



# TREE PROTECTION NEWS

Newsletter of the Tree Pathology Co-operative Programme - University of Pretoria

NO 2

NOVEMBER 2001

It will not be the first time, or no doubt the last, that I say that writing a few words for the front page of Tree Protection News instils a sense of panic in me. This is because I know that I need to end *by wishing our members of the Tree Pathology Co-operative a happy holiday season.* Yes! the end of yet another year is racing towards us. And what a hectic year this has been for all of the TPCP team. The thought that we are not that far from the time to hold another annual meeting is almost "scary". But this is the way of the world, and as the oldest member of the team, I suppose that I feel the passing of time more vividly than some of our younger members. After all, it is said that one's perception of time is a factor of the time that has passed in ones life.

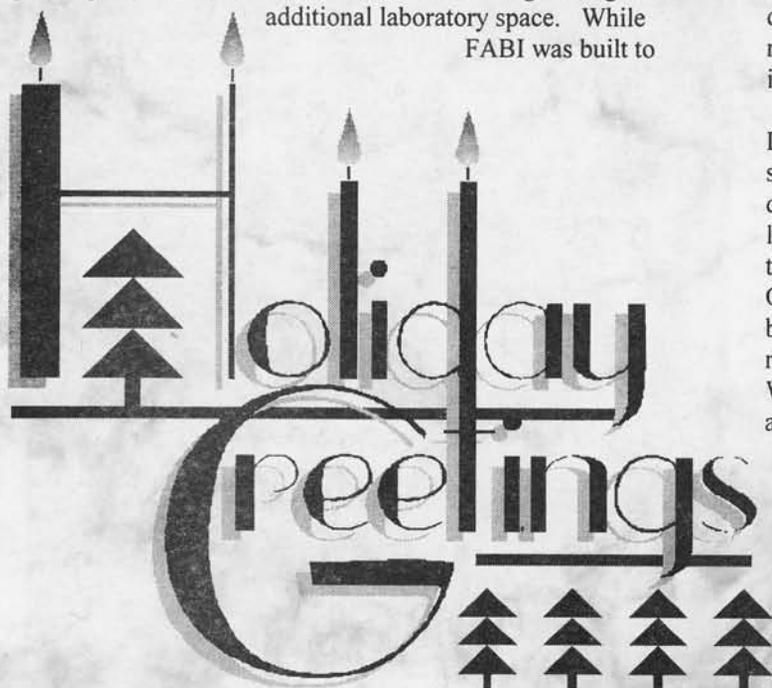
I have promised Jolanda Roux, who compiles our news letter and who is well-known to many of you as our manager of field services, that I will keep this editorial short. This is a promise made at least twice each year, and not one that is easy to keep. This is mainly because there is so much activity in the TPCP that I feel compelled to share details with you. On the other hand, in this newsletter, you will once again read details of many of our activities and there is certainly no need for me to be repetitive.

Within the University of Pretoria environment, and specifically in FABI, some members of the TPCP team are spending considerable time and effort negotiating for additional laboratory space. While FABI was built to

## From the desk of the Director

accommodate what we thought would be at least five years of growth, our facilities have filled rapidly. Indeed, within just three years, and after adding office and other space on the University of Pretoria experimental farm, we find ourselves facing a major space shortage. This is a serious problem as we have reached a point where it is virtually impossible to accept new students into the programme, without concurrently having to consider space constraints. The good news is that the University of Pretoria is very sympathetic to this problem and recognises the tremendous growth and accomplishments of FABI. Thus, we are very optimistic about our plans to double the floor space of FABI in the next year. In this regard, TPCP members and the South African forestry industry will benefit greatly.

Living in an environment and working in a field that is strongly technology driven is exciting, but also highly demanding. The growth of biotechnology and opportunities linked to DNA based techniques are having a huge impact on the fields of forest pathology and forest entomology. Opportunities and impacts have only just started to be felt. I believe that we can hardly imagine what the biological research environment will be like just ten years from now. What is essential is that we remain abreast of developments and that we maintain ready access to key equipment. In this regard, the TPCP has been most fortunate. Just in the last year, we have taken delivery of two major items of laboratory equipment worth some R4million. These are a new, high throughput automated DNA sequencer to replace one of our three year "OLD" machines (three years is really old in this environment!!) and a DNA microarrayer. Both pieces of equipment will bring many new opportunities to the TPCP.



The recent establishment of the African Centre for Gene Technology (ACGT), which is part of the alliance between the University of Pretoria and the CSIR will also have a very positive impact on the TPCP and associated programmes. This new research unit is rapidly growing to provide new opportunities to South African enterprises, to ensure critical mass of scientists working in the field of biotechnology and to "capture" major funding opportunities from government and international organisations. There is no doubt in my mind that ACGT will provide many exciting opportunities for entrepreneurial young South Africans. And the South African forestry industry will also gain greatly from this new centre.

During the course of the last year, we have continued to build our programme in forest entomology. This process is not one that can move particularly fast. As I have said in the past, once human capacity is lost, this is regained only slowly and with great difficulty. It is hard for me to imagine that just 20 years ago, we had approximately 15 people working in forest entomology. Today we do not have access to even one fully trained forest entomologist. But we are working feverishly to change this situation. Prem Govender is making great progress with his work and we have a small but fine team of students working with us to be

able to provide the support and services that the industry deserves. Although I, for one, feel somewhat uneasy about our lack of capacity in this field, I understand that the growth process demands patience and effort. I lack the first of these characteristics but we are making considerable effort and will reach the point in some years where our entomology and pathology programmes are both internationally recognised for their accomplishments.

Forestry is changing in South Africa and the TPCP is changing with it. Privatisation of SAFCOL, one of our key members is a matter that will touch the activities and structure of all general forestry research providers. The establishment of the new Forestry South Africa will also herald in a new era for our industry. The TPCP and its rapidly crystallizing entomology wing, will soon enter a process of financial restructuring. This will need to occur in a fashion that does not allow corrosion of capacity and that also enables us to capitalise on major matching funding opportunities. A change of structure will also hopefully enable us to bring smaller forestry companies into the fold of the TPCP, rather than having to continue providing services to them at the cost of other members. I look forward to sharing some of the details with you at our next TPCP meeting during the first week of March 2002.

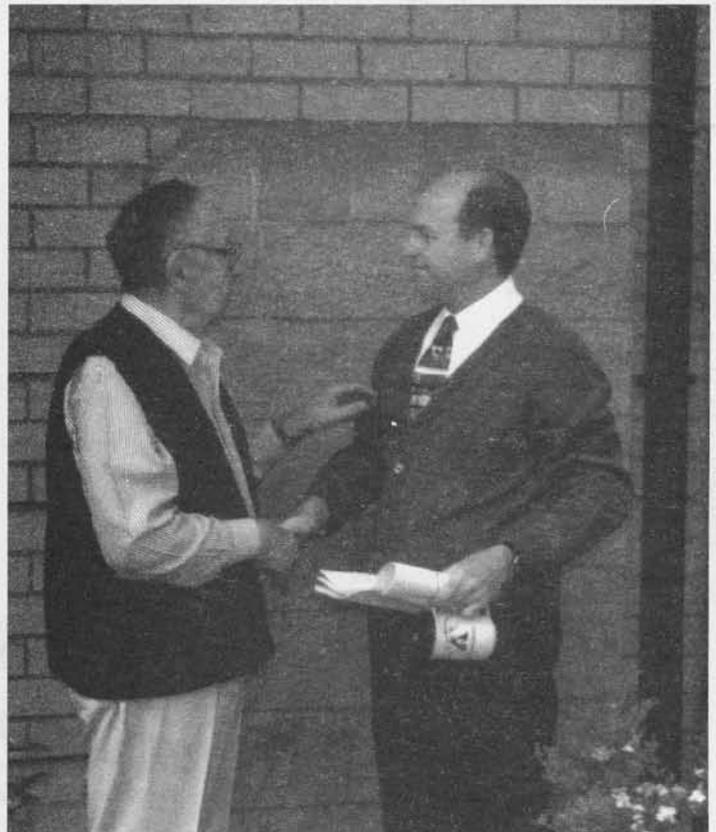
The success of the TPCP lies fully on a close working relationship and collaboration between members and the team. In all of the 11 years of the formal existence of the Programme, we have enjoyed close friendships and cordial working associations with many hundreds of South African foresters and forest managers. Each year at this time, I take the opportunity to thank you all for your support and commitment to assisting us in our focussed goal of

"KEEPING TREES HEALTHY"

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*Mike Wingfield thanks Prof van der Walt for the donation of his books to FABI during our annual spring day tea*



# FABI online

It is not every day that one stumbles onto a website that tells you exactly what the company is about, shows you pictures and bio's of the employees and offers unique information that is available nowhere else. Most companies do strive to provide at least some of these features on their websites... and end up overloading the user with graphics that take ages to download.

Fortunately, with the recent redesign of the FABI web pages, we have been able to take all of this into account and ended up with a professional and visually pleasing site that offers a treasure cove of information at the user's fingertips. Whether you would like to know what the conidia of *Cryphonectria cubensis* look like, find out what research programmes we have, or even if you would just like to chat to some

## Professor J.P. van der Walt donates books to FABI

Prof. Johannes van der Walt is a "Professor Extraordinary" appointed for a three-year term to FABI by the University of Pretoria. He is unquestionably the most famous living Yeast Mycologist and Zymologist in the world. He has authored many important books on yeasts and also authored hundreds of internationally acclaimed articles. Recently he donated the core of his working collection of books to FABI so that they would be available on a day-to-day basis to students and staff. These books have always been important to him and to honour him and this donation, FABI celebrated the occasion at our annual spring day. A bronze plaque was placed next to the collection and it reads as follows:

"The books in this collection were the personal property of the internationally acclaimed yeast taxonomist, Professor J.P. van der Walt. The collection was donated to FABI by Prof. van der Walt after his retirement and at the time that he served the University of Pretoria as an Extraordinary professor. This gift of books is a token of a special friendship between a great scientist and many FABIANS."

FABLans online – you can get it all from the FABI website.

This, of course, has all been made possible by the endless help and dedication of FABLans, who believe that a shining website reflects a shining FABI. The website team currently consists of Albe van der Merwe, Eduard Venter, Juanita de Wet, Bongani Maseko, Cassi Myburg, Mariette Truter and Gina Swart – but these are only the people that are directly involved in the website itself. Every FABIAn has helped to make the website excellent; whether it was checking up on spelling errors, providing pictures or information, we thank you all.

## Log on to

<http://www.up.ac.za/academic/fabi>

and enjoy a FABI experience!



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

The research team of the Tree Pathology Co-operative Programme is varied. It includes full time staff of the University of Pretoria (Prof MJ Wingfield, Director and Mondri Professor, Prof B Wingfield, Prof TA Coutinho, Dr J Roux, Dr O Preisig and Mr P Govender), Rosemary Visser, Shazia Shaik, Sonja de Beer, Elizabeth Attinger, Eva Muller and Brett Hurley, colleagues and students attached to other organizations such as the ICFR, technical assistants funded by the University or through membership fees and post graduate students who are mainly funded by the NRF. Staff from various Departments in the University obviously provide advice and support where this is required.



# SPOOF

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*Society for the Presentation of Outrageous Findings (SPOOF)*

*By Eduard Venter, Karen Surridge, Dirk Swanevelder and Riana Jacobs*

**You are cordially instructed to read the review of this year's prestigious meeting of the Society for the Presentation Of Outrageous Findings (SPOOF).**

"SPOOF is dedicated to the proposition that no research is too trivial, mundane, useless, misguided, asinine or insane to deserve a forum for intellectual discourse. Reports of original or partly original research, real or fabricated, from any

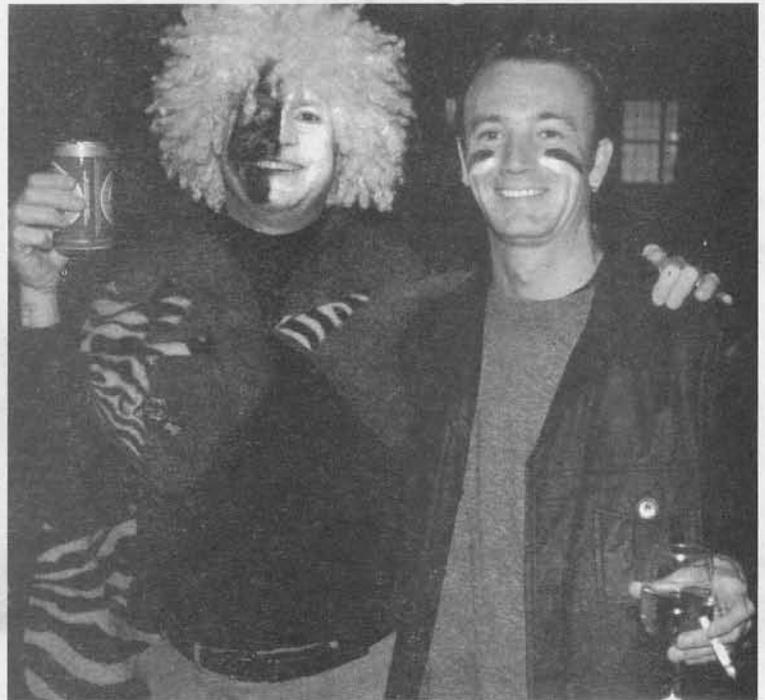
discipline or from those totally disciplined are invited." (Epsilon chapter, 1998). George Hudler (PhD), who invited himself as the keynote address, founded the Epsilon chapter in 1997. He ventured into the African plains, and like Livingstone himself, converted all of the Bloemfontein. Miffmechanix. This in itself is no mean feat, and in honour of his great achievements SPOOF has been transfected from Bloemfontein to Pretoria, like the Voortrekkers of old.

This revelatory roosting took place on the glorious afternoon/evening/morning of XVII Augustus MMI in the FABI kraal. Dressed to the theme, "Deep Dark Africa", the members of this esteemed society, were treated to a magnitude of senseless research or lack thereof. Several keynote addressees were not invited and therefore did not attend, so novices ran the whole meeting. However, the esteemed speakers, skilled in the art of doing squat (and actually getting something published) stunned the audience with their mundane research. Due to the unconscious nature of the audience we shall not mention the names of the speakers as they had passed into oblivion half way through their individual presentations. We can, however, apologise for the Big Kahuna (and his dead sidekick!), as they disrupted the proceedings at regular intervals. This is in compliance with his normal *modus operandi*. We fear that this person has some deep-seated level of

anarchistic behaviour. In his defence, the interludes provided a means of timing for the proceedings.

One of the highlights of the evening was the end thereof. Others include, human cloning (which is the talk of the day/afternoon/evening/midnight/early morning/...?), botanical taxonomy, roman take-aways (which did not include slaves, gladiators or wild animals) and the effects of thin-walled plastic probosci on the consumption of ethyl alcohol. This last talk went down extremely well with demonstrated interest during the break. With some nuclear disasters still fresh in memory, the talk on heavy water was not taken lightly. After the meeting everyone was so confused that cloning in general has been set back by three generations.

On the travel grant front, we can attest that all students attending the meeting must be either extremely rich, very fit, lazy (the hand-written, triplicate 20-page motivation could also have done the trick) or are running some underground commercial cloning project, as no applications were received. Scientific paraphernalia dominated the refreshment table and drew scientists like bees to honey. We also discovered that the nametags the



scientific folk wear are only there so that the organising committee can know to which abode the debauched need to be purveyed. This statement does not hamper their constitutional right to publish; in fact it might even aid and abet it.

The *sangoma* presiding over the *indaba* did so with the ease of any duly designated member of our wondrous rainbow nation. Once again we have to apologise on behalf of the Big Kahuna (and his dead sidekick!) for raining on the *sangoma's* parade. After having his future read in the bones, he has pledged that next year he will behave. However, he can not vouch for his dead sidekick!

All said, the meeting went down successfully and has left a lasting indentation on the peasantry. True to form they came, they saw (we hope that they laughed), they drank and they left. But first, they paused at the tables groaning under the weight of tasty delicacies. A redundantly creative team of laymen/women spearheaded the fourth meeting of the epsilon chapter of SPOOF. They can, however, not be blamed for the huge success of this meeting, as the FABillous peasantry *toi-toied* the night away under the great African sky.



*Testing some of the interesting scientific beverages.*

## **Congratulations to the following students on graduating with a PhD**

**Henk Smith** - A comparison of the biology and taxonomy of *Botryosphaeria dothidea* and *Sphaeropsis sapinea* in South Africa

**Percy Chimwamurambe** - Molecular plant-pathogen interactions with special reference to *Eucalyptus grandis* PGIP's and fungal polygalacturonases

### **CONGRATULATIONS !!!!**

# FABIans in Paris! – MYCOVIRUS RESEARCH AT FABI

One of the focus points of research conducted by the TPCP during the past few years has been to develop alternative control strategies for South African forestry pathogens by using fungal viruses (mycoviruses). It has been shown, by researchers in the Northern Hemisphere, that some mycoviruses may result in the reduction of virulence (hypovirulence) of fungal pathogens. Once such example is the mycovirus infecting the chestnut blight pathogen, *Cryphonectria parasitica*. This virus greatly reduces the virulence of this pathogen and has been successfully deployed in natural populations of *C. parasitica* in Europe to control chestnut blight.

A research team at FABI, lead by Dr. Oliver Preisig, has over the last few years, screened populations of the South African forestry pathogens, *Cryphonectria cubensis* and *Sphaeropsis sapinea*, for the prescence of mycoviruses that may be used in the management of the diseases caused by these two pathogens. This research has lead to the discovery of a number of interesting viruses and they have also expanded their research to include a fungal pathogen of stone fruits, *Diaporthe ambigua*, a close relative of *Cryphonectria* spp.

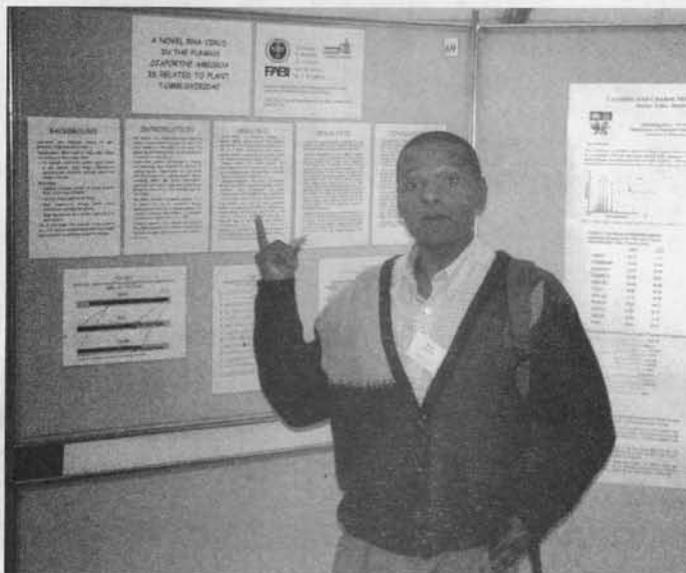
In May, Oliver and two students working with him, Ntsane Moleleki and Schalk van Heerden, had the pleasure to represent FABI at the SIXTH INTERNATIONAL SYMPOSIUM ON POSITIVE STRAND RNA VIRUSES, which was held in

Paris from the 28<sup>th</sup> of May till the 2<sup>nd</sup> of June. Approximately 400

virologists attended this international meeting, all of them working on RNA viruses. Most of the attendees work on human (eg. dengue virus, yellow fever virus and poliovirus) and animal viruses (eg. foot and mouth disease virus). The presence of plant virologists was significant too, with many working on different toombusviruses (like turnip crinkle virus). Each of the FABIANS presented a poster about their work on the *C. cubensis* mitoviruses and the *D. ambigua* RNA virus (have a look at their abstracts). They were the only virologists presenting research on fungal viruses. This is due to the fact that most fungal viruses are double-stranded RNA viruses! Even more exotic, they were the only virologists that would like their viruses to be more effective. Keep in mind their aim is to reduce the virulence of fungal pathogens by infection with these mycoviruses. Although exotic, plant virologists working with toombusviruses were very interested in their work, especially in DaRV, because the genome of this virus resembles a truncated form of a toombusvirus genome. In the meantime a few of them asked for Ntsane's infective DaRV clone to test whether it might replicate in plant cells and if so, with what effects. They learnt a great deal about other virus host systems and the hot topic 'gene silencing' at the symposium.

Beside the scientific experience that they gained at the meeting, they also enjoyed the magnificent city of Paris. They also had some time to explore the main attractions of Paris. Survival consisted of staying in a small hotel, in an even smaller room, close to the Institute Pasteur, where the symposium was held. They also ate huge amounts of fresh baguettes and delicious French cheeses (but Swiss cheese is still better according to Oliver), which is the most affordable staple food in this city, where wine and coke are nearly priced the same.

**Ntsane  
Moleleki**  
explains a  
crucial point  
on his poster.



## Abstracts – 6<sup>th</sup> International Symposium on Positive Strand RNA viruses

### STUDIES OF THE EFFECTS OF *DIAPORTHE AMBIGUA* RNA VIRUS (DaRV) ON NATURALLY INFECTED AND TRANSFECTED ISOLATES OF *DIAPORTHE AMBIGUA*

The fungus *Diaporthe ambigua* is an important canker pathogen of stone fruits and apples in South Africa. The symptoms associated with *Diaporthe* canker include sunken, pointed lesions with longitudinal cracks on infected trees. Different isolates of *D. ambigua* differ in morphology and display varying degrees of pathogenicity. Hypovirulent isolates were found to be infected by a 4113 bp positive-strand RNA virus that has been named *Diaporthe ambigua* RNA virus (DaRV). This mycovirus has significant potential as a biocontrol agent of *Diaporthe* and other plant pathogenic fungi. The aim of this study was, therefore, to consider whether the hypovirulence in naturally infected isolates of *D. ambigua* is solely the result of the resident DaRV, or whether other factors are necessary to confer hypovirulence on the host. DaRV was thus cloned in a pGEM T-Easy vector using RT-PCR. The T7 polymerase was used to produce, *in vitro*, RNA from DaRV cDNA clone. The RNA was used to transfect by electroporation, virus-free isolates of *D. ambigua*. The transfected *D. ambigua* isolates did not display any morphological changes. Further studies are underway to consider the effects on pathogenicity and other traits found in naturally infected isolates of the fungus.

### A NOVEL RNA VIRUS IN THE FUNGUS *DIAPORTHE AMBIGUA* IS RELATED TO PLANT *TOMBUSVIRIDAE*

RNA viruses infect a wide range of fungi. They are often detected as dsRNA elements in nucleic acid isolations or as virus-like particles by electron microscopy. Positive-stranded RNA genomes have been found for a few mycoviruses. The effects of mycoviruses on their hosts vary from lytic to cryptic. In some fungi, for example the chestnut blight pathogen *Cryphonectria parasitica*, virus infection drastically reduces the virulence. Viruses mediating hypovirulence have potential as biological control agents. In this study, a dsRNA element was isolated

and characterized from a hypovirulent isolate of *Diaporthe ambigua*. This fungus is a serious canker pathogen of pome and stone fruit trees. The dsRNA represents the replicative stage of a positive-stranded RNA virus, which we have named *D. ambigua* RNA virus (DaRV). The genome sequence contains 4113 bases and has a GC content of 53%. Two large ORFs are present in the same reading frame. The second ORF is most probably translated by readthrough of an UAG stop codon of the first ORF. This results in a protein with a predicted molecular mass of 125 kDa. Significant homology can be found to the non-structural proteins of carmoviruses of the positive-strand RNA virus family *Tombusviridae*, which also contain the RDRP domain. The coding regions for coat proteins of *Tombusviridae* are usually situated at the 3' end of their genomes, but such an ORF is not present in the case of DaRV. Therefore, DaRV is most probably not encapsidated but might occur as RNA-RDRP complexes. DaRV is a mycovirus with a unique genome organization and is distantly related to the plant virus family *Tombusviridae*.

### CHARACTERIZATION OF MITOVIRUSES ASSOCIATED WITH THE FUNGAL PATHOGEN, *CRYPHONECTRIA CUBENSIS*

The fungal pathogen, *Cryphonectria cubensis* causes considerable damage to *Eucalyptus* plantations worldwide. It is, therefore, essential to develop an effective control strategy against *Cryphonectria* canker. Planting of disease tolerant clones is one approach. The potential to reduce the virulence of the pathogen through virus-mediated hypovirulence is also an attractive option. In this study slow growing isolates from a South African *C. cubensis* population were screened for the presence of dsRNA using CF11 cellulose column chromatography. Some of the isolates were found to contain dsRNA elements with a size of ca. 2.5 kb. Sequence analysis of cDNA produced randomly from the dsRNA revealed that these elements represent at least three genomes related to mitochondrial mitoviruses (*Narnaviridae*). These are similar to those found in *C. parasitica* and the dutch elm disease fungus, *Ophiostoma novo-ulmi*. Northern blot hybridization was performed to determine whether the virus genome predominantly occurs in the + or – single stranded RNA or ds RNA form. Results indicate that the mitoviruses associated with *C. cubensis* are positive ssRNA genomes. A greenhouse inoculation trial was conducted to determine whether the isolates containing the mitovirus display reduced virulence. No reduction in virulence was observed for these isolates. It is, therefore, unlikely that these viruses will be useful as biological control agents although further studies will be necessary to better understand their role.

# ABSTRACTS FROM THE 13<sup>TH</sup> CONGRESS OF THE ENTOMOLOGICAL SOCIETY OF SOUTHERN AFRICA

## Distribution of bark beetles and their associated fungi on exotic plantation pines in the Southern Hemisphere.

*Pinus* spp. from the Northern Hemisphere are widely used for the establishment of plantations in the Southern Hemisphere, especially in South Africa, Brazil, Australia, New Zealand and Chile. Bark beetles are important pests on pine in their native range and they thus threaten plantations in the above-mentioned countries. Five exotic bark beetle species, *Hylurgus ligniperda* Fabr., *Hylastes ater* Payk., *Hylastes angustatus* Herbst, *Orthotomicus erosus* Wollaston, and *Ips grandicollis* Eichhoff, have been introduced to pine-growing countries in the Southern Hemisphere over the past 120 years. These beetles have been responsible for considerable damage during the feeding and breeding stages of their life cycle. They are

furthermore, known to vector pathogenic fungi, in the genera *Ophiostoma*, *Ceratocystis* and *Leptographium*. Some of these fungi can lead to serious tree diseases, and the majority cause sapstain of timber. Few studies have been conducted on these bark beetles in either their native or exotic environments. Furthermore, virtually nothing is known regarding the fungi that they carry. To date, at least 27 ophiostomatoid species have been reported and many of them are the associates of pine-infesting bark beetles that have been accidentally introduced into new environments. We believe that this is a relatively conservative estimate. Further investigations are underway to better understand the composition of fungi associated with bark beetles, both in their native and exotic situations. Knowledge regarding bark beetles and their associated fungi is necessary to fully understand their spread in new environments and their likely quarantine importance.

## Molecular and morphological approaches to forest whitegrub (Scarabaeidae) identification

The larvae and adults of certain Scarabaeidae species feed on the roots, stems, fruit or foliage of many crops. Collectively these pest scarabs are known as whitegrubs (larvae) and chafers (adults). The South African Forestry Industry incurs considerable financial losses from whitegrub and chafer damage. Ten field trials were conducted at six sites, between 1990-1993 in the Natal Midlands to determine factors leading to poor establishment of commercial *Acacia meamsii* and *Eucalyptus grandis* seedlings. Limited taxonomic research and expertise hampered this study, as the whitegrubs could not be identified. Species level identification is important as whitegrubs differ in their biologies, pest status, and sensitivity to insecticides. Non-identification thus impacts negatively on efforts to implement an Integrated

Pest Management Program against forest whitegrubs. However, three main problems confound obtaining, matching and naming whitegrubs and chafers. These are (i) difficulty in rearing adults from larvae, (ii) problems in distinguishing between morphologically similar larvae, (iii) and complexities of accurate adult identifications. We propose a combined molecular and morphological approach to solve these problems. Whitegrubs and chafers have thus been collected from outbreak areas and preserved for molecular and morphological work. The development of molecular tools to match larvae to adults and distinguish between morphologically indistinguishable larvae is underway. Standard morphological techniques are also being used to identify or describe the whitegrubs and their adults. Early results indicate that, molecular techniques can circumvent some of the above-mentioned problems, while standard morphological techniques will allow for the subsequent identification or description of whitegrubs.

## Biological control of *Gonipterus Scutellatus* (Coleoptera: Curculionidae) by *Anaphes nitens* along an altitude gradient.

*Gonipterus scutellatus* was introduced into South Africa from Australia around 1916 and caused extensive damage to *Eucalyptus* trees. As a consequence, the egg parasitoid wasp *Anaphes nitens* (Hymenoptera: Mymaridae), was successfully introduced into South Africa as a biocontrol agent. The efficacy of *A. nitens* has been observed to decrease on marginal high altitude sites. The aim of this study was to investigate the temporal biological control of *G. scutellatus*, along an altitudinal and a corresponding mean monthly minimum temperature gradient. *Eucalyptus dunnii* trees were selected from various sites and surveyed during the year 2000. The growth and damage to trees, the population density of *G. scutellatus* and the percentage

parasitism of *G. scutellatus* egg capsules were measured. Percentage parasitism decreased during the autumn to winter period, except at the low altitude site (800 - 1000 m.a.s.l.). The decrease in percentage parasitism occurred earlier at the higher altitude sites (> 1200 m.a.s.l.). Percentage parasitism then sharply increased in October, except for a higher rainfall site, where the increase occurred in September. Most sites experienced a significant drop in percentage parasitism shortly thereafter in November and this trend was observed at most sites. A high correlation between damage and number of egg capsules was observed, and this has useful monitoring possibilities. Percentage parasitism generally appears to peak early in summer and then late in summer. High altitude sites and especially sites that receive late spring rains, experience an extended period of lower parasitism.

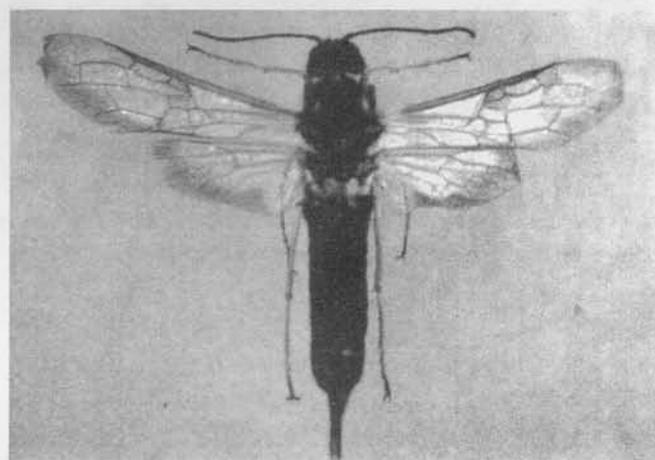
# SIREX ALERT! ———

The woodwasp *Sirex noctilio* and its symbiotic fungus *Amylostereum areolatum* pose a serious threat to forestry in South Africa. In Australia, potential losses caused by *Sirex* were estimated at A\$1 billion to A\$4 billion loss over a 30-year rotation period. Fortunately, the prompt introduction of control measures into South Africa has limited the damage of *Sirex*, and its distribution. However, *Sirex* has recently been recorded in Knysna, approximately 165 km further eastwards than recorded just 2 years previously. This finding gives rise to new **concern** and consequent **action**.

Adult wasps of *Sirex noctilio* are attracted to stressed pine trees. *Sirex* are strongly attracted to *Pinus radiata*, but have also been recorded on *P. elliottii*, *P. patula*, *P. pinaster*, *P. pinea* and *P. taeda*. Numerous holes are drilled into the wood by the wasp, depositing its eggs in all the holes, except one. This hole is injected with phytotoxic mucus and the symbiotic fungus, *Amylostereum areolatum*. The mucus results in a reduction of radial growth, serious chlorosis and loss of needles, increased respiration and a reduction in leaf



Top left: Male



Top right: Female

pressure. Resistance of the tree is decreased by the action of the mucus, allowing *A. areolatum* to grow slowly through the wood, drying out and killing the sapwood. In combination, the mucus and fungus are lethal to the tree. Larvae of *Sirex* feed on the fungus and pupate just under the bark.

Native to Eurasia and North Africa, *Sirex* was first recorded in South Africa at Tokai plantation, near Cape Town, in April 1994. *Sirex* was also introduced into Australia, New Zealand, Tasmania, Uruguay, Argentina, Brazil and Chile. Isolates of *A. areolatum* from South America and South Africa are very similar. Consequently, it is likely that *A. areolatum*, together with *Sirex*, was introduced from South America or that *A. areolatum* from South Africa and South America was introduced from the same source. Soon after *Sirex* was discovered in South Africa, control measures, previously established in Australia, were introduced into South Africa. These included the parasitic nematode, *Deladenus siricidicola*, and the parasitic

wasps, *Ibalia leucospoides* and *Megarhyssa nortoni*. The spread of *Sirex* was monitored and during 1999 *Sirex* was discovered to have spread as far as Riversdale, Western Cape.

This year (2001) SAFCOL plantations from Port Elizabeth (Eastern Cape) to Wolsely (Western Cape) were monitored for the presence of *Sirex*. This survey led to the unexpected finding of *Sirex* at Jonkersberg plantation (just west of George) and on private land at Brenton-on-Sea (near Knysna). The occurrence of *Sirex* in the Knysna area was especially unexpected, as this is approximately 165 km from the last recording, just two years previously.

The spread and containment of *Sirex* is of serious concern to the entire forestry industry, as the primary biocontrol agent, *Deladenus siricidicola*, is only licensed in the Cape provinces (below 32°S). The migration of *Sirex* into the forestry areas of KwaZulu-Natal and Mpumalanga could result in serious

economic losses within the pine industry. The *Sirex* wasp can be spread either through natural migration or through the movement of infested pine logs, as was the case with the introduction of *Sirex* into South Africa. Great care should be taken not to move logs suspected of infestation to non-infested areas of the country.

The TPCP has taken various steps in an attempt to avoid the potential crisis. These include monitoring the spread of *Sirex* on an annual basis and evaluating the status of the biocontrol agents currently in use. This monitoring will enable us to be aware of which areas require an introduction or re-introduction of the biocontrol agents. The first step in this process was undertaken in July 2001, when the first annual survey was undertaken in the Cape Province. Infested logs have been collected and all emerging adults of *Sirex* and the parasitic wasps, *Ibalia leucospoides* and *Megarhyssa nortoni*, will be counted and evaluated. The *Sirex* wasps will also be dissected and examined for the parasitic nematode, *Deladenus siricidicola*.

If the forestry industry is to successfully manage this threat, all involved parties have to ensure that all aspects of the management process is functioning optimally. This includes careful monitoring of pine log movement from infested areas, something that all traders in wood should be involved in.

## Joint meeting held in Salt Lake City

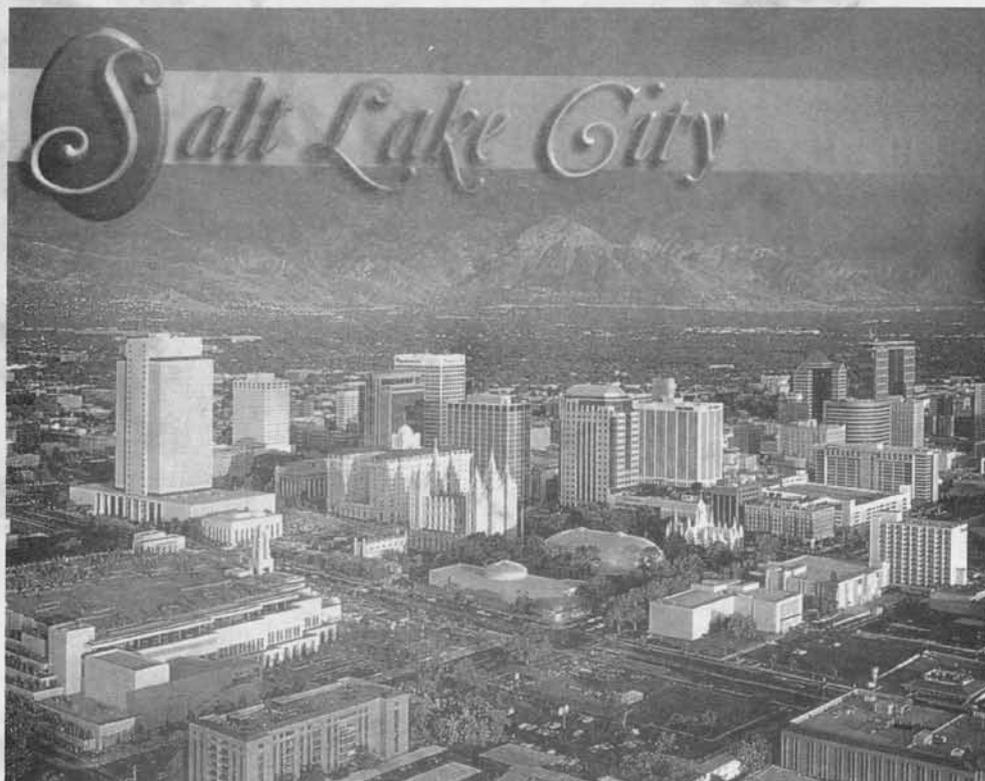
Salt Lake City, host to the 2002 Winter Olympic Games was the venue for the 2001 APS/SON/MSA Joint Meeting. As thousands of skiers will take to the slopes of one of North America's finest ski destinations in February 2002, plant pathologists (APS), nematologists (SON) and mycologists (MSA) from all over the world congregated in the Winter Olympic City from 25 - 29 August. Usually these three societies meet separately, but in line of the great overlap often experienced between the disciples of plant pathology, mycology and nematology, the societies decided to this year encourage communication between scientists and to bridge interdisciplinary boundaries. Almost 2000 delegates from all major continents and numerous different disciplines participated in this event. Twelve scientists, of whom four are current Fabians (Brenda Wingfield, Jolanda Roux, Juanita de Wet and Martin Coetzee) and two are ex-FABI students (Emma Steenkamp now at the University of York as researcher and Karin Jakobs in Ottawa, Canada as researcher) represented South Africa.

Salt Lake City lies in the heart of Utah surrounded by the majestic Wasatch Mountains and adjacent to the Great Salt Lake. It is being described as a hub for excursions to local canyons and national parks, which include the well-known Bryce Canyon National Park, Grand Canyon National Park, and Yellowstone National Park. The Mormon faith (Church of Jesus Christ of Latter-day Saints) dominates in Salt Lake City and dates back to 1847 when the city was founded by a group of Mormon pioneers led by Brigham Young.

The conference kicked-off with the usual Forest Pathology field trip and MSA foray. Forpathers experience the scenic Logan Canyon and the even more scenic lodgepole pine dwarf mistletoe, annosus root disease, aspen cankers

(*Ceratocystis* and *Phellinus*) and tomentosus root disease in Engelmann spruce. Eager mycologists took to the Uinta Mountains. Very dry conditions limited their mushroom findings, but not their spirits. The rest of the scientific programme was filled with four days of stimulating, thought provoking science.

In the Joint Plenary session for the societies presentations were focussed on interdisciplinary co-operation and science communication. Dr. Clutter from the US National Science Foundation especially, emphasised the importance of a "cohesion of sciences" and a "disappearance of borders". She stated that the only successful research groups will be interdisciplinary and will realise the importance of being virtual and visible on the internet. Twentieth century barriers ("boxology") should be overcome and scientists and the industry should communicate outside of their little boxes. New models of co-operation between industry and science are needed for the future. Dr. Clutter further touched on the should include the funding of both infrastructure and initiatives. Successful future research programmes will consist of a close co-operation between government, industry, scientists/education centres and international partners. It was refreshing to realise, listening to Dr. Clutter and other speakers, that this is exactly what FABI and the TPCP is all about: Co-operation and communication.



A broad range of topics was addressed during the meeting which include biology of plant pathogens, diseases of plants, epidemiology, ecology, environmental plant pathology, molecular/cellular plant-microbe interactions, nematology and plant disease management. In a joint MSA/APS session the controversial issue of "What is a fungal species?" was also addressed. FABI made an impression with four

presentations and five posters that elicited various questions regarding diseases and pathogens in South Africa.

Just as plant pathologists and geneticists know how important equilibrium in a pathogen population is, they know how important it is to balance work and play. Salt Lake City provides a wide variety of cuisine, pub breweries selling unique homemade ales and entertainment options. Foreigners can, however, experience Utah's liquor laws as peculiar and strict. Alcoholic beverages can only be served with a meal after 12 noon provided you are at least 21 (obtaining a beer can be a potential problem if you do not look your age). Many clubs are also "private", meaning only members are allowed or you can buy a temporary 2-week membership for \$5. These laws did, however not stop conference delegates from going out and having a couple of brews after a hard day of science (is that why Juanita ended up in a cowboy jail?).

The 2001 APS/SON/MSA Joint Meeting was extremely well organized and a huge success. Seeing and meeting the world's most renowned scientists and exposing your research to international evaluation is a great learning curve in any student's science career. We can only thank and encourage those involved in sending students to international conferences and come to the conclusion that the 2001 APS/SON/MSA Joint Meeting was an unforgettable, highly recommended experience.



*Top: Juanita de Wet experiences some alternative western accommodation – is this as a result of the strict alcohol laws Jua?*

# PG-PGIP interactions

## PG-PGIP interactions

### A pathogen perception-based plant defence system.

Plants are constantly under pressure from biotic and abiotic stresses. Unfortunately they cannot move (run away) to escape these challenges. Additionally, they do not have a cellular immune system similar to the one in vertebrates. However, plants have developed a complex series of mechanisms to protect themselves from these challenges. One of the mechanisms is based on their capacity to perceive pathogenic attacks and adapt accordingly by employing various defensive protocols. The study and utilisation of these natural plant defence mechanisms is one of the most active and exciting research areas in agriculture and also forestry. One of the fastest developing areas is the study of the endopolygalacturonase (PG) and polygalacturonase inhibiting

proteins (PGIP's) in fungi and plants respectively.

Plant pathogenic fungi secrete cell wall degrading enzymes, especially PGs, as a way of circumventing the barrier that is presented by the pectic cell wall of the plant. These enzymes degrade the cell wall polymeric chains to small assimilable sugars, thereby providing the nutrition that is required by the fungus. To counteract, plants secrete PGIPs that specifically and effectively bind to and inhibit the action of these PGs. PGIPs belong to the super family of proteins called Leucine-Rich Repeats (LRRs). These are involved in plant defence signalling. DNA sequences of more than 120 PGIP's have been deposited in Genbank (international DNA sequence data base), mostly from dicotyledonous plants.

The mechanism of action of PGIPs in plant defence is not yet fully elucidated, but there is enough evidence to suggest that there is a highly specific interaction of fungal PGs and the PGIPs. In this interaction, PGs are inhibited to such an extent that the rate of degradation of pectin is performed in such a way that there is an accumulation of elicitor active

oligogalacturonides. These oligogalacturonides then form egg-like complexes with  $Ca^{2+}$  ions. It is these complexes that are thought to trigger defensive responses in plants. Conversely, if this specific recognition, at the PG-PGIP level, does not occur, then the pathogen evades plant defence system. In this interaction, PGs have an antithetical role in pathogenesis, firstly as pathogenic factors and secondly as defence pre-elicitor biomolecules. Clearly, it is important to fully understand these interactions before attempting to utilize them in a genetic engineering programme to improve disease resistance in plants. Cloning and sequencing of the genes responsible genes in these interactions is a crucial starting point.

In the Forest Molecular Biology Co-operative (FMBC) group, we have cloned several PGIP genes from *Eucalyptus* species and PG genes from fungal pathogens. Furthermore, we have investigated the relevance of this interaction with regard to two *Eucalyptus grandis* clones (one highly disease susceptible) and four fungal pathogens namely *Cryphonectria cubensis*, *Botryosphaeria dothidea*, *Coniothyrium zuluense* and *Phytophthora cinnamomi*. Our results, show that it is only in the case of the disease

susceptible clone, that a transgenic approach of over-expressing PGIPs against an endoPG-mediated damage by *Coniothyrium zuluense* may be used. Research in this aspect of transgenic over-expression of PGIPs is in progress. It was clear that in the rest of the fungal pathogens there is no sufficient specificity between the PGIPs of the two clones and the fungal PGs to initiate a PG-PGIP associated defence. It has previously been shown that even one amino acid difference can influence specificity of the interaction. These differences can even be extended to include glycosylation patterns of the mature polypeptide PGIPs.

In conclusion, it is necessary to make case-by-case investigations of the biological relevance of these molecular interactions before selecting or neglecting them in the modern approaches of developing more disease tolerant plants. Over-expression of PGIPs has been successfully utilized in a few research groups to generate disease tolerant transgenic plants. Since PGIP research is relatively young, more success stories can be expected. More and more research groups are initiating PGIP-PG research with a view to developing plants that are more disease tolerant.

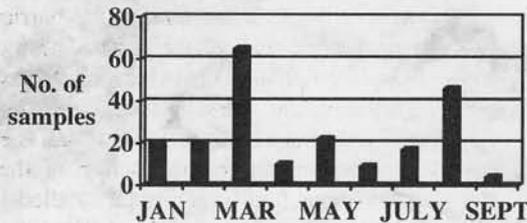
# Diagnostic clinic 2001

For the period January to September 2001, a total of 213 disease and 45 entomological samples was received by the TPCP diagnostic clinic (Fig. 1). Of the disease samples 62% were from pine, 25% from eucalypts, 5% from wattle and 8% from either wood, water or bark media (other in pie chart) samples (Fig. 2). The majority of pine samples were either

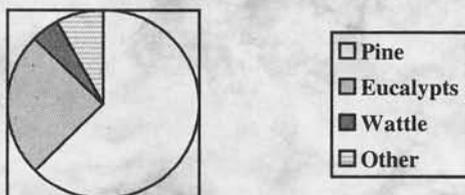
from nurseries or from newly established plantations. The most common pathogen responsible for death of seedlings was *Fusarium circinatum*, the causal agent of pitch canker.

However, outbreaks of *Sphaeropsis sapinea* did occur occasionally. In the case of samples received from eucalypts, bacterial blight, *Botryosphaeria dieback* and *Mycosphaerella* leaf blotch were the most common diseases. A *Botryosphaeria* sp. was common on the wattle samples received. For the entomological samples a total of 47% were from pine, 29% from eucalypts, 20% from wattle and 4% from other samples, including furniture and other tree species. Various samples of *Pissodes nemorensis* (pine weevil), *Gonipterus scutellatus* (eucalypt snout beetle), wattle mirids, whitegrubs and bagworm were received.

**Figure 1**  
NUMBER OF SAMPLES RECEIVED  
JAN TO SEPT 2001



**Figure 2**  
NUMBER OF SAMPLES RECEIVED  
FROM DIFFERENT HOSTS



All your diagnostic questions  
can be addressed to:

Pathology – Dr. Teresa Coutinho

012-420 3934/8/9;

teresa.coutinho@fabi.up.ac.za

Entomology – Mr. Brett Hurley

012-420 3938/9; 0824508941;

brett.hurley@fabi.up.ac.za

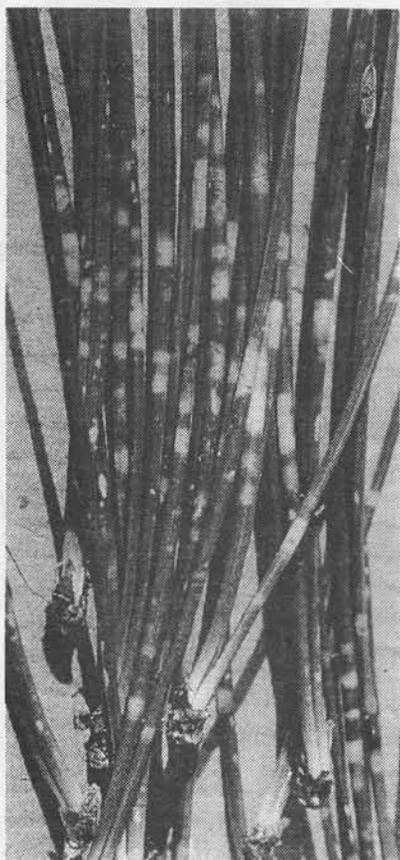
Field Services – Dr. Jolanda Roux

012 420 3938/9; 082 3728350;

jolanda.roux@fabi.up.ac.za

**Dothistroma needle blight** is the most important fungal disease of *P. radiata* in countries such as New Zealand and Chile, where this tree is grown in intensively managed plantations. These countries spend millions of rand annually for chemical spray programmes to reduce the impact of Dothistroma needle blight. The disease, caused by *Dothistroma septospora*, also commonly known as red band needle disease, infects a wide range of *Pinus* spp, although *P. radiata* is one of the most susceptible. Thus, in various parts of Africa, this disease has led to the termination of *P. radiata* planting programmes.

*Dothistroma septospora* is a primary pathogen that infects needles of healthy vigorously growing trees, in the absence of wounds. It requires host tissue for survival and high humidity for infection. Symptoms of infection include the formation of yellow to orange/brown bands on infected needles. The distal ends of infected needles, above the lesions turn brown and die. The disease is easily identified by the formation of small black fruiting bodies (stromata) produced within the bands of discoloured tissue. Infection is first visible on the lower needles on the stems of trees. From this point, infection spreads upwards and outwards, to affect needles on the distal ends of branches, and in severe cases, the entire tree. The formation of necrotic lesions on the needles results in needle drop, with



# DOTHISTROMA NEEDLE BLIGHT ON PINUS SPECIES IN SOUTH AFRICA

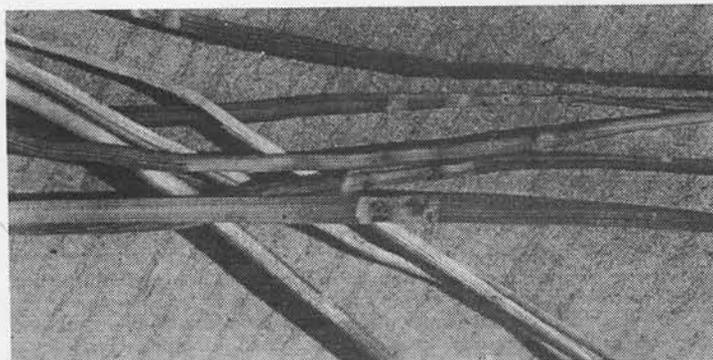
infection of more than 30% of the live crown resulting in reduced growth and malformation of the stems of trees.

The Dothistroma situation is enigmatic. The disease was recorded for the first time in the early 1960's in the eastern Cape on *P. radiata*. However, the disease has never moved beyond this area. This is despite the fact that *P. radiata* is grown in other parts of the country that appear to have ideal conditions for infection. At present, Dothistroma needle blight is especially abundant on *P. radiata* in the Hogsback area of the Eastern Cape. There have been a number of other reports of this disease from other parts of the country, but in every case where suspected infections have been examined, the cause has not been *D. septospora*. One species that has been suspected of being infected by Dothistroma in South Africa is *P. greggii*. This is of special concern as this species is one that is being planted with increasing frequency. It is also the basis of a number of very relevant breeding programmes. However, recent examination of purported infections led to the conclusion that lesions were caused by factors other than Dothistroma.

As with other diseases, responsible diagnosis is of extreme importance. A number of symptoms, caused by agents such as insects and secondary pathogens are easily confused with Dothistroma needle blight.

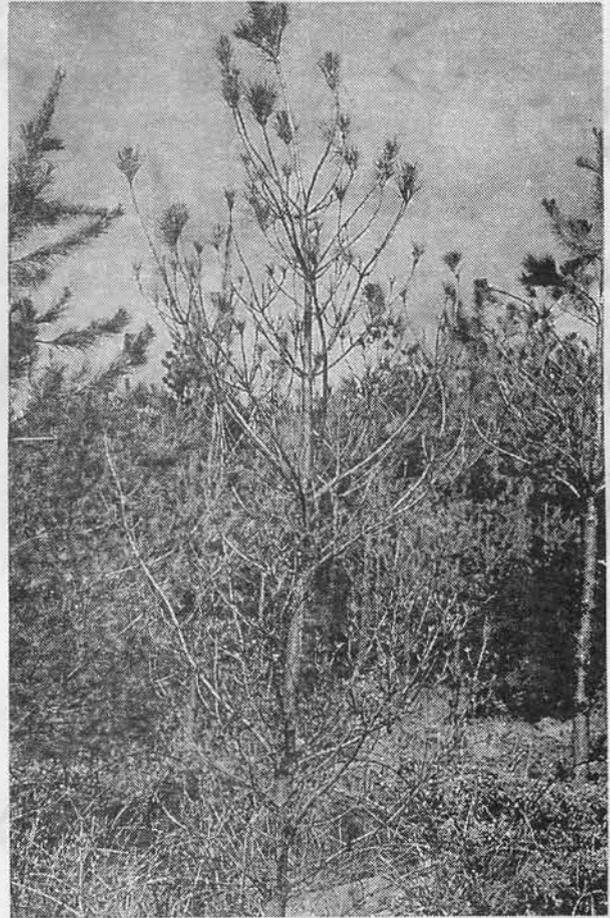
**Left:** Spots caused by the pine needle aphid, lack the absence of fungal fruiting bodies.

**Bottom:** Small black stromata of *D. septospora* on *P. radiata* needle.



Symptoms caused the pine needle aphid, is especially commonly confused with Dothistroma needle blight. It remains important to investigate all disease symptoms on trees and to send material to the TPCP diagnostic clinic for detailed analysis. Dothistroma needle blight can easily be spread with infected nursery stock and its occurrence and future status in Southern Africa could be of great economic importance.

Successful management of Dothistroma needle blight has been achieved in Chile and New Zealand with the implementation of chemical spray programmes. Despite the costs involved, these spray programmes remain profitable to these countries, where potential losses are enormous. Spraying does not need to be conducted on all stands every year and application of sprays is determined after careful stand surveys. Such surveys inevitably also generate costs but trees also gain a level of resistance as they age. Much progress has been made in breeding for Dothistroma resistance in *P. radiata* and consequently, the costs of spray applications have been reduced. If Dothistroma needle blight should develop as a problem in South Africa in the future, significant knowledge, including those from studies of SAFCOL in the eastern Cape will be available to reduce its impact.



**Left:** Severe infected tree showing needle drop.

## **IMPORTANT : PLEASE READ THIS**

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

### **POSTAL ADDRESS**

**Tree Pathology Co-operative Programme  
Att: Prof. Michael J. Wingfield  
Mondi Professor of Forest Pathology and  
Director of FABI  
Forestry and Agricultural Biotechnology  
Institute (FABI)  
University of Pretoria 0002**

### **STREET ADDRESS FOR DELIVERIES**

**74 Lunnon Road (between  
Tindall and Herold Street)  
Hillcrest  
Pretoria**

Tel: 012-420 3939 Fax: 012-420 3960 e-mail: [Mik@Wingfield@fabi.up.ac.za](mailto:Mik@Wingfield@fabi.up.ac.za)  
Web page: <http://www.up.ac.za/academic/fabi/tpcp>

Compiled by Jolanda Roux

Design and Layout by Susan Christie