tree pathology news

NEWSLETTER OF THE TREE PATHOLOGY COOPERATIVE PROGRAMME - UFS

NO 15 MAY 1997

COMMENTS FROM THE DIRECTORS DESK

The annual meetings of the TPCP have just past which means that the deadline for posting our first Newsletter of the year is approaching rapidly. As many readers of Tree Pathology News will now know, two issues are produced each year and these are dispatched in mid May and in mid November. In recent times, we have had a great deal of positive feedback from readers of Tree Pathology News. This has not only been from members of the TPCP but from people in a wide range of countries that visit our Web site and read the document there.

ANNUAL MEETING

The annual meeting of the TPCP has grown in size each year since the inception of the Programme seven years ago. This year the meeting was attended by almost one hundred representatives of the TPCP member companies. For those of you that have attended the meetings before, and were not able to join us this year, you can imagine that the traditional Beef Baron dinner required that we take over the entire restaurant! One has to wonder what we will do if attendance continues to grow. Perhaps Lance and his team will need to provide their excellent services at some other venue.

That's why I never walk in front

As has been true in the past, our annual meeting was typified by students and staff of the TPCP presenting short papers illustrating their research results. This year we were also privileged to have various colleagues from other parts of the world to share some of their experiences with us. Thus Tom Harrington, who is visiting our team from Iowa State University, presented a fascinating lecture on root and butt rot diseases, George Carroll from the University of Oregon shared some of his vast knowledge of fungal endophytes and Karin Wikler from the University of California, Davis, illustrated how the Pitch Canker disease is threatening *Pinus radiata* in California. Not to forget South Africa's own, Charlie Clarke of Sappi shared the stage with Francois Wolfaardt to discuss techniques that they have developed to evaluate the impact of stem canker diseases of eucalypts in Zululand.

GROUP ORGANISATION

The TPCP has grown considerably during the past year and the team has spent a great deal of time streamlining various functions. We have thus defined major roles in the programme and assigned these to Programme Managers. The impact and efficiency of this streamlining has been most positive with TPCP members being able to obtain support even more efficiently. Thus Teresa Coutinho has assumed responsibility for the Diagnostic Clinic and Jolanda Roux is the TPCP Manager of Field Services. Brenda Wingfield fills the role of Manager of New Technologies, Susan Christie acts as curator of the culture collection and Chrissie Moolman and her team support the group in an administrative role.

MONDI CHAIR

An exciting development this year has been Mondi's announcement that it will continue to support the Forest Industry through their endowed chair in forest pathology. The initial term of this endowment was three years and this has now been extended by a further three years. John Quy, who opened this years TPCP meeting announced Mondi's decision to renew its endowment and noted some of the accomplishments that have resulted from this investment in the past. He also noted that the endowment had been increased in value and that it would amount to an investment of approximately R700 000 over the next three years. As director of the TPCP I feel proud to be able to continue occupying this endowed chair and to be able to carry Mondi's name alongside my own. Perhaps more importantly, Mondi's investment has contributed very significantly to the training of students and to the development of disease free plantation trees.

(Top):
Mr John Quy congratulate Prof G. van Wyk, Dean of Science on the renewal of the Mondi Chair
(Right):
Mr Peter Keyworth signing the contract joining CTC to the TPCP

NEW MEMBERS

By now, most readers of TPN will be aware of the fact that CTC/ NCT/ TWK have joined the TPCP. In addition, the Department of Water Affairs and Forestry (DWAF) has announced that it will join the Programme this year. These are exciting developments as it will now mean that the TPCP serves virtually the entire South African forestry industry. Given the fact that many disease problems are of interest to all players in the Industry, it makes a great deal of sense for all to share in the initiatives of the TPCP.

CTC and the forestry groups associated with it are somewhat different to the past members of the

TPCP. The major difference is that this group represents a large number of medium and small timber growers. While the task of making contact with such a large group might seem daunting, the TPCP team views this as an exciting challenge. We have dealt with samples from NCT/ CTC and TWK for many years now. It is good to have these friends as legitimate partners of the TPCP and we look forward to providing them with the best service that we can possibly muster.

At this stage, membership of DWAF has not been finalised. What remains to be done is to sign the various contracts and then to implement initiatives to serve the unique interests of DWAF. This might require the appointment of additional staff to deal with work in more distant areas and to promote DWAF's desire to include a focus on indigenous trees. We look forward to discussing progress with these initiatives with you in future editions of TPN.

THE YEAR AHEAD

The TPCP has had many successes in its short term of existence. New diseases have been recognised, the impact of many diseases has been substantially reduced, new strategies to deal with disease have been introduced and new technologies have been brought to the South African Forestry Industry. Perhaps more mundane, but equally important has been the implementation of a field monitoring and survey system which is, in our opinion the first effective and sustainable initiative of its kind in South Africa. Likewise the establishment of a high quality diagnostic service, with associated field follow -up facilities and the preservations of samples and cultures for longer term reference has been an important development.

In years to come, we expect the TPCP to serve its members with increasing efficacy. It is a pleasure to have the opportunity to work with, and support interested and committed friends. Our task is, in every respect a team effort, achieved together with foresters and forest managers in South Africa. As I have said many times in the past, we appreciate having the opportunity to work with you - in our common goal of **KEEPING TREES HEALTHY**.

Diagnostic Clinic 1996

The Diagnostic Clinic functioned relatively well during 1996. There were occasionally a few hiccups but on the whole the Clinic functioned effectively. Over the past three years, there has been a steady increase in the number of samples we receive. During 1996 approximately nine samples were received per month as opposed to six samples per month sent in 1995. A larger number of samples are sent when the environmental conditions favour disease development, that is, when it is warm and wet. Thus there is seasonal variation in the number of samples we receive, with the greatest number sent in summer (Fig. 1). The samples received were either from eucalypts or pine with some from wattle and other tree crops (Fig. 2).

Please remember -

- *When you are about to send a sample please phone the TPCP beforehand. Occasionally, the samples go missing en route to Bloemfontein. It also gives us*

enough time to make up the required media.

- *Please use a courier and not the fast mail service offered by the Post Office. The sample is sent to the Post Office on campus and can remain there until they remember to phone us to come and collect the parcel. In December last year one sample remained there until mid-January.*
- *Please do not send dead material. It is impossible to identify the causal agent as saprophytes are abundant on the material. Rather send dying material which includes some healthy tissue.*
- *If you want a water sample analysed for the presence of pathogens, please inform us well in advance. The isolation procedure has to take place on site.*

Don't hesitate to send us samples....

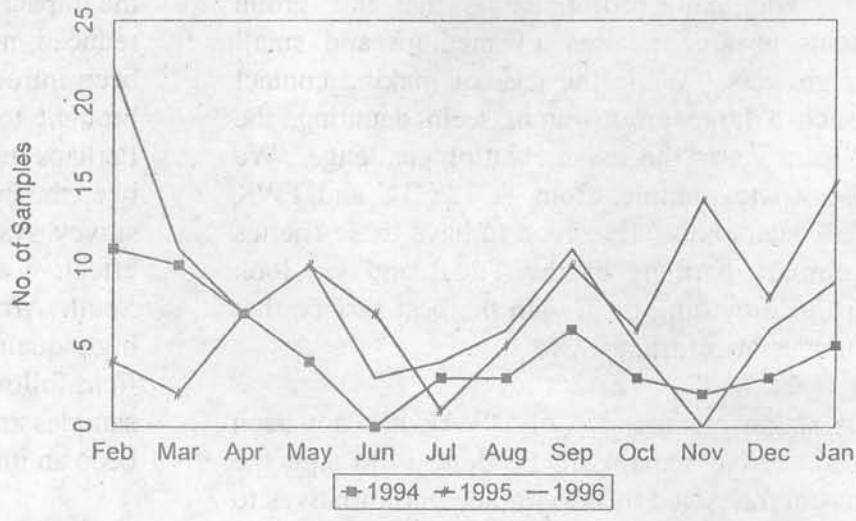

Fig. 1 Seasonal variation in the numbers of samples received by the Diagnostic Clinic in 1994,1995, 1996

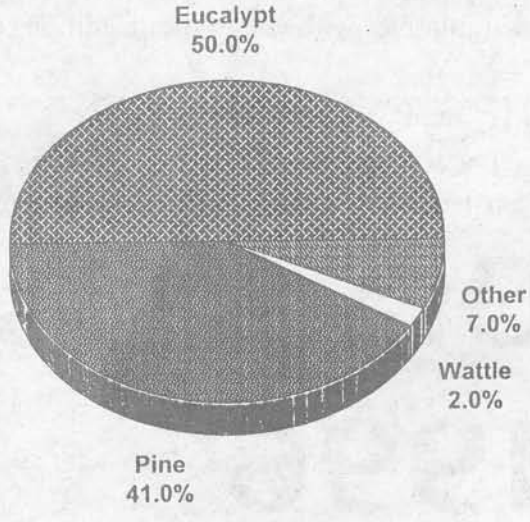

Fig. 2 Source of samples received by the Diagnostic Clinic during 1996

TPCP and the WWW

Mid-1996 we decided to create our own web site on the Internet. The web site, which is said to be "colourful", "young" and "jazzy" has been operational since then. Information we have included is on a range of topics such as mug shots of the permanent staff, the research projects, diagnostic aids (pamphlets), this newsletter, etc. To date over 1000 people have accessed our web site and their comments have been both interesting and useful. We learnt that somewhere out there is another Michael J.

Wingfield! If you have access to the Internet please look us up - our address is:.... http://www.uovs.ac.za/nat/mkboc/tpcp.htm

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AN UPDATE ON THE ACTIVITIES OF THE TPCP FIELD SERVICES

Over the past few months the TPCP has maintained a high presence in the field. These visits form part of regular visits to trial sites and nurseries and include visits to specific sites for the identification of disease problems. With the appointment of Jolanda Roux who is responsible for field work and extension, such regular visits, especially to problem sites, have been made easier. The activities of the field services manager is closely associated with the activities of the Diagnostic Clinic, the two interacting closely to ensure effective disease identification and follow-ups.

Diseases caused by stress related pathogens have again shown how serious their effect can be on tree survival. Many trees were reported to be dying after hot winds, frost or hail damage. Mainly *Botryosphaeria dothidea* on *Eucalyptus* spp. and *Sphaeropsis sapinea* on *Pinus* spp. caused severe losses, causing the death of trees that might normally have recovered. These two pathogens show how important it is to match the site to the tree species planted. There is no way of controlling these diseases once established, prevention is thus the only viable option.

Nursery management is the most critical factor in insuring the supply of disease free, healthy trees to the field. Ensuring that the water, seed, containers and media used for planting is clean reduces the possibility of a disease problem almost entirely. This will also save money on expensive chemicals for control of a problem after it has emerged. Sending out healthy, strong trees to the field will ensure a higher survival rate. During December and January, there was a new report of an outbreak of the pitch canker pathogen. The disease started in a nursery and spread to the field where diseased trees had been planted. This pathogen and most others can be controlled in the nursery by ensuring strict sanitation measures.

The TPCP aims to keep your trees healthy. To do this we need the help of every forester, since it is you who come into contact with the trees every day, and thus are the first to notice any new or strange diseases. The sooner we can identify a disease, the better the possibility of controlling it. Effective disease monitoring

is also important for the quick identification of any new disease that may appear in South Africa. Let us know what's going on out there and if possible we will visit the site.

ABSTRACTS OF RECENT CONGRESS PAPERS

INFLUENCE OF MONOTERPENES ON WOOD COLONIZING FUNGI

Monoterpenes are important components of passive defense mechanisms where wounding and fungal invasion are involved. They inhibit wood colonizing fungi and are also important in biotechnological processes such as biopulping. On pine wood, monoterpenes were found to be more important than fungal contamination, in the inhibition of white-rot fungi. The inhibitory effect of monoterpenes occurring in pine wood was, therefore, tested on different groups of wood colonizing fungi. Thirty fungal strains were tested representing 5 groups, Basidiomycetes that colonize (1) living softwoods, (2) living hardwoods and (3) dead wood; and Ascomycete related fungi that colonize (4) living softwoods and (5) living hardwoods. These fungi were ground on petri dishes in monoterpene (α-pinene, β-pinene, ρ-cymene, limonene and myrcene) saturated environments in 2L flasks and incubated for 4-5 days. Colony diameters on agar were measured and inhibition calculated as a percentage of the growth on control plates. The effect of monoterpenes on fungi was compared using the T-method for contrasts. Ascomycetous fungi were less affected by monoterpenes than Basidiomycetes. Basidiomycetes isolated from living softwoods were inhibited less than saprophytic Basidiomycetes. Comparison of Basidiomycetes from hardwood with other Basidiomycetes produced inconclusive results. We conclude from this study that the habitat from which fungi are isolated is directly related to their tolerance of monoterpenes and that Ascomycetes are more tolerant than Basidiomycetes. This implies that Ascomycetes from living wood will be most useful in pretreatment of softwood biopulping.

Prof. Mike Wingfield and his team in front of the Venture 1997!

TOWARDS A FUNCTIONAL CHARACTERISATION OF dsRNA ELEMENTS IN THE PINE PATHOGEN *SPHAEROPSIS SAPINEA*

In some plant pathogenic fungi, the presence of double-stranded RNA elements (dsRNA) is associated with hypovirulence. A 4kb, linear dsRNA element has recently been reported in

some South African isolates of *S. sapinea.* This fungus is an opportunistic pathogen on pine species world wide, but it can live as a symptomless endophyte in healthy pine cone tissue and seems to remain latent until the onset of stress. A preliminary study has suggested that there is no significant association between the presence of dsRNA and hypovirulence. However, the nature and function of this conservatively transmitted dsRNA genetic element remains a tantalizing question. The project is focused on further characterising dsRNA elements in South African isolates of *S. sapinea.* In more practical sense, the potential for biological control of the pathogen will be considered. To obtain a tool for the characterisation of this dsRNA the production and subsequent cloning of cDNA is essential. The reverse transcription of the denatured *S. sapinea* dsRNA is complicated by the fact that there is no poly A at the 3' ends of this dsRNA element. Thus the commonly used oligo dT primer for the cDNA production is not applicable. We are attempting to link a specific oligonucleotide to the 3' ends of the RNA using T4 RNA ligase and to subsequently use a complementary oligonucleotide for reverse transcription. In this manner full length cDNA clones will be obtained and we thus hope to make progress in the functional characterisation of the *S. sapinea* dsRNA.

CHARACTERISTICS OF SOUTH AFRICAN *ARMILLARIA* ISOLATES USING RFLP's

Species of the genus *Armillaria* are important tree pathogens that occur on a wide range of hosts. The genus has been well studied in the northern hemisphere but has received minimal attention in Southern Africa. Two species, *A. mellea* and *A. heimii*, have been reported to occur in South Africa and are associated with Armillaria root rot on commercially important *Pinus* and *Eucalyptus* trees. The use of the names, *A. mellea* and *A. heimi*, has been a source of much confusion for plant pathologists and the taxonomic disposition of these fungi remains doubtful. The aim of this study was to identify isolates of *Armillaria* from Southern Africa using RFLP's. Isolates were collected from infected trees in the Western Cape, Mpumalanga, KwaZulu-Natal and Northern provinces using established isolation techniques. The intergenic spacer region (IGS) between the large subunit (LSU) and the 5S ribosomal RNA gene was amplified in all isolates. Primers CLR12R and 0-1 were used for the isolates originating from the Western Cape province while primers P-1 and 5S-2B were used for isolates from Mpumalanga, Northern province and KwaZulu-Natal. The PCR products were digested with the restriction endonuclease *Alu*I to detect RFLP's. Differences between the RFLP patterns for the different *Armillaria* isolates suggests that two species of *Armillaria* occur in South Africa. Isolates originating from the Western Cape appear to belong to *A. mellea* or a closely related species. RFLP profiles of isolates originating from other parts of Southern Africa are dissimilar to authentic *A. mellea* and *A. heimii* RFLP profiles. These isolates may, therefore, represent a new species of *Armillaria* in Southern Africa.

GENETIC DIVERSITY OF *BOTRYOSPHAERIA DOTHIDEA* AND *SPHAEROPSIS SAPINEA* IN SOUTH AFRICA

Botryosphaeria dothidea and *Sphaeropsis sapinea* are taxonomically and ecologically similar fungi that cause serious canker and die-back diseases of *Eucalyptus* spp. and *Pinus* spp. respectively. These fungi cause disease when trees are stressed or physically damaged by factors such as hail, wind, frost or insects. Recently, we have shown that both fungi exist as symptomless endophytes in healthy trees. The aim of this study was to determine the degree of genetic diversity within the endophytic populations of these fungi in South Africa. Isolates of *B. dothidea* from healthy *Eucalyptus grandis* leaves, as well as isolates of *S. sapinea* from a hierarchical sampling of *Pinus patula* cones, were examined for the presence of vegetative compatibility groups (VCG's). The genetic diversity within the *B. dothidea* population was high. In contrast, the *S. sapinea* population was genetically uniform and tended towards clonality. These findings are consistent with the fact that *S. sapinea* is an introduced pathogen, with only asexual reproduction. This is in contrast to *B. dothidea* that is found on native woody and commonly undergoes sexual reproduction. Contrary to our previous hypotheses, we believe that *S. sapinea* has been introduced into South Africa in a limited fashion and that excellent opportunities exist for its biological control.

POPULATION DIVERSITY OF THE *AMYLOSTEREUM* SYMBIONT OF *SIREX NOCTILIO* IN SOUTH AFRICA

Sirex noctilio is a destructive wood boring wasp on exotic conifers and was reported for the first time in South Africa in 1994. Favourable bioclimatic conditions and large stands of *Pinus radiata* in the Western Province have aided the successful establishment and steady spread of this pest. *Amylostereum areolatum*, the mutualistic fungal symbiont, has been introduced into South Africa together with *S. noctilio* and is an essential part of the pest complex. A large number of *A. areolatum* isolates were obtained from infected wood and mycangia of female wasps. Vegetative Compatibility groups (VCG's) and RAPD markers on the genomic DNA were used to determine the population diversity represented by these isolates. Preliminary results suggest that the South African population of *A. areolatum* is highly uniform and tends towards clonality. This should favour the biological control of *S. noctilio* by the fungus feeding, infectious nematode *Deladenus siricidicola*.

Bongani Maseko collecting soil samples for the isolation of *Pythium* and *Phytophthora*

PHYTOPHTHORA CINNAMOMI, AN INTRODUCED PATHOGEN TO SOUTH AFRICA

The origin and genetic structure of *Phytophthora cinnamomi* populations in South Africa and globally have always been a matter of debate with little or no genetic evidence to clear up confusion. Using isozymes as genetic markers on a *P. cinnamomi* population consisting of 135 isolates from South Africa, we have found low levels of gene and genotype diversity in combination with a high clonal fraction. Low levels of genetic differentiation were identified between the two regional *P. cinnamomi* populations examined (Cape and Mpumalanga) (Gst=0.14) and between populations collected between 1977-1986 and 1991-1993 (Gst=0.13). This indicates a uniform population in the two regions and a stable pathogen population over time. High levels of genetic differentiation between mating type populations (Gst=0.45), significant differences in allele frequencies, significant Hardy-Weinberg disequilibrium of genotype frequencies, a high fixation index, and a high clonal fraction, all indicate the absence or rare occurrence of sexual reproduction in the South African *P. cinnamomi* population. The observed low levels of gene and genotypic diversity and the lack of sexual reproduction, provide unambiguous evidence for an introduced population, that mainly reproduces asexually. The lack of sexual reproduction, and the uniformity of the population over time and between regions, are important findings for breeding and selection in *Eucalyptus* for resistance against *P. cinnamomi*.

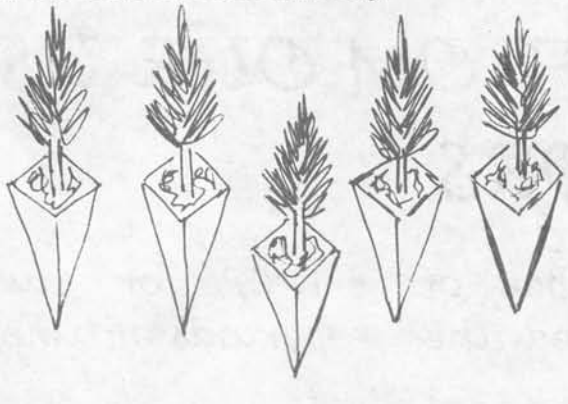
SCREENING OF TWO COLD TOLERANT *EUCALYPTUS* SPP. FOR TOLERANCE TO *PHYTOPHTHORA CINNAMOMI*

Phytophthora cinnamomi is one of the best known and most notorious root pathogens of woody plants. This fungus has an extremely wide host range and, among these hosts, are *Eucalyptus fraxinoides* and *E. smithii*. Both species are cold tolerant and could be

commercially established in areas not suitable for *E. grandis*. They have excellent pulp qualities and a fast growth rate but are both highly susceptible to infection by *P. cinnamomi*. The aim of this study was, therefore, to select families of *E. fraxinoides* and *E. smithii* with tolerance to Phytophthora root rot. In greenhouse trials, eucalypt seedlings were inoculated with a virulent strain of *P. cinnamomi,* and lesion lengths measured after three weeks. Field trials were conducted at one site in Mpumalanga and two sites in KwaZulu-Natal. Three-year-old *E. fraxinoides* and six-year-old *E. smithii* trees were inoculated with *P. cinnamomi* and lesions measured after six weeks. Mortality of individuals in families in breeding seed orchards were also recorded at two sites planted to *E. fraxinoides* and one planted to *E. smithii*. Comparison of the three sets of data showed promising results that will facilitate selection of tolerant families for commercial propagation.

BOTRYOSPHAERIA DOTHIDEA, A PATHOGEN OF *ACACIA MEARNSII* IN SOUTH AFRICA

Botryosphaeria dothidea has recently been isolated from diseased *Acacia mearnsii* in South Africa. Symptoms from which isolations were made included pith discoloration, stem and branch cankers and tip die-back. The fungus has been isolated from diseased trees in all the major wattle growing areas, as well as from the western Cape. Isolates from *A. mearnsii* in the Western Cape, KwaZulu-Natal and South Eastern Mpumalanga Provinces, and from *Eucalyptus* spp. were inoculated into 18-month-old *A. mearnsii* of trees in the field. Inoculations were made both into the main stems of trees, as well as into side branches and branch tips. Extensive lesions developed within six weeks of inoculation. This suggests that *B. dothidea* plays a significant role as a pathogen of *A. mearnsii* in South Africa. The possible endophytic existence of *B. dothidea* in healthy trees is currently under investigation and this fungus will be included in a programme to produce disease tolerant planting material for the local wattle industry.



EUCALYPTUS RUST: A DISEASE WITH SERIOUS INTERNATIONAL IMPLICATIONS

Eucalyptus spp. are propagated extensively in many parts of the world. A number of diseases result in serious losses to this economically important forest resource. Eucalyptus rust, caused by *Puccinia psidii*, is one such example. Symptoms initially appear on the tender organs of the plant, such as, the primordial leaves, their petioles and branch tips. In severe cases, the main branches and stems of the trees are infected. Trees have been found to be most susceptible to infection when they are younger than two years of age. The fungus has a microcyclic life cycle and is considered to be autoecious. Ten genera in the Myrtaceae are reported to be naturally infected by *P. psidii*. Cross inoculation studies have shown considerable physiological variability in this pathogen, but studies to delineate races of *P. psidii* and their host range have yet to be undertaken. Such knowledge will be necessary in order to successfully implement a resistance breeding programme. This fungus occurs predominantly in Central and South America but there are unconfirmed reports of its occurrence in Taiwan and South Africa. Comparisons have been made between material collected from these two countries. Morphologically, the uredia and urediniospores were identical. Teliospores were, however, absent making it difficult to identify the fungus to genus level. Comparisons at the molecular level will be needed to resolve the identity of these collections. *Puccinia psidii* is considered to be a serious threat to eucalypts in Australia where these trees are native, as well as to plantations in many parts of the world. It is thus deserving of intensive study, preferably through an internationally coordinated effort.

TWO NEW *OPHIOSTOMA* SPECIES FROM *EUCALYPTUS* IN THE SOUTHERN HEMISPHERE

Ophiostoma stenoceras (Robak) Melin & Nannf. is well known for causing a mild blue stain on a range of softwoods in the Northern Hemisphere.

During a survey of Ophiostomatoid fungi occurring in South Africa, a number of isolates resembling *O. stenoceras* were found on *Eucalyptus* timber as well as on *Acacia mearnsii* wood chips. All isolates originated from either KwaZulu-Natal of Mpumalanga. During the same period of time, a similar set of isolates was collected from *Eucalyptus* in Brazil and Colombia. The characteristics of the teleomorph and *Sporothrix* anamorph of both the South African and South American isolates closely resemble those of *O. stenoceras*. As is the case of *O. stenoceras*, all *isolates* were homothallic. The South African isolates, however, differ from authentic *O. stenoceras* isolates and the South American group in that annuli frequently occur on the perithecial necks. Furthermore, both the South African and South American produce secondary conidia, while this is not typical of *O. stenoceras*. The fact that the isolates from the Southern hemisphere were collected only from hardwoods, together with morphological differences, lead us to conclude that both the South African and South American groups represent new *Ophiostoma* species.

A PHYLOGENETIC STUDY OF *CRYPHONECTRIA CUBENSIS*

Cryphonectria cubensis is the causal agent of a serious canker disease of *Eucalyptus*. The pathogen occurs in many tropical and subtropical areas where *Eucalyptus* trees are cultivated. *Cryphonectria cubensis* was originally described in the genus *Endothia havanensis*. *Cryphonectria cubensis* was also known as *Diaporthe cubensis*, but was later transferred to *Cryphonectria*. Other related genera include *Cryphonectria parasitica* (causal agent of chestnut blight), *Endothia eugeniae* (causal agent of die-back on clove) and *Endothia gyrosa* (causal agent of pin-oak blight). Morphological, isozyme and pathogenicity studies have shown that these pathogens are similar and closely related. Confusion, however, still exists concerning the taxonomic placement of these genera. The aim of this study was to determine the phylogenetic relationships between *C. cubensis, C. parasitica, E. eugeniae* and *E. gyrosa*. This was achieved by PCR-amplification and sequencing of the internal transcribe spacer region, ITSI and ITS2, and the conserved 5.8S gene of the ribosomal RNA operon. Sequence analysis was done using PAUP (Phylogenetic Analysis Using Parsimony). Preliminary sequence data indicate that *Cryphonectria cubensis* represents a well defined species and supports its synonymy with *Endothia eugeniae*.

INDUCTION OF POLYGALACTURONASE BY POLYGALACTURONIC ACID IN *CONIOTHYRIUM ZULUENSE*

Coniothyrium zuluense causes a serious stem canker disease on *Eucalyptus* in subtropical areas of South Africa. The mode of infection of this pathogen, as well as resistance mechanisms in trees are, therefore, of interest. Plant cell wall polysaccharide-degrading enzymes have been reported to be a determining factor in fungal pathogenesis. These enzymes are known for their ability to macerate plant tissue and also to release endogenous elicitors of plant defense responses. Extracellular polygalacturonase (PG) production was estimated *in vitro* for twenty isolates of *C. zuluense*, using a cup plate assay and an assay of reducing sugars. Experimental assays demonstrated that PG activity, determined as an increase in deducing sugar activity, was correlated with the virulence of *C. zuluense* isolates on *Eucalyptus* clones. Preliminary results also suggest that PG's are involved in the production of disease symptoms on *Eucalyptus*.

CONTRIBUTIONS FROM OUR USA VISITORS

In the past six months a number of scientists from the USA have joined the TPCP for various periods of time. In

September last year, Prof. George Carroll from the University of Oregon, began his 15 months stay with us. Early this year, Prof Tom Harrington from the Iowa State University, joined us for a short six months stay. Karen Wikler, a PhD student in the laboratories of Prof. Tom Gordon at the University of California, spent six weeks with us.

The baffling secret of room 54.....

Karin Wikler, Prof George Carroll and Prof. Tom Harrington

Karen had the following to say about her visit: "When Mike (Wingfield) learned of our failed attempts to cross the causal agent of pitch canker, *Fusarium subglutinans* f.sp. *pini*, in California he invited me to South Africa to work in his laboratories as much success has been achieved crossing *Fusarium* isolates here. During my stay Henriette (Britz) and I have been crossing isolates collected in Mexico with the South African mating testers. These crosses should compliment Henriette's work characterising the different populations of this fungus. Successful crosses have started to develop and I am still baffled as to why the South African isolates appear to be so much more fertile than isolates collected in the United States. I am looking forward to Henriette's visit to our laboratories next year where we will examine the fertility of the fungus in California.".

The following from TOM HARRINGTON.....

I have had a long connection with Mike and Brenda Wingfield and the TPCP and I share many research interests with the group. I came here for my 6 month sabbatical to pursue some of these common

interests, including *Armillaria*, woodwasp symbionts, and blue stain. My strongest interests here, however, are with the biology, evolution and biogeography of *Ceratocystis* species. These fungi are important tree pathogens throughout the world, especially in the Americas, causing oak wilt, mallet canker on *Prunus* species, and canker on *Populus* in North America. These fungi are most prevalent in South America, causing serious diseases on palms, *Gmelina*, Acacia, rubber tree, Cacao, banana and coffee. Species of *Ceratocystis* have been introduced to other parts of the world on South American plants, such as rubber tree, taro and sweet potato. Currently, there is a major epidemic on plane tree in southern Europe. A few Australasian species, including one on *Eucalyptus*, have recently been recognised.

In South Africa *C. albofundus* is a newly recognised pathogen on black wattle and it may prove to be the most important disease on this host. I am working with Jolanda Roux and Mike Wingfield in trying to determine the extent of this disease and to see if it is a native pathogen or introduced. Mike and I also hope to complete our studies on the conifer-inhabiting species of *Ceratocystis*, including some blue stain species and those few species that are associated with tree-killing bark beetles. I am continuing work with Brenda Wingfield and a graduate student here, Corli Strydom, on the molecular biology of mating type switching in *Ceratocystis*, which appears to be important in the rapid evolution of new, host - specialised species. These studies are being supported in part by a fellowship from the Ernest Oppenheimer Trust and a cooperative agreement between my laboratory and the TPCP supported by the United States Department of Agriculture.

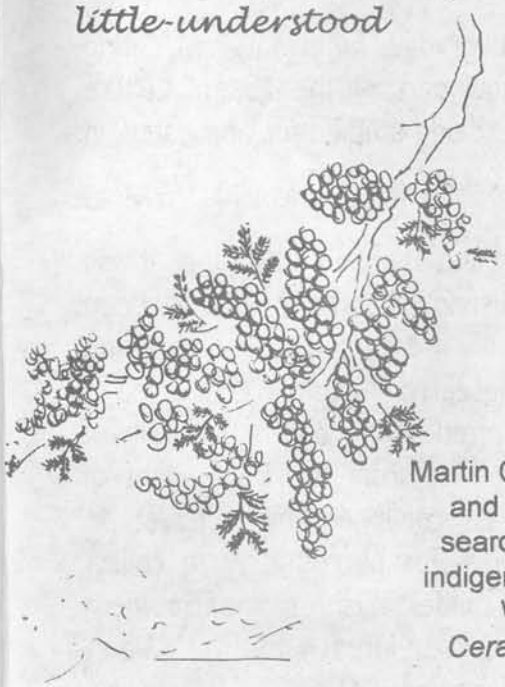
Fungal endophytes - a contribution by George Carroll

Fungi occur in almost every habitat on the face of the earth that has been seriously investigated. Among such fungal niches are the interior tissues of healthy plants, particularly tissues of shrubs and trees. Fungi living asymptomatically within healthy plants are called

endophytes, and they have been cultured from bits of surface-sterilized leaf and stem from virtually every plant that has been investigated. Typically a single plant may play host to a dozen or more species of fungal endophytes, and typically individual infections within the plant are microscopic.

The role of these ubiquitous plant symbionts has been much studied. Some, such as *Botryosphaeria* in *Eucalyptus*, are latent pathogens. Others, such as seed-borne endophytes in grasses and *Phomopsis* in elms, are clearly mutualists, producing a variety of chemical defenses against insect and vertebrate herbivores and (in the case of grasses) conferring little-understood increases in vigor and drought resistance on the host plant. Many endophytes probably exert little effect on their hosts, merely "hitchhiking" in plant tissues until a preferred substrate appears again in a variable habitat such as a successional forest.

Certain Ascomycete genera and their associated asexual stages almost always appear when surface-sterilized plant tissues are cultured. The fungal genus *Phyllosticta* is principally composed of endophytes, and species have been reported as leaf spots from a huge number of host plant. The prevalence of these fungi on vascular plants suggests that the association is an ancient one and that *Phyllosticta*-like fungi may have been associated with living plants since the time when fungi and plants first appeared in terrestrial habitats. In the past possible instances of co-speciation, as between the rusts and their hosts, have been inferred entirely on the basis of conventional analyses of fungal structure. More recently,



Martin Coetzee, Jolanda Roux and Prof Tom Harrington searching for the possible indigenous host of the Black Wattle pathogen *Ceratocystis albofundus*.

advances in the sequencing of nucleic acids have allowed the construction of phylogenetic trees based on similarities in nucleic acid sequences. If phylogenetic trees based on nucleic acid sequences between fungal endophytes and their hosts are congruent, one might conclude that these fungi have been long associated with their host plants. South Africa provides numerous opportunities to look at this question. It has a distinctive native flora which is heavily infested with endophytes, and Phyllosticta is widespread. The presence of endemic genera with numerous species such as cabbage trees (Cussonia) and cycads (Encephalartos) allows one to look for divergence in Phyllosticta nucleic acid sequences among isolates from different species of the same host genus. Over the longer term I hope to use the same approach to ask whether endophytes from related but geographically separated hosts are more closely related to each other than a random selection of isolates. Put another way, did species of Phyllosticta inhabit Rhus species before continental drift separated today's major land masses from Africa? If so one might expect Phyllostictas from North American and African Rhus species to be more related to each other than are Phyllostictas from taxonomically unrelated hosts in Africa. Until January I spent most of my time isolating Phyllostictas. More recently I have been (occasionally) amplifying and sequencing DNA. Tune in next year for some answers.

SAPPI SPONSORS COURSE ON MUSHROOM IDENTIFICATION

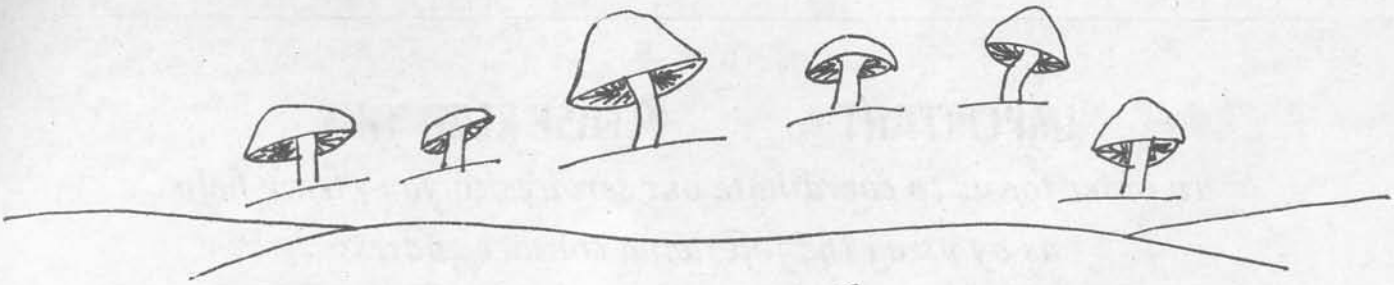
A unique course in mycology and sponsored by Sappi was held recently. The aim of the course, held at Sappi's Glenthorpe estate, was to teach students the ecological role of the fungi as well as the required skills for their collection and identification. A useful survey was also made of the fungi in the plantation. The students were taught how to isolate strains of the mushrooms or bracket fungi, grow them on nutrient media and maintain them in pure culture. Twenty-six cultures of wood degrading fungi obtained during the course are now part of the "Sappi Culture Collection" which is being studied for application in biotechnological processes.

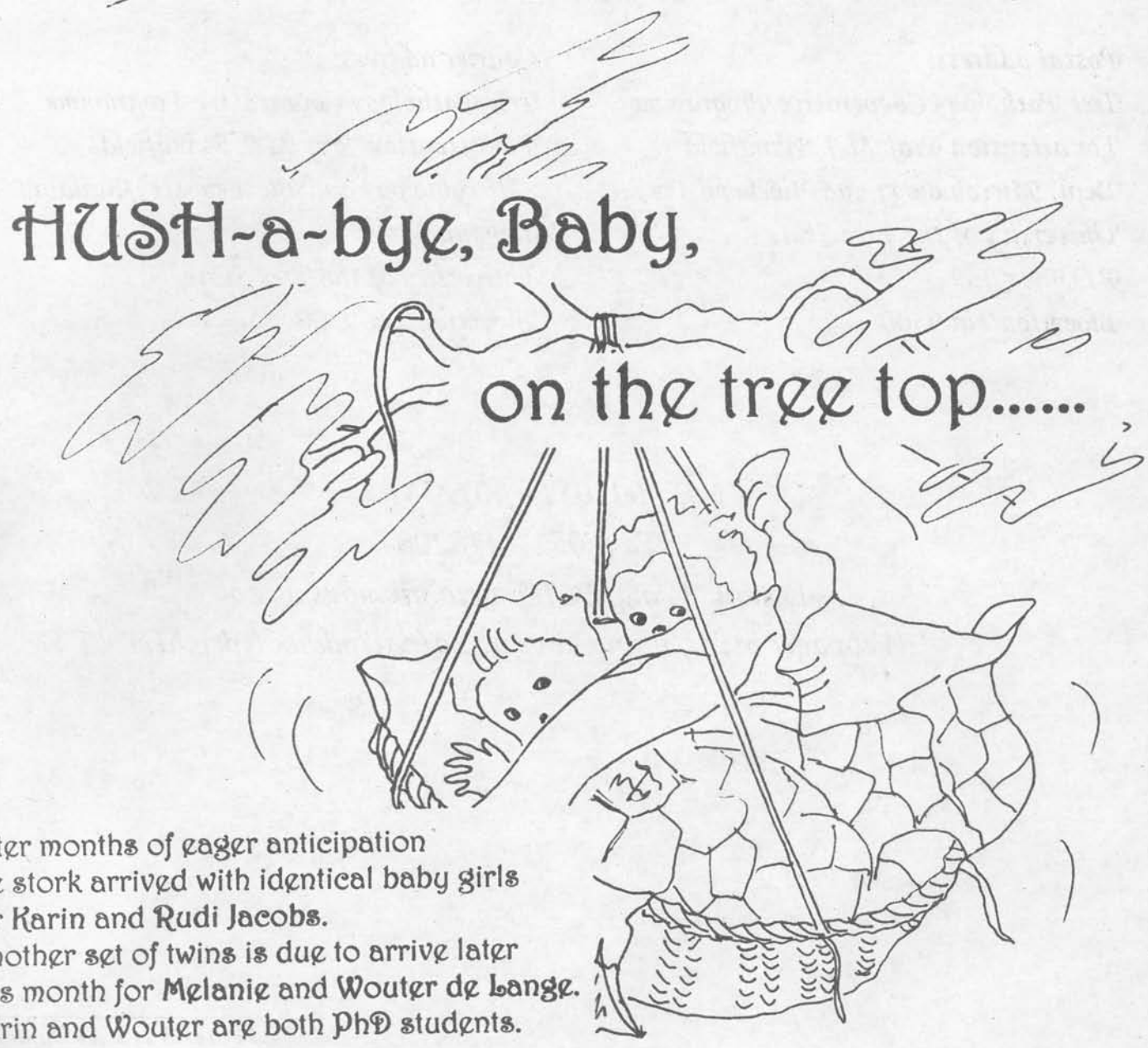
Although organised and sponsored by Sappi, it was presented by the distinguished mycologist Dr. Deon van der Westhuizen. Research into wood-rotting fungi at the Plant Research Institute in Ottawa led to his doctorate, conferred on him by the University of Pretoria. Since retiring from the Department of Agriculture he has combined his interest in mycology with a love for photography to collect material for a field guide "Mushrooms of Southern Africa", which he co-authored with Prof. Albert Eicker (Struik Publishers, 1994).

The five-day course was attended by students from the TPCP. In future this course could, however, be expanded to include participants from a wider

background. Due to the restricted accommodation, only 8 students could be accommodated. This number could be increased in future if larger facilities are made available to the organisers.

The program included three slide shows; on fungi growing on wood, fungi growing on dead plant material and fungi involved in symbiotic relationships. Students also had the opportunity to collect fungi, observe them under a microscope and identify them. The 142 collections which were made, included 56 fungal species. Lectures provided theoretical background on the collection, identification, photography and the maintenance of fungal material in a herbarium. Planning has already started for a second course in 1997 and the intention is to make this an annual event. Interested persons can contact Francois Wolfaardt at (051)--4013051.





After months of eager anticipation
the stork arrived with identical baby girls
for Karin and Rudi Jacobs.
Another set of twins is due to arrive later
this month for Melanie and Wouter de Lange.
Karin and Wouter are both PhD students.

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, Prof B. Wingfield, Dr T.A.Coutinho, Me Jolanda. Roux and Mr Francois Wolfaardt), 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

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.....Edited and compiled by Susan Christie