TREE PATHOLOGY NEWS

NEWSLETTER OF THE TREE PATHOLOGY COOPERATIVE PROGRAM - U.O.F.S.

NO 8 NOV 1993

MESSAGE FROM THE DIRECTOR

1993 has been a most eventful year for the Tree Pathology Cooperative Programme (TPCP) and once again it seems to have passed all too quickly. On the other hand, given the very difficult financial problems experienced due to the protracted drought, some folks are probably pleased to be looking forward. Indeed, all indications are that the drought has broken and that, at least in this regard, things are looking up.

Drought and its influence on the disease situation has been one of the issues that the TPCP has had to consider. As explained in our last newsletter, the influence of drought on disease development is not a simple matter. Simply put, some pathogens are more serious under drought conditions whereas others are less serious when trees are stressed. Thus, Cryphonectria canker and Pythium root disease have been less problematic for us during the last year. In contrast, Botryophaeria cankers have been much more common and serious during this period.

Christmas Greetings from TPCP Team Members

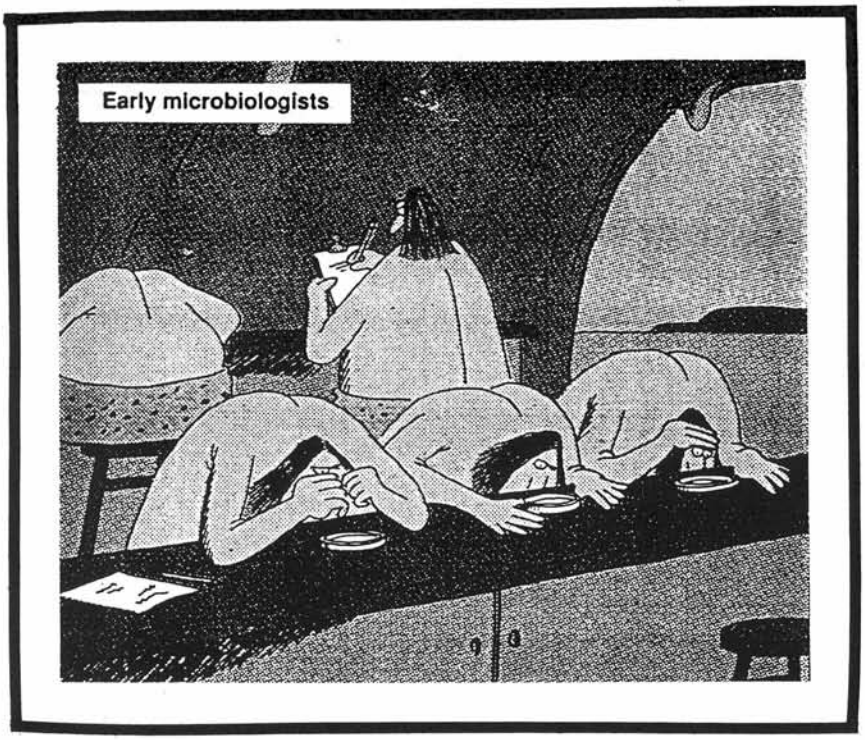
It is the nature of the TPCP that we all travel a great deal to assists with disease problems and to conduct field experiments. Through this regular travel, we are able to visit with forestry friends regularly and also establish new friendships. We also depend on many of you for assistance with the setting up of various field trials, monitoring disease development and field surveys. We would thus like to thank one and all for your support and interest in the Tree Pathology Programme. We thank you most sincerely for your friendship and in many cases hospitality and wish you and yours a most joyous Christmas. We also wish you a happy, healthy and prosperous 1994.

One of our greatest concerns during the past year has been Coniothyrium canker of eucalypts in Zululand. This disease which was only identified for the first time four years ago has increased very substantially in its distribution. One of the most important questions that we have had to contend with regarding this disease has been how it might be influenced by stress.

There can be little question that the very severe drought has led to trees exuding great amounts of kino and thus looking particularly bad. On the other hand, we believe that infection itself is not linked to stress. On the contrary, infection occurs in young green tissue and probably requires some moisture to occur.

Our current impression is that resistance in clones to infection due to *Coniothyrium* is breaking down and this is obviously of concern. We base this view on the fact that clones previously known to be tolerant to infection are now becoming infected. Obviously this situation is complex and will require in depth investigation. However, all evidence points to the fact that the disease appeared recently and is spreading and adapting to the environment.

Concern for the Coniothyrium situation has led some foresters to be despondent and to feel that it will not be possible to grow eucalypts in certain areas in the future. In contrast, we believe that there is substantial cause for optimism.



We have spent considerable time examining provenance and clonal trial in Zululand and have already identified a great deal of material that is healthy. The aim now must be to capitalise on this material and bring it into production. In the longer term, it will be very valuable to us in our breeding trials.

In previous reports, I have discussed the recent appearance of the Pitch Canker pathogen, *Fusarium subglutinans* f.sp. *pini* in South Africa. During the last year, we have completed further tests to determine with certainty that our isolates are definitely of this notorious pine pathogen. I can thus report that every study that we have completed on this material confirms our preliminary observations. Our objective must now be to select pines and particularly *P. patula* exhibiting tolerance to this pathogen.

In this report, we are including a brief overview of the concept of hypovirulence in fungi. This condition is the result of viral infections of fungi and leads them to exhibit reduced virulence. The upshot of this is that we have the capacity to capitalise on these infections to reduce the impact of our more important tree pathogens. This field of research is thus one of the exciting new areas that we are including in the programme of the TPCP.

As most of you are now well aware, research projects of team members of the TPCP are very varied and include many different pathogens of interest to us. Briefly, we are currently investigating the following:

1. Cryphonectria canker of eucalypts. In this project we have four post graduate students actively considering various aspects of the disease.

2. *Coniothyrium* canker of eucalypts.

3. Pitch canker of pines.

4. Eucalypts leaf pathogens.

5. *Sphaeropsis* die-back of pines.

6. Fungi associated with blackening of pine seed.

7. *Ceratocystis* wilt of black wattle.

8. *Phytophthora* and *Pythium* diseases.

9. *Botryosphaeria* canker of eucalypts.

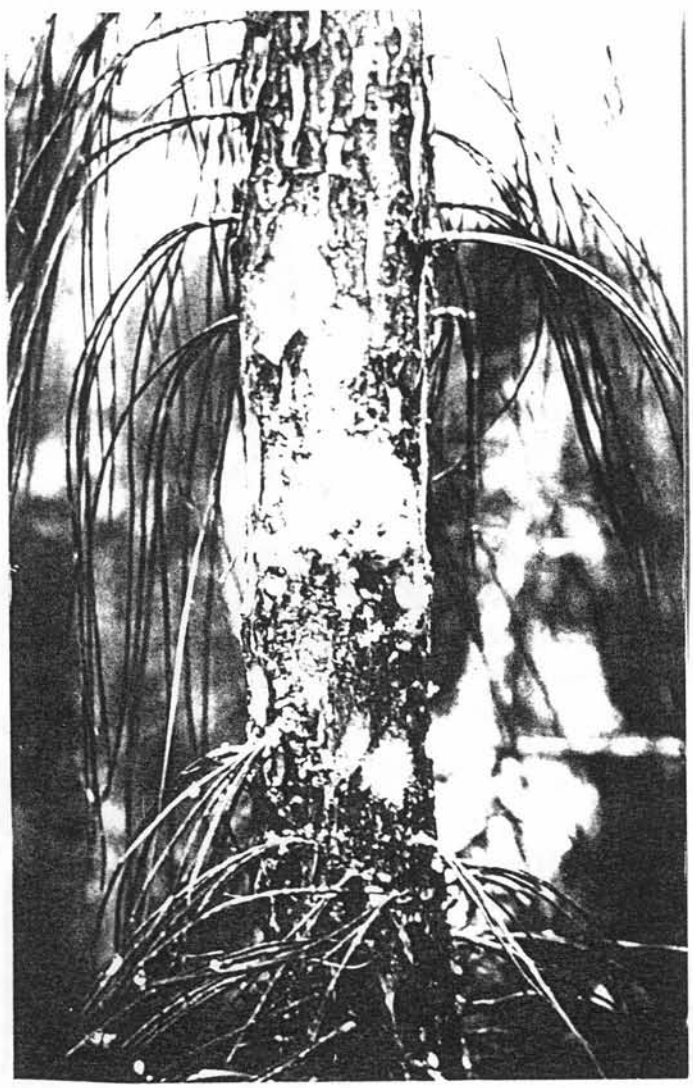
10. *Acremonium* wilt on eucalypts.

In addtion to the above, we are obviously dealing with a number of other diseases in a more ad hoc fashion. We also maintain very close contacts with colleagues in other countries and thus attempt to remain up to date with developments on an international scale.

All in all, the TPCP has been active and dynamic in the past year. As pathologists, we consider the disease problems intersting and exciting. We are often thought of as rather morbid by our forestry friends - particularly when a shriek of excitement is exclaimed when an inoculation has caused a gaping bleeding hole in the

stem of a tree. Obviously if we were not excited about these problems we would not take them sufficiently seriously. On the other hand, I must assure you that our aim is clearly set on the goal of planting trees tolerant to infection and avoiding disease at all costs. Perhaps our greatest excitement has been about the successes we have had in achieving this goal in recent years. We want you to know that without your support, this could not have been possible.

SYMPTOMS TYPICAL OF PITCH CANKER



Four-year-old *Pinus patula* tree inoculated with *Fusarium subglutinans* f.sp. *pini* showing symptoms typical of pitch canker.
Left: Heavy flow of resin on the main stem.
Right: Below the bark, the canker is soaked with pitch, often to the centre of the stem.

HYPOVIRULENCE
Fungi have diseases too!

Hypovirulence or reduced pathogenicity associated with the presence of double-stranded RNA (dsRNA) has been reported in many fungi. Possibly the best known case of hypovirulence is that found in *Cryphonectria parasitica* the well known chestnut blight fungus. In this fungus the presence of dsRNA causes a decrease in virulence as well as decreased growth. In fact you could say that the fungus is ill as a result of the infection by the dsRNA. The dsRNA infection is in many ways similar to a viral infection.

This dsRNA is passed from one fungus to another by anastomosis. This is a process whereby fungi can exchange some of their cell contents. Through this process dsRNA can thus be transferred from hypovirulent fungi to virulent fungi. The virulent fungi then through the dsRNA infection become hypovirulent.

The biological control possibilities of this dsRNA associated hypovirulence are obvious. In fact in the case of *Cryphonectria parasitica* (which has devastated the chestnut trees in North America) a natural biological control due to dsRNA infection exists in certain parts of Europe. In these areas it is possible to grow chestnuts.

Spreading this dsRNA infection is unfortunately not as easy as it may first appear. Only certain types of fungi can undergo anastomosis with another. Therefore in a genetically diverse population spreading an infected hypovirulent strain would only result in the infection of a small percentage of the existing fungi. However, the use of molecular techniques and potentially cloning these dsRNA offers the possibility of using the dsRNA genes for disease control.

The present excitement in our laboratories is that we have found that some isolates of *C. cubensis* (which is closely related to *C. parasitica*) also contain dsRNA. *C. cubensis* causes a devastating disease we are starting to see more commonly in South Africa, Canker of Eucalyptus. From studies in our laboratories we think that this fungus has only recently been introduced to South Africa and most probably has a fairly narrow genetic basis. Therefore, we are potentially in the ideal position to use hypovirulence caused by the presence of dsRNA which we have found in *C. cubensis* in order to ascertain that it is causing hypovirulence and to understand more about how this dsRNA causes this hypovirulence before we can start infecting *C. cubensis* with the dsRNA.

ENDOTHIA GYROSA ON *EUCALYPTUS* IN SOUTH AFRICA

Endothia gyrosa, along with its *Endothiella* anamorph is a canker pathogen of tree species in many parts of the world. It has a wide host range, but is known primarily as a pathogen of pin oak (*Quercus palustris* Muenchh) causing pin oak blight. In Virginia (USA), *E. gyrosa* has been found inciting disease or inhabiting moribund tissue of sweet gum (*Liquidamber styraciflua* L.), northern red oak(*Q. borealis* Michx. f.), water oak (*Q. nigra* L.), willow oak (*Q. phellos* L.) and silver maple (*Acer saccharinum* L.). *Endothia gyrosa* occurs as a canker pathogen of *Eucalyptus* in Australia and Portugal, and has recently also been found in South Africa.

On *Eucalyptus*, *E. gyrosa* causes stem cankers similar to, but less severe, than those caused by the well known and serious pathogen of *Cryphonectria cubensis*. These cankers were exemplified by cracked and slightly swollen areas on the bark, and were more superficial than cankers caused by *C. cubensis*. Cankers occur over the entire surface of the bole but are most prominent at the base. Instead of long-necked pychnidia typical of *C. cubensis*, bright orange-brown stromatic pycnidia and perithecia were present. Perithecia were embedded in the stromata, with necks protruding from the surface. Asci were unitunicate anon-amyloid apical refractive ring, and contained eight ascospores. In culture on MEA-plates, *E. gyrosa* colonies are initially white, turning orange after six to ten days.

E. gyrosa appears to have a wide host range among *Eucalyptus* species, as it has been found on*E. grandis*, *E. nitens* and *E. urophylla* as well as hybrids of *E. grandis* with *E. camaldulensis* and *E. urophylla* during preliminary surveys. Numerous trees in certain clonal plantations are heavily infected by the pathogen and this could have serious implications for the South African forestry industry. Unlike *C. cubensis*, that is restricted to warm, high rainfall areas of South Africa *E. gyrosa* appears to have a much wider distribution.

Stress conditions, such as water stress, is known to increase the susceptibility of trees to colonization by *E. gyrosa*. Periodic droughts occur in South Africa. and this could enhance the damage caused by *E. gyrosa*. Henk Smith from our group, is also currently looking at the effect that disease caused by other pathogens such as *Botryosphaeria dothidia* have on *E. gyrosa*. Further studies including screening of *Eucalyptus* clones are urgently needed in order to alleviate future losses associated with *E. gyrosa*.

'N NUWE SIEKTE VAN EUCALYPTUS *IN SUID AFRIKA*

"SIRKELS IN DIE BOS"

"Daar sal altyd nuwe siektes wees", is 'n stelling wat al verskeie kere in die verlede gemaak is. Die stelling is weereens waar bewys met die verskyning van "Sirkels in die Bos". Die naam spreek vanself en die siekte veroorsaak letterlik sirkels in plantasies van ongeveer 30 meter in deursnit.

Die siekte word veroorsaak deur die swam *Pseudophaeolus baudonii* wat ook soos "Pink Disease" aan die paddastoelfamilie behoort. Die kenmerkendste eienskap van die siekte is die voorkoms van geel tot goudbruin vrugliggame (paddastoele) aan die basis van dooie bome. Die vrugliggame kan wissel in grootte en sommige met 'n deursnit van tot 30 cm is al waargeneem. Die sirkels word gevorm deur 'n ry van tot 5 dooie bome langs mekaar in plantasies. Waarnemings toon dat die sirkels besig is om groter te word. Baie interessant asook belangrik, is die feit dat die swam net in Afrika voorkom en ook al aangeteken is as 'n patogeen van *Pinus* en *Eucalyptus*. Die verspreiding van die swam in Suid-Afrika is bekend in Kwambonambi. Verkenningsloodse het wel soortgelyke sirkels in die Mtubatuba en Mtunzini omgewing opgemerk. 'n Verdere waarneming is dat die sirkels tot dusver slegs voorkom in kompartemente waar *Eucalyptus maculata* en *E. paniculata* voorheen geplant was.

DIE DONKER WOLK HANG OOR DIE KOUE TOLERANTE EUCALYPTUS *SPECIES IN SUID-AFRIKA*

Die toekoms van die koue tolerante *Eucalyptus* species in Suid-Afrika is weens patologiese redes in die weegskaal. Die species sluit in *E. fastigata*, *E. fraxinoides*, *E. smithii*, *E. macarthurii* en *E. dunnii*. Tot 'n mindere mate word *E. nitens* deur die probleem geraak. Weens die wortelvrot-kompleks word *E. fastigate* en *E. fraxinoides* op 'n klein skaal indien enige nog aangeplant. Alles dui daarop dat *E. smithii*, *E. mararthurii* en *E. dunnii* dieselfde roete gaan volg.

In die verlede is die wortelvrot-kompleks toegeskryf aan 'n kompleks van patogene wat *Phytophthora* spp., *Endothia gyrosa*, *Botryosphaeria dothidea* asook 'n *Fusarium* insluit. Dit is welbekend dat die bogenoemde patogene met die uitsondering van *Phytophthora* spp. almal stresverwante patogene is. Opnames het getoon dat dit slegs *Phytophthora cinnamomi* is wat konstant met die wortelvrot-kompleks geassosieer word in die geval van *E. fastigata*, *E. fraxinoides*, *E. smithii* en *E. macarthurii*. Die wortelvrot wat voorkom by *E. dunnii* word veroorsaak deur *P. cinnamomi* basiskankers waarna die *Fusarium* sp. sekondere kankers hoër op die stamme veroorsaak. Die basiskankers wat voorkom op *E. smithii*, *E. macarthurii* en *E. dunnii* word primer veroorsaak deur *P. cinnamomi* waarna daar addisionele kankers ontwikkel wat geassosieer word met *B. dothidea* en *E. gyrosa*. Omdat die wortels van die bome geinfekteer word deur *Phytophthora* spp. word die opname van water en voedingstowwe beinvloed. Dit gee aanleiding tot stres in die bome waarna die stresverwante patogene 'n kans kry om hulself te vestig.

SELECTED ABSTRACTS FROM PRESENTATIONS BY THE TPCP TEAM MEMBERS AT RECENT OVERSEAS CONFERENCES

FUSARIUM STEM CANKER OF *EUCALYPTUS FASTIGATA* IN SOUTH AFRICA

The cultivation of *Eucalyptus fastigata* in the south eastern Transvaal Province of South Africa, has been stopped due to its high susceptibility to *Phytophthora cinnamomi*. Wilting and die-back of one-year-old *E. fastigata* is exclusively associated with *P. cinnamomi*. *However, when the trees reach two years of age, basal cankers as well as multiple stem cankers have been observed. Stem cankers extend to the tops of trees and are associated with resin exudation.* *Phytophthora cinnamomi* was isolated from basal cankers but could not be isolated from cankers in the upper parts of ;the stem. A *Fusarium* species was, however, consistently isolated from the pith and cankers on the upper parts of the stem. Artificial stem inoculation with the *Fusarium species on 4-year-old E. fastigata*, resulted in extensive lesions extending from the point of inoculation to the top of the tree after 5 weeks. Cankers typical of those observed under natural conditions also developed on the stems 6 months after inoculation. Results of this preliminary study suggest that *P. cinnamomi* is not the sole factor involved in deaths of *E. fastigata* in South Africa. Rather, a *Fusarium* species appears to be an important component in this disease complex.

FUSARIUM SUBGLUTINANS F. SP. *PINI* ASSOCIATED WITH *PINUS PATULA* SEEDLINGS IN SOUTH AFRICA: CHARACTERIZATION AND IDENTIFICATION

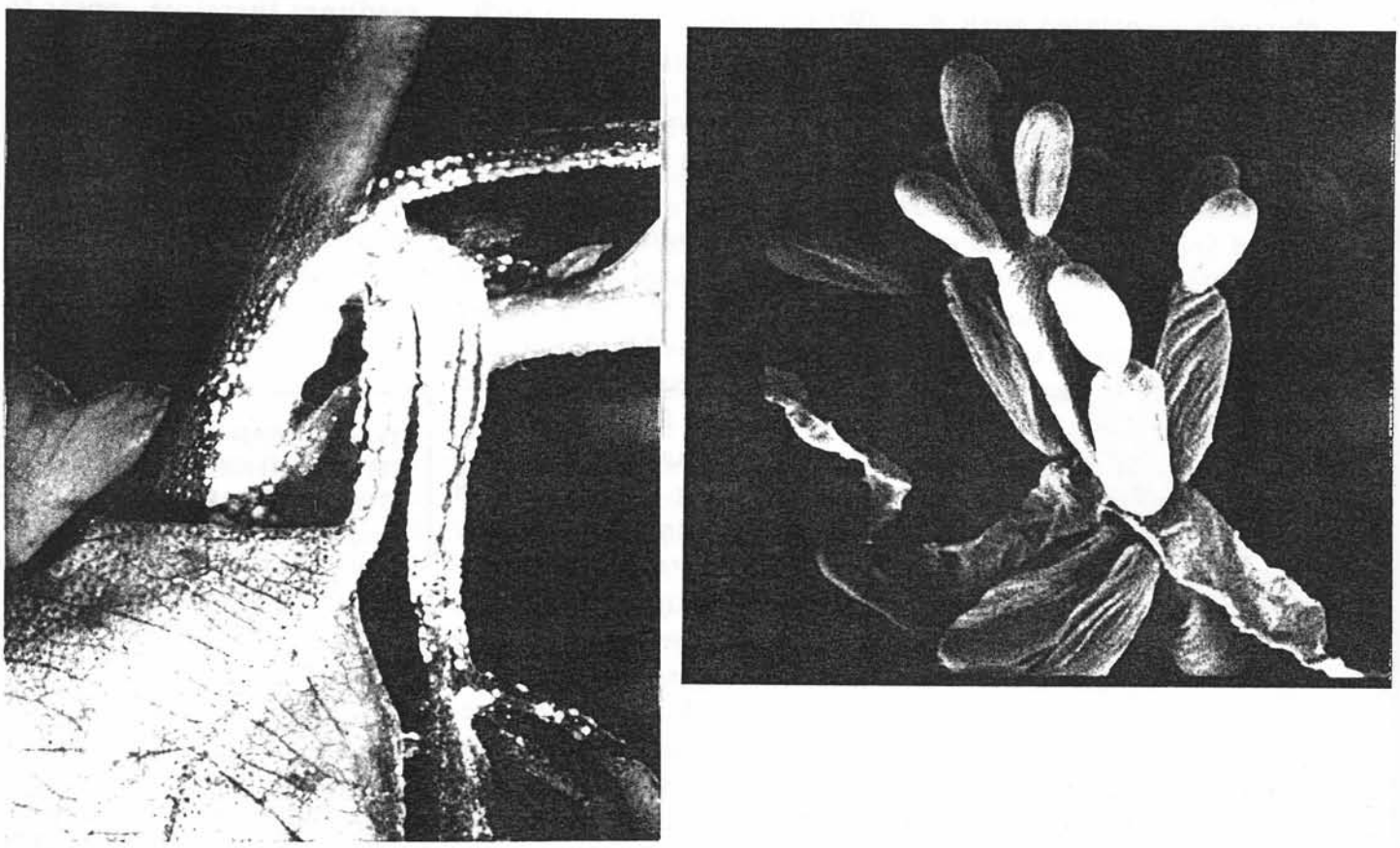
Fusarium subglutinans f. sp. *pini* is the causal agent of the notorious pitch canker disease of pines in the United States. This fungus has recently been responsible for large-scale losses of *Pinus patula* seedling due to root rot in South Africa. During pathogenicity tests under growth room conditions, *F. subglutinans* f. sp. *pini* caused significantly more damping-off of newly germinated *P. patula* seedlings and root rot of 2-mo-old *P. patula* seedlings than died *F. oxysporum*. The fungus also yielded symptoms reminiscent of pitch canker on stems of 3-yr-old *P. patula* trees. Detailed studies were undertaken to compare the South African isolates of *F. subglutinans* f. sp. *pini* with pitch canker isolates and *F. subglutinans* isolates from hosts other than pine. The South African isolates were found to be similar to the pitch canker isolates, but differed somewhat from the other *F. subglutinans* isolates in cultural and morphological characteristics. Only isolates obtained from pines were pathogenic to newly germinated *P. patula* seeds and 1-yr-old *P. patula* saplings. The South African isolates from diseased roots of pine seedlings therefore appear to be related to the pitch canker isolates which cause cankers of fully grown trees. We also support the view that isolates of *F. subglutinans* pathogenic to pines should be distinguished from the rest of the isolates as *F. subglutinans* f. Sp. *pini*.

THE RESEARCH TEAM OF THE TREE PATHOLOGY COOPERATIVE PROGRAM

The research team of the Tree Pathology Cooperative Program is varied. It includes full time staff of the University of the Orange Free State (Prof M.J. Wingfield, Dr W.J. Swart, Dr B. Wingfield and Mr G.H.J. Kemp), collegues 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 Department in the University obviously provide advice and support where this is required.

SPOROTHRIX *EUCALYPTI*, A SHOOT AND LEAF PATHOGEN OF *EUCALYPTUS GRANDIS* IN SOUTH AFRICA

A species of *Sporothrix* was consistently isolated from leaf spots and serious shoot infections on a clone of *Eucalyptus grandis* in northern Natal, South Africa. A whitish mycelial growth containing masses of conidia wasassociated with necrotic areas on leaves. No *Sporothrix* sp. has previously been described from *Eucalyptus*. The fungus was morphologically distinct from other species in the genus and has consequently been described a new taxon. *S. eucalypti*. *Sporothrix eucalypti* was shown to be highly virulent in pathogenicity tests on a number of . *grandis* clones. Significant differences were also detected in the relative susceptibility of clones to the pathogen. *Sporothrix eucalypti* represents a new pathogen of *Eucalyptus* that has the potential to cause substantial damage in plantations of *E. grandis* in South Africa and probably other eucalypt growing countries.



Infection of young *E. grandis* shoot by *Sporothrix eucalypti* (LEFT) as well as a magnified view using Scanning Electron Microscopy of spores of the fungus

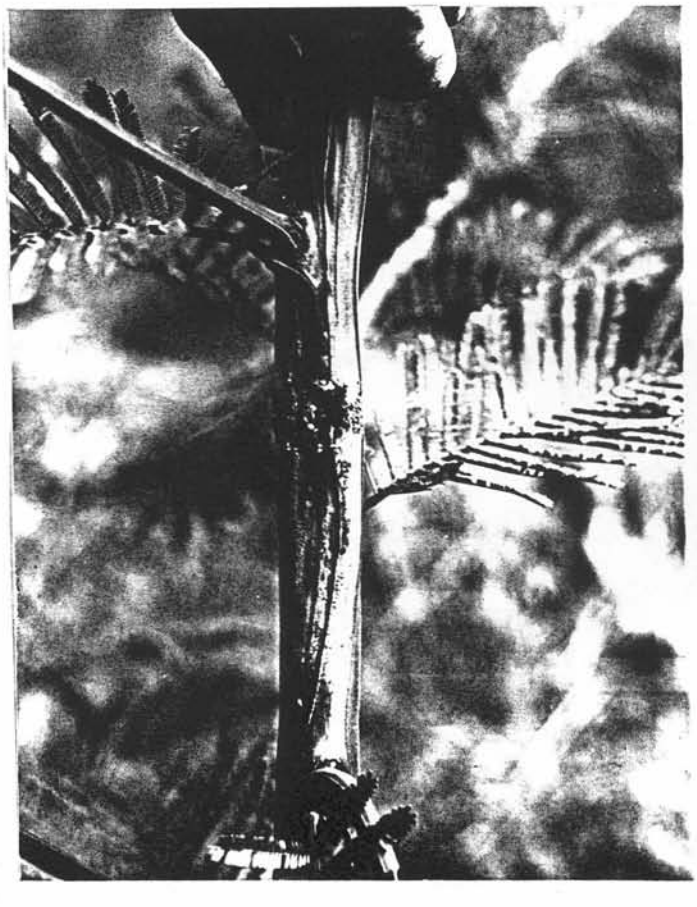
SAPPI/UOVS FOREST PATHOLOGY LABORATORIES

As many of you are aware, we are now in the midst of a major building programme. More specifically, what we have under construction is a laboratory/greenhouse facility which is specifically for research on tree diseases. The establishment of such a facility has been a major objective of ours for the past five years. The extremely high cost of building, and particularly building specialised facilities has been the major obstacle in establishing this complex. A very generous gift of R500 000 from Sappi has however made it possible for us to reach this objective.

The research facility which will be known as the Sappi/UOVS Forest BIOTECHNOLOGY FACILITY will include six controlled environment greenhouse units. These will be supported by two preparation and isolation laboratories as well as a walk-in growth chamber and microscope room. All in all, this facility will cost approximately R1 million and it is our hope that the building will be ready for occupation by the end of December. The building project will also include some minor modifications to the existing laboratory facilities of the TPCP which will add a computer room to the overall facility.

At this stage the team is experiencing considerable frustration with a portion of our current facilities closed due to the building programme. There is also a profusion of dust in the air and this of course seriously affects our ability to isolate fungi without contamination. On the other hand, the excitement of having high quality greenhouse space in close proximity to our laboratories outweighs all frustration.

Obviously the type of research conducted by the TPCP requires facilities to grow trees under controlled conditions. Such facilities are available on our campus but highly oversubscribed and have, in some cases been built with the support of other interest groups. They are also displaced from our laboratories which has been frustrating and inefficient. The new facilities will thus provide a tremendous boost to the activities of the TPCP in coming years. We very much look forward to sharing these facilities with you.



Disease symptoms on *Acacia mearnsii* (black wattle) inoculated with a *Ceratocystis* sp.

Relative susceptibility of Mexican and other Pine species to *Sphaeropsis Sapinea*

The South African Forestry Industry is currently searching for alternative pine species which are adaptable to marginal sites. Pine species from countries having similar site and environmental conditions to those in South Africa are most suitable and species from Mexico are particularly well-adapted. A very important requirement of pine species considered for planting in the summer and constant rainfall areas of the country, is a high level of tolerance to the important pine pathogen *Sphaeropsis sapinea*. In cooperation with Mondi a study was conducted to compare the relative susceptibilities to *S. sapinea* of various provenances of *Pinus greggii* and *P. leiophylla* with local selections of *P. patula*, *P. elliottii*, *P. taeda* and *P. kesiya*. This screening was done in the glasshouse by artificially inoculating one-year-old potted pine seedlings with *S. sapinea*. Evaluation of the results was performed 2 weeks after the inoculations. By this time the foliage of most plants had wilted due to girdling of the stem by the pathogen. Distinct cambial lesions below the bark surrounding the inoculation point were used as a parameter for measuring the effect that *S. sapinea* had on the inoculated plants. Significant differences between species and provenances were observed. All provenances of *P. leiophylla* showed the highest degree of resistance to *S. sapinea*. Previous studies we have conducted have shown that *P. taeda* is the most resistant species currently cultivated in South Africa. This was confirmed in this study together with the fact that three provenances of *P. leiophylla* had a similar or greater degree of resistance than *P. taeda*.

Certain provenances of *P. greggii* differed significantly in their susceptibility to *S. sapinea* but the results of this trial suggest that it is generally more susceptible to *S. sapinea* than *P. patula* which in this study did not differ in susceptibility to *S. sapinea* from *P. elliottii* and *P. kesiya*. Preliminary conclusions that can be drawn from this study are that *P. leiophylla* is a very promising species for cultivation in South Africa as far as its tolerance to *S. sapinea* is concerned. On the other hand, the potential of *P. greggii* is considerably less. It is, however, important that these glasshouse results are confirmed by means of field trials before firm conclusions are made.

IMPORTANT : 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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