TREE PATHOLOGY NEWS

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

Tree Pathology Cooperative Programme
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In order for us to coordinate our services to you please help us by using the abovementioned contact address

Typical malformation of young *Eucalyptus grandis* stems due to canker formation by *Botryosphaeria dothidea*, following hail damage.

DIRECTORS MESSAGE - THE YEAR DRAWS TO AN END

In terms of diseases, this last year has continued to challenge us greatly. In most areas the drought has continued to be severe and the pundits are predicting that we might expect the same for the foreseeable future. Depressing news indeed. Of course this has a significant effect on the opportunistic diseases that we have to deal with. We are also finding that drought and other stresses result in symptoms that we have to deal with. We are also finding that drought and other stresses result in symptoms of some disease being more dramatic. In this case, I refer specifically to those pathogens such as *Coniothyrium* that appear to appear to infect trees irrespective of drought.

Although some of you might find it rather tiresome, our message in every newsletter requesting that samples or enquiries be directed to a single contact point, remains oblivious to some. Let me explain this need for this request. Housing of the Tree Pathology Cooperative Programme (TPCP) in a University Department means that requests can go astray. We believe that it is desirable for members to be assured of an response even when one or more of us are "in the forests" or otherwise occupied by lectures and other duties. We have thus arranged that one phone number will always be assured of a reasonably rapid response. We also try to ensure that every sample submitted for diagnosis is logged into a database ensuring that we can track down these samples in the event of an enquiry. We would thus be most obliged if you would spread the word concerning the official contact address of the group. In the end, this will ensure that we are able to serve you more efficiently.

As many of you are aware, both Brenda and I have been on sabbatical leave during the course of the past eight months. It would be wholly impossible to summarise our scientific experiences in this newsletter without making it long and cumbersome to read. Suffice to say that we really appreciate having had the opportunity to expand our knowledge and to learn new techniques. In this regard we owe thanks to many people and organisations. Firstly, the University of the Orange Free State for granting us six months of study leave and some financial support: the Foundation for Research Development who awarded both of us study bursaries, Mondi Paper Co. who awarded me a study bursary and the Ernest Oppenheimer Trust who also awarded me a bursary. In addition to the above, many of you had to deal with us via fax, e-mail or even snail mail. This was at times inconvenient and we sincerely appreciate your patience!! Finally many TPCP team members including students and particularly Gert Kemp had to assume extra duties. These were executed most competently and we really appreciate this support very much.

While on the subject of sabbatical leave, I might mention that Brenda has returned with some wonderful techniques to identify specie of the important tree pathogen *Armillaria*. This is going to provide us with enhanced insight into the fungus and its biology in South Africa in the near future. We will be communication more on this subject both in this newsletter and in the future.

In this newsletter we will treat a number of topics that have been raised by many of you during the course of this year. Perhaps one of the most important of these is "What do we really know about *Coniothyrium*?" Another topic of current and intense interest concerns the biology of *Botryosphaeria* and *Sphaeropsis*. In both these cases we have accumulated convincing evidence that these pathogens are able to live in a latent (non-infective) phase in healthy trees. This of course has a very substantial impact

on how we view the associated diseases and on how they might be managed. Another topic of current concern relates to our projects and activities as a whole. Because of the severe impact of some of certain eucalypt diseases especially in Zululand, there is an unfortunate misconception that most of our energy and time is spent in that area. Nothing could be further from the truth. Indeed it would be fair to say that our time and activity is quite broadly spread across the forestry areas of South Africa and there remains a very substantial input into pine diseases. In order to assure those of you working in areas other than in Zululand, I am including a break-down of our projects and activities in this newsletter.

And finally - this is the second and last newsletter of the year. It must therefore serve to convey our thanks to all of you that have assisted us during the course of the year with collection of data, setting up of field trials and in many other ways. We appreciate this assistance most sincerely. We also all join in wishing you a very happy and joyous festive season and a great 1995.

TISSUE CULTURE: A TOOL FOR SCREENING PLANTS FOR DISEASE RESISTANCE

When sections of a plant such as pieces of leaf or root material, shoot nodes or embryos are placed on nutrient media, containing plant hormones, callus is formed. Callus is a mass of undifferentiated, dividing cells, and can be subcultured on to fresh media producing genetically-identical material. By altering the plant hormones and their concentration in the medium, root and shoot induction will take place. The procedure takes a relatively short space of time when compared to "normal" propagating methods. The ideal stage to screen for disease resistance is when the callus has formed. A number of methods can be used and two will be described here.

The easiest method is to inoculate the callus with fungal spores. One would expect that in the susceptible interaction, colonization of the callus will take place which would not occur in the resistant/tolerant interaction. The success, however, will depend on the particular fungus/host examined.

Another method would be to either expose the callus cells to a fungal toxin directly (inoculate callus with the toxin) or indirectly (grow the callus on media containing the toxin).

One of the essential factors with screening plants for disease resistance using tissue culture is to correlate results with field studies. For example, a tree with thick bark will have a natural defence mechanism not expressed in tissue culture.

The TPCP is currently considering making use of tissue culture. It would be used as a screening technique for a range of plant pathogens. In this way, resistant or tolerant material would be rapidly identified.

POPULATION DIVERSITY AMONG BRAZILIAN ISOLATES OF *CRYPHONECTRIA CUBENSIS*

Cryphonectria cubensis is one of a notorious group of canker pathogens of *Eucalyptus* trees and causes serious stem cankers in Brazil and many other tropical areas of the world. This pathogen has limited the development of plantations of susceptible *Eucalyptus* spp. in areas where climatic conditions favour disease development. The disease is favoured by high rainfall (2000-2400 mm/yr.) and temperatures above 23 C. In the cooler, drier areas of Brazil infection rates are much lower as is the extend of canker development.

Results of a recent TPCP study on *C. cubensis* suggested that isolates of *C. cubensis* in South Africa represent a uniform population structure. This is indicative of an introduced pathogen. Therefore, the aim of this study was to determine the genetic variability amongst Brazilian isolates of the pathogen. Isolates of *C. cubensis* were collected throughout the *Eucalyptus* growing areas of Brazil and were compared based on their vegetative compatibility reactions. Based on the number of vegetative compatibility groups (VCG's) found in this study, there appears to be a high degree of diversity in the Brazilian population of *C. cubensis*. This was not unexpected given the abundant presence of the teleomorph in Brazil as opposed to in South Africa. These results suggest that *C. cubensis* has been present in Brazil for an extended period of time and that the fungus has only recently been introduced into South Africa.

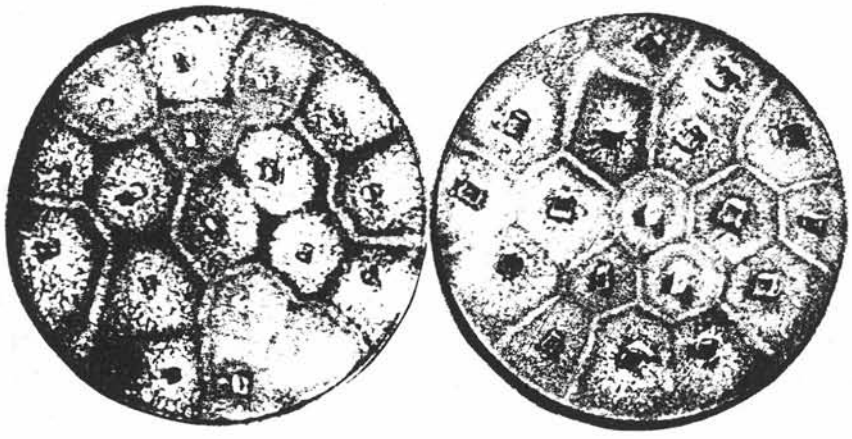


Fig 1: At the end of the incubation time, mycelia that were vegetatively compatible had grown together, forming a confluent mycelium. Incompatible mycelia had grown to a meeting point in the agar, but remained separated by a "barrage-like" reaction line formed along the line of contact between paired colonies.

SIREX WOOD WASP - THE FUNGAL COMPONENT

As many of you are now aware, the potentially very serious wood wasp *Sirex noctilio* has recently been discovered in South Africa by Geoff Tribe and his staff at the Plant Protection Research Institute, Rosebank, Cape. This insect pest has caused tremendous damage to plantations of *Pinus radiata* in Australia and New Zealand in the past and is currently active in Argentina and Brazil. We have predicted and feared its introduction into South Africa for many years and its appearance her now is most discomforting.

One of the most fascinating aspects of this pest, at least from the pathologists viewpoint, is the fact that it lives in a strict symbiosis with a fungus. Indeed it is the fungus and a mucous deposited by the wasp that is believed to ultimately kill the trees although this is perhaps a moot given the fact that the two are fully dependent on each other. The fungus, *Amylostereum areolatum* is a Basidiomycete. This means that it is related to the mushrooms as opposed to, for example *Cryphonectria* which is in the distantly related Ascomycete class.

Amylostereum areolatum is transmitted from tree to tree by female Sirex wasps. This fungus is carried in specialised "transport sacs" known as mycangia which are associated with the ovipositor. Thus when the insect lays her eggs by means of boring into the bark of trees, the fungus is also transmitted. The fungus then has an association with a mucous substance that is deposited with the eggs and then grown into the wood, blocking the vessels and resulting in wilting of infected trees.

A very successful strategy for managing *Sirex* infestations is through biological control of the insect with a parasitic nematode. This nematode which has been used very successfully in Australia and New Zealand is known as *Deladenus siridicola*. Female wasps infested with the nematode lay eggs that carry the namatodes which are then transmitted to trees. These nematodes then feed on the fungus *Amylostereum areolatum* and multiply rapidly in trees. Later an infective stage of the nematode infects wasp larvae which are in effect rendered sterile. Once again, the fungus has a very important role to play in the biological control of the wasp.

A fascinating aspect of the wood wasp in areas where it has been introduced as an exotic relates to the genetic diversity of the wasp. Logically, a more diverse population in the wasp will be less intensively affected by the nematode in prospective biological control programmes. One rather fascinating approach to measuring the diversity of the wasp population will be through a study of the fungus which it carries. Genetic diversity of fungal populations are relatively easily studied and the technology to deal with such questions has been developed by researchers in the TPCP in recent years. We therefore hope to capitalise on this experience and, in collaboration with Geoff Tribe of the PPRI, consider genetic diversity (as well as other aspects) of *Sirex noctilio* in South Africa.

THE 1994 AMERICAN PHYTOPATHOLOGICAL SOCIETY'S ANNUAL MEETING -TPCP INVOLVEMENT

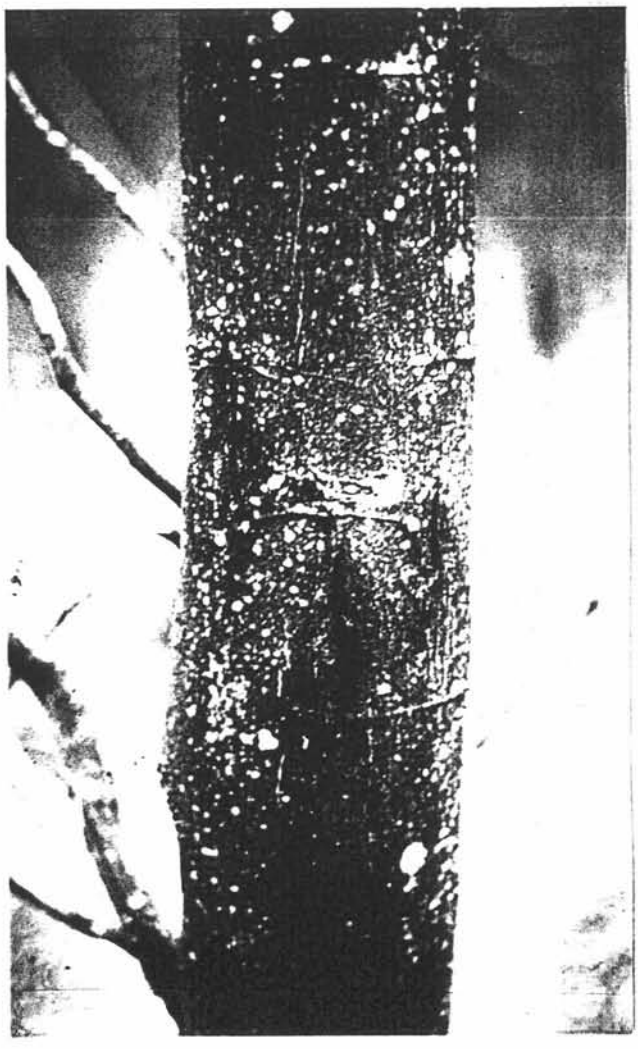
The annual meeting of the American Phytopathological Society (APS) is the largest annual gathering of plant pathologists in the world with approximately 2000 plant scientists from all over the world attending annually. The cultural mix of attendees with diverse backgrounds and various interests makes the meeting unique. This year's meeting took place in Albuquerque, New Mexico's largest city, from 6-10 August. The theme ;for the meeting was "Pro Plant Pathology". The word "pro" reinforced the positive, proficient, professional attitude of the plant scientists who gathered at Albuquerque.

The TPCP was represented at the 1994 APS annual meeting by mr Gert Kemp and dr Wijnand Swart. The meeting was preceded by a one-day forest pathology tour organised by the Forest Pathology Committee of the APS. The tour travelled north of Albuquerque through pueblo country through the Jemez Mountains to Bandelier National Park. Pathology topics mainly focused on riparian tree health, dwarf mistletoe management, hazard tree management and stump removal trials for Armillaria root disease control.

Topics in the technical programme of the APS meeting reflected many new and exciting developments in the science of plant pathology. The Forest Pathology component of the meeting included two poster sessions comprising 17 posters in total and two oral sessions with 23 papers in total. The TPCP made four contributions to this programme. A paper by A.J. Cilliers, W.J. Swart and M.J. Wingfield entitled, "Infection of *P. elliottii* seed by *L. theobromae* provided for some very stimulating discussion. Poster contributions were by: I.P. van der Westhuizen, M.J. Wingfield, W.J. Swart and G.H.J. Kemp entitled, "Effect of water stress on pathogenicity of two *Eucalyptus* canker pathogens"; I.P. van der Westhuizen, W.A. Smit, M.J. Wingfield and G.H.J. Kemp entitled, "Hypovirulence associated with dsRNA discovered in *Cryphonectria cubensis*"; and W.J. Swart, W.C. Saaiman and W-M. Botes entitled, "Use of the isolated root cap cells and cell cultures from *Pinus* spp. in an assay for toxin production by *Sphaeropsis sapinea*. Two very enlightening discussion sessions were also included in the Forest Pathology programme namely, "The application of GIS/GPS technologies to Forest Pest Management" and Molecular Techniques in Forest Pathology"/ The annual APS meeting also provided an opportunity for the annual meeting of the APS Forest Pathology Committee. Both Gert and Wijnand, who is a member of this committee for the next three years, attended this meeting which provided an interesting insight to the activities and concerns of forest pathologists in the USA.

BLACK BUTT OF *ACACIA MEARNSII*

Black butt disease is characterised by black discoloration of the bark at the base of black wattle trees. This symptom is accompanied by the cracking of the bard and the exuding of gum (Fig. 1). The disease occurs in all wattle growing areas in the country. The causative organism was identified as *Phytophthora parasitica* (Dastur.) Waterhouse in the past, although it was generally recognised that this is a more complex problem. In many cases the black discolorations spreads higher up the stem of infected trees towards the terminal growth points (Fig. 2). Isolations from these advanced lesions have failed to yield *P. parasitica*. Various secondary pathogens have however been isolated in our preliminary surveys. At this stage we believe that *P. parasitica* infects trees through the roots or base of the tree, causing entry sites for opportunistic pathogens to infect. Secondary lesions then spread higher up the tree. At present we are conducting inoculation to determine the sequence of events associated with the development of black butt disease. This will include inoculations with various fungi that have been isolated. We hope then to also develop strategies to select trees that are able to tolerate the problem.



SELECTED ABSTRACTS FROM PRESENTATIONS BY MEMBERS OF THE TPCP AT RECENT CONFERENCES

BOTRYOSPHAERIA DOTHIDEA ENDOPHYTIC ON *EUCALYPTUS NITENS* AND *EUCALYPTUS GRANDIS* IN SOUTH AFRICA

Species of *Botryosphaeria* are well recognised as opportunistic, wound related pathogens with a wide host range. In South Africa, *B. dothidea* is an important pathogen of *Eucalyptus*, causing serious die-back and canker diseases. These diseases have been associated with trees suffering from severe environmental stress, such as drought. Although *B. dothidea* is a wound related pathogen, drought stress usually does not result in visual wounds. The aim of this study was to determine whether *B. dothidea* might be endophytic, in the leaves and xylem of healthy *E. nitens* and *E. grandis* trees, only becoming active at times of stress. Fungal isolates representing many genera were isolated from leaf and xylem segments, with *B. dothidea* being the only dominant species known to be a serious pathogen. Results clearly indicate that *B. dothidea* is an endophyte of *E. nitens* and *E. grandis* and its association with disease development in drought-stressed trees requires further investigation.

PYTHIUM IRREGULARE ASSOCIATED WITH *PINUS* SEEDLING DEATH ON PREVIOUSLY CULTIVATED LANDS

A serious root disease of *Pinus patula* seedlings has occurred in the northeastern Cape forestry region of South Africa during the past 3 years. Mortality as high as 100% was experienced 4-5 months after seedlings were planted on previously cultivated agricultural lands. No mortality, however, occurred in plantings on virgin lands. Where seedlings survived on previously cultivated lands their growth compared poorly with those on virgin lands. *Pythium irregulare* was consistently isolated from diseased roots of *P. patula* as well as from soil of previously cultivated lands. *P. irregulare* was highly virulent when artificially inoculated onto 4-mo-old *P. patula* and 2-mo-old *Eucalyptus grandis* seedlings. *P. irregulare*, therefore, appears to be an important factor associated with deaths of *P. patula* on previously cultivated agricultural lands.

CHARACTERIZATION OF A *FUSARIUM* SP. FROM GLADIOLUS PATHOGENIC TO PINES

Fusarium subglutinans is a well-known pathogen of many crop plants. The fungus also causes a serious disease of pines known as pitch canker. It has been proposed that isolates from pines be designated _F. subglutinans_ f.sp. _pini_ (FSP). An enigma regarding this fungus is that some FSP isolates have been reported to induce decay of gladiolus corms. Isolates of _F. subglutinans_ from gladiolus were found to be weak to moderately pathogenic to pine seedlings. In this study we re-examined previously studied isolates from gladiolus in the U.S.A. that had been treated as _F. subglutinans_ in the literature. We found that these isolates were typical of _F. proliferatum_. They were mildly pathogenic on pine seedlings and significantly less virulent than an isolate of FSP. Inoculation of gladiolus corms with these isolates of _F. proliferatum_ from gladiolus, _F. oxysporum_ from pine seedlings and _F. subglutinans_ from pines (FSP), mango, maize and pineapple indicated that only isolates of _F. proliferatum_ were moderately pathogenic to gladiolus corms. This study therefore confirms that isolates of _F. subglutinans_ from pines represent a specific _forma specialis_ within the species.

EFFECT OF WATER STRESS ON PATHOGENICITY OF TWO *EUCALYPTUS* CANKER PATHOGENS

Eucalyptus species are extensively planted by the South African forestry industry. _Cryphonectria cubensis_ and _Endothia gyrosa_ are two closely related canker pathogens of _Eucalyptus_. _Cryphonectria cubensis_ is restricted to areas of high rainfall and temperature whereas _E. gyrosa_ has a wider distribution. The aim of this study, was to determine whether a relationship exists between virulence of these fungi and water stress. Artificial inoculation trials were conducted with both pathogens on trees under stress as well as under conditions of normal water availability. Stress conditions were regulated by using a pressure bomb. Trees were more susceptible to _C. cubensis_ under non-stressed conditions while the opposite was true for _E. gyrosa_. This is consistent with field observations where _C. cubensis_ is more severe in areas of higher rainfall and _E. gyrosa_ under conditions of drought stress.

IMC 5

The Fifth International Mycological Congress was held in Vancouver, Canada from the 14th until the 21st of August 1994 at the campus of the University of British Columbia. Among the attendants were the following from the UOFS: Prof. M.J. Wingfield, Dr. B.D. Wingfield, Mr. Gert Kemp, Adriaan Smit (Infruitech), Christa Visser, Christopher Viljoen and Corli Strydom. Papers were presented by the above attendants as well as various students (Celeste Linde, Altus Viljoen and Henk Smith) in absentia.

As one of the delegates I found the congress an incredible learning experience. It was also very rewarding to learn from internationally known researchers that our work is on a par with theirs even though we are somewhat isolated.

HYPOVIRULENCE ASSOCIATED WITH dsRNA DISCOVERED IN *CRYPHONECTRIA CUBENSIS*

Cryphonectria cubensis is responsible for serious losses in commercial *Eucalyptus* plantations around the world. Currently, the only means of controlling the disease is by resistance breeding and selection. The virulence of certain fungal pathogens can be reduced biologically through hypovirulence, as is true in the closely related pathogen *C. parasitica*. Isolates of *C. cubensis* were screened for the presence of dsRNA and other hypovirulence associated traits. Pathogenicity tests were also conducted to link the presence of dsRNA with hypovirulence. Various *C. cubensis* isolates from South Africa were found to contain dsRNA. These were hypovirulent in comparative pathogenicity tests and also displayed other hypovirulence associated traits such as a reduction in oxalic acid production and laccase activity. Results further indicate that naturally occurring hypovirulence might play an important role in reducing the impact of Cryphonectria canker in South Africa.

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