TREE PATHOLOGY NEWS

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

NO 7 MAY 1993

A NEW YEAR AHEAD

As has become custom, the beginning of the year was packed with furious activity for the TPCP. One of the most important events of our year is the annual congress of the South African Society for Plant Pathology which is traditionally held during the third week of January each uear. Thus the New Year had hardly passed when most of the research team re-assembled at Bloemfontein to put the finishing touches to posters and slide presentations for the congress held this year at Club Mykonos in the Cape.

Our sojourn to Langebaan started off with one car and three combi minibusses departing from Bloem very terribly early on the morning of Sunday 16th January. All told, about 22 team members made the trip to present the results of their years efforts in the interests of the South African forestry industry. Certainly a far cry from the "old days" when a single presentation concerning forest disease was considered rather an oddity!! There is little question that today, tree diseases are well known and nothing strange to local plant pathologists irrespective of whether their interest lies with diseases of pumpkins or pineapples.

Returning from National Congresses is always beset with mixed blessings. Exchange of ideas and rekindling of acquaintances always leaves one feeling elated and ready to tke on the world. But the reality of fungi needing attention. uncompleted experiments and the beginning of the academic year, with its accompanying demands, always help to bring us all down to earth.

Shortly after our return from congress, we all started to prepare for the Annual Meeting of the Board of the TPCP. This year this event was to take a new form. It was to be attended not only by Board members BUT also by selected staff of each of the members. In all, we were preparing to share our previous years' work with about 20 colleagues - a mini congress to say the least!!

The mini symposium and board meeting of the TPCP was held at Bloemfontein on Thursday and Friday 18th and 19th of February. The meeting started off with a light finger lunch followed by a welcoming introduction. This was followed by a series of lectures in short succession consuming all of Thursday afternoon and most of Friday morning. After all the presentations and a fair share of vigorous discussion, board members broke away from scientific discussions for their annual meeting. Finally, after the formalities were over, we all enjoyed a light lunch in the garden.

From the above discussion, it would almost seem that there was little time for research during the first few months of this year!! It is however surprising what a bit of "midnight oil" will do for getting work done!! Thus, despite all the presentations and meetings, our first field trip of the year to read the results of field inoculations and to establish new screening programmes, occurred on schedule. Our field trials and surveys dealing with *Cryphonectria* canker, *Phytophthora/Pythium* root diseases, the newly discovered pitch canker of pines, *Bothryosphaeria* canker, *Endothia* canker, *Coniothyrium* canker and more are therefore all well cared for and results are pouring in at a somewhat unmanageable rate.

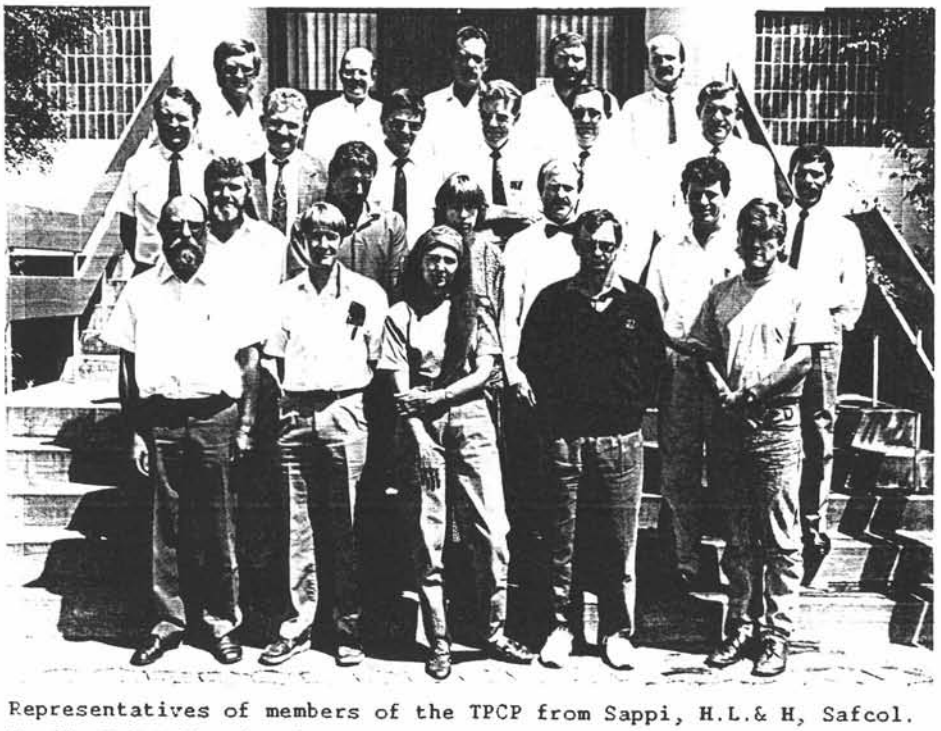
The year ahead is likely to be as hectic as usual. At this stage, our services have become a little too well known and samples for routine examination pour in to the lab virtually every day. Although taking care of these samples can become a tremendously time consuming operation. they are essential if we are going to detect new and serious diseases timeously. We thank all of you that survey plantations and nurseries for apparently new diseases and encourage you to continue to submit samples to us.

On the research side, we continue to pursue projects on the major plantation and nursery diseases. Some new diseases that will have to receive attention are wilt of black wattle and an apparently new stem canker disease of eucalypts. We are also excited about a new project where we will study the potential for controlling *Cryphonectria* through hypovirulence.

Hypovirulence refers to reduction in virulence of a pathogen resulting from it becoming infected with fungal viruses. We believe that there is tremendous potential for applying this form of biological control in South African forestry and will be reporting to you in more detail on this at a later date.

Research team members will continue to be active on the extension/education side during this year. On the tertiary education side, the annual tree pathology course for forestry students will be held during the second half of the year at Stellenbosch. Field days and lectures will also be held for those who request them and at appropriate places and times. Furthermore, our routine trips to plantations will also continue to occur at about two monthly intervals. These are always intespersed with ad hoc visits to forestry areas to deal with specific problems and it certainly should not be difficult to match our 290 or so person days in the forests this year!!

We once again thank you for your support in our efforts to ensure that diseases do not result in unacceptable losses in our plantations. We furthermore look forward to hearing from you whenever problems do arise and also to seeing you in the forests.



Representatives of members of the TPCP from Sappi, H.L.& H, Safcol. Mondi, U.O.F.S and I.C.F.R., who attended the annual meeting of the programme at Bloemfontein.

FINGERPRINTING EUCALYPTUS CLONES USING RAPDS

An effective programme for clonal propagation of forest trees requires sophisticated means to characterise clones. This fact is rapidly being recognised by scientists dealing with a wide diversity of agricultural crops. It is also currently an area of feverish activity in animal husbandry circles where characterisation of many different domestic animals such as race horses is underway. The scientific jargon being used to describe this area of technology is FINGERPRINTING.

This term "fingerprinting" is used very loosely and can be misleading. The basis of this distinction could be protein banding patters, different DNA restriction fragments, isoenzyme patterns, differential hybridisation of probes or even at perhaps a less powerful level, the presence of unique metabolites.

In a highly complex organism such as a eucalypt tree there are many different cell types involved in a variety of complex processes. However, every cell contains the same genetic material, the same DNA. Despite the level of differentiation or effect of the environment the DNA in every cell remains the same throughout the lifetime of the plant. Therefore, fingerprinting using DNA is the best method on which to base characterisation as material can be collected and analysed no matter how old a tree is or where it is growing. Obviously the most accurate way of distinguishing between different clones would be to sequence the entire genome. This would be much too complicated to consider for every individual. Another approach would be to obtain a map of all the DNA using the different restriction enzymes that cut the DNA into different size fragments. However, the size of the genetic material is far too large to consider such a task.

A novel method to fingerprint large genomes was developed in 1991. This method, commonly referred to as RAPDS (Random amplified Polymorphic DNA) makes use of PCR (Polymerase Chain Reaction) and random primers. PCR is a technique whereby large amounts of DNA can be amplified from exceedingly small amount of starting material. Using random primers (short sequences of DNA) to start the PCR reaction it is possible to specifically amplify specific size fragments of DNA from the genome of an individual. These different size fragments can be separated electrophoretically to produce a "fingerprint" or "bar code". Because the primers are random sequences each pattern that is produced is specific to each individual. This technique also has the advantage that many different primers can be used. In this way an almost infinite number of different pattern can be produced for any one individual, and thus more characteristics on which to base comparisons between individuals. RAPDS are not only useful in distinguishing between individuals. Each "band" in the fingerprint pattern represents part of the genome of an individual and can thus be considered to be the same as a characteristic or character. It is possible to find linkage between these bands and desirable characteristics such as drought resistance, disease resistance and rate of growth to name just a few. Such a linkage has the potential that young trees can be identified (with a high degree of probability) as having a desirable characteristic long before reaching maturity. The advantages to a breeding program of such early predictions are obvious.

One of the most exciting aspects of fingerprinting is in the identification of trees resistant to disease and finding some markers linked to this resistance. This may enable the elucidation of the actual mechanism of resistance. This would then allow researchers to genetically

modify trees so that they tolerate disease.

In our laboratories we have been successful in obtaining such RAPD fingerprints with *Eucalyptus grandis* clones. Using just two different primers we have succeeded in distinguishing between many clones. Many more clones and primers need to be tested before this technique can be used commercially by the forestry industry. It is however, an extremely powerful technique which will allow the resolution of many identification problems. It also has the potential to increase our understanding of disease resistance. Ultimately this will lead to the genetic modification of clones to produce superior trees.

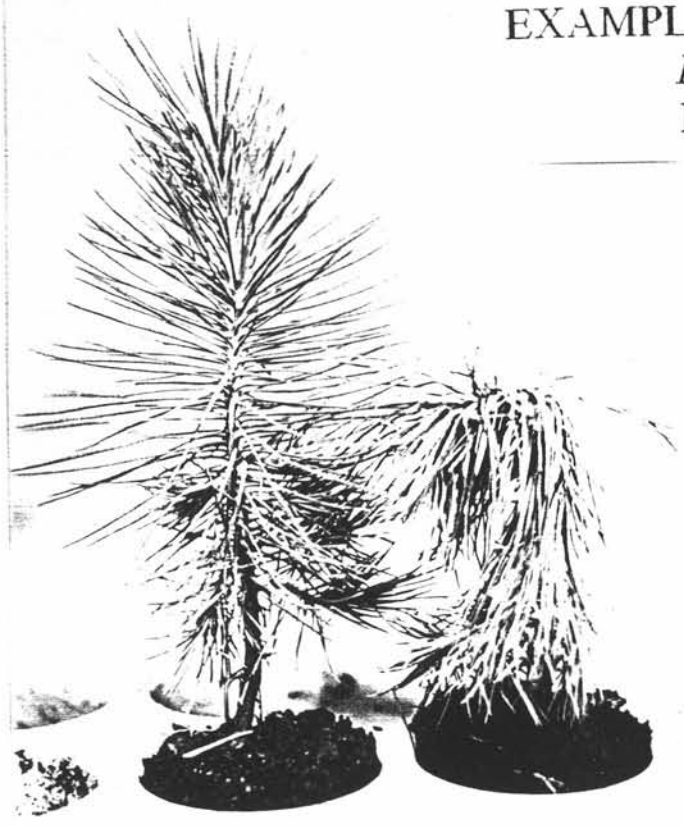
BLACK SEED DISEASE

The phenomenon of Black Seed Disease of *Pinus elliottii* was previously reported in Tree Pathology News. Results which have been obtained thus far strongly suggest that the disease, which results in reduced germination percentage jof pine seeds, is related to cone harvesting and seed extraction procedures. The fungus, *Lasiodiplodia theobromae* which is commonly associated with the disease, thrives under wet or humid conditions and temperatures above 25°C. These conditions can inadvertently be created during the collection and pre-curing of cones prior to seed extraction. Cones which are collected too early and stacked in hessian bags will result in heat and moisture build-up which will promote fungal activity. Seeds will then be infected by penetration of the pathogen through the ovary wall and seed coat or through natural openings.

A trial has been initiated in cooperation with Mondi, Sabie to investigate the effect of storage under various environmental conditions on the incidence of Black Seed Disease. The cones of three susceptible and three resistant clones were collected during March at the optimum stage for harvesting, i.e. when "cracking" commenced. One batch of cones was placed in hessian bags and stacked as tightly as possible to prevent free movement of air between bags and the bags were subsequently wet thoroughly. A second batch was spread on kiln trays to allow maximum air movement and drying. Cones from each batch were sampled at harvesting and again at two weekly intervals. They are then sent to the Department of Plant Pathology at the UOFS in Bloemfontein where seeds from each cone are then screened for the presence of *L. theobromae*.

Results thus far have shown a definite increase over a four week period in the percentage of seeds infected with *L. theobromae* originating from cones stored in hessian bags. It is significant however that even cones that were newly harvested revealed the presence of *L. theobromae*. This fact therefore strongly suggests that cones become infected by the fungus when they are still on the tree. This hypothesis is substantiated by the fact that although cones from the second batch that were spread on kiln trays showed lower percentages of *L. theobromae* than those in hessian bags, the fungus was nevertheless still present. The preliminary results of this experiment therefore strongly suggest that the control of Black Seed Disease must be tackled both in the seed orchards and during storage.

EXAMPLES OF DAMAGE DONE BY *FUSARIUM SUBGLUTINANS* F. SP. *PINI*



Fusarium subglutinans f. sp. *pini* resulted in serious losses of *Pinus patula* seedlings (left) during the last number of years. The pathogenicity of the fungus can be clearly illustrated on the photo below. On the left, newly germinated seedlings were completely killed by damping-off, while all the seedlings survived during control inoculation on the right.

NUUSBROKKIES

Nuwe baba: Gert en Benita Kemp het in Januarie 'n seun, Garrett ryker geword. Baie Geluk aan die trotse ouers!

Huwelik: Die huweliksklokke het gelui vir twee van ons personeellede. Eerstens het Theresa Filmalter in die huwelik getree met Leon Mouton en tweedens was dit Sakkie van der Westhuizen en Lisl van den Berg wat die groot stap geneem het. Ons beste wense vir die toekoms aan hulle!

SELECTED ABSTRACTS FROM PRESENTATIONS BY THE TPCP TEAM MEMBERS AT A RECENT CONFERENCE

CERATOCYSTIS FIMBRIATA FROM BLACK WATTLE IN SOUTH AFRICA COMPARED WITH ISOLATES FROM OTHER CONTINENTS

*Ceratocystis fimbriata*is a cosmopolitan plant pathogen causing diseases on a wide variety of hosts. The fungus was recently isolated from *Acacia mearnsii* in the Natal midlands of South Africa causing dieback and gummosis. South African isolates of the fungus differ morphologically from those collected elsewhere in the world. The aim of the study was to compare these isolates by means of light and electron microscopy. Perithecial bases of *C. fimbriata* from wattle are a yellow colour and unlike others that have dark bases. Hat-shaped ascospores of the South African isolate also have a double brim which is in contrast with the single brim found in other isolates. Results of this study, based on morphology, suggest that *C. fimbriata* from wattle in South Africa probably represents a distinct species. Comparisons at the molecular level are however underway to test this hypotheses.

SUSCEPTIBILITY OF *EUCALYPTUS* SPECIES TO *CONIOTHYRIUM* STEM CANKER IN SOUTH AFRICA

Coniothyrium stem canker is a serious disease of *Eucalyptus* trees in South Africa, with the highest disease incidence occurring in Zululand. *Eucalyptus grandis* clones and hybrids vary in susceptibility to this disease and therefore breeding for resistance is an effective strategy for disease avoidance. Genetic information concerning species used in hybridization is inadequate. The aim of this study was to evaluate the susceptibility to *Coniothyrium* stem canker of different *Eucalyptus* species used in hybridization. Artificial inoculations were made on one-year-old trees and lesion development was measured after three months and one year. Susceptibility was relatively uniform in most species but was highly variable in *E. grandis* and *E. nitens*. Therefore, in resistance breeding, it will be necessary to carefully select species and even individuals within species known to be resistant to this disease

COMPARISON OF BAITING TECHNIQUES FOR ISOLATING PYTHIUM AND *PHYTOPHTHORA* SPECIES FROM SOIL IN *EUCALYPTUS* AND PINE PLANTATIONS

Various species of *Pythium* and *Phytophthora* are associated with serious root diseases of *Eucalyptus* and *Pinus* species. These are soil-borne fungi and their presence in soil is difficult to determine without effective baiting techniques. The aim of this study was to identify a suitable baiting technique to isolate *Pythium* and *Phytophthora* spp. from South African forestry soils. Baiting with *Eucalyptus* and *Citrus* leaf discs was compared with that using lupin roots. Soil pre-infested with low concentrations of *Pythium splendens*, *Pythium irregulare*, *Phytophthora cinnamomo* and *Phytophthora parasitica* were used to compare the efficacy of the baiting techniques. Lupin baiting was the poorest technique for isolating the test fungi. *Eucalyptus* and *Citrus* leaf discs were equally effective in isolating the two *Pythium* species. *Citrus* leaf discs were most effective in isolating the *Phytophthora* species. The results of this study have shown that baiting techniques vary considerably in their efficacy and should be carefully evaluated before field surveys are conducted. In the case of South African forestry soils, baiting with *Citrus* leaf discs is recommended.

POPULATION STUDY ON SOUTH AFRICAN ISOLATES OF *Cryphonectria cubensis*

Cryphonectria cubensis is a serious canker pathogen of *Eucalyptus* trees in South Africa. To minimize damage due to Cryphonectria canker, clones are screened for relative susceptibility before large scale planting is undertaken. The aim of this study was to determine the genetic variability amongst South African isolates of the pathogen. Isolates of *C. cubensis* were collected throughout the *Eucalyptus* growing areas of the country and these were compared based on their vegetative compatibility reactions. Results of this study suggest that *C. cubensis* represents an extremely uniform population structure indicative of an introduced pathogen. Therefore, inoculation trials to select for disease tolerance probably do not include the broader range of genetic diversity associated with the pathogen in its native habitat.

VARIATIONS IN ALLOZYME PATTERNS IN ISOLATES OF *Sphaeropsis sapinea* OF DIFFERENT GEOGRAPHICAL ORIGIN

The range of isozymic diversity among isolates of *Sphaeropsis sapinea* from various parts of the world was investigated by means of starch-gel electrophoresis. Gene products of 14 enzyme-encoding loci were detected of which three loci were monomorphic for all isolates. From the resultant genotype information, allele frequencies and genetic distances were calculated. Only two loci, Acp-1 and Pep-GL, differentiated clearly between type A and B isolates were, however, found from other parts of the world. The general pattern of isozymic diversity reflected relatively high levels of genetic variation within local populations but lack of sharp dissimilarity between geographic populations. Isolates of *S. sapinea* from *Pinus* spp. could not be differentiated from one another on the basis of host species.

SPECIES OF *Mycosphaerella* PATHOGENIC TO *Eucalyptus* LEAVES IN BRAZIL AND SOUTH AFRICA

Mycosphaerella spp. are recognised as some of the most important pathogens of Eucalyptus leaves. Eleven *Mycosphaerella* spp. are known to be pathogenic to *Eucalyptus* worldwide. Two species, *M. nubilosa* and *M. molleriana* have been reported from South Africa. Based on type, culture and germination studies, *M. nubilosa* was shown to be conspecific with *M. molleriana*. Two new *Mycosphaerella* species distinct from all others occurring on Myrtaceous hosts have recently been found on *Eucalyptus* in Brazil. Both species are associated with prominent leaf spots of several economically important *Eucalyptus* spp. in that country. Preliminary surveys indicate that there are probably additional *Mycosphaerell* spp. occurring on *Eucalyptus* in Brazil, whereas only one is known from this host in South Africa.

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), colleagues and students attached to other organisations such as of 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 of the Departments in the University obviously provide advice and support where this is required.

A RETROSPECTIVE ANALYSIS OF MYCORRHIZAL RESEARCH : WHERE HAVE WE BEEN, WHERE SHOULD WE BE GOING

A detailed analysis of MYCOLIT, a comprehensive bibliographic database of mycorrhizal research containing almost 12 000 references, showed some interesting trends over the past 40 years. During the last four decades, the average numbers of papers published per year were 84, 110, 214 and 488, respectively. The current rate of publication is about 700 papers per year. Much research has focussed on nutrient dynamics, inoculum production, mycorrhiza formation, and morphology and physiology of both vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizae (EM).

Attention has shifted significantly from EM to VAM since 1970. Very little work has been done on the other kinds of mycorrhizae. Barriers to progress in some areas, such as our inability to grow the Glomales in axenic culture, have stimulated large numbers of methodological papers over the past decade, while the genetics and molecular biology, especially of VAM, have been neglected. Ecological studies have been more numerous than any other kind. However, there was a bias towards laboratory studies as opposed to field studies. Field studies have focussed on agricultural ecosystems, while neglecting disturbed systems. Unfortunately, natural ecosystems have been largely ignored. This analysis suggests that we may know less than we think about mycorrhizae, since we have consistently based broad hypotheses and conclusions on studies of a small number of taxa. Future research should be directed to correcting the various imbalances revealed by this study.

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

Fusarium subglutinans (Wollenw. & Reinking) Nelson, Toussoun & Marasas causes disease of rice, maize, sugarcane, pineapple as well as the notorious pitch canker of pine trees. It has recently been proposed that the pitch canker pathogen be designated as *F. subglutinans* f. sp. *pini*. Isolates of *F. subglutinans* causing root disease of *Pinus patula* seedlings in South Africa were compared with four pitch canker isolates and six isolates from hosts other than pine. Detailed comparisons of the cultural characteristics, morphology and pathogenicity of these isolates were made to determine the exact identity of the South African isolates. Local isolates were similar to those associated with pitch canker in culture and morphology, and also with regard to pathogenicity. Only pine isolates were able to cause disease to pine seedlings and saplings. We therefore support the view that isolates of *F. subglutinans* pathogenic to pine represent a disticnt forma specialis whithin the species.

PYTHIUM and PHYTOPHTHORA SPP. associated with EUCALYPTUS plantations

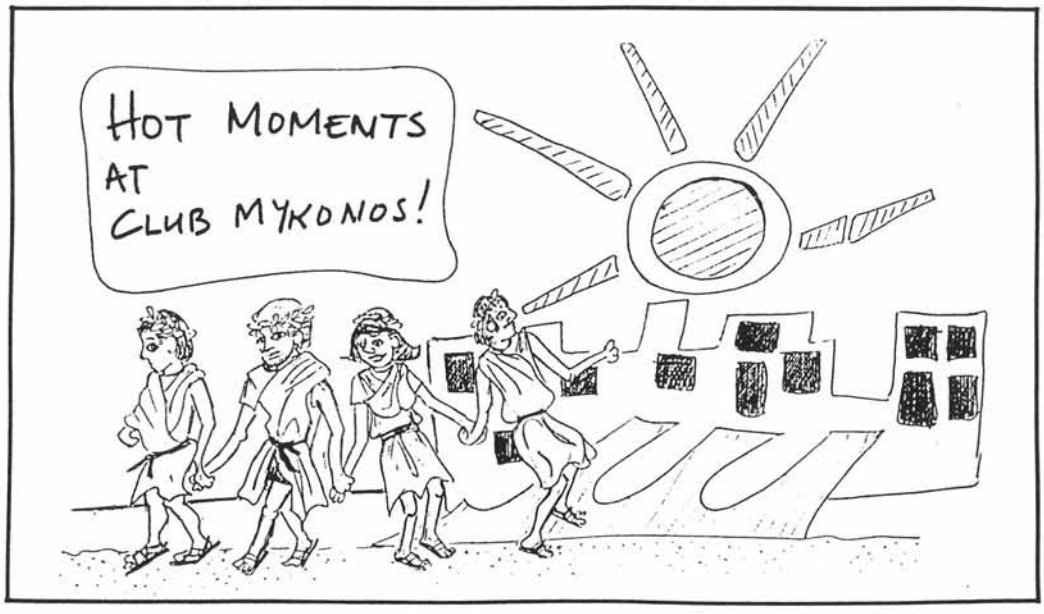
Pythium and *Phytophthora* spp. are well-known pathogens of *Eucalyptus* and *Pinus* spp. *Phytophthora cinnamomi* for example is the causative agent of littleleaf disease of pines in the U.S.A., *Pinus radiata* decline in New Zealand, and the spectacular root disease of various *Eucalyptus* spp. in Australia. The fungus is also well-known in South Africa where *Eucalyptus fraxinoides* and *Eucalyptus fastigata* are very susceptible to *P. cinnamomi* root disease. However, information regarding the presence of *Phytophthora* and *Pythium* spp. in South Africa is lacking. Surveys were done to determine which species of *Pythium* and *Phytophthora* are present in South African forestry plantations.

Areas which were specifically investigated were Zululand (root disease of one to two-year-old *Eucalyptus grandis* trees); South eastern Transvaal (*Eucalyptus fastigata* dieback, as well as *Eucalyptus dunnii*, *Eucalyptus macarthurii*, *Eucalyptus smithii* and *E. grandis*-hybrid root diseases); Eastern Transvaal Lowveld and Natal Midlands (general root disease problems).

Twenty-one *Pythium* spp. and two *Phytopthora* spp. were isolated and identified. Nine of these *Pythium* spp. were recorded for the first time in South Africa. Most of the *Pythium* spp. isolated are common soil saprophytes and not pathogenic to *Eucalyptus* or *Pinus* spp. under field conditions. However, *Pythium irregulare* was frequently isolated from roots of diseased trees and is seen as important in the root disease complex. *Pythium splendens* was frequently isolated from *E. grandis* in Zululand and pathogenicity tests confirmed its virulence. This is the first time that a *Pythium* spp. were also isolated but occurred at low frequencies and are unlikely to cause disease of *Eucalyptus* and *Pinus* spp. under field conditions.

Phytophthora cinnamomi was frequently isolated from all the *Eucalyptus* spp. examined in south eastern Transvaal. *Phytophthora boehmeriae* was also frequently isolated from *E. dunnii*, *E. macarthurii* and some of the *E. grandis* hybrids.

The presence of high concentrations of known pathogens such as *P. cinnamomi*, *P. boehmeriae*, *P. irregulare* and *P. splendens* from soil associated with diseased trees, suggests that they are an important component of the mortality of forest trees. A detailed study of their population dynamics is currently being undertaken by the TPCP. The specific role of all the species in root disease development is also being examined.



IMPORTANT : READ THIS

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