tree pathology news

NEWSLETTER OF THE TREE PATHOLOGY COOPERATIVE PROGRAMME - UOFS

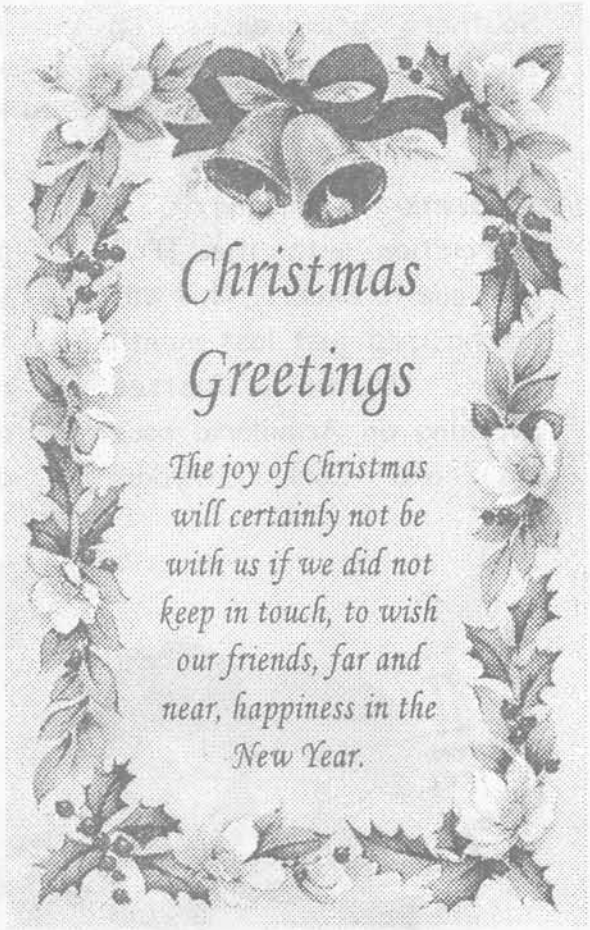
NO 14

NOVEMBER 1996

DIRECTOR'S MESSAGE

It is truly remarkable how quickly the TPCP years pass. The Programme appears to become busier by the month with new diseases being found, old diseases appearing in unexpected situations and novel solutions to such problems continuously being generated. In this second and thus last Newsletter for 1996, we will once again attempt to share some of the highlights of the past six months with you.

The last issue of Tree Pathology News appeared shortly after the annual meeting of the Programme. In the Editorial column, I made reference to the newly inaugurated SAFCOL Botryosphaeria project. I also suggested that more details would be provided in the body of the Newsletter. We then failed to include the promised column - one of the gremlins that must plague professional Editors continuously. In this issue,



we are reproducing a column from the Free State University publication "Bult" that focuses on the topic fully.

Another column in our last issue aimed to draw your attention to the fact that the TPCP was in the process of developing an exciting and interactive World Wide Web site. Through the very able efforts of Teresa Coutinho, this is now functional and most impressive. Those of you that have access to a Netscape or alternative "browser" should visit the TPCP home page. Complete lists of projects, all the diagnostic pamphlets (with colour illustrations) and even the latest copy of Tree Pathology News can be read there. What is most amazing to us is the very high number of people that have visited the "site" since its completion and the

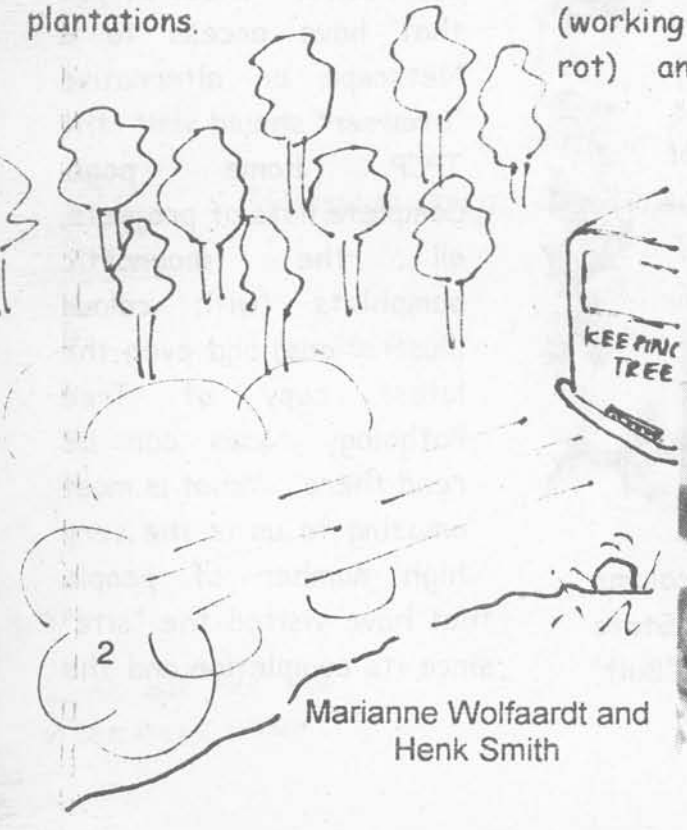
installation of a counter. About 83 visitors in less than three weeks. Visit us if you can!!. Drop messages and give us your views on how we might improve the pages and make them more useful to you.

One of the most exciting developments in the TPCP during the course of the past few months has been the appointment of a person to further expand the Programme's field extension and disease monitoring activities. Here the Forest Owners Association have provided funding for this important activity. Jolanda Roux who is well known to many of you will fill this position. Given the fact that she is still in the process of completing her Doctoral studies, she will be assisted by a full time technician to enable her to spend more time in the plantations.

One of the very exciting aspects of our research Programme during the course of the past six months pertains to our studies on the susceptibility of *Pinus gregii* to disease. Of course the disease of greatest interest here is Diplodia blight caused by *Sphaeropsis sapinea*. Many interesting observations have been made and these will be presented to you in various ways as data become available. Perhaps most exiting to us is the fact that the Northern Province of *P. gregii* appear to have very high degrees of tolerance to the pathogen. Southern provenances, on the other hand, are highly susceptible to infection.

Students working in association with the TPCP continue to excel. This was emphasized just last month when Martin Coetzee (working on Armillaria root rot) and Corli Strydom (working on Ceratocystis wilt pathogens such as the wattle wilt organism) travelled to the United States to attend the annual meeting of the American Phytopathological Society, held in Indianapolis. At this meeting Corli won the prize for the best forest pathology presentation and Martin won the award for the second best poster. An amazing accomplishment indeed. Abstracts of both these presentations are included in this document.

As has become tradition, this second issue of Tree Pathology News is also the last for the year. We would therefore, like to take the opportunity to wish you all a very happy Christmas and a most prosperous and happy 1997. We also thank all of you that have helped us with our research and extension activities during the course of the last year.



Marianne Wolfaardt and
Henk Smith

ONE STEP AHEAD

by Justus Visagie

The forest industry has been losing some sleep recently. The fungus *Botryosphaeria dothidea* has emerged as a major threat to the industry: killing eucalyptus trees or rendering them useless on a large scale

Fortunately scientists at UOFS Microbiology and Biochemistry Department have been researching this disease since its appearance in 1994, discovering promising solutions. And in April this year the forestry company SAFCOL signed a contract with the UOFS to find ways to counter this devastating disease. (The UOFS is still the only university in South Africa to research tree diseases such as *Botryosphaeria* canker.)

Since a sick tree cannot be healed, researches attempt to cultivate eucalyptus clones or hybrids capable of resisting attacks from the *Botryosphaeria* fungus.

"*Botryosphaeria* is an opportunist" says Mr. Henk Smith, a Ph.D-candidate who has studied the fungus since working on his honours degree. As soon as a eucalyptus plantation is under stress, it strikes. Under conditions of late frost, hot or cold winds or drought the tree tops die, providing the fungus with a foothold. It subsequently infects the pith (centre) of the tree, often killing the whole tree. Insect damage or branch pruning also makes a tree vulnerable. In these cases *Botryosphaeria* infects tree branches, causing stem cankers and making the trees useless for saw timber production.

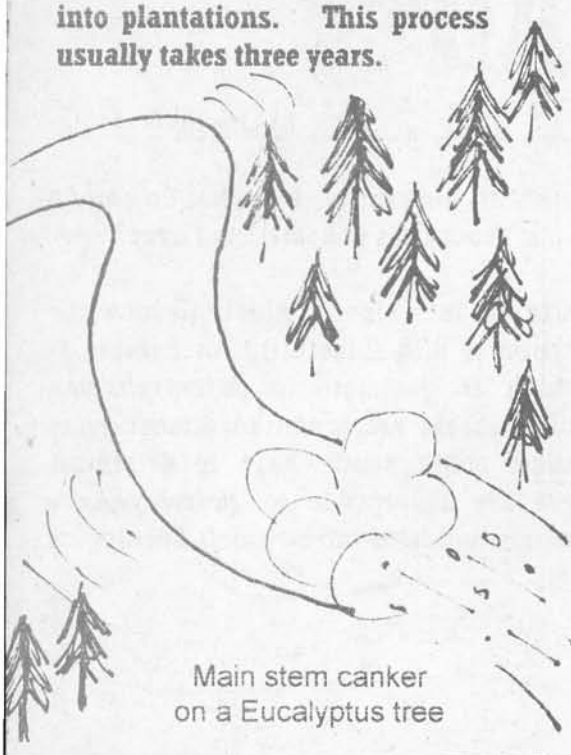
"What makes the disease so disastrous is that Eucalyptus trees intended for saw timber have to reach an age of 25 to 30 years and trees planted for pulp are harvested at eight years. If healthy trees are then attacked by the *Botryosphaeria* fungus and killed or damaged after, say, five or ten years, the industry suffers massive losses"

Now if SAFCOL was a vegetable farmer, it could have covered its crops to protect them against frost or hail, or sprayed them with fungicide. But what do you do when your crop is thousands of hectares of trees?

The answer is to produce clones, which all have the same genetic code or DNA fingerprint, or hybrids of Eucalyptus which are resistant to *Botryosphaeria*. New clones and hybrids are produced almost every day. Scientists subsequently have to determine which of them are susceptible to *Botryosphaeria* infection. One method is to infect young Eucalyptus

Ruined. The awkward growth in this tree was caused by a *Botryosphaeria* infection

trees, which are planted in experimental plots with *Botryosphaeria*. The trees that do not develop lesions are considered immune and released into plantations. This process usually takes three years.



Main stem canker on a Eucalyptus tree

Unfortunately, just as the human race is battling with bacteria and parasites like TB and malaria that become resistant to antibiotics and prophylactics, *Botryosphaeria* too learns to outsmart the clever scientists. Because the fungus is a dynamic organism it overcomes the resistance of the new hybrids and clones with time.

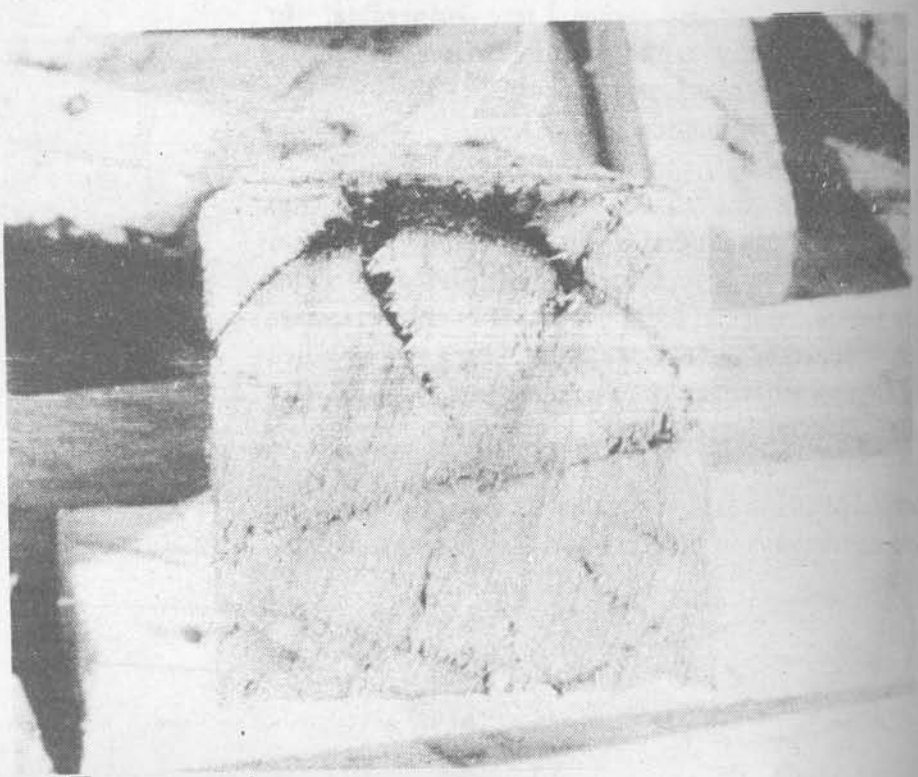
For this reason the SAFCOL project has to stay one step ahead in producing new hybrids and clones to replace the older ones that can no longer fight the disease. If scientists can therefore establish within three months instead of three years which hybrids and clone are immune and which of them are not, they can produce sufficient new clones and hybrids. Which means even if some are overcome by the disease, there are enough trees capable of resisting *Botryosphaeria* until they are ready for harvesting.

To achieve this, researcher Dr. Marianne Wolfaardt will use the following method. She will first determine the amount of polygalacturonase inhibiting protein (PGIP) in each clone. (Researchers at the UOFS have discovered that this protein defends the tree cells under attack of the enzymes in *Botryosphaeria.* The higher the level of PGIP, the more immune the clone is.)

The next step would be to raise a number of clones, inoculate them, and to see which of them form lesions. Dr. Wolfaardt can now infer what level of PGIP is needed to ward off the disease and thus which of the clones are immune.

In the end researchers will no longer need to inoculate trees in the field. New clones that a forestry company consider planting can simply be measured in a laboratory to determine whether they have enough PGIP

Timber intended for sprags (in mines) will be crushed due to a structural weakness from earlier *Botryosphaeria* infection

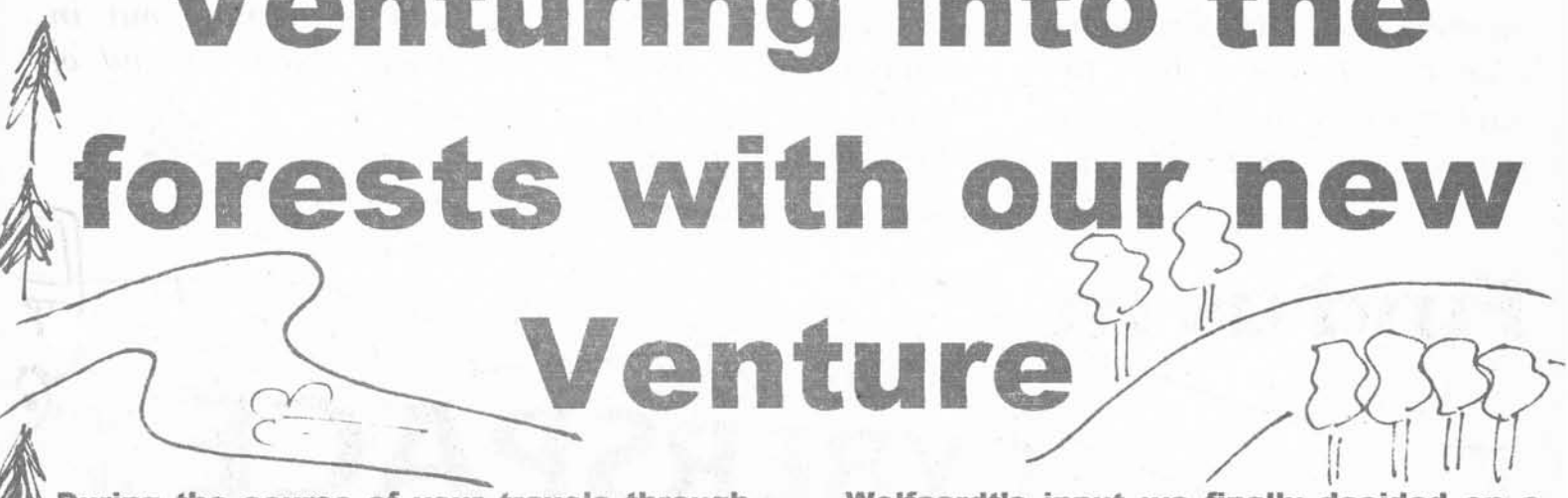


to be immune.

A giant leap in streamlining the recognition of immune clones would be to attain DNA fingerprints from immune and susceptible clones. Ultimately this will enable researchers to say beforehand what DNA fingerprint a clone needs to be immune.

"There's no reason why we shouldn't be able to achieve this," - Mr. Smith says.

Venturing into the forests with our new Venture

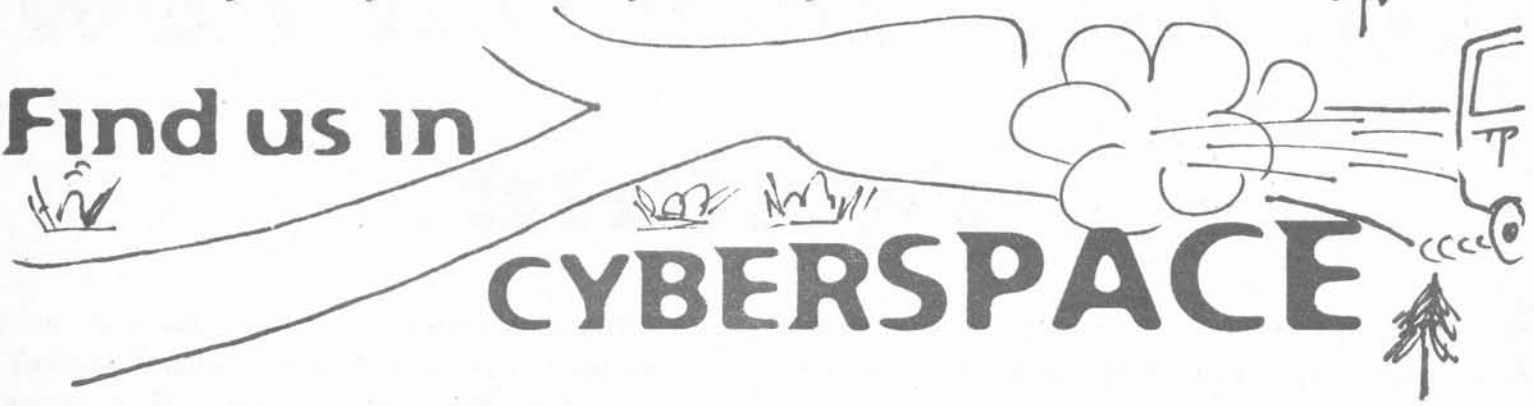


During the course of your travels through the forestry areas of South Africa, you will occasionally notice a packed white Toyota Venture colourfully decorated with the TPCP logo. We have been debating purchasing a car to use on field trips for a couple of years. Due to high rental costs, we finally decided that buying a car would be the only solution. Heated discussions centred around which type of car to purchase and thanks to Francois Wolfaardt's input we finally decided on a Venture. The colour was another matter - Mike Wingfield had visions of a bright yellow car zipping through South Africa but this was strongly vetoed by the group (and luckily he felt that waiting for one on order would take too long). So, when you see us on the road don't forget to wave and if you need any assistance with a pathological problem we will be more than willing to stop and help!!

Switzerland, Sweden, the Netherlands, the U.S.A. and Norway - all meet at the TPCP

The number of foreign visitors has steadily increased over the course of 1996. Dr. Oliver Preisig ("Swiss cheese" as he is fondly referred to by the group), from Switzerland, joined us as a postdoctoral

fellow at the end of last year. He is working on Sphaeropsis sapinea and will be with us for two years. Daniel Dalevi, from Sweden, and Karin Overkamp, from the Netherlands, joined us more recently. Daniel is here for part of his B. Sc. degree and Karin as part of her Masters degree. They are both with us until the end of 1996. Prof. George Carroll from the University of Oregon is on sabbatical and will be in the Department for 15 months. His speciality is endophytes. Last but not least is Hanna Solheim from Norway. She has just finished her schooling and is in Bloemfontein as part of an "African experience". She will be helping out in the group until the end of the year.



If you have access to cyberspace, please look up the TPCP webpage..... our address is:

http://www.uovs.ac.za/natwet/mkboc/tpcp.htm

In this webpage we have included permanent and student members of the TPCP, projects, all recently published diagnostic pamphlets, recent publications, our newsletter and structure and future plans for the webpage. Look us up and find out more about our Programme!!!

NEW PAMPHLETS ON PINE DISEASES

Mondi kindly sponsored the publication of three new pamphlets on pine diseases. These are on *Armillaria, Sphaeropsis sapinea* and *Rhizina*. The production of diagnostic pamphlets form an integral part of the TPCP. Through the distribution of these pamphlets, foresters can be **made aware of diseases that can be responsible for substantial losses and report**

their occurrence as soon as early symptoms become apparent. In this way, the TPCP will be able to "Keep trees healthy"!!!

The TPCP also published two disease alerts - one on pitch canker and the other on Eucalyptus rust. Both diseases will have a substantial impact on forestry in South Africa should they appear in plantations. For this reason, foresters should be aware of the symptoms such as those presented in the pamphlets and report them to the TPCP. Early recognition will facilitate the development of effective management practices.

If you would like a copy or a number of copies of these pamphlets for distribution, please contact us and we will post them to you.

MSc thesis of Wouter de Lange

The focus of my MSc research was on *Cryphonectria cubensis*, a serious canker disease of *Eucalyptus* in South Africa. Double stranded RNA (dsRNA) was previously found to be associated with this fungus, and my studies involved investigations of this phenomenon. The initial part of my project was concerned with the development of two novel techniques. The first was a modified isolation technique that allowed for dsRNA isolation in less time than with previously used techniques. The second was an adaptation of a technique used with other fungi. In this case, apples were successfully used as the host in virulence testing. These two techniques were used in the remaining part of my study, where the virulence of the 24 South African isolates of *C. cubensis*, and the presence of dsRNA in the isolates was determined. The variation amongst the 24 South African isolates of *C. cubensis* was not reflected in their geographical distribution, nor in the presence of a small sized dsRNA fragment. The results indicate a relatively homogeneous population of *C. cubensis* in Southern Africa. This is possibly the result of a recent introduction of this pathogen. The presence of dsRNA in *C. cubensis* isolates is of special interest in the light of biocontrol. For this reason it is suggested that future research be concentrated on detecting this phenotype. The research accomplished will be of value in accomplishing this goal.

ABSTRACTS OF TWO TPCP STUDENTS AT THE AMERICAN PHYTOPATHOLOGICAL SOCIETY/MYCOLOGICAL SOCIETY OF AMERICA JOINT ANNUAL MEETING, INDIANAPOLIS, INDIANA

IDENTIFICATION OF SPECIES *OF CERATOCYSTIS SENSU STRICTO* USING RFLP AND DNA SEQUENCE DATA

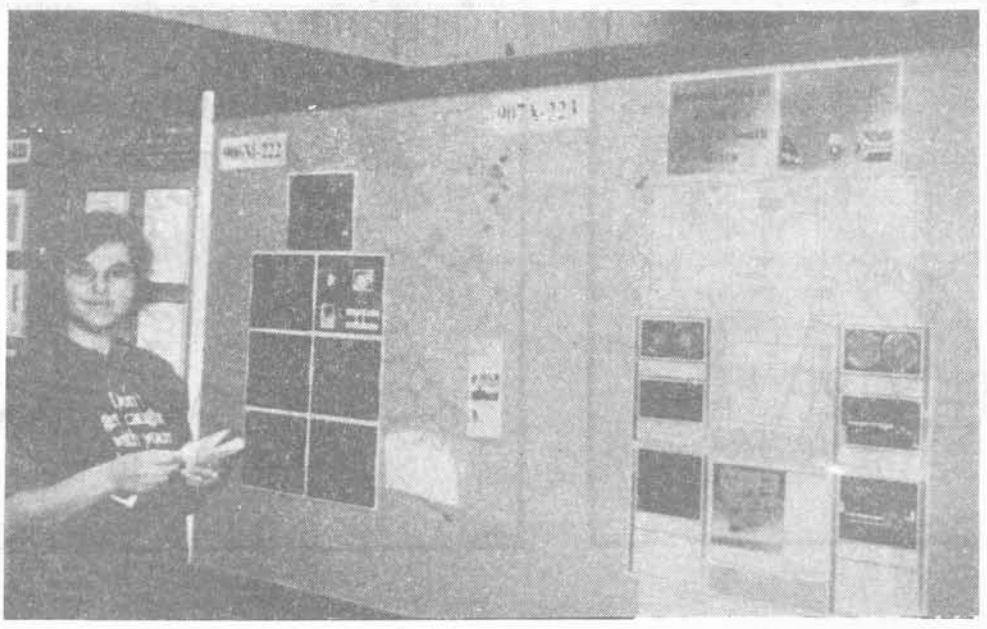
Corli Strydom

Most species of *Ceratocystis sensu stricto* are virulent pathogens of a wide variety of plants including forest and fruit trees, sweet potatoes, pineapples and sugar cane. These organisms are difficult to identify. In this study we have developed a quick and reliable method to distinguish between 10 different species of *Ceratocystis*. A 1600 base pair fragment within the ribosomal DNA operon was amplified using the polymerase chain reaction (PCR). The amplification was done directly from living fungal tissue without extracting DNA. The amplified fragment included part of the small and large sub-unit rRNA genes, the 5.8S rRNA gene and the internal transcribed spacers (ITS) 1 and 2. We sequenced both strands of the 1600 base pair PCR product of one isolate of each of the species. Phylogenetic relatedness was determined using Phylogenetic Analysis Using Parsimony (PAUP). We were unable to distinguish most of the *Ceratocystis* species based on the unique restriction fragments produced by *Alu*I. RFLP and sequence data were thus used in the development of a rapid and accurate diagnostic tool to identify species of *Ceratocystis*.

IDENTIFICATION OF *ARMILLARIA* IN SOUTH AFRICA

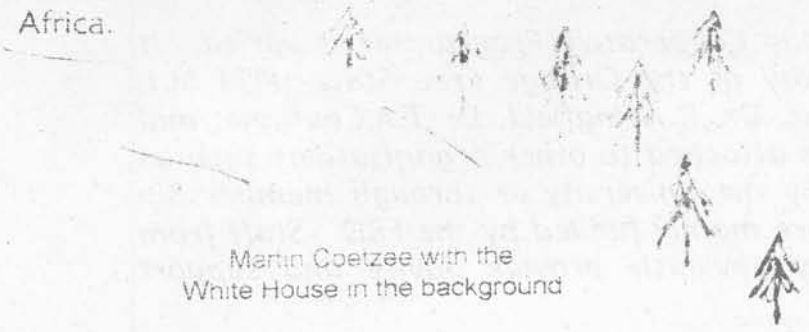
Martin Coetzee

Armillaria spp. are important pathogens of trees throughout the world. The genus has been well studied in North America and Europe, but has received minimal attention in Africa. *Armillaria mellea* was first reported to occur in South Africa in the early 1900's, associated with root rot of *Pinus* and *Eucalyptus*. The taxonomic disposition of the fungus, however, remains doubtful although the name *A. heimii* has recently been used in some reports. The aim of this study was to examine isolates of *Armillaria* from Southern Africa. Thirty one isolates, originating from different regions in Southern Africa, were obtained using established isolation techniques. These were compared using cultural morphology, basidiomes



Corli Strydom in front of the posters presented by her and Martin Coetzee

produced *in vitro* and on the basis of RFLP'S. Our preliminary results suggest that at least two species of *Armillaria* occur in South Africa.



Martin Coetzee with the White House in the background

A COMPARISON OF ISOLATES IN THE *CERATOCYSTIS COERULESCENS* COMPLEX USING DNA SEQUENCE DATA

Corli Strydom

Ceratocystis sensu stricto includes numerous species of insect-vectored, wood-staining and plant pathogenic fungi. *Ceratocystis coerulescens* is the cause of blue-stain in spruce and pine. Previous investigations, using morphological characteristics and isozyme comparisons, have shown that *C. coerulescens* encompasses at least five morphological types. The aim of this study was thus to compare isolates of *C. coerulescens sensu lato* and morphologically similar species, including *C. laricicola*, *C. polonica* and *C. virescens*. Using the polymerase chain reaction (PCR), a 600 base pair fragment within the ribosomal DNA operon was amplified. The amplification was performed directly from living fungal tissue without the extraction of DNA. The amplified fragments included part of the large sub-unit rRNA genes, the 5.8S rRNA gene and the internal transcribed spacers (ITS) 1 and 2. Both strands of the PCR products were sequenced. Phylogenetic relationships were determined using Phylogenetic Analysis Using Parsimony (PAUP). The five morphological types in the *C. coerulescens* complex had unique ITS sequences and appear to represent distinct taxa. *C. laricicola* and *C. polonica* have similar ITS sequences and may be synonymous.

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 T.A.Coutinho, and Dr W.J. Swart), 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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Look out for me in the next issue!!

.....Edited and compiled by Susan Christie