tree pathology news

NEWSLETTER OF THE TREE PATHOLOGY COOPERATIVE PROGRAMME - UOFS

NO 11

MAY 1995

DIRECTOR'S REPORT

The TPCP has been abuzz with activity since the issue of our last report in November. To start with, we enjoyed a day long visit from Mr Piet Odendaal of SAFCOL and his senior field staff early in November. This month and early December also marks the height of our field inoculation work to test the susceptibility of trees to various pathogens. The annual congress of the Southern African Society for Plant Pathology was held in the second week in January and, shortly after Christmas, most of us set to work to produce papers and posters for this important event. Some of the abstracts of papers presented will be included in this Newsletter. Finally, and as many of you will know, we have just held the inauguration of the new Sappi Forest Biotechnology facility and the annual meeting.

Christmas holidays.....

From the foregoing summary, you will realise that the Christmas period is hardly a time for rest and reflection within the ranks of the TPCP. I must confess that this situation does result in a modicum of "conflict of interest" in our greater Department. For example, it was always the philosophy of our former and visionary head of Department, Prof Piet Lategan that the Department should close for a few weeks over Christmas. The logic here was that, after the pressures of exams and the completion of final reports, staff tended to be somewhat tense. A time of relaxation before the new influx of students, new curricula, new projects seems sensible. For us in the TPCP, however, it is the trees that count. This time of the year is not only the height of the growing period, but also the optimal time for infection by most of our important disease-causing agents. In tree pathology terms, a brief respite in June would seem most acceptable.

It was a great pleasure to host Piet Odendaal and his team (eleven people in all) to a day of forest pathology lectures early in November. I find that people tend to forget that students and researchers enjoy the opportunity to present the results of their work. Such opportunity provides us with feed back on our work and this is often also from the user group. In addition, we strive to maintain a balance (often with difficulty) between immediate problem-solving research and research that will place us in a position to understand the pathogens in the slightly longer term. Visits such as the one from SAFCOL and the annual TPCP meeting, give us the opportunity to place these components of our work in perspective. I must also admit, however, that a great deal of work goes in to these gatherings and the period of preparation is seldom free of a few frayed edges.

Thaba Nchu Sun.....

The annual congress of the Southern African Society of Plant Pathology is the premier gathering of people dealing with plant disease problems in Africa. The Society is by far the largest and most active in Africa and there is currently a very sincere effort being made to build bridges and incorporate other groups, at least in Southern Africa. This year, our annual meeting was held at the Thaba Nchu Sun Hotel just outside Bloemfontein. The meeting was attended by approximately 200 people and we were fortunate to have; no less than four "overseas"

Visit by SAFCOL Management team to the TPCP

guests in attendance. Amongst these guests was Prof. Tom Harrington from the United States who is one of the world's foremost forest pathologists. Presentations from students and staff attached to the TPCP were numerous - short of making an exact count, approximately 30. Indeed, the forest pathology component of the meeting has become substantial. What a change from fifteen years back when a single presentation was made each year!

Inauguration.....

Many of you would have recently attended the inauguration of our new Sappi laboratories and forest pathology growth rooms. This event was the culmination of many months of planning and preparation. It ultimately turned out exactly as I would have hoped. The aim here was not to focus solely on the new facilities, but to hold an "open house" event where friends and colleagues would have the opportunity to view the activities of the "greater" department. Here, it must be remembered that the successes of the TPCP are dependent, not only on those of us working specifically on tree disease problems. Our achievements are rather, dependent on various other scientists in the Department of Microbiology and Biochemistry, many of whom are world leaders in specific aspects of microbiology. Thus a key component of the Inauguration was the morning "open house" where friends could "pry" into the cupboards and see experiments in progress.

The inauguration had many components including an afternoon of lectures and presentations. The key speakers here were Dr Linda Kohn, a leading plant disease specialist from Toronto who was brought in specifically for this occasion and Prof Trevor Britz of our own Department. Minister Stella Sigcau, Minister of Public Enterprises and Mr Andre Vlok one of the Executive Directors of Sappi also made presentations. The new building was formally opened and a tree was planted to mark the occasion.

The annual meeting of the TPCP was held on the day following the inauguration. This year, the meeting was attended by 51 staff of Mondi, Sappi, H.L. & H., SAFCOL and the ICFR. Almost double the number of people that attended our 1994 meeting! The lecture room of the Department has recently been "rationalised" to seat 80 people, and even then, accommodating all was rather a squeeze. Having said that, the meeting was exciting, vibrant and packed with new information, and necessary feedback from field foresters. This gathering must have become one of the largest "non-society" meetings of its kind in South Africa.

At the time of writing this note, we are well into the last month of the quarter of 1995. It is hardly appropriate to view the year ahead. We are well and truly at "cruising altitude" with much experimentation, laboratory diagnosis of submitted samples and many days of field work already behind us.

***Top* and *Left*: Mr Vlok of Sappi planted a tree and received a gift from the U.O.F.S.**

Three of our postgraduates doing inoculations as part of pathogenicity studies conducted in our new glasshouses

As is true each year, we have various new comers to the group and I will introduce these people to on a later occasion. We have also established a number of new and important projects which will be shared with you in future Newsletters of the TPCP. All in all, the year has started with great gusto - **WE ARE STRIVING TO KEEP TREES HEALTHY!** - Director.

A NIGHT AT THE BEEF BARON

(from a student's point of view)

After two exhausting days of opening speeches, research presentations, refreshments, lunches, a buffet and much more, one would expect everybody to be too tired to really look forward to yet another meal. Contrary to normal expectations, however, this was not the case - not for the students, anyway. There are a few good reasons for this:

Firstly, the opening of the Sappi Biotechnology facility and the TPCP annual meetings might have been physically exhausting, but for any student interested in tree pathology, it was an intellectually stimulating adventure. There is always much to learn from the annual meetings and presentations. Perhaps we do not learn as much from the presentations themselves (since we know what the others in the group are doing) than we do from the questions and remarks from colleagues in the industry. So we definitely were not too tired for another get together with the men and women of forestry.

Secondly, all the meals and refreshments of the two day might have been a bit much for somebody used to regular business meals, or to those fortunate enough to get home after work each day to a well-prepared meal. But most students cannot afford to dine out regularly, neither do most have a person at home cooking for them. So for a tree pathology student dining at the Beef Baron once a year is no small item, and definitely something they look forward to.

Lastly, and most importantly, for a tree pathology student night at the Beef Baron means meeting and communicating personally with people from the industry. Much has been said about applied versus basic research, but it is occasions such as these, where there's personal communication, that contribute to bringing applied and basic research closer to each other and that help scientists like us to understand the problems and needs the people working in the field. Formal presentations serve their purpose well, but students are usually a bit nervous and sometimes find the experience a little intimidating. Therefore the value of informal discussions should not be underestimated. Not only because the student learns the needs of the industry, but most, it not all, students find exposure of this kind highly stimulating. It really serves to help them become more enthusiastic about their particular field of research, and it often changes the direction of their research to make it more practical.

We do realise that an evening at a good steak house such as the Beef Baron is costly and we really do appreciate the hospitality. We, the students involved in the TPCP programme would, therefore, like to once again thank HL&H, Mondi, SAFCOL and Sappi for their contribution to making this evening possible. We hope, however, that this was not only of benefit to us, the students, but that it was also a worthwhile investment for the industry

Who says apples are for eating only!

See page 6.....

TWO REMARKABLE VISITORS

This year, we have already had two remarkable overseas visitors to the TPCP. Both are leading figures in the field of plant pathology and both have had considerable experience working with aspects of fungal pathogens of interest to the South African Forestry Industry. They are Dr Tom Harrington, Chair of the Department of Plant Pathology at Iowa State University, U.S.A., and Dr Linda Kohn, a Professor in the Department of Biology at the University of Toronto, Canada

TOM HARRINGTON is without question, one of the world's leading figures in the field of Forest Pathology. He worked on an insect-associated tree decay problem as part of his M.Sc. degree at Washington State University under the guidance of the famous C., Gardner Shaw II and went on to complete a Ph.D. on Black Stain Root Disease of conifers at the University of California at Berkeley. His research on Black Stain Root Disease caused by *Leptographium wageneri* is the authoritative work on this subject. After completing his studies he joined the University of New Hampshire where he established a noteworthy programme studying various root decay problems on the Eastern seaboard of North America. Some of his most interesting and noteworthy work at that time was to bring some perspective to the relative importance of the so called "Acidic Precipitation" problem being experienced there. More recently, he accepted a term as Chairperson of Plant Pathology at Ames, Iowa, where he has also continued his research on root decay pathogens such as *Armillaria* and *Leptographium*, and the Ceratocystis wilt pathogens.

Tom Harrington's visit to South Africa was supported in part by Sappi, SAFCOL, H.L. & H., Mondi, the Foundation for Research Development and the Southern African Society for Plant Pathology. During this brief visit, he presented a keynote address at the plant pathology congress, met with various TPCP students to discuss collaborative research and presented a lecture to Mondi staff at Zululand. He also had the opportunity to inspect various field trials and disease problems and to offer his opinion and advice on these.

LINDA KOHN has also had an illustrious research career. She obtained a Ph.D. degree from Cornell University under the tutelage of the now retired and very famous mycologist Richard Korff, studying the root pathogen, *Sclerotinia sclerotiorum*. The focus of her more recent work has been in establishing appropriate technologies for the molecular characterisation of plant pathogens. Indeed it is in this field that she has gained much notoriety - leading also to her receipt of the coveted Alexopolous award for young mycologists some years ago. Another current thrust of her research is to develop new concepts and understanding of the population dynamics of plant pathogenic fungi. This is a critically important area of study with a multiplicity of practical consequences, but one that is only now being fully appreciated. Linda, is a leading figure in bringing us new concepts for appreciating pathogens in populations. She is thus enabling us to select plants (trees) tolerant, not only to an individual, but to the entire population of the pathogen.

Linda Kohn's visit to South Africa was specifically arranged to coincide with the inauguration of the new TPCP facilities. Funding for this visit came from the UOFS Foundation and from the Department of her presentation at this function she presented three lectures, at Bloemfontein, Stellenbosch and Johannesburg on clonality in plant pathogen populations. We were thus entertained to a highly stimulating and academically superb lecture "The clonal dynamic in agricultural plant pathogen populations".

Dr Linda Kohn

Those of you that had an opportunity to meet Linda will have no doubt about Linda Kohn's academic excellence. Discussions with students and researchers brought a great deal of depth and new thinking to those of us working in the TPCP.

Bringing scientists and visitors to South Africa is a time consuming and an expensive undertaking. The wealth of knowledge and experience that we gain from such visits is, on the other hand, invaluable in any research programme. Those of us that had the opportunity to listen to, and meet with Tom Harrington and Linda Kohn were left with a great deal of inspiration and new knowledge. We are grateful to all who made these visits possible.

SOME RECENT PUBLICATIONS

Morris, M.J., M.J. Wingfield and C. de Beer. 1994. Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. Plant Pathology 42: 814-817.

Viljoen, C.D., B.D. Wingfield and M.J. Wingfield. 1994. Comparison of *Seiridium* isolates associated with cypress canker using sequence data. Experimental Mycology 17: 1-6.

Linde, C., G.H.J. Kemp and M.J. Wingfield. 1994. *Pythium* and *Phytophthora* species associated with eucalypts and pines in South Africa. European Journal of Forest Pathology 24: 345-356.

ABSTRACT OF CONGRESS PAPER

SCREENING *PINUS RADIATA, P. PATULA* AND *P.TAEDA* CALLUS FOR RESISTANCE TO *FUSARIUM SUBGLUTINANS* F.SP. *PINI*

Fusarium subglutinans f.sp. *pini* (FSP) causes pitch canker of pines and has recently been recorded in South Africa for the first time. In order to prevent possible large-scale damage to forests in the future, screening *Pinus* families for resistance to this pathogen is necessary. Tissue culture provides an ideal means of studying host-pathogen interactions. It was therefore decided to screen pine callus for disease tolerance. A culture filtrate was prepared by growing an FSP isolate (MRC 6213) in 2% malt extract medium for eight days. The medium was centrifuged, they supernatant decanted and filter-sterilised before use. Pieces of callus were placed in test tubes containing the filtrate. The callus was broken into single cells using a vortex mixer and 25ul samples removed 2,4,6,8,and 24 hours after treatment. No filtrate was added to the controls that contained either the callus growth or the malt extract media. Samples were stained with fluoresein diacetate and viewed with a Zeiss Axioskop microscope fitted with epifluorescence equipment. A cell death index was determined at each time interval for the *Pinus* species examined. *P. patula* was found to be more susceptible than both *P. radiata* and *P. taeda* to FSP culture filtrates with *P. taeda* the most resistant. These initial results appear to reflect seedling inoculation data and suggest that screening of callus will be useful in studying pitch canker in the future.

CERATOCYSTIS WILT OF BLACK WATTLE

Ceratocystis wilt of black wattle was first reported in 1990 in the Natal Midlands, where trees that had been mechanically damaged were dying of an unknown disease. Symptoms of the disease included wilting and die-back, discoloured lesions on the stem as well as the discolouration of the wood. Isolations were made from the diseased trees and a fungus, identified as *Ceratocystis fimbriata* was identified as the causal agent of this disease, thus the name Ceratocystis wilt.

The fungus isolated from the black wattles was, however, found not to be typical of *C. fimbriata* since it's distinctive sexual structures, known as perithecia, have white perithyecial bases and not the typical black bases. This lead to further studies and, with the use of molecular techniques, it was proven that the wattle isolates are in fact unique.

In artificial inoculations, this newly discovered *Ceratocystis* species proved to be pathogenic and capable of killing trees. The good news is that family trials have shown that there is resistance to the fungus among different families of black wattle. Eighteen months after the first family trials had been conducted, there were both healthy and dead trees.

During further studies of this new species, it was determined that both self-fertile and self-sterile isolates exist. The self-fertile isolates are capable of producing sexual fruiting structures, while the self-steriles are not. Preliminary pathogenicity tests using self-sterile and self-fertile isolates showed that the self-fertiles are pathogenic, while the self-steriles are not. This phenomenon is of importance since it can be used in increasing our understanding of the biology of this fungus. If self-sterility can be promoted, it might be possible to reduce the impact of this pathogen in the field.

MONOTERPENES AND DISEASE RESISTANCE IN TREES

*Monoterpenes are ten carbon compounds that consist of two isoprene units joined head to tail (**Fig. 1**). They are natural products produced mainly by plants and are responsible for the characteristic fragrances and flavours of many plants i.e. the smell of pinewood and the smell of oranges. Plants produce monoterpenes as a defence mechanism against invaders. Monoterpenes form for instance an important fraction of the resin produced by Pinus species upon wounding or invasion by insects or fungi.*

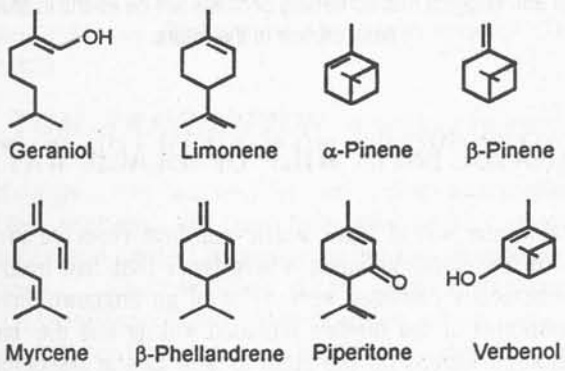

Fig. 1. Structures of some monoterpenes

Although various researchers have studied the monoterpene response of trees and the effect of monoterpenes on fungi the importance/exact role of monoterpenes in disease resistance is not yet clear. Different tree species produce different monoterpenes and varying quantities of monoterpenes. The total amounts of monoterpenes in the trees always increase upon wounding or invasion and sometimes there are also changes in the monoterpene composition. There are also differences in the monoterpene response of individuals in a species.

It is also apparent from the literature and from our own work that the toxicity of different monoterpenes to different fungi vary. Some fungi are very sensitive to monoterpenes while others can grow in atmospheres saturated with certain monoterpenes. No work has yet been done to understand the mechanism of the monoterpene tolerance of certain fungi. To complicate matters further, some monoterpenes are bark beetle pheromones produced by the beetles or their symbiotic fungi. It appears, however, unlikely that a fungus will be pathogenic to a tree that produces large quantities of a monoterpene that is very toxic to this fungus.

(Editors note:- It is clear that the function of monoterpenes in trees is complex. Our efforts in the TPCP and especially through collaboration with Dr Martie Smit, we hope to understand these compounds and their role in disease resistance better.)

"GOING APPLES" - Testing *Cryphonectria* virulence

No, we've not changed our focus to the fruit industry, neither do we have a problem finding catchy titles, we are actually going apples. We, in the TPCP, have purchased over 2 000 apples, but not for eating. Along with the overpowering fragrance of Golden Delicious, and all in the interest of forest pathology in South Africa, progress in science is being made.

In order to test the pathogenicity of a fungus, i.e. the ability of the fungus to infect and kill a tree, we normally inoculate seedlings or established trees. After some time, the lesions formed by the fungus are measured, and this then used as an indication of the relative virulence of the pathogen. In order to achieve this, we must plant and cultivate seedlings, and wait for them to grow to an appreciable size. This takes a great deal of time and money. We desperately need a rapid technique to screen large numbers of *Cryphonectria* isolates for virulence and hypovirulence.

So we decided to "go apples". Now in the morning I "pop" over to the market, buy a few boxes of apples, and take them to the laboratory. I punch holes with a cork-borer, and insert the fungus, replace the apple-plug, and the experiment is on its way. Two weeks later I measure the lesions, and the experiment is done. If at any stage I need to redo, or the need to test the pathogenicity of a new fungus, I just "pop" over to the market, and .... science progresses.

The results are still a little premature, but they look very promising. We have a good correlation between *Cryphonectria* lesion sizes from experiments done with seedlings and trees, and those done on apples. This will enable us to test virulence in a much shorter time than was possible before, and at a much lower price. Results will probably need backup from tests on trees, but for quick screening, this technique is excellent.

CLINTON PLANS TO GREEN US TIMBER

Cheaper recycling and faster growing trees are among the targets of a huge programme of research agreed by the Clinton administration to

make the American wood and paper industry leaner and more environmentally friendly. The question still to be answered, however, is whether the Republicans who now control Congress will agree funding for the project.

The programme spells out the kinds of research the industry needs, such as sustainable forest management, pollution control, energy efficiency and recycling. It will guide government agencies in their allocation of research funding.

The American industry needs to cut production costs because it faces stiff competition from Brazil and South Africa, says Robert Williams, chief executive of James River Corporation, a forest products company in Virginia. The chosen areas of research should help to close "technology gaps", he says.

For example, it is not feasible at present to recycle many paper products, because recycling them takes more energy than making them from scratch. The industry hopes that new technology will bring down the cost of recycling, and that genetic engineering will produce faster-growing trees. "We need to grow trees faster. We need to grow more trees, and we need to use fewer acres," says Williams.

Fresh approaches are also needed to improve pollution control, which costs the industry about $1 billion a year. Present methods have reached their limit, says Williams, and further improvements to them would be prohibitively expensive.

The fly in the ointment for the scheme is whether the Republicans will consent to spend money on a programme devised by a Democratic administration. The energy secretary Hazel O'Leary believes she will be able to convince Congress to back the programme, but tight government finances may make this hard to do.

Taken from New Scientist , December 1994: page 10.

RESISTANCE SELECTION TO *PHYTOPHTHORA CINNAMOMI* IN HIGH ALTITUDE *EUCALYPTUS* SPECIES

Planting of high altitude *Eucalyptus* spp. in South Africa namely *Eucalyptus fastigata* and *Eucalyptus fraxinoides*, has been limited due to the soil-borne pathogen *Phytophthora cinnamomi*. Unfortunately, other high altitude *Eucalyptus* spp. such as *E. smithii* and *E. dunnii* can also be seriously damaged by this pathogen. High altitude *Eucalyptus* spp. are important species for planting in specific areas and drastic action must be taken to select for resistance within these *Eucalyptus* populations.

First of all, we are working not only with a diverse genetic pool within the *Eucalyptus* seedlings, but also with a constantly changing and diverse genetic populations within the pathogen, *P. cinnamomi*. An aggressive and virulent isolate of *P. cinnamomi* that is representative of the population, must be selected before any pathogenicity tests and thus resistance selection can be made. In order to select such an isolate or set of isolates, many isolates from a diversity of South African situations were selected and inoculated on *E. smithii*. These isolates more or less represent the current genetic abase within the South African *P. cinnamomi* population. Currently and through extensive research efforts, we have a collection of virulent isolates of *P. cinnamomi* and all that remains to be done is to inoculate the desired *Eucalyptus* trees to select those with high levels of tolerance to *P. cinnamomi*. As a subsequent strategy breeding can be applied to achieve the desired volume and other desired properties.

A problem that must not be forgotten is the fact that *P. cinnamomi* is represented by two mating types, thus male and female isolates in South Africa. Thus the population of the pathogen is constantly changing. Therefore, the isolates that are currently the most virulent may not be so in five years' time. The selection for virulent isolates for screening purposes is an ongoing process and we must continually be sure that trees selected for resistance in 1995 remain resistant, for instance in the year 2000, when new aggressive isolates of the pathogen will most probably exist.

ENDOPHYTES IN FOREST TREES: A NEW CONCEPT

Considering the sheer number of niches occupied by fungi, it is perhaps not surprising to find them surviving and living within healthy plant tissue. The fungi capable of infecting healthy plants without causing lesions or symptoms are known as endophytes. They exist entirely within the host tissue and play important roles in the biology of the plant. These fungal inhabitants of plants are thought to protect them against herbivore grazing and insect attack by producing toxins. They may also play a role in leaf and branch senescence, thus assisting in natural pruning. This endophyte ability of fungi has, however, also partially being adapted as a strategy by plant pathogenic fungi. These pathogens are not true endophytes as they are endophytic only for parts of their life cycle. They are latent pathogens causing symptomless infections, which later develop into full-blown diseases.

Two serious and well-known pathogens of pines and eucalypts in South Africa have recently been shown to occur as symptomless endophytes in leaves and wood of plantation trees. Sphaeropsis sapinea (=Diplodia) is a serious die-back and canker pathogen of pines associated with hail damage. This pathogen has been shown to occur associated with latent infections in healthy cones and wood of various pine species in South Africa. Botryosphaeria dothidea is a similar and serious die-back and canker pathogen of eucalypts associated with environmental stress. This pathogen has also been shown to occur associated with symptomless infections in healthy leaves and wood of various eucalypts species in South Africa.

Why are these findings important to forestry in South Africa? Both above mentioned fungi are stress related pathogens. They can apparently remain in healthy trees and then rapidly colonize

these trees after hail or other stresses. Screening for tolerance to disease might more logically measure latent infections than disease itself. The level of latent infections in tissue might also provide us with a measure of relative tolerance to these pathogens. These and other question relating to latency in Sphaeropsis and Botryosphaeria are currently being investigated by the TPCP. The importance of a stress-associated pathogen already established within healthy unstressed trees is enhanced when one considers the recurrent drought conditions experienced in South Africa.

"Poplar Hypovirulence"

Sphaeropsis sapinea (= Diplodia pinea) **is one of the most important pathogens of pines in South Africa. This fungus is associated with many different disease symptoms such as collar rot of seedlings, cankers associated with resinosis, blue stain, shoot blight and root disease. In South Africa its most serious manifestations occurs as die-back after hail damage. Hail associated die-back of pines due to *S. sapinea*, can cause losses estimated at more than R10 million per year.**

Some isolates display characteristics such as reduced growth, reduced virulence lack of pigmentation, altered colony morphology and suppressed conidiation. These characteristic have previously been linked to the presence of double-stranded RNA and hypovirulence in the chestnut blight fungus, *Cryphonectria parasitica*. The presence of double-stranded RNA causes an attenuation of pathogenicity on chestnuts, in the hypovirulent strains of *C. parasitica*. Natural occurring hypovirulent strains of *C. parasitica* led to the successful biological control of chestnut blight in Europe.

Recently it was discovered that certain strains of *S. sapinea* contained double-stranded RNA. The strains containing dsRNA also displayed hypovirulence associated traits. These findings introduced a new avenue for studying *S. sapinea* and its pathogenicity on pines. It may ultimately result in the successful biological control of diseases associated with *S. sapinea*.

.....GORILLA WARFARE.....

There is a fungus that lives among us!
A silent killer, while we consider:
Sex and compatibility, breeding variation-
Natural selection for geographic acclimitization.
Trees fighting the silent intruders
Slowly girdled and strangled, often the losers!
Ah! But what is this ahead I see!
Pathogens Beware! Here comes the TPCP

Chris Viljoen 1995

THE RESEARCH TEAM OF THE TREE PATHOLOGY COOPERATIVE PROGRAMME

The research team of the Tree Pathology Cooperative Programme is varied. It includes full time staff of the University of the Orange Free State (Prof M.J. Wingfield, Director and Mondi Professor, Dr. B. Wingfield, Dr W.J. Swart and Dr T. Coutinho), colleagues and students attached to other organisations such as the ICFR, technical assistants funded by the University or through membership fees and post graduate students who are mainly funded by the FRD. Staff from various Departments in the University obviously provide advice and support where this is required.

IMPORTANT : PLEASE READ THIS

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

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