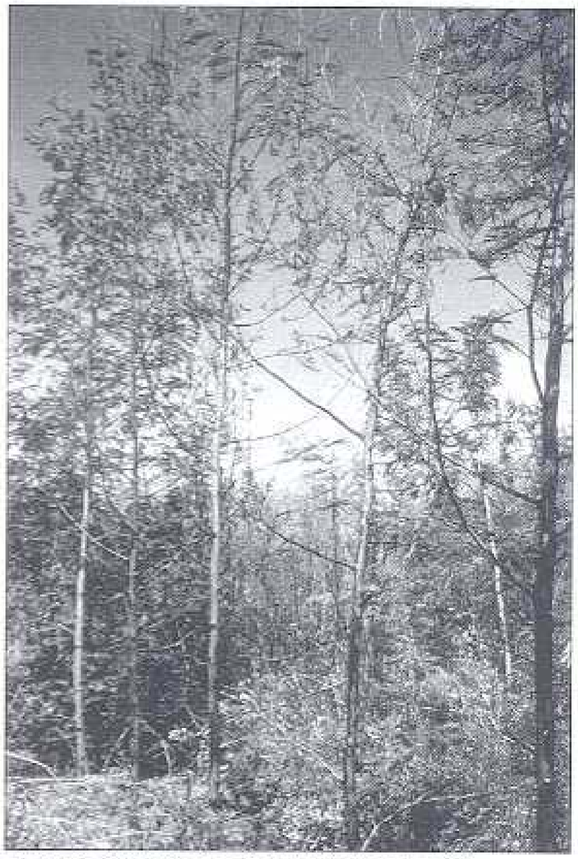
Diseases of *Acacia mearnsii*

The *Acacia mearnsii* (black wattle) industry forms an important component of South

African Forestry. This tree is planted for its high quality pulping timber and its bark from which tannins are extracted. It is also valuable as a nitrogen fixer and many farmers utilize it for soil improvement in rotation with other crops. There are, however, a number of diseases that reduce the success of *A. mearnsii* in plantations.

Ceratocystis wilt, caused by *Ceratocystis albofundus*, is considered to be the most serious disease affecting *A. mearnsii*. This pathogen is capable of killing healthy trees within a short period of six weeks after artificial inoculation. It is currently known only from *A. mearnsii*, *A. decurrens* and native *Protea* spp. in South Africa. Management of this disease relies strongly on management practices in which wounding is kept to a minimum. Investigations into the possible insect vectors of this fungus, as well as its host range and possible impact on other tree species (including native species) are underway. Screening trials of elite *A. mearnsii* families, conducted by the TPCP, have shown that there is great potential for the selection of disease resistant planting material. Research has also been undertaken to develop molecular markers to rapidly identify disease resistant trees.

The most common disease of *A. mearnsii* is caused by *Botryosphaeria dothidea*, the pathogen also found on *Eucalyptus* spp. It is closely associated with environmental stress on the trees, especially frost, and is capable of causing the death of entire stands of susceptible trees. The management and control of this disease is of utmost importance to the TPCP and Industry. *Acacia mearnsii* also suffers from a root disease caused by *Phytophthora nicotianea*. Strategies to manage these three important diseases, and to identify other possible pathogens, are of priority to the TPCP.



Typical symptoms of Ceratocystis wilt

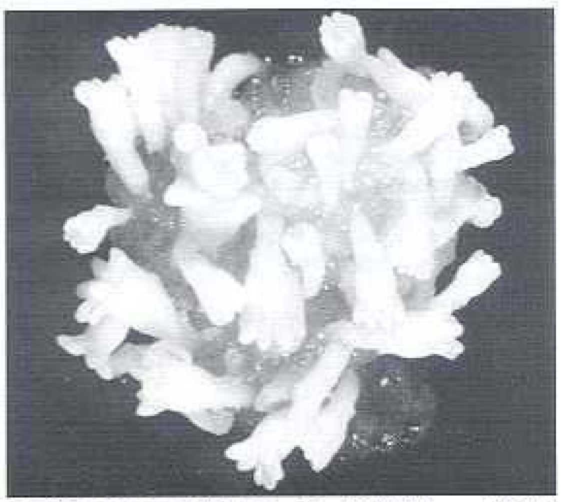
Forest Biotechnology

Anna-Maria Oberholster

Forest trees present a special challenge to the geneticist because of their long reproductive cycles. The capture of genetic gain via asexual reproduction offers a desirable option. In forest tree improvement, clonal propagation is applied using a program of selection, progeny testing and controlled breeding. Conventional methods of tree improvement and selection offer only limited possibilities of meeting growing demands. New and innovative techniques for the creation of new hybrids, early selection and testing of desirable genotypes and the vegetative propagation of selected genotypes must, therefore, be developed to achieve these goals. Vegetative propagation has a clear edge over sexual reproduction as a means of achieving both rapidity of propagation and assured maintenance of the genetic composition of the progeny. Mass propagation of forestry species is mainly achieved via micropropagation (production of whole plants or multiple shoots) and somatic embryogenesis (production of multiple emblings making use of immature zygotes).

Micropropagation is probably the most effective way of producing numerous clones of a desirable genotype. Rooting, however, sometimes proves problematic. Rooting is genotype specific and tissue age also influences the process. Longterm storage of desirable genotypes also poses difficulties. The micropropagation of *Eucalyptus grandis* clones is one of the main objectives of the ongoing forest biotechnology program. The process consists of the initiation, multiplication and rooting of superior clones. Initiation and multiplication of the clones commence on solidified medium. Rooting is achieved in liquid culture, which not only improve the rooting of recalcitrant clones, but also facilitate the hardening-off of plantlets. Screening of the clones to select for DNA and other markers linked to rooting ability and pathogen resistance is also in progress. An indirect correlation has been observed between the secretion of secondary products and poor rooting in some of the genotypes.

Somatic embryogenesis has the advantage that rooting occurs as part of a process that is analogous to the development of zygotic embryos. The embling has the potential to grow into a whole plant, much as a seedling would. Properly controlled somatic embryo-genesis carries a low risk of genetic instability, guarantees juvenile plants of normal growth habit, and is amenable to mass production and planting processes. Storage and multiplication of the genetic material through cryopreservation also proved to have few pitfalls. The emblings, however, need to be tested for desirable traits before they can be commercially applied. The program focuses mainly on the somatic embryogenesis of coniferous species, e.g. *Pinus elliottii* X *P. caribaea* var. *hondurensis*, *P. patula*, *P. radiata*, and makes use of *Picea abies* as a model system. Somatic embryogenesis consists of a number of different stages, which include initiation, proliferation, maturation and hardening-off of the plantlets. To date, the programme has managed to produce numerous *P. abies* emblings and some *P. radiata* emblings. Highlights of the program include the first report of embryonal mass initiation from immature seed outside the so called "window period", which is one of the major obstacles in the process, making the process less seasonally dependent. The first transient expression of marker genes in *P. patula* and *P. radiata* has also been accomplished. This work is done in collaboration with the South African Forestry Company Ltd. (SAFCOL).



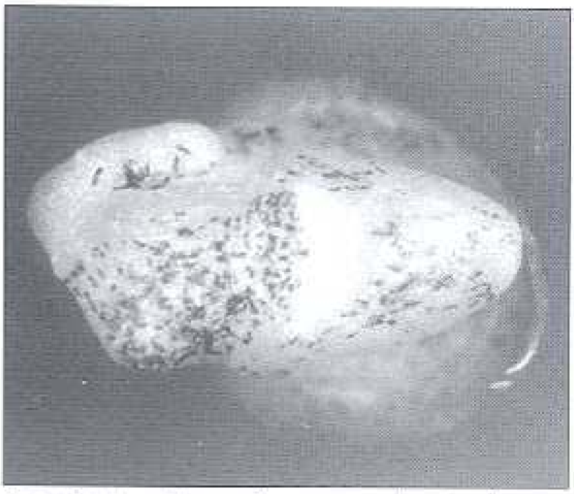
Coniferous emblings in culture. These emblings will grow into mature trees.

Wheat Genomics

Anna-Maria Oberholster

Plant Breeding is essential for the maintenance of the worlds' food supply. Population growth requires the production of more and more food from an environment with a reducing capacity to support plant growth. Genetic improvement of plants is the only option available to supply this demand. Genetic improvement of crops can be accomplished through the application of marker technology to aid breeding programs, and through direct transfer of specific genes. The program focuses mainly on disease and pest resistance in wheat. The main objective of the program is the saturation of the wheat map that will enable us to follow a map-based cloning approach. Screening of suitable cultivars for DNA markers linked to genes that confer resistance to Russian wheat aphid and leaf rust is ongoing. Applied technologies include PCR-RFLPs, RAPDs, AFLPs and microsattelites. Also, preparation and screening of cDNA and gDNA libraries to obtain expressed sequence tags (ESTs) and the genes that confer resistance, is in progress.

Highlights of the program include the identification of several RAPD and SCAR markers linked to Russian wheat aphid resistance and leaf rust resistance. Partial mapping of these markers has also been accomplished. The transformation and regeneration of a large number of South African wheat cultivars have been completed. Recently, we became part of the International Triticale Expressed Tag Initiative (ITEI) and the International Triticale Mapping Initiative (ITMI).



Transient anthocyanin expression in immature wheat embryos, 48 h after bombardment

Forest Molecular Biology Co-operative Programme (FMBC)

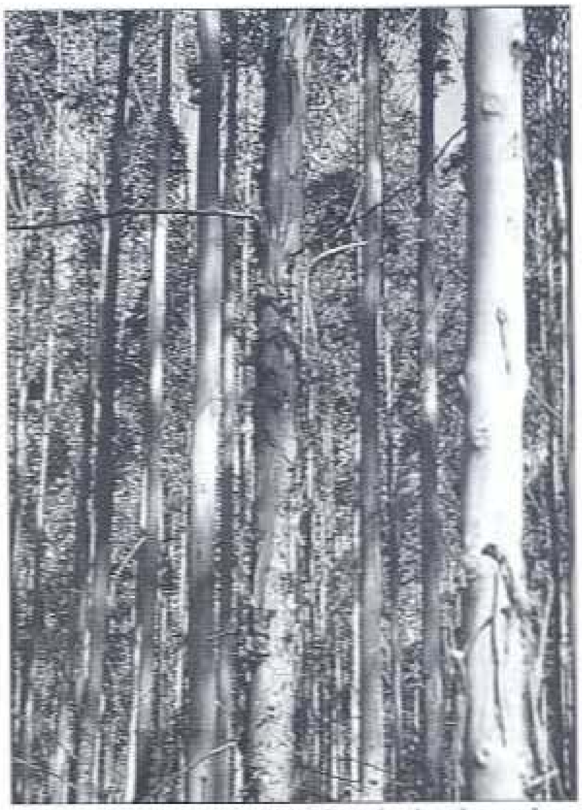
Brenda Wingfield

The FMBC was started in 1994 as an iniative to develop the techniques and technologies to use molecular markers in tree breeding. It is funded by all the major Forestry companies in South Africa. At that stage it was decided to concentrate on a potential marker linked to disease resistance against the canker pathogen *Cryphonectria cubensis*.

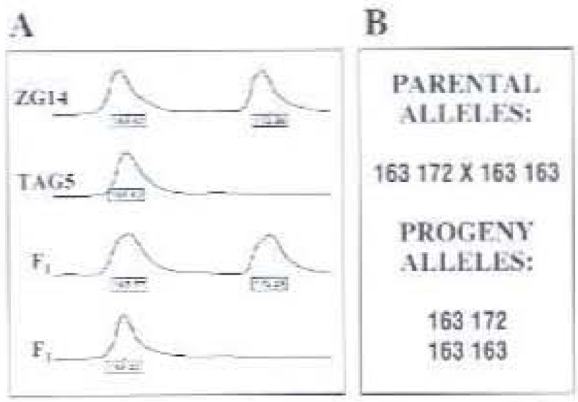
Since the start of the FMBC, we have investigated the use of Random Amplified Polymorphic DNA Sequences (RAPDS) Amplified Fragment Length Polymorhisms (AFLPs) and Micro-satellite or SSRs as potential fingerprinting tools for clonal material. This technology has been well established in a number of laboratories. We have also developed our own markers for South African material.

A number of pedigrees have been developed to provide material on which to develop and test these molecular markers. These include a three generation pedigree which is related to much of the breeding material currently used by Forestry companies in South Africa. There is also a "mini" pedigree which involves a controlled cross between a clone that is highly susceptible to the canker pathogens *Cryphonectria cubensis* and *Coniothyrium zuluense* and one that is tolerant to these fungi.

This year the FMBC has become involved in an international initiative to develop micro satellite markers to fingerprint *Eucalyptus* species. We now hope to develop an internationally recognised set of primers to identify the most commonly used commercial species.



Tolerant and susceptible *Cucalyphs* clones in a plantation



(A) Amplified micro-satellite alleles (FMRSA3) from the parents (TAG5 and ZG14) and from two F1 progeny, visualized with the Genotyper 2.1 software
(B) Parental genotypes as well as the two possible progeny genotypes

Molecular Systematics of Microorganisms
(specifically Forest Tree Pathogens)

Brenda Wingfield

Molecular Diagnostics. There is no question that using molecular techniques to study and identify microorganisms has become the fastest growing area in Microbiology and Plant Pathology. Molecular techniques offer rapid and unambiguous identification criteria for all microorganisms. In the field of Forest Pathology, the need for these techniques is as great as other areas of Plant Pathology or Microbiology

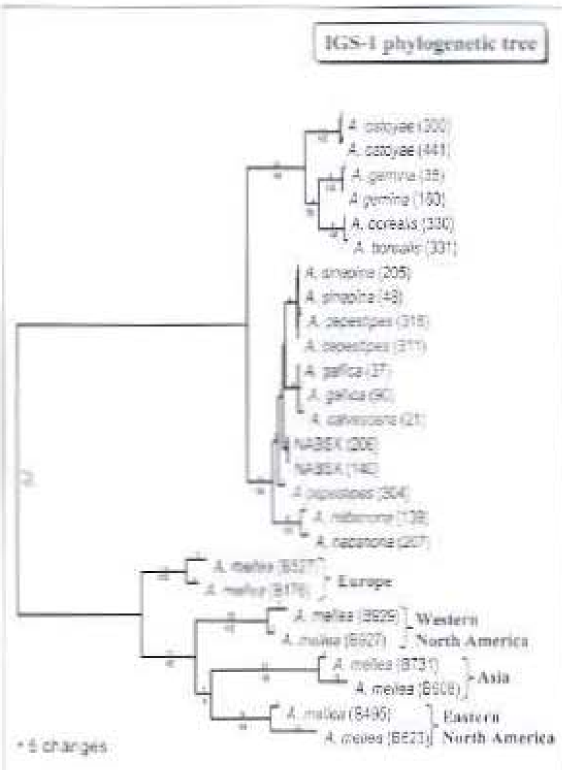
As with any identification technique it is necessary to be sure that the technique can distinguish between a potential plant pathogen and its closest relatives, which may or may not be pathogens. It is, therefore, necessary to study not only the potential pathogen, but also a large number of its closest relatives. This necessitates access to a significant culture collection of live fungi. This is without question FABI's greatest asset, one of the biggest and best collections of tree pathogenic fungi, in culture.

The technique that we have established as being most effective for identification of tree pathogens is a combination of PCR amplification and restriction enzyme digestion of these fragments. The most important first step is to establish which gene sequence is sufficiently variable to allow distinction between the species of interest. In many cases, we have found that the ITS1 and ITS2 regions of the rRNA operon is ideal. However, in the case of very closely related fungi we have obtained better results using the Histone 3 gene or the beta Tubulin gene.

Having determined which gene region is the most appropriate for identification purposes the next step has been to find the most useful restriction enzymes enabling distinction between the species. The ideal situation is when only a single enzyme allows differentiation of all the species in the group. In many cases this has not been possible and at least two enzymes need to be employed to distinguish between species.

In the last year we have established definitive molecular identification techniques for *Ceratocystis* (Witthuhn *et al* 1998, 1999), *Cryphonectria* (Myburg *et al.*, 1999), *Cylindrocladium* (Schoch *et al.*, 1999) *Fusarium* (Steenkamp *et al.*, 1999) and African *Armillaria's* (Coetzee *et al.*, 1998). These genera all contain species that are significant tree pathogens in many parts of the world. These techniques are now being used routinely in our tree diagnostic clinic and in many other laboratories throughout the world.

Molecular Phylogeny. Using the currently available molecular tools, establishing meaningful fungal phylogenies is now possible. This has resulted in a much improved understanding of fungal relationships and is leading to more relevant taxonomy of plant pathogens. Establishing the species concepts in fungi is becoming an essential part of disease management. This is only possible if the molecular basis of what determines certain fungal species has been established.

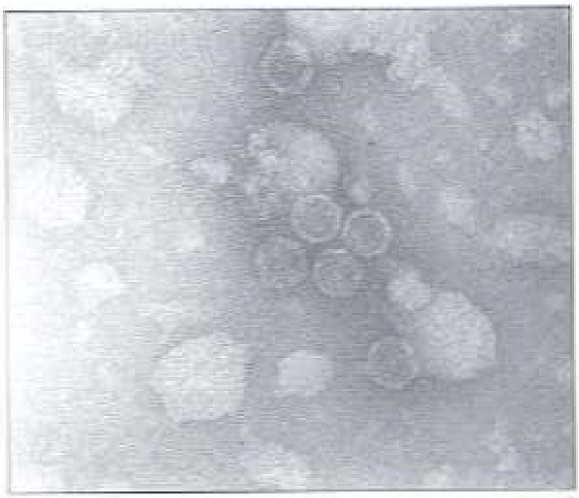


An example of a phylogenetic tree which was constructed from DNA sequences of *Armillaria* spp.

The first step in any molecular phylogeny is to establish the region of the fungal (or bacterial genome) which will be most useful to compare species. Regions of genes that are unduly conserved are uninformative and areas that are overly variable do not provide an appropriate platform for comparison. Thus far, we have used the rRNA Operon, Histone 3 gene and the beta tubulin gene quite successfully for fungal phylogenies. More recently we have began to investigate the usefulness of the mating genes (*MAT*) in these phylogenies. Understanding the *MAT* genes better will also potentially allow for a more definitive distinction between species based on the biological species concept. In the case of fungi that have no apparent sexual state, studying the *MAT* genes may also answer many of the questions as to whether this stage actually does not occur or whether it only occurs infrequently. During the past year we have constructed the first molecular phylogenies for a number of important tree pathogens [*Ceratocystis* (Witthuhn *et al.*, 1998, 1999), *Cryphonectria* (Myburg et *al.*, 1999), *Cylindrocladium* (Schoch *et al.*, 1999) *Fusarium* (Steenkamp *et al.*, 1999) and African *Armillaria*'s (Coetzee *et al.*, 1998)].

Myc viruses and Hypovirulence. Hypovirulence associated with dsRNA viral genomes is arguably the ultimate form of bio-control. Generally fungal viruses have been poorly studied and their role in fungal biology is not well understood. The potential to use these viruses to control tree pathogenic fungi drives our current research.

The first step in our research has been to identify and characterise dsRNA viruses in the fungal tree pathogens that we study. We have identified a number of mycoviruses and are in the process of cloning and characterising them further. Thus far, two entire viral genomes have been sequenced from *Sphaeropsis sapinea* (Preisig *et al* 1998). Previous studies have shown that dsRNA genomes are present in *Cryphonectria cubensis* and *Diaporthe ambigua*.



Viral particles within the mycelium of *Sphaeropsis sapinea*

Large brown conidia of *Sphaeropsis sapinea*

Panana Wilt of Bananas

Altus Viljoen

Fusarium wilt or Panama disease of bananas, caused by *Fusarium oxysporum* f.sp. *cubense* (*F.o. cubense*) is considered to be one of the most destructive diseases of agricultural crops in recorded history. Since its discovery in Australia in 1874, the disease has been reported from almost all banana-growing regions in the world. It became notorious when it decimated over 40 000 hectares of the cultivar Gros Michel in central America before 1960, thereby almost destroying the entire export industry. The trade was only rescued by the timely conversion to varieties of the Fusarium-wilt resistant Cavendish subgroup. In the subtropics, however, race 4 *of F.o. cubense* attacks all Cavendish varieties. Major losses have already occurred in Australia, South Africa and Taiwan, and there is concern that the tropically grown Cavendish bananas may eventually be affected. No effective chemical control or fumigation exists to control the disease. Furthermore, neither rotational nor other cultural practices that would be feasible in the country offer a solution, due to the length of time that the fungus can survive in soil. Panama disease, therefore, heralds the end of the productive life of a banana plantation, and it is essential to apply the strictest sanitation methods in order to keep uninfected land disease-free and healthy.

The history of Panama disease in South Africa is not well documented. The first verified case of the disease occurred in 1941 when wilted Cavendish plants were observed in a localized area 25 km south-west of Durban, and shortly thereafter at Pinetown and at Anerly on the South Coast. Regular reports of new outbreaks were received from the South and North Coast since 1966. Panama disease is, however, more severe in Mpumulanga than in KwaZulu-Natal. In Mpumulanga, the disease occurs in the Kiepersol area, where it has been present since at least 1970. Almost a third of the area once planted with bananas has now been forced out of production. Thus far, it has not been found in the Letaba, Levubu and Underberg production areas.

FABI became involved in Panama research at the beginning of 1999. Our main research goal is to understand the biology and epidemiology of *F.o. cubense* on banana in South Africa, and to implement a sustainable, practical strategy to manage the disease. Breeding of new, resistant banana cultivars, and the development of genetically modified plants acceptable to the South African market, are long and arduous processes. We, therefore, attempt to develop short and medium term solutions, apart from sanitation measures, as part of an integrated disease management programme to reduce the effect of the disease. As first steps to achieve this, a thorough analysis of the South African population of *F.o. cubense*, complete epidemiological study of the disease, and systematic search for factors responsible for disease development is being undertaken. The nature and occurrence of the disease in the various production areas of South Africa can contribute significantly in our analyses, and will be investigated. New developments in Panama research in other parts of the world will also be included.

Typical symptoms of Panana wilt of banana

Pathogens of Indigenous Food Crops Research and Development Programme

Terry Aveling

Various studies conducted in South Africa have emphasised the role of indigenous plants in providing many of the basic needs of rural people. The rural disadvantaged usually rely on energy-rich carbohydrate as a stable diet. In order to balance the diet, a protein must be added. The Vegetable and Ornamental Plant Institute (VOPI) is investigating the use of indigenous seed and leafy vegetable crops which are good sources of protein. Such crops must also be drought resistant and able to grow in poor soils, which make them suitable for production in areas of low agricultural potential. Potentially suitable crops include bambara groundnut, cowpea and *Amaranthus* sp. (marog). The indigenous African population have utilized these plants as food in times predating the colonial era. Cowpea, for example, is an important food crop for small scale farmers in the rural areas. Production in South Africa is mainly confined to Northern Province, Mpumalanga, North West Province and KwaZulu/Natal. Cowpea seeds have a high protein content (22-24%) and also contain fats (1.5%), fibre (7.3%), calcium iron, vitamins and most of the essential amino acids. Fresh immature pods and leaves are also boiled as a vegetable providing an important protein source.

Symptoms of *Colletotrichum dematium* on cowpea stems (arrow)

Anything that affects the health of plants is likely to affect their growth and yield and thus seriously reduce their usefulness to humans. Plants suffer from diseases caused by fungi, bacteria, mycoplasmas, viruses and nematodes. Despite a large and rapidly growing amount of literature, there is a dearth of authoritative reviews or research on the pathology of indigenous food crops as most are not of commercial economic significance. As researchers, we need to respond to the need for reduced usage of pesticides for plant disease control, especially by small scale farmers who cannot afford these chemicals. The pathogens of indigenous food crops project concentrates on research on the mechanisms of disease induction and resistance. A further focus is on breeding resistant varieties by developing basic epidemiological information on pathogen survival, infection and spread. Currently, most of the research is on cowpeas and this information is made available to the Grain Crops Research Institute, VOPI and Roodeplaat Grassland Institute, the centres of cowpea knowledge in South Africa.

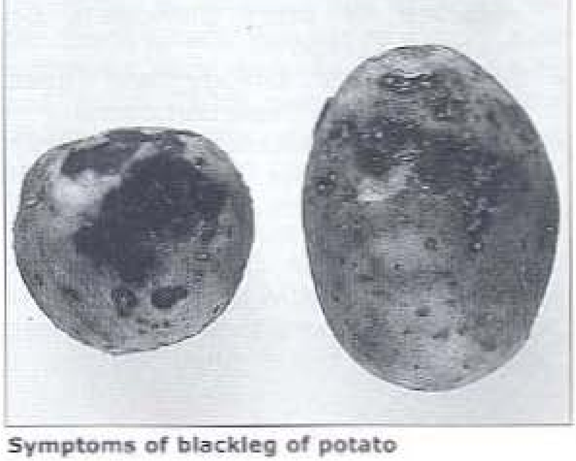
The aims of our programme are to improve the quality of indigenous food crops by:

- Surveying for plant pathogens on indigenous food crops
- Collecting, isolating and identifying those plant pathogens reducing yields of indigenous crops
- Providing a reference collection resource of plant pathogens on indigenous crops
- Studying the biology and seed transmission of these pathogens
- Training and educating students with relevant expertise to do extension work in disadvantaged communities.

Potato Research Programme

Lise Korsten

Increased international concern over the excessive use of pesticides and its detrimental effects on human health and the environment, have become a reality fruit producers can no longer ignore. Disease management has become a key phrase in disease control programmes. Disease forecasting has subsequently become a viable option to better manage disease based on sound basic pathological principles. Disease forecasting systems, if developed and implemented correctly, can offer the farmer a reduced, more tailor made and better targeted pesticide spray programme. In order to develop an effective forecasting system, one needs to identify the target pathogen amongst a conglomerate of diverse fungal spores. One accurate, inexpensive method of differentiating between spores, is monoclonal antibodies and subsequent application in immunofluorescence or spore spot immunobinding. *Alternaria* is an important pathogen on both citrus and potato causing extensive financial losses if not adequately controlled. The possibility of developing monoclonal antibodies against field isolates and using these antibodies to develop quick diagnostic assays for disease forecasting systems, have been successfully evaluated for other crops. This approach is currently being used in our research programme. Once effective field monitoring and detection systems have been developed, a computer aided disease forecasting system can be developed to accurately predict spore release with subsequent implementation of appropriate control measures.



Symptoms of blackleg of potato

Early blight of potatoes, caused by *Alternaria solani*, is an economically important disease in South Africa and globally. Control of the disease is best obtained by spraying protectant fungicides. Traditionally, spraying commences when symptoms appear and subsequent sprays are applied every 7-10 days. This strategy provides adequate disease control, but results in application of sprays even when the threat of disease outbreak is minimal. Fungicide use can, however, be minimized with the correct use of reliable disease warning systems or decision support systems. One such system is PLANT-*Plus*, which was originally developed by DaCom, Automatisering, Netherlands, for the prediction of late blight in potatoes. Another PLANT-*Plus* model was recently developed for early blight of potatoes. Development of the disease is calculated based on temperature, relative humidity, wind speed, wind direction, radiation and rainfall. The advisory module of PLANT-*Plus* can be split into two main parts, namely the (un)protected part of the crop and the disease cycle of the fungus. The (un)protected part of the crop is determined by new growth of leaves and deterioration of the sprayed product. The disease cycle of the fungus is split into four main phases: sporulation, spore dispersal, germination, penetration and incubation. Spraying advice is based mainly on a graph that provides information required by the producer. The model is being tested for epidemiological soundness and accuracy of predictions under South African conditions.

The importance of potato seed tubers as primary inoculum source for blackleg disease caused by *Erwinia carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* has been well documented. Extensive surveys have demonstrated that a high proportion of seed stocks may be contaminated with both pathogens. The pathogens may also be present on tubers obtained from any potato crop even when the incidence of blackleg is very low. Furthermore, in the process of multiplication of seed stock derived from stem cutting operations, it is extremely difficult to prevent recontamination. It is also difficult to distinguish the subspecies of *E. carotovora* on the basis of symptoms in stems or tubers.

Infected propagative material is a source of primary inoculum for most of the important phytobacteriological diseases of major crops around the world. Production of pathogen-free plant material is an effective means of control. Detection of bacteria is dependent upon the development of appropriate optimized isolation procedures and confirmation of pathogen identity and its pathogenicity. These procedures are interdependent and must be developed and evaluated simultaneously to ensure the reliability of the final detection method.

New outbreaks of *Erwinia* diseases in South Africa have renewed interest in this group of pathogens. The incidence of this disease pre- and postharvestly is greatly influenced by environmental conditions prior to and following harvest, as well as storage conditions of the seed potatoes. Survival of *Erwinia* in association with potato tubers is the most important aspect of their ecology when considering control strategies. The presence of the pathogen is regularly screened for in European countries, on all potato tubers before seed certification. However, very little is known in SA concerning the pathogens and its potential threat to our industry. It is therefore important to have a better understanding of this disease concerning the epidemiology, detection and control.

A survey was done in all potato growing regions of SA to determine the status and economic importance of *Erwinia*. Isolates have been identified, pathogenicity confirmed and compared using standard physiological tests, as well as ELISA protein profile patterns and RFLP. Diagnostic tests have been developed for commercial indexing of potato seed material with the industry.

Early blight symptoms on potato leaves in the field

Integrated Disease Control on Citrus

Lise Korsten

Guignardia citricarpa causes black spot on all commercial citrus cultivars and is of major economic importance in South Africa. According to the literature, losses of up to 80% have been recorded in untreated orchards. *Alternaria alternata*, which causes brown spot is also of economic importance in certain areas, with losses of up to 50% reported from unsprayed orchards. Control of these diseases has been achieved mainly with preharvest fungicidal sprays. However, build-up of resistance to benomyl has been reported for *G. citricarpa*. Furthermore, increasing awareness of environmental pollution has resulted in re-evaluation of basic plant protection strategies. One alternative, which is currently pursued with great success, is biological control. Utilising this natural control strategy in preharvest field applications has proven effective in controlling of *A. alternata* on other crops. Applying antagonists at a preharvest level has also proven effective in controlling postharvest diseases such as soft brown rot and anthracnose on mango and anthracnose and stem-end rot on avocado.

To control any plant disease, an obvious approach would be to interrupt the life cycle of the pathogen. *G. citricarpa* has a potential weak link that can be targeted for effective control. Pycnidia develop on litter under trees and provide inoculum that can infect leaves and fruit. If leaf degradation can be accelerated and pathogen development curtailed, the disease can be controlled. Four approaches can be evaluated to achieve these goals; ultra low volume (ULV) spray, sprinkle irrigation applications of antagonists to leaf litter, an antagonist enriched compost mix or a combination of these strategies. Over the past three years, we have developed an additive product from waste material for the citrus industry. Citrus peel has effectively been turned into natural compost. Additive value can be given to the natural compost mix by adding selected antagonists. The antagonists can be added with a carrier product to ensure antagonist survival and to give it a competitive advantage against the microbes already present in the compost mix. The aim of this facet of the project is to evaluate the above mentioned disease control strategies for control of black spot.

Several postharvest pathogens of citrus can infect fruit through the stylar or stem end. Infection already takes place in the orchard during flowering. Control of such diseases thus starts in the orchard, with preharvest fungicidal sprays. The success of any biocontrol programme depends strongly on the effective application of the antagonist to the target site. It has been shown that antagonists can be applied to apple and pear flowers using bees, for control of fireblight. Because of the foraging habit of bees, the antagonists can be deposited on the flower soon after it opens. With the second facet of the project, we aim to establish viable antagonist populations on the stamen within citrus flowers, in order to prevent the establishment of *Alternaria* during the critical infection period. Natural antagonists previously shown to be effective against pre- and postharvest diseases of avocado and mango (*Bacillus subtilis*) will be used in this project, as well as selected second generation antagonists.

Postharvest diseases of oranges (arrow)

In 1993, South Africa produced 837 344 tons of citrus of which 19% was sold on the local market and 50% was exported. Of the eight million cartons of Valencia fruit exported, actual waste due to postharvest decay was determined at 0.54 percent. Fungicides have traditionally been used to control postharvest diseases of citrus. However, regulatory restrictions by the US Environmental Protection Agency, makes re-registration of existing fungicides and registration of new products increasingly difficult. With the development of new export markets, the need to extend shelf life of fruit and satisfy quality demands has increased. Consumers now rate quality in terms of appearance, texture, flavour, and absence of fungicides. Thus, there is an urgent need to develop alternative decay control strategies such as postharvest biocontrol.

With the third facet of the project we aim to evaluate national and international commercially available biocontrol products, as well as using it as selected second generation antagonists in packhouses. Integrated control strategies will also be evaluated where antagonists can be combined with either hot water, disinfectant or reduced chemical concentration treatments. New innovative strategies using anti-fungals and elicitors in disease control is an alternative option that is being pursued.

Disease-resistance resulting from the induction of plant defensive systems by treatment with certain microorganisms and bioelicitors has been shown to offer an important manageable form of plant protection and has been documented in different host/pathogen systems. Recently, it has been suggested that antagonistic yeasts are capable of inducing disease resistance in harvested tissue. Preliminary studies have shown that biocontrol activity of selected antagonists may be due in part to their ability to induce several defense reactions in harvested tissue. We are studying the possible induction of anti-fungal hydrolases (chitinase, §-1,3 glucanase, and chitosanase), phytoalexins, and structural barriers by selected antagonists and antifungal additives.

With this postharvest research programme we have established a strong core focus within the University on postharvest technology. A new MSc degree in postharvest technology has been introduced as an inter-department and inter-disciplinary focus within the University and CSIR alliance.

Laboratory where testing of potential biological control agents to prevent postharvest diseases is undertaken

Stress Tolerance in Plants

Karl Kunert

Both abiotic and biotic stresses adversely affect the growth of crop plants. The search for novel DNA sequences involved in stress tolerance is one of the most critical inputs to enhance stress tolerance in plants using a plant genetic engineering approach.

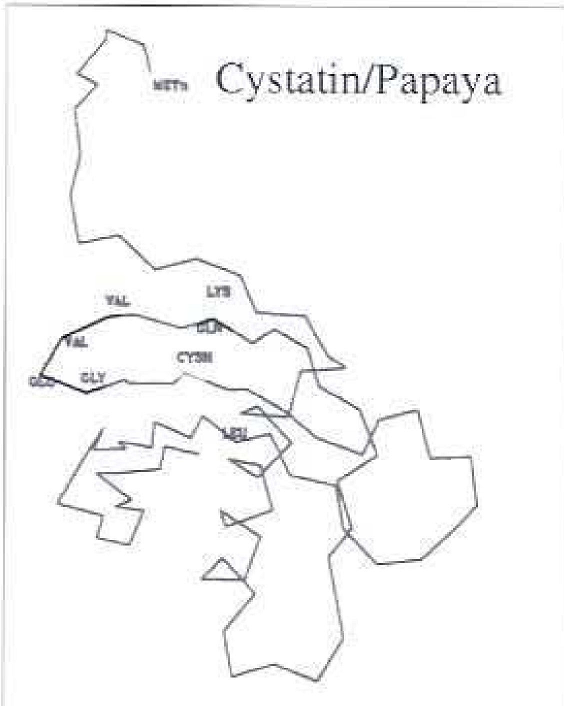
Abiotic and biotic stresses severely limit the extension of planting areas for crops. The creation of stress-tolerant plants for crop production would, therefore, have a high economic value. However, the number of genes with a defined role in stress tolerance are limited. Most of the physiological/biochemical studies previously carried out have not proved to be of direct importance for plant genetic engineering for the isolation and engineering of useful genes. Investigations on the molecular mechanism for stress tolerance, including the use of genetically manipulated plants, have therefore attracted extensive research activities worldwide.

Cystatins and Stress Tolerance

We are using plant cystatins, which are cysteine proteinase inhibitors, as a group of possible useful genes. Cystatins are essential regulators of cysteine proteinases, which play a prominent role in stress-induced phytotoxic processes. Proteinase functions have been identified as being fundamental in the control of programmed cell death. To understand the role of cystatins in stress tolerance we are using cold stress as a model stress. Tools of plant physiology/ biochemistry and plant molecular biology are integrated into our current research activities.

We have produced transformed tobacco plants expressing the rice cystatin OCI to evaluate cold tolerance provided by cystatins in genetically engineered plants. We have discovered in a first pilot experiment with transgenic plants that total cysteine proteinase activity is significantly decreased under cold stress in transgenes expressing OCI. Lower cysteine proteinase activity seems further to be directly related to prevention of cold-induced leaf chlorosis (Figure 2). Transgenes might allow the identification of both specific cysteine proteinase action and the role of cystatins in stress tolerance. A molecular approach to plant improvement using plant transformation and expression of a proteinase inhibitor might finally provide a powerful biological protection system against plant stresses.

We have also isolated and cloned a papaya cystatin (PCI, Figure 1), which is expressed after exposure of papaya plants to environmental stresses. This cystatin has a unique cysteine in the cystatin amino acid consensus region, which is absent in all other known plant cystatins. Reduced glutathione possibly keeps PCI in its active monomer form. This seems necessary for interaction with pathogenic proteinases by preventing PCI dimerization. We have started to replace the cysteine by tyrosine, which is normally found in the consensus region of cystatins, by using a site-directed mutagenesis program. Use of mutated cystatins might provide insight into the function of single amino acids in cystatins and their interaction with proteinases under stress conditions.



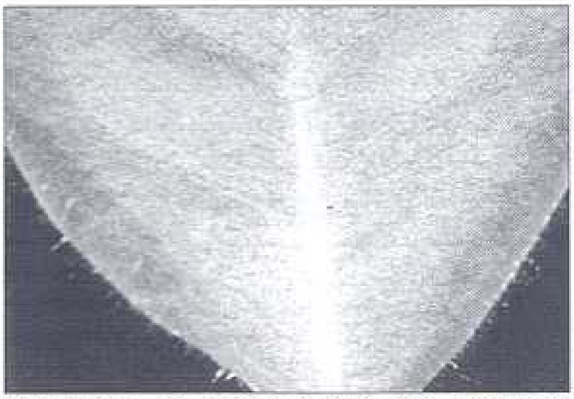
Molecular model of the papaya cystatin under investigation showing the location of the unique cysteine in the cystatin consensus region.

Novel DNA Sequences for Stress Tolerance

Representational Difference Analysis (RDA) is a method where large portions of genomes can be compared in a single experiment. Essentially, the method consists of a subtraction of all sequences that are held in common between two individuals, which might be morphologically identical, but differ, for example, significantly in their tolerance to an environmental stress. Together with Prof. C. Cullis at Case Western University, Ohio, we have extensively investigated the potential of the technology to differentiate closely related plants. From our gained experience with banana and date palms, from which we could isolate various different products, we are confident that the technology will also be applicable to isolate new and unique DNA sequences from cold tolerant plants. In a first step, we are currently investigating indigenous grasses, which are morphologically identical, but differ in their tolerance to low temperatures. Isolated DNA differences might ultimately be used to identify or to screen for DNA sequences related to cold tolerance in a variety of plant species, which also includes economically important crops.

Outcome

We expect that our research might ultimately allow the creation of new and commercially interesting cell lines with increased stress tolerance. Such plants might greatly supplement current plant breeding programs aimed to extend crop production into environmentally less favorable areas. Our developed technology might find immediate application in plants of concern to the local plant and fruit production industry.



Prevention of cold-induced leaf chlorosis studied in transgenic tobacco plants. Leaf chlorosis in non-transgenic plants (top) is not found in transgenic plants expressing the rice cystatin OCI (bottom).

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1998/1999

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Dr. D. Wilson
Endophytic fungi associated with eucalypts
Dr. T. Burgess
Population genetics of *Sphaeropsis sapinea*
Dr. Hong Li
Identification of Mycosphaerella leaf blotch pathogens of eucalypts
Dr. J. Chen
Selection of disease (especially *Ceratocystis albofundus*) tolerant wattle
Dr. A. Gaur
Studies on *Ceratocystis* spp. infecting different crops

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Phytophthora root rot associated with cold tolerant eucalypts in South Africa
Bernard Slippers
The taxonomy, phylogeny and ecology of Botryosphaericeous fungi on selected woody hosts
Christelle Klopper
Studies on the rooting ability of *Eucalyptus grandis*
Eduard Venter
Host resistance in South African *Pinus* spp.

Marius Boshoff
Epidemiology and control of bacterial black spot of mangoes

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

Henriette Britz
Taxonomic, genetic and molecular study on *Fusarium subglutinans*

Karin Jacobs
Re-evaluation of the genus *Leptographium*

Henk Smith
Sphaeropsis die-back of pines and Botryosphaeria canker of eucalypts

Martin Coetzee
Molecular characterisation of *Armillaria* (Basidiomycetous Agaricales Tricholomycetaceous)

Ntsane Moleleki
Hypovirulence in the pine pathogen, *Sphaeropsis sapinea*

Len van Zyl
Coniothyrium canker of eucalypts

Robert Mokgatla
Isolation and characterisation of mating genes of *Sphaeropsis sapinea* - a paradox

Percy Chimwamurombe
Molecular plant-pathogen interactions with special reference to *Eucalyptus* PGIP and fungal PG proteins

Schalk van Heerden
Studies on *Cryphonectria cubensis* in South Africa

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

Gina Swart
Comparative study of *Colletotrichum gloeosporioides* from avocado and mango

Lynelle Lacock
Sequence expressed Tags in wheat

Cassi Myburg
Cryphonectria canker of eucalypts

Yolisa Pakela
Interaction between cowpea and *Colletotrichum dematium*

Marinda Visser
Population biology of the banana panama wilt pathogen, *Fusarium oxysporum f.sp. cubensis*

Chantel Botha
A genetic study on resistance of wheat to the Russian wheat aphid, using Expressed Sequence Tags

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

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

Anita Severn-Ellis
Mechanisms of resistance in Cavendish banana cultivars to subtropical race 4 of *Fusarium oxysporum* f.sp. *cubense*

MSc Students

Albé van der Merwe
Population diversity studies on the *Eucalyptus* canker pathogen, *Cryphonectria cubensis*

André Pretorius
Population diversity studies on the pine needle pathogen, *Dothistroma septospora*

Christiaan Troskie
Identification and characterisation of markers linked to the leaf rust resistance gene *Lr37*

Edzard Grimbeek
Management strategies for panana wilt disease of banana in South Africa

Juanita de Wet
Diversity of the pine pathogen *Sphaeropsis sapinea* and its association with dsRNA

Jo-Marie Lottering
Identification and characterisation of markers linked to the leaf rust resistance gene *Lr37*

Marieka Venter
A taxonomic evaluation of the *Eucalyptus* canker pathogen, *Endothia gyrosa*

Nonnie Geldenhuis
Population diversity studies on the wilt pathogen, *Chalara elegans*

Oliver Dickens
Somatic embryogenesis in the *P. elliottii* x *P. caribiae* hybrid

OT Shakwane
Investigations on the potential use of biological control agents against Phytophthora root rot of citrus

Riana Jacobs
The genus *Phialocephala* - a taxonomic study

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

Mfuna Chunda
Characterisation of dsRNA isolated from *Leucostoma persoonii*

Ronelle Koekemoer
Host induced resistance in pine and wheat

Wayne Rathbone
Development and assessment of a biotechnology curriculum for high school scholars

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

Lindi Mabena
Studies on the bacterial diseases of cowpea

Jacky Doyle
Determining linkage and parental contribution in *P. elliottii* x *P. caribaea* hybrid pine

Tessa Bandounas
Interaction beteen *Albugo tragopogonis* and sunflower

Noelani le Grange
A new disease of cowpea caused by *Alternaria cassiae*

Anton Jordaan
Molecular manipulation of roses

Christell Bester
Influence of abiotic stresses on the expression of sex in plants

4th Year and Honours Students

Shalati Shiburi
Gavin Hunter
Irene Vincent
Angeline Jacoby
Bafana Khoza
Lerato Mmatsaungane
Shilo Loots
Raksha Bhoora
Duria Rutkoska
Juan Vorster

Student Assistants

Solomon Mabapa
Karin Surridge
Ferdi Geldenhuis
Jake Busang
Naphtaalian Makoe
Shalati Shiburi

Overseas Visitors

Mr. Markus Weber (University of Berne, Switzerland)
Dr. Chris Cullis (Case Western Reserve University, Cleveland, Ohio, USA)
Dr. Michael Milgroom (University of Cornell, USA)
Dr. Andre Drenth (University of Brisbane, Australia)
Mr. Aaron Maxwell (Murdoch University, Australia)
Dr. John Leslie (Kansas State University, USA)
Dr. Suzy Bently (CSIRO, Australia)
Prof. Frans Oberwinkler (Tubingen, Germany)
Mr. Dolor Marco (Madagascar)
Dr. John Fryer (ACIAR, Australia)
Dr. Ken Old (CSIRO,Australia)
Dr. Eric Boa (IMI, UK)
Dr. Gary Kong (DPI, Australia)
Dr. E. Bekele (Addia Ababa University, Ethiopia)
Dr. D. Guest (Melbourne University, Australia)
Dr. T.Gulya (USDA-ARS, USA)

Recent Graduates

Ph.D.

Celeste Linde
Corli Witthuhn
Francois Wolfaardt
Jolanda Roux
Zachee Ngoko
Neil Theron
Fanus Swart

M.Sc.

Schalk van Heerden
Bernard Slippers
Bongani Maseko
Ntsane Moleleki
Eduard Venter
Lynelle Lacock

Prestigious NRF Bursary Holders

Henriette van Heerden
Martin Coetzee
Eduard Venter
Lynelle Lacock
Gina Swart
Marinda Visser

Mellon Foundation Grants

Robert Mokgatla
Ntsane Moleleki
Bongani Maseko
Martin Coetzee
Bernard Slippers
Jacquie Smith

Management

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Professor B.D. Wingfield
Assoc. Professor K. Kunert
Assoc. Professor L. Korsten
Assoc. Professor A-M. Oberholster
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Mr. P. Govender

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Professor A. Neitz, Head of the Department of Biochemistry
Professor P. Hammes, Head of the Department of Plant Production
Professor C. Scholtz, Head of the Department of Zoology and Entomology
Professor F.J.C. Swanepoel, Head of the Postgraduate School for Agriculture and Rural Development
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Prof. R. Crewe, Dean of the Faculty of Natural, Agricultural and Information Sciences
Dr. Z. Ofir, Director of Research
Prof. M.J. Wingfield, Director of FABI

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Aveling T.A.S., Smith J.E. and Korsten L. (1998) Seed treatment of cowpea seed to control fungal stem pathogens. **Proceedings of the 5th International Congress of Plant Pathology, 6 - 12 August, 1998. Edinburgh, Scotland, UK.**

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Korsten L. (1998) Biological control potential against *Colletotrichum gloeosporioides*. **International Workshop on Host Specificity, Pathology and Host-Pathogen Interaction of *Colletotrichum*, Jerusalem, Israel, 29 August – 1 September 1998.**

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Van Zyl A., van der Westhuizen A.J. and Botha A-M. (1998) Improving growth in suspension cultures of *Pinus radiata*. **South African Association of Botanists, University of Cape Town, 12-16 January 1998.**

Venter E. and Botha A-M. (1998) Characterization of two fragments related to Russian wheat aphid resistance genes, *Dn2* and *Dn5*. **South African Association of Botanists, University of Cape Town, 12-16 January 1998.**

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Seminar Presentations

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

Special Seminars

Prof. Lise Korsten
July 1998
The status of plant pathology in Australia

Prof. Franz Oberwinkler
September 1998
Molecular taxonomy of Basidiomycetes

Prof. Wally Marasas
October 1998
Mycotoxins in food in Africa

Prof. Callie Pistorius
October 1998
Dynamics of technological change

Prof. Robin Crewe
October 1998
How to lose the queen caste and remain social: co-operative breeding in ants

Prof. Carla Falkson
November 1998
Medical oncology: Past, present and future

Prof. John Leslie
November 1998
Vegetative compatibility: fungal recognition and death

Dr. Susan Bentley
November 1998
Structure of a world-wide collection of *Fusarium oxysporum f.sp. cubensis* isolates

Prof. John Leslie
November 1998
Effective population number and AFLP approaches to the analysis of fungal po-pulations

Prof. Gerry Adams
November 1998
Creating mysteries and solving theoretical problems using heterokaryons of *Fusarium*

Prof. Karl Kunert
November 1998
The old and new fascination about glutathione

Dr. Andrew Morris
January 1999
An overview of Sappi Forests research

Prof. Eugene Cloete
February 1999
Multimedia and microbiology

Prof. Hauke Hennecke
March 1999
Oxygen control of respiration, nitrogen fixation and chaperonin synthesis in *Bradyrhizobium japonicum*

Dr. Kurisu Fukoshi
March 1999
Microbial community analysis of a thermophilic contact oxidation process

Dr. Ken Old
April 1999
Eucalypts and eucalypt diseases: a Ken Old perspective

Dr. Barbara Huckett
April 1999
The development of an expressed sequence Taq database for applications in sugarcane biotechnology

Mr. Aaron Maxwell
May 1999
Mycosphaerella leaf blotch: an emerging problem in west Australian eucalypt plantations

Ms. Suzette Hugo
June 1999
How to present a seminar and chair a session successfully

Prestigious Seminars

Ntsane Moleleki (M.Sc. cum laude)
July 1998
The involvement of cytochrome P-450 monooxygenasas in the hydroxylation of monoterpenes by yeasts.

Martin Coetzee (M.Sc. cum laude)
October 1998
1001 nights with *Armillaria*

Yolisa Pakela (M.Sc.)
February 1999
Development of rural oyster mushroom (*Pleurotus* spp.) industry in South Africa

Robert Mokgatla (M.Sc. cum laude)
February 1999
Mechanisms of increased tolerance of Salmonella from a poultry abattoir to hypochlorous acid

Bernard Slippers (M.Sc. cum laude)
May 1999
The *Amylostereum* symbiont of *Sirex noctilio* in South Africa

Schalk van Heerden (M.Sc. cum laude)
May 1999
Pathogenicity and variation amongst South African isolates of *Cryphonectria cubensis*

FABI TEAM

5th Row: Jinbiao Chen, Oliver Dickens, Chris Visser, Edzard Grimbeek, Schalk van Heerden, Andre Pretorius, Christiaan Troskie, Len van Zyl, Aaron Maxwell, Wilhelm de Beer

4th Row: Annelie Rabie, Prem Govender, Karin Jacobs, Eduard Venter, Altus Viljoen, Jo-Marie Lottering, Lynelle Lacock, Ronelle Koekemoer, Cassi Myburg, Jolanda Roux, Hong Li, Jacquie Smith, Tessa Bandounas, Shalati Shiburi

3rd Row: Christelle Klopper, Riana Jacobs, Martin Coetzee, Gerry Adams, Anna-Marie Oberholster, Chris Bornman, Brenda Wingfield, Gina Swart, Emma Steenkamp, Anupama Gaur, Oliver Preisig, XuDong Zhou, Magriet van der Nest, Shaiza Shaik, Sonja de Beer

2nd Row: Teresa Coutinho, Vivienne Clarence, Mike Wingfield, Terry Aveling, Kaylene Maasdorp, Robert Mokgatla, Percy Chimwamurombe, Dennis Wilson, Yolisa Pakela, Marvelene Molema, Rose Visser

Front Row: Nonnie Geldenhuis, Henriette van Heerden, Marieka Venter, Dolor Marco, Bongani Maseko, Albe van der Merwe, Juanita de Wet, Awelani Mutshembele, Bernard Slippers