TREE PROTECTION NEWS

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University of Pretoria

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From the Director's desk

It never ceases to amaze me how one's ideas can become fixed in a certain direction and that this may not always represent the full story. One of these pre-conceptions has been my view that the great threat (in terms of disease) to commercial forestry in South Africa lies with the introduction of new pathogens into the country. Certainly, there are very many really serious pests and pathogens of pines, eucalypts and wattles in their areas of origin that would do terrible damage locally. But it has been a great surprise to me how many of our most serious forest pathogens are turning out to be native. It has been well known for many years that numerous of the insect pests damaging plantations particularly of pines and acacias are native to South Africa. But somehow, I would have expected the pathogens to be different. A higher degree of specificity in pathogens as opposed to insects is a fair perception. However, data emerging from the application of our new DNA-based tools are showing us clearly that some of the most important pathogens that we are dealing with are most probably native.

A question in my mind is what principles might emerge from our understanding that many of our key pathogens are native fungi. Thus far, the best examples of host jumps for the key pathogens appear to be on eucalypts and acacias. This might have been expected as South Africa has many native Myrtaceae and native *Acacia* species. Perhaps it is reasonable to expect that we will not find many examples of diseases moving to pine from native South African plants. But then we have the curious situation with insects, where some of the more important pests of pines are native insects. It is difficult to guess what might emerge in the future. But we are clearly strongly in the learning phase and there are many surprises that await us. What will certainly not be a surprise is an increasing level of damage to plantations by pests and diseases.

A new year has rushed upon the TPCP team remarkably fast. This seems to be truer every year that passes. When talking with the FABI team this week, I remarked on the new influx of students and how they become younger

every year. It is of course we that are becoming older and the newly enrolled students remain in the domain of 18-years-old. The gap gets wider and it is said that time passes more rapidly as one ages.

Other than having to gaze upon an apparently ever-younger influx of students, the beginning of each year is marked by our participation in a number of key scientific congresses. For the TPCP team, these include the meetings of the Southern African Society for Plant Pathology, the South African Association of Botanists, the Systematics Society and others. Thus preparing our papers and posters makes FABI a hive of activity at this time of the year. This also represents an important learning exercise for TPCP students who have the opportunity to present new research to peers. While this can be an intimidating exercise, it is a crucial part of the education and mentoring process. Once again, TPCP students were lauded for the quality of their work and the presentation thereof.

I was delighted to see that the ICFR will begin a symposium series that will enable scientists in specialist disciplines to showcase some of their presentations at prior meetings. While I believe that the intention is for these to be from international meetings, there is certainly equally important material that has been presented at local congresses that might be included. Either way, the TPCP will enjoy this opportunity to meet with other South African forestry colleagues to share experiences and research.

Of all the diseases and pest problems facing South African forestry, the two currently of most concern are the Sirex wood wasp and damage due to the pitch canker fungus. Much time and effort has been expended on these two problems during the last year. Damage due to these non-native alien invasive species is of great concern and every effort is being made to reduce this. Various new initiatives have been launched to achieve this goal and we believe that they are already showing signs of success. But to be realistic, the onslaught against these problems will be an ongoing and often difficult process. We certainly require focused activity but also patience. This is perhaps one of the frustrations of working with biological systems. Much as is the case with for example genetics, progress is slow and it requires multiple approaches and a deep understanding of the factors associated with the system in question.

One of the exciting events marking the start of this year was the formal launching of the Department of Trade and Industry (DST)/ National Research Foundation (NRF) Centre of Excellence in Tree Health Biotechnology (CTHB). This launch was attended by many of our friends from the South African Forestry Industry and members of the TPCP. This was sincerely appreciated as the new CTHB must strive to provide synergy for the TPCP and likewise, the TPCP should synergize the CTHB. While the focus of the research in the CTHB will be on the health of native Southern African Trees, it is well known to TPCP members that there is substantial overlap between these domains. I return to the issue raised at the outset of this commentary that many diseases of plantation trees appear to be caused by pathogens originating on indigenous trees. We are thus delighted at the opportunity to be able to integrate the TPCP and CTHB in coming years.

The annual meeting of the TPCP is rushing towards us. This year, the meeting will be held on Tuesday 18th and Wednesday 19th April. The meeting will be the first where we begin to integrate the TPCP and the CTHB. Our plan is thus to treat this meeting as a joint annual meeting of the two closely linked programmes. I believe that this will add depth to our understanding of pests and diseases of forest trees and in general tree health in South Africa. We look forward to being able to share this exciting event with you and to your participation in our efforts to *"KEEP TREES HEALTHY"*

Mike Wingfield,
Mondi Professor of Forest Pathology, Director of the TPCP and the DST/NRF CTHB

…..The GMO debate in Forestry…..

Genetically modified organisms (GMOs) have been a hot topic for discussion the past few years. In South Africa this topic, particularly in agriculture received lots of coverage in 2004. Agriculture, however, is not the only sector looking at GMOs. Below is a short extract from a recent press release on GMO trees. A webpage is also given for more information on the subject.

Extract from ISIS Press Release 28/02/05
Complete article available from http://www.i-sis.org.uk/GMFTTUT.php

World status of GM forest trees

Most genetic modification of forest trees have been done by *Agrobacterium*-mediated DNA transfer; but bombardment with DNA-coated particles, or 'biolistic transformation', has also been used. Of the 205 permit applications listed at the end of 2003, 73.5% originated in the USA, 23% in ther OECD member nations (in particular, Belgium, Canada, France, Finland, New Zealand, Norway, Portugal, Spain and Sweden) and 3.5% elsewhere (Brazil, China, Chile, South Africa and Uruguay). Four traits account for 80% of the permit applications: herbicide tolerance (32%), marker genes (27%), insect resistance (12%), and lignin modification (9%). Of the tree species involved, *Populus*, *Pinus*,

Liquidambar (Sweet Gum Tree) and *Eucalyptus* account for 85% of applications.

Although commercial interest was low during the first ten years of GM trees development, it has steadily increased since the late 1990s. By the end of 2003, 45% of the permits submitted were from industry, mostly for transgenic poplars. But to-date there has not been a concerted push for commercialisation of GM trees except in China, where more than one million GM trees have been planted in "reforestation" initiatives since commercialisation was approved by The Chinese State Forestry Administration in 2002 (see "GM trees get lost", this series).

Several companies, including Weyerhaeuser, Shell and Monsanto, at one time involved in GM tree research, have since pulled out because it was not economically attractive. However, the decision reached in December 2003 at the ninth Conference of the Parties to the UN Framework Convention on Climate Change to allow Northern companies and governments to establish plantations of GM trees in the South under the "Clean Development Mechanism" might be the subsidy that GM proponents need to make GM trees seem economically attractive.

The IV Asia-Pacific Mycological Congress (AMC) and IX International Marine and Freshwater Mycology Symposium (IMFMS)

The IV Asia-Pacific Mycological Congress (AMC) and IX International Marine and Freshwater Mycology Symposium (IMFMS) was held in Chiang Mai, Thailand from the 14th to the 19th of November 2004. A total of 325 delegates attended the congress from 25 different countries representing 5 continents. Both poster and oral presentations were presented. Four Fabians had the privilege to attend the meeting. They were Proff. M.J. Wingfield, B.D. Wingfield, Dr. X.D. Zhou and Marelize van Wyk. All four are part of the Tree Protection Co-operative Programme (TPCP).

The meeting was opened with thunderous drumming and dancing, typical of the Northern Thailand culture. There were 10 symposiums held with six keynote speakers. The 10 symposiums consisted of (1) Forest Pathology, (2) Towards understanding fungal diversity, (3) Fungal Phylogeny, (4) Plant Pathology, (5) Basidiomycete biography and Systematics, (6) Biotechnology, (7) Some enzymes from fungi, (8) Mycorrhizal fungi and cropping systems, (9) Diversity and utilization of edible mycorrhizal fungi and (10) Recent progress on taxonomy and diversity of plant parasitic fungi in Asia. Of the group of Fabians attending, Mike Wingfield was a Chair for the symposium on Forest Pathology, Brenda Wingfield presented an oral presentation entitled “Genetic boundaries of plant pathogens confuse plant pathologists” while both Zhou and Marelize presented both oral presentations and posters. For Zhou the oral presentation was entitled “Phylogeny of species in the *Ophiostoma galeiforme* complex” while he had a poster on a survey of *Ophiostoma* and *Ceratocystioposis* species associated with conifer-infesting

Prof. Brenda Wingfield, Prof. Mike Wingfield, Marelize van Wyk and Dr. Zhou

bark beetles in China. Marelize's presentation was "*Ceratocystis polychroma*, a potentially important pathogen of clove" and her poster on a first report of *Ceratocystis fimbriata* on *Eucalyptus* trees in Indonesia".
On the social calander at the AMC and IMFMS, delegates were treated to a welcoming reception at the swimming pool that is situated on the seventh floor of the Lotus Hotel, Pang Suan Kaew. Snacks and wine was offered at the poolside with live Thailand music playing in the background. This was the perfect setting for colleagues and old friends to get together again as well as for new friends and collaborations to be made. The theme for the congress dinner was "Khan Tok". This was a dinner in the true Thailand style. We sat on little pillows on the floor with our feet underneath us and a very low table. The food served was traditional Thai food, and while eating we were entertained by some traditional Thai dances. The AMC and IMFMS congress in Chiang Mai, Thailand was a great success, and I learned a lot from this international congress experience as well as met many of the famous names of forest pathology and mycology. Thank you to the TPCP and FABI for funding my attendance at the congress.

Contribution by Marelize van Wyk

CONGRATULATIONS

The following students obtained degrees in the last 6 months. Congratulations on your achievements!!

B.Sc Honours

James Mehl – Detection of the pitch canker fungus, *Fusarium circinatum*, in pine seeds

Masters

Draginja Pavlic (Cum Laude) - *Botryosphaeria* species on native South African *Syzygium cordatum* and their potential threat to *Eucalyptus*.

RESEARCH ON BARK BEETLE-FUNGUS ASSOCIATIONS

My sabbatical visit to South Africa

Bark beetles are small insects in the subfamily Scolytinae that feed and breed in the phloem layer that lies directly between the outer bark and sapwood of trees. While most of the 3000+ species of bark beetles do not typically kill trees, several species are very aggressive and can develop extensive outbreaks where tens to hundreds of thousands of trees can be killed over the span of just a few years. These tree-killing bark beetles, when in their native ranges, can be viewed as affecting the forest in two ways. Firstly, they are among some of the most important forest pests worldwide contributing greatly to economic losses in the wood products industry, and secondly, they act as natural disturbance agents that maintain natural forest structure, composition, and function. Outside their native ranges, such as in the plantation forests of South Africa and other parts of the world, their role is strictly that of a potentially economically important pest.

Figure 2. Bark beetle pupae in fungus-lined pupal chambers

All bark beetles, regardless of whether they kill trees or not, are associated in some manner with fungi, especially *Ophiostoma* and *Leptographium* species. How the beetles interact with their symbiotic fungi varies greatly and can range from obligate to facultative, and beneficial to highly antagonistic. Beneficial fungi typically provide critical nutrients to the beetle and support rapid development and population growth. In contrast, antagonistic fungi severely reduce beetle brood survival which can cause beetle populations to crash. Thus, for many beetles, fungal associates can have strong effects on beetle population dynamics and success. Unfortunately, interactions among most beetles and their fungi remain unstudied.

Apart from bark beetle research and learning about PCR and sequencing, Prof. Six also found time to acquire some new skills in other fields, such as at the recent SASPP congress in January.

The primary focus of my research program is on the ecology and management of tree-killing bark beetles; the interactions the beetles have with their symbiotic fungi is of particular interest. At least some bark beetles are dependant upon their fungal symbionts, and thus may provide a "weak link" in the biology of the beetles that can potentially be exploited in their management. As with any pest, the key to developing effective management techniques and tools lies in an accurate understanding of the biology and ecology of the pest and its interactions with its environment. In fact, many management failures of the past can be directly attributed to an inadequate understanding of the pest's biology. This has been particularly true for bark beetle management where all too often there has been a "manage first-understand later" approach.

My work at FABI and with the TPCP team involves increasing knowledge of bark beetle-fungus associations especially those occurring among *Dendroctonus* species and their *Ophiostoma/Leptographium* associates. The genus *Dendroctonus* contains some of the world's most destructive forest pests, however, surprisingly, very few of the fungal associates of these beetles have been described. For a portion of each of the last ten years I have traveled throughout North and Central America to collect the various *Dendroctonus* species and isolate their fungi. Now, at FABI, using a combination of morphology and DNA sequencing, I have been working to identify the fungi in these collections. Not surprisingly, many of the fungi isolated from previously unstudied *Dendroctonus* beetles are new species.

The knowledge of the fungi associated with various *Dendroctonus* species can be used in various ways. For example, *Dendroctonus valens* has recently been introduced into China where it is killing thousands of trees annually. Information on the fungi associated with this beetle in its home range helped inform managers in China of the potential fungal species that may have been introduced with the beetle, in this case, two tree pathogens, *Ophiostoma ips* and *Leptographium terebrantis*. Surveys of *D. valens* in China are now in progress to determine whether either of these pathogens were introduced along with the beetle and whether they are playing a role in the aggressiveness of the beetle in its new habitat (the beetle typically does not kill trees in its native range).

The next step in my work at FABI will be to use DNA sequences to develop a phylogeny for the fungi that can then be compared with a phylogeny of the host beetles. By detecting points of congruence, or similarity, between the fungus and beetle phylogenies

we can begin to identify which fungi are likely to have had long evolutionary histories with their hosts, and thus are most likely to be obligate mutualistic partners.

Obligate mutualistic symbionts have often been considered weak links in the biology of pest organisms and targeting these organisms may provide a novel approach for the management of bark beetles. Interestingly, the idea of targeting symbiotic fungi to manage bark beetles is not new. For many years, it was thought that the fungi associated with bark beetles provided benefit to the beetles through their pathogenic effects on the host tree. In other words, the beetles used pathogenic fungi to help them kill trees. Other non-pathogenic fungi were also known to be associated with the beetles. These fungi provide nutritional benefits important in beetle development and reproduction, but were often dismissed as unimportant because they lacked pathogenicity. Because of how the roles of the fungi were perceived at the time, it was suggested that by reducing or eliminating the 'beneficial" pathogens that beetle populations would be greatly reduced. More recent studies, however, have shown that, at least in some cases, the pathogenic fungi are highly antagonistic to the beetle and can act as a major factors causing of the decline of beetle outbreaks. Therefore, targeting the pathogenic fungi could have actually aided the beetle and resulted in even more problems. This example further stresses the need for strong basic research on the biology of a pest if management is to be effective.

As my six months sabbatical leave at FABI nears its end I would like to take this opportunity to thank everyone in the TPCP for their help and friendship during my stay. FABI is truly an exceptional place full of exceptionally wonderful people and I will be very sad indeed to leave. Thanks!!

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CONGRESS REPORTS

Southern African Society for Plant Pathology (SASPP)

This year the annual Southern African Society for Plant Pathology (SASPP) congress was held in the fair Cape at the Hartenbos Beach Resort from 23 to 26 January. The congress was held in conjunction with the African Mycological Association and Medical Mycology in Africa meetings, and was preceded by the International Grapevine disease symposium. All these parallel meetings lent a truly international flavor to the congress, with many guests and presenters from foreign countries. Delegates were from various countries as far a field as South and North America, Europe, Australia, the Near East and Africa. The meeting covered a wide variety of topics, including sessions on disease control and management, disease detection and losses, host-

Professor Teresa Couthino accepting her publicity award.

pathogen interactions, pathogen identification and characterization, and disease resistance. This was supplemented by the concurrent sessions of the African Mycological Association.

The SASPP congress consisted of 74 oral presentations and 70 poster presentations. The TPCP/FABI group was very well represented with 26 talks and 21 posters presented. Keynote presentations were on *Mycosphaerella* phylogenetics, *Botrytis* ecology on plant surfaces, biogeography and taxonomy of wood-inhabiting *Basidiomycetes*, sustainable control of grapevine powdery mildew and pathogenecity in fungus-bark beetle symbioses. Prof. Mike Wingfield of the TPCP gave the annual Ethel Mary Doidge Memorial Address on forest pathology and forest pathogens in South Africa.

As usual, several awards were made at the congress. A colleague and former PhD student of Mike Wingfield, Prof. Pedro Crous, was awarded the prestigious Hendrik Persoon Gold Medal for his contributions to plant pathology in South Africa. This is the highest award that can be bestowed on any one by the society and it is only presented when a worthy recipient has been identified. Other honors achieved by TPCP members included a student merit travel award by Marieka Greyzenhout and the publicity award won by Prof. Teresa Coutinho for hosting the International Fusarium Workshop in 2004.

The congress was much more than just the attendance of seminars and poster presentations. The social events during the evenings gave ample opportunities to interact with colleagues on a relaxed social footing, and to further the exchange of ideas and develop collaborations. The picturesque setting of the congress venue next to the Hartenbos beach only contributed to the success of the meeting. As is tradition the society also held its annual "Mildenhall" Beer drinking competition with which some foresters will be familiar with from attending our annual TPCP meetings in Pretoria. Here, Dr. Jolanda Roux continued her winning streak by taking the ladies title, while Prof. Diana Six, one of our sabbatical visitors gave her tough competition for the title. Fitting to the occasion, the congress was ended with a FABI braai on the beach. This brought us to the end of a highly successful congress where the TPCP once again left its mark as a leader in Forest Pathology, both nationally and internationally.

Oral presentations at the recent SASPP Congress

Cylindrocladium pauciramosum, dominant in South African *Eucalyptus* nurseries

L. Lombard, M.J. Wingfield and P.W. Crous

In South African *Eucalyptus* nurseries, damage to plants by *Cylindrocladium* is common and reflected by a number of disease syndromes. *Cylindrocladium* was first recorded on *Eucalyptus* in South African nurseries in 1988 where it appeared to damage hybrid cuttings. Subsequently, several *Cylindrocladium* spp. have been encountered in local *Eucalyptus* nurseries. A survey conducted on *Eucalyptus* cuttings, and hedge plants in hydroponics have shown that *C. pauciramosum* is the dominant species in nurseries. This pathogen has been found to be dominant in *Eucalyptus* nurseries in Australia, Europe and South America. Thus, this is clearly a common pathogen in the Southern Hemisphere. Most records of *C. scoparium* in fact reflect the presence of C. *pauciramosum*. Although populations from different continents are sexually compatible, DNA data have shown that these represent different phylogenetic species, and thus a species complex. More attention, therefore, needs to be given to more accurately characterize the species in this complex.

Multi-gene phylogeny for species of *Mycosphaerella* occurring on Eucalyptus leaves

G. C. Hunter, M. N. Cortinas, M. J. Wingfield, B. D. Wingfield and P. W. Crous

Leaf damage caused by *Mycosphaerella* species reduces the growth and commercial viability of *Eucalyptus* plantations. Identification of these fungi is most commonly based on teleomorph and anamorph characteristics and DNA sequence data for the Internal Transcribed Spacer (ITS) region of the rDNA operon. However, interspecific variation is commonly observed between *Mycosphaerella* species, resulting in uncertainty regarding species concepts. For this reason, there is a need for robust comparisons based on a suite of appropriate genes or gene regions. We have, therefore, constructed a multiple DNA sequence data set from nuclear and protein coding genes for *Mycosphaerella* species occurring on *Eucalyptus*. Type or ex-type cultures of *Mycosphaerella* species from *Eucalyptus* were obtained from various culture collections. Following growth of pure cultures, DNA was extracted, amplified and sequenced. DNA sequences were subjected to phylogenetic analysis using Parsimony and Bayesian inferences. DNA sequence data from the ITS, Large Subunit (LSU), ATP-6, Elongation Factor 1-? and Actin genes support the existing hypothesis that *Mycosphaerella* is monophyletic and heterogeneous, with species generally grouping according to their anamorph genera. The use of multiple DNA data sets has substantially increased the resolution of species concepts within *Mycosphaerella* leading to a robust and reliable foundation for future species identifications.

Discovery of novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis*.

M. Gryzenhout, B. D. Wingfield and M. J. Wingfield

The pathogen *Chrysoporthe cubensis* (formerly *Cryphonectria cubensis*) is best known for the canker disease it causes on *Eucalyptus* species. This fungus is also a pathogen of *Syzygium aromaticum* (clove), which is native to Indonesia and, like *Eucalyptus*, resides in the *Myrtaceae*. Furthermore, *C. cubensis* has been found on native trees belonging to the *Melastomataceae* in various parts of South and Central America, i.e. *Miconia* spp. Recent surveys have yielded collections of *C. cubensis* isolates from new hosts that have been characterized based on DNA sequences for the ITS and ?-tubulin gene regions. These hosts include native *Clidemia sericea* and *Rhynchanthera mexicana* (*Melastomataceae*) in Mexico and exotic *Lagerstroemia indica* (Pride of India, *Lythraceae*) in Cuba. Isolates from these hosts and areas reside in the sub-clade of *C. cubensis* accommodating the South American collections of the fungus. New host records from South East Asia included exotic *Tibouchina urvilleana* from Singapore and a native *Melastoma* sp. (*Melastomataceae*) from Indonesia. Isolates from these plants and areas reside in the Asian sub-clade of the fungus. *Chrysoporthe cubensis* thus occurs naturally on hosts in three different families of the *Myrtales*, in addition to the five families reported to be susceptible in artificial inoculations. *C. cubensis* is hypothesized to either have originated in South East Asia or in South/Central America on native trees. However, since this fungus has native hosts in both South East Asia and South/Central America, its origin cannot be resolved based only on its occurrence on native plants.

Chrysoporthe species in Eastern and Southern Africa

G. Nakabonge, R.N. Heath, M. Gryzenhout, M.J. Wingfield and J. Roux

Chrysoporthe cubensis and *Chr. austroafricana*, previously known as *Cryphonectria cubensis*, are important pathogens of *Eucalyptus* spp. (Myrtales) worldwide. Previous studies have suggested that *Chr. austroafricana* occurs only in South Africa, while *Chr. cubensis* occurs in South East Asia, Central Africa, East Africa and South and Central America. In South Africa, *Chr. austroafricana* occurs on *Eucalyptus* and *Tibouchina* spp. Recently it was also found on native *Syzygium cordatum* (Myrtales) trees, leading to the hypothesis that it is

native to Africa. On the contrary, *Chr. cubensis* is thought to be introduced into Central Africa and is known on exotic *Eucalyptus* and *Syzygium aromaticum* (clove). The aim of this study was to determine the distribution of *Chrysoporthe* spp. on exotic *Eucalyptus* spp. as well as on native Myrtales in Southern and Eastern Africa. Isolates were collected from as many trees as possible and characterised based on their morphology and DNA sequence data for two gene regions. Results show, for the first time, that *Chr. cubensis* occurs in Mozambique, Kenya and Malawi on exotic *Eucalyptus* spp. In Malawi, it was also found on native *S. cordatum*. *Chr. austroafricana* was found for the first time in Mozambique and Zambia on *Eucalyptus* spp. and *S. cordatum*. In South Africa, *Chr. austroafricana* was also collected for the first time from areas south of Durban, as well as in the Mpumalanga Province. Further studies on the genetic diversity and structure of *Chrysoporthe* populations from Southern and Eastern Africa are underway to achieve an extended understanding of the origin and movement of *Chrysoporthe* spp. in Africa.

Effect of inoculum concentration, plant vigour and wounding on infection of *Pinus patula* by *Fusarium circinatum*

A. Hammerbacher, T.A. Coutinho, B.D. Wingfield and M.J. Wingfield

Fusarium circinatum is an economically important pathogen of pine seedlings and cuttings in South Africa. Yet, little research has been conducted on the epidemiology of the fungus in South Africa. The aim of this study was to consider the effects of wound type, spore concentration, environmental stress as well as a fungicide treatment on disease development and symptom expression. Seedlings were inoculated using four different wounding methods and six spore concentrations. After inoculation seedlings were incubated under optimal environmental conditions for the host, sub-optimal conditions for the host as well as sub-optimal conditions combined with a fungicide treatment. Results showed that the mean percentage disease caused by increasing spore concentrations can be described by the Michaelis-Menten function. The gradient of the function as well as the asymptotic maximum level of disease was dependant on the environment and physiological state of the host, as well as the wounding method. The highest rate at which spore concentration influenced disease incidence was observed in stressed seedlings. However, vigorously growing seedlings reached the same maximum disease incidence as stressed seedlings. Fungicide treatment did not influence the rate of disease incidence, but significantly lowered the asymptotic maximum level of disease incidence. Seedlings with stem wounds displayed the highest disease incidence, when compared to other wounding methods.

Poster presentation at the recent SASPP congress

Detection of the pitch canker fungus, *Fusarium circinatum*, in pine seeds

J. W. M. Mehl, A. Hammerbacher, T.A. Coutinho, M.J.Wingfield and B.D. Wingfield

The pitch canker fungus, *Fusarium circinatum*, is one of the most important pathogens in South African pine nurseries. The fungus is known to be seed-borne and this is, without doubt, the primary manner by which it has been disseminated into new areas. Consequently, there is a need to evaluate seedlots for the presence of the fungus. This is typically done by direct isolations onto selective media, a time consuming practice that is also often ineffective. Erroneous results typically arise from the fact that other fungi and contaminants out-compete *F. circinatum* on isolation plates. There is, therefore, an urgent need for a rapid and accurate means to screen pine seeds for the presence of the pitch canker fungus. In this study, we have developed a DNA-based method to achieve this goal. DNA was isolated from two batches each of approximately 60 seeds. Seeds were freeze-dried and ground to a fine powder in liquid Nitrogen. The DNA was then amplified using PCR with species specific primers previously developed to amplify the IGS region of *F. circinatum.* The resultant bands were purified and sequenced. These corresponded to those that have been deposited on Genbank indicating the presence of *F. circinatum*. This investigation has, therefore, enabled us to rapidly screen pine seeds for the presence of the pitch canker fungus.

Contributions to this issue by:

Diana Six, Almuth Hammerbacher, Lawrie Wright, Mike Wingfield, Jolanda Roux, Solomon Gebeyehu, Brett Hurley, and Marelize van Wyk.

WELCOME TO TPCP AND FABI!!

Visitors

Prof. Dale Bergdahl from Vermont and **Prof. Diana Six** from Montana in the USA, as well as **Dr. Ursala Heiniger** from Switzerland are three scientists who visited the TPCP in the last six months for their sabbatical periods. It was a special treat for all in TPCP to have them here as we all learned a lot from them. Read more about some of their research projects in this and the following TPCP news issues.

Post Doctoral Fellows

Dr. Jane Wright joins us from the UK. She completed her PhD at the University of York where she worked on the possible role of choanoflagellates in the evolution of ancestral animals. For her post doc with FABI she will be looking at a global perspective for the population biology of the pine pitch canker fungus.

Students

Makhado Ndivhuho A. Rebecca joins the TPCP for her M.Sc. degree. She will be working with Prof. Teresa Coutinho on the isolation of the bacterial endophytes and epiphytes from healthy plants of *Eucalyptus* to see if the leaf blight pathogen *Pantoea ananatis* exists as endophyte and/or epiphyte.

Wilhelm Dreyer and **Buyi Mtalane** are third year forestry students at Saasveld which falls under the Technological University of the Nelson Mandela Metropole. They joined the TPCP for their 6-month third year practical of their forestry diploma. Wilhelm is working with Brett Hurley and Hardus Hatting on the Sirex project, while Buyi is working on a project to evaluate *E. nitens* against diseases and characterise a new disease outbreak with Dr. Jolanda Roux.

Sirex Update:
Inoculation Results

In 2004, over 1700 pine trees were inoculated with the nematode *Beddingia siricidicola* in the Western and Eastern Cape and KwaZulu-Natal. These inoculations marked the beginning of the re-introduction into South Africa of the primary biological control agent against the woodwasp *Sirex noctilio*. Such mass releases of the nematode will occur on an annual basis, in an effort to manage the drastically increasing Sirex population. The majority of the nematodes were released on the Sirex front in KZN. To assess the success of the inoculations, wasps emerging from sampled inoculated logs were dissected and examined for the nematodes in their ovaries or testes.

The techniques used to inoculate trees with nematodes are being investigated to determine possible improvements that will increase nematode survival and spread.

Drastic differences in parasitism rates between logs inoculated in KZN and the Eastern Cape and logs inoculated in the Western Cape were obtained. Parasitism in the Western Cape was close to 50% (i.e. about 50% of the wasps emerging from the logs were parasitised). These results are very positive for the first year's inoculations and improvement of inoculation techniques should push parasitism to over 80% in the near future. Parasitism in KZN and the Eastern Cape was less than 5%! These results are very disappointing and of great concern to the industry if such low parasitism persists in future inoculations.

The TPCP, together with the ICFR and the forestry industry has immediately started to examine possible causes for the low parasitism in KZN and Eastern Cape and the differences in parasitism between these two provinces and the Western Cape. The transport and storage of the nematode, differences in establishment of the nematode between *P. patula* and *P. radiata*, the effect of the different climates on the establishment of the nematode and problems with the inoculation technique are a few of the areas that will be investigated. We hope that these investigations will soon provide the cause of and solutions to the low success rate of the inoculations in KZN.

Woodchips are sampled from inoculated trees to investigate to what extent the nematodes survive and spread in the tree after inoculation

QUAMBALARIA LEAF BLIGHT OF *EUCALYPTUS*

In the 1980's a mysterious new disease resulting in leaf and shoot blight of *Eucalyptus* grandis clones was reported from a nursery in the Kwambonambi area. The disease was characterized by white powdery spots on affected leaves and shoots. Interestingly this disease, that was causing significant damage to plants, was restricted to two *Eucalyptus* clones.

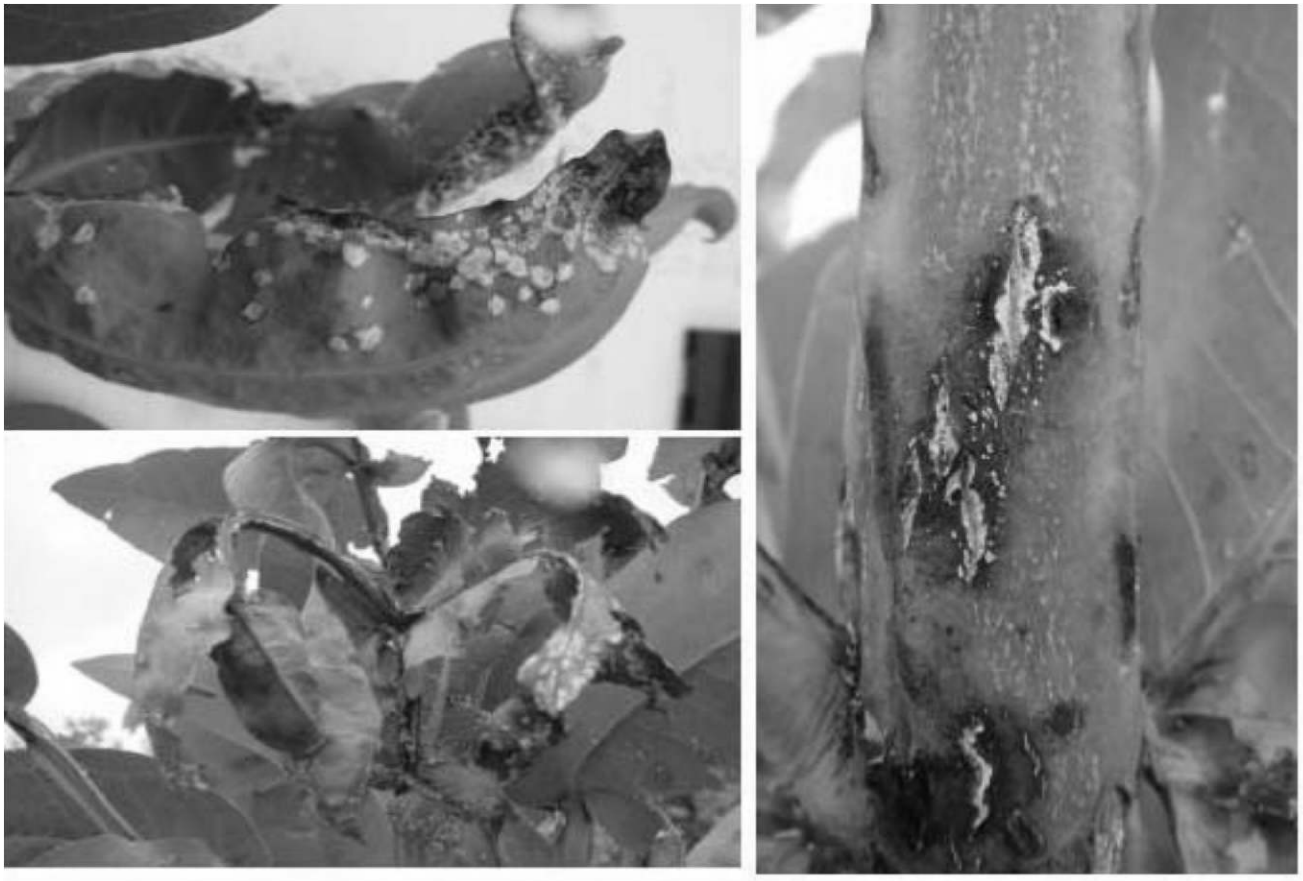
The mysterious disease of Eucalyptus had never before been seen in South Africa and symptoms were different to anything similar elsewhere in the world. The causal agent was studied and a new species, *Sporothtrix eucalypti*, was described for the pathogen. Once the susceptible clones were removed from the affected nursery, the disease appeared to disappear, as mysteriously as it had appeared. It was not seen again until the late 1990's, this time from a different nursery in the Kwambonambi area and on hybrids of *E. grandis* and *E. urophylla*. Again it affected young shoots and leaves in the clonal hedges, resulting in malformation and death of shoots grown for cuttings. Members of the TPCP team referred to this disease as Sporothrix blight, and recongised that it was negatively affecting nursery production.

Despite its dramatic appearance and serious damage that Sporothrix blight could cause when present, the disease has remained at relatively low levels in South Africa. It

has, however, also been reported from South America (Brazil and Uruguay) causing similar problems in nurseries. A growing interest in the disease has led TPCP researchers and colleagues elsewhere in the world to consider it more carefully and to recognise that the pathogen was very similar to one known as *Ramularia pitereka* in Australia. However, in Australia the disease situation is very differenct where it causes serious damage to young trees in forests and plantations. In that situation, however, the tree most seriously affected is spotted gum, *Eucalyptus maculate*, now residing in the genus *Corymbia* as *Corymbia maculate*. Intriguingly, *E. grandis* and *E. urophylla* are not affected. The disease in Australia is sufficiently serious to have led to the suspension of planting spotted gum in New South Wales and in Queensland. Research in Australia suggests that the pathogen is also an endophyte (infects trees without causing immediate symptoms) as researchers have isolated it from apparently healthy shoots.

Relatively recent research in Australia has led to *Ramularia pitereka* being transferred to a new genus known as *Quambalaria*. Three, species, *Q. pitereka, Q. eucalypti* and *Q. pusilla* are regonised. The fungus in South Africa represents *Q. eucalypti* and this is the same fungus that infects plants in Brazilian nurseries. *Q. pusilla* is less well known and occurs in Thailand. At the time of describing *Quambalaria*, it was suggested that the fungus might be a basidiomycete (related to mushrooms) and TPCP research has confirmed this fact.

In early 2005 Dr. Jolanda Roux (Field Extention Officer of the TPCP) noticed leaf and stem spots on one-year-old *E. nitens* trees in the Carolina area of South Africa. Initial investigation suggested that the stem wounds were hail damage and the leaf spots caused by *Mycosphaerella* spp. Closer investigation and study of additional trees, however, showed the presence of white powdery spores in young leaf and stem lesions. The cause of these disease symptoms has since been identified as a *Quambalaria* sp. This is the first report of this fungus in the highveld and on *E. nitens.* It is also the first occurrence of the disease on established field grown trees in South Africa. Damage in this compartment ranged from leaf spot and malformation to sunken branch and stem cankers resulting in dead branches and growth tips.



Disease symptoms caused by Quambalaria infection: ***Top left****, Leaf blight* ***Bottom left****, shoot death.* ***Right****, stem cankers*

The TPCP is currently investigating Quambalaria blight in more detail. There are various important questions to be answered. The species of *Quambalaria* needs to be identified. For example, it is unclear whether the fungus in the highveld represents *Q. eucalypti.* The team is also trying to determine the impact of this disease on forestry operations to obtain more information on its threat to the industry. Any sightings of the disease would be appreciated. Please contact Jolanda Roux (0829093202; jolanda.roux@fabi.up.ac.za) if you believe that you have seen this disease. It is potentially extremely threatening to South African forestry especially given the importance of *E. nitens* and its hybrids.

LAUNCH OF THE DST/NRF CENTRE OF EXCELLENCE IN TREE HEALTH BIOTECHNOLOGY

The DST/NRF Centre of Excellence in Tree Health Biotechnology (at FABI) hosted by the University of Pretoria, is one of the six successful research groups to have been allocated status and funding as centres of excellence by the DST and NRF. The official launch of this Centre of Excellence took place on Monday, 31 January 2005 in the Sanlam Auditorium at the University of Pretoria and was attended by approximately 100 people. The guests and students were welcomed by the Principal and Vice-Chancellor, Prof Calie Pistorius. Other speakers included the Vice-Principal: Research, Prof Robin Crewe who spoke on the Centres of Excellence and Research at the University; and Dr Rob Drennan of the NRF who congratulated Prof Mike Wingfield, Director of the new Centre of Excellence and his team, on the honour of being one of the six groups to be charged by the DST and NRF with taking research to new heights.

From l to r: Prof Mike Wingfield, Prof Robin Crewe, Dr Rob Drennan, Prof Calie Pistorius, Prof Braam van Wyk and Prof Brenda Wingfield

After a most enjoyable musical interlude provided by "The Notables", a string quartet from the University of Pretoria, the guest speaker, Prof Braam van Wyk, gave an interesting and stimulating talk entitled "Southern African trees: outstanding features". Since the new Centre is to concentrate its research efforts on the health of "indigenous" or "native" trees, Prof van Wyk's talk emphasized the rich diversity of southern African trees and highlighted specific regions where the trees display particularly noteworthy features.

He emphasized the importance of plants and trees as they are able to exist without animals and humans, but neither animals nor humans can exist without them as they provide habitat, shelter, food, materials and medicines, to name but a few of their uses. Plants are often perceived to be inanimate and thus their value and vulnerability are not always fully appreciated.

Among southern African tree species, there are not many, other than the mopane, that are wind pollinated and therefore few native trees dominate any one vegetation type. This accounts for the diversity and richness of tree and plant species in southern Africa as compared with many parts of the Northern Hemisphere, for example Europe. One of the important tasks, therefore, of the new Centre of Excellence will be to attempt to identify and, where appropriate, control and eradicate diseases of plants and trees so as to conserve this rich heritage and resource.

Prof van Wyk referred to four of the Centres of Plant Endemism in Southern Africa that are known for the interesting features of their trees and other plants, some of which have potential to form the basis for research projects in the new Centre.

In the **Kaokoveld Centre of Endemism**, located in the far northwestern corner of Namibia and adjacent southwestern Angola**,** he referred to the mopane, which is one of the few wind pollinated trees in Southern Africa and which does dominate, in the form of mopaneveld, about 50 000km^2 of the subcontinent. The corkwoods (*Commiphora* spp.) are an example of a group of trees, which has many species, restricted to the Kaokoveld Centre, but where the species differ dramatically in leaf and bark morphology from one another. This makes it a very interesting group to discover why there is such diversity among the species and whether this has not perhaps resulted from "attempts" to counter attack by different herbivores or even pathogens.

In the **Sekhukhuneland Centre of Endemism**, a region centred on the towns of Steelpoort, Roossenekal and Burgersfort and spanning the border between Limpopo and Mpumalanga, there are not only many existing thorntrees (Acacia spp.), but this appears to be a region in which the group still undergoes active diversification. Over the last ten years no less than five new species of *Acacia* have

been identified in this area, which is only about 250 km from Pretoria. If new trees are still being discovered so close to a major urban area, how many new species must still await discovery in more remote areas of southern Africa. The importance of fire for the survival of savanna trees was emphasized. Fire affects the thickness of the bark, seed germination and results in the strengthening of the root system of these trees. In savanna areas subjected to frequent wild fires, the below ground portion of the trees are often much older than the above-ground stem and canopy. This extensive root system provides the trees with a buffer against drought and stress conditions. Research needs to be done on this phenomenon and on the species' adaptation to fire.

An interesting feature of the **Maputaland Centre of Endemism** in northeastern KwaZulu-Natal and southern Mozambique is the so-called underground trees, of which *Parinari capensis* is an example. These are woody perennial plants that spread underground by means of an intricate system of branched stems and roots. Plants with this growth form are particularly well represented in sub-Saharan Africa and particularly noticeable in the coastal or woody grasslands of Maputaland. The canopy of the trees only emerges in spring as annual or short-lived perennial shoots, and then die down after fire or frost in winter. Under ground, each tree spreads wider and wider and these essentially immortal plants are thought to be among the oldest living organisms in southern Africa, with ages of more than 10 000 years for some of these clones not being improbable. On the High veld, the "ploegbreker" (*Erythrina zeyheri*), a type of underground coraltree, is a well-known example of an underground tree.

The **Pondoland Centre of Endemism**, a region spanning the border between KwaZulu-Natal and the Eastern Cape, contains relic tree species, which botanists believe were previously more widespread in southern Africa. Many of these trees are palaeoendemics ("living fossils") and present a challenge for research into tree health. Some of these trees might be susceptible to disease and reproductive failure, perhaps reflected by the extreme rarity and poor regeneration of some of the species. An example is *Manikara nicholsonii* where all or most flowers turned into sterile galls due to the activity of a still unknown pathogen or insect. It is here where man can step in and, through research, attempt to rectify or counter what appears to be genetically mediated reproductive failure and high disease susceptibility. Thus Pondoland, which harbours more than thirty narrow endemic trees, many of which are apparently palaeoendemics, offers much opportunity for research by the new Centre of Excellence.

Prof van Wyk then addressed the subject of diseases and compared the death process of both trees and animals. Trees can still survive even though certain branches or other parts have died. Animals, on the other hand, are either dead or alive, not both at the same time. Diseases are known to have spread from animal to man, but never from plants/trees to animals or man. This observation serves to emphasize the considerable difference between these two life forms, a difference that is not always fully appreciated. To this day the training of biologists tend to be strongly zoocentric or anthropocentric in approach. Since man and animals cannot exist without plants and trees, it is essential that research be done on the diseases of plants and trees.

Prof van Wyk concluded his talk by saying that the new Centre of Excellence in Tree Health Biotechnology should aim to care for the survival of all species of plants and not only those that are of economic value at the present time. It is a fact that obscure and little known indigenous tree species may harbour pathogens that could have devastating effects on commercial tree crops. Likewise, pathogens introduced with commercially grown trees may spread to native trees, resulting in their demise. The Centre is a manifestation of the foresight and need to take care of the future of these living organisms with which we share the planet.

Prof Wingfield's team at FABI, are well experienced in researching diseases of commercial forest plantation trees through the long standing and very successful Tree Protection Cooperative Programme (TPCP). Prof Wingfield highlighted some of the successes of this Programme and was confident that the solid research base and expertise established through the TPCP would assist in developing the new research foci of the Centre of Excellence (CoE). He stressed that the objectives of the new CoE did not pose a threat to the work of the TPCP. He was confident that the research efforts and foci of the two programmes would complement each other and he looked forward to the new challenge that the establishment of the DST/NRF Centre of Excellence in Tree Health Biotechnology presented to the group at FABI.

The formal proceedings of the launch were followed by a most enjoyable cheese and wine party in FABI Square, where the guests were again entertained by "The Notables".

TPCP undertakes to continue research on the Eucalyptus Snout beetle

The Eucalyptus snout beetle, *Gonipterus scutellatus* (Coleoptera: Curculionidae), is native in Australia but has become established in several countries outside its native range, including South Africa. In countries where *Eucalyptus* spp. are propagated as non-native plantation trees, the insect can cause serious damage. South Africa was the first country ever to report the accidental introduction of *G. scutellatus* where it had become established and became an important pest of commercially grown *Eucalyptus* spp. The pest was subsequently introduced to the United States as well as countries in Europe and South America, where it has in many cases become established forming an important component of

the pest complex damaging commercially important plantations of non-native *Eucalyptus* spp. in plantations.

Shortly after its first detection in 1916 in Newlands, Cape Province, *G. scutellatus* became widespread and began to result in serious losses in plantations. Both the adult and larvae of this insect feed on young Eucalyptus leaves, resulting in severe defoliation and thus in stunting of growth. In some cases, defoliation can be so severe as to result in death of young trees. In fact, damage resulting from this insect was so severe that in 1925 the Eucalyptus-growing industry needed to take immediate steps to combat it. The well-known South African entomologist, Dr. F.G.C. Tooke thus travel ed to Australia to seek a possible bio-control agent that might regulate the population of *G. scutellatus* in its native range. The egg parasite, *Anaphes nitens* (Hymenoptera: Mymaridae), was thus discovered at Penola, South Australia.

The egg parasite, a thenundescribed species in Australia was transported to South Africa and it was subsequently described as *Anaphes nitens*. It was mass-reared and released in Eucalyptus plantations in South Africa where damage from the weevil was severe. Most fortunately, the parasite was able to reduce damage by *Gonipterus scutellatus* to economically acceptable levels. This remarkable result led to repeated mass-releases of the parasite in many parts of the country over several years until it was established successfully. The result was that the weevil was brought under control in most areas planted to susceptible *Eucalyptus* spp.

Although biological control of *G. scutellatus* has been remarkably successful in South Africa, there are areas where control has not been sufficiently effected. This is particularly in colder, high altitude sites where the parasite performance is generally not satisfactory. This is mainly due to the weevil undergoing a dormant stage for several months in winter that results in absence of host eggs for the parasites to continue their development and to maintain a viable population. Absence of host eggs coupled with the cold temperatures results in a severe depression in the parasite population. When the insect host resumes its activity as temperatures rise after winter, it takes the parasites a great deal of time to catch up and to achieve acceptable levels of parasitism.

The outstanding performance of *A. nitens* in controlling *G. scutellatus* in South Africa is considered as one of the notable examples of a successful biological control programme in Southern Africa and also elsewhere in the world. Other countries where *G. scutellatus* has been accidentally introduced such as the United States have made use of the South African experience and have introduced *A. nitens* from this country. They have thus benefited immensely by establishing a biological control system by introducing and releasing the egg parasite from South Africa.

To date *A. nitens* remains the only means of managing *G. scutellatus* in South Africa. While high altitude *Eucalyptus* plantations have occasionally suffered some serious infestations by *G. scutellatus*, plantations in warmer areas and the coastal regions are generally well protected by *A.*

An adult Gonipterus on a Eucalyptus shoot

nitens. Nevertheless, in recent years, there have been new reports of the resurgence of the weevil population in previously well-protected areas. Reports are emerging that outbreaks of the pest are being experienced even in plantations in warm areas such as those on the Zululand coast.

Researchers working within the Tree Protection Co-operative Programme (TPCP) at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, have begun a new thrust of research on *G. scutellatus* and its egg parasite. The primary aim of this work will be to understand why the pest has increased in importance in recent years. We also wish to understand why the parasite has failed to keep pace with the exploding populations of the weevil. Major emphasis will be given to the following areas:

- A survey of the distribution, damage levels and *Eucalyptus* species most preferred by the weevil will be undertaken in selected regions to establish the current state of the pest and its egg parasite.
- Species trial sites and seed orchards as well as gene banks will be used to understand the response of various genotypes to attack by the pest and the level of parasitism by the egg parasite.
- Mass-rearing and releasing of the parasite by way of augmentation in selected plantations, followed by monitoring and evaluation of levels of parasitism will be undertaken.
- Investigations will be undertaken into factors responsible for failure of the parasite to afford acceptable levels of control of the pest in selected warmer areas.
- A search for other alternatives to biological control, such as screening of bio-pesticides in the laboratory, that are acceptable in a forestry setting and that are in line with FSC regulations will be done. Promising candidates are envisaged for possible use in an Integrated Pest Management (IPM) strategy.

THE RESEARCH TEAM OF THE TREE PROTECTION CO-OPERATIVE PROGRAMME

The research team of the Tree Protection Co-operative Programme is varied. It includes full time staff of the University of Pretoria (Prof MJ Wingfield, Director and Mondi Professor, Prof BD Wingfield, Prof TA Coutinho, Dr J Roux , Brett Hurley, Rosemarie Visser, Eva Muller, Hardus Hatting, Helen Doman, Valentine Nkosi, Martie van Zyl, Pritty Khumalo, Lydia Twalo, Kgosi Mongwaketsi and Martha Mahlangu). Colleagues and students attached to other organizations such as the ICFR, technical assistants funded by the University or through membership fees and post graduate students who are mainly funded by the NRF. Staff from various Departments in the University provides advice and support where this is required.

Contact numbers and Web address

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IMPORTANT : PLEASE READ THIS

In order for us to coordinate our services to you please help us by using the following contact address:

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