

May 2010

FROM THE DIRECTOR'S DESK

VOL: 19

As I prepared to write this short introduction to Tree Health News, I had the latest issue of Nature beside me. I could not help but be drawn to the lead article "The human genome at ten. Growing pains of the genomics age". The "ten" of course is the ten years post the first publication of the human genome. A project that was set to take decades when it was first conceived, and one that was completed in a fraction of the time. It is also a project that has turned the world of Science and in particular the biological sciences on its head. Forestry and the fields of forest pathology and forest entomology are no exception.

In just ten years, the cost of sequencing a million base pairs has dropped from US\$ 10 000 to about US\$1!! Our colleagues raved about sequencing a genome and now we see projects set to compare fifty genomes. An issue of Nature just the other day compared a suite of human genomes, interestingly including that of Dr. Desmond Tutu and the winner of the US\$ 1000 per human genome competition must surely be in sight.



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Yet, sequencing genomes is just a small step in understanding what these mega datasets tell us. The process of annotating the genomes and extracting

their jewels remains a long and tedious process. Thus, when we decided to sequence the genome of the pitch canker pathogen, Fusarium *circinatum*, it was not surprising that that part of the work would be completed in



Investigating the death of native Euphorbia ingens in Limpopo

not much more than a month. The alignment and annotation process is now well on its way, but this has been a hugely time consuming project for a team of people. It will continue to be so for some time to come.

However, we have already extracted many exciting blocks of information that will help us to work with and understand one of the most serious pathogens to have challenged pine plantation forestry in South Africa.

We are now well into the first half of 2010 and there is much that is exciting happening in our world of tree health and where "keeping trees healthy" is a driving passion. Field extension trips leave FABI for the plantations virtually every week, samples pour into the diagnostic clinic, students are working on their projects and nematodes for the biological control of *Sirex* are being

produced in large numbers. Just yesterday I was out at the FABI farm facilities (greenhouse, insectarium etc) and I had the opportunity to visit the team that were inoculating pine seedlings as part of the pitch canker screening programme. What was remarkable was to see blocks of dying pine plants, families that are clearly highly susceptible to infection by the pitch canker fungus.

It is only a few moths back that we had the first report of the Eucalypus gall wasp, *Leptocybe invasa* in Zululand forests. The fact that the pest would spread, and probably rapidly, was well recognised, but the reality of the growing invasion was sobering. Just yesterday, I saw a mail from Ian Harrison of Mondi, letting us know that this minute, the nasty insect was becoming much more evident in Zululand. But there is good news too. The biological control programme for the pest is

starting to gain

traction. During my

nursery visit yesterday,

Gudrun

showed me evidence of the

first completion

of the life cycle of three

biological control

parasitoids in

our quarantine



Leaf galling on the midrib of a eucalypt leaf caused by *L. invasa*

facility. This is more than good news. It is truly splendid news! We are far from the point of being able to release the biological control agents, but to have an active culture of the pest in the greenhouse is a key first step. This will allow us

> to undertake the risk assessment tests that are a pre-requisite to a legal release of the biocontrol agents.

> As I write what was intended to be a short introduction to our newsletter, I realise that I am set to write an entire newsletter! If I were to continue to provide examples of some of the exciting results that emerge from our days in the tree protection projects in FABI, the pages would soon fill beyond logical. But there are other avenues that will allow me to share some of our products. In less than six weeks, many readers of Tree Health

News will be in Pretoria for the annual meeting of the Tree Protection Programme and the DST/NRF Centre of Excellence in Tree Health Biotechnology. The programme for the meeting our 20th meeting and the 21st Birthday of the TPCP - is packed with interesting items. We have about ten overseas visitors here for the meeting and we are very much looking forward to engaging with members of the TPCP to review work of the past year and to consider what lies ahead in our quest to ensure the health of South Africa's forests and the sustainability of commercial plantation forestry in the country.

Best wishes Mike



The Eucalyptus gall wasp, Leptocybe invasa, rapidly spreading in RSA

ILLEGAL PET TRADE THREATENS FORESTRY SECURITY!

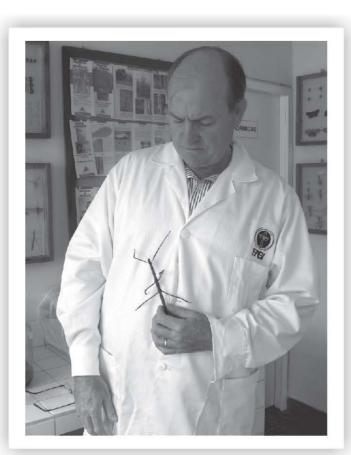
It was recently brought to the attention of the TPCP that there is a thriving trade in South Africa of nonnative animals that could pose a serious threat to the sustainability of our plantation resource. industry is the trade in non-native stick insects. The cryptic and bizarre appearance of stick insects makes them a particularly fascinating group of insects and appealing to many insect-enthusiasts. In fact, the order of stick insects (Phasmatodea) is

Most of us are probably familiar with the trade in non-native pets. Monkeys, lizards, snakes and fish are some of the more popular groups of non-native pets. But - as strange as it may seem for some - there is also a growing interest in keeping non-native insects. For example, Madagascan Hissing Cockroaches are becoming increasingly popular, perhaps in part because of their high novelty a n d entertainment value, and low maintenance requirements. And some people simply enjoy the creepy-crawly, bizarre things in life.

This fascination with

nature and biology is great provided the introduction and trade of these animals is done through the appropriate legal channels. Permits are required to introduce non-native organisms into the country, and these permits require a risk assessment to ascertain if the introduced organism could pose a threat to the environment or economy of the country should they escape (which is highly likely to occur at some time). Unfortunately, a component of the pet trade in South Africa does not follow these legal channels.

One example of illegal pet trade in South Africa that should be of particular concern to the forestry



Mike Wingfield with a stick insect which is causing considerable damage to plantations in Colombia derived from the Greek word *phasma* which means ghost, due to the insect's ability to 'disappear' in its natural habitat. Stick insects are herbivorous and in some countries they have become serious pests on commercial crops, including forestry species such as *Pinus* and *Eucalyptus*.

It is with this in mind that the TPCP became alarmed when it learnt that *Eucalyptus*-feeding stick insects were being imported and sold as pets in South Africa. These insects, in the absence of natural enemies, could cause serious damage to *Eucalyptus* trees, including those planted

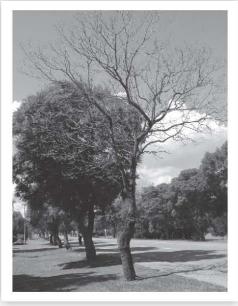
for commercial gain, should they escape and become established in the country.

Various bodies are in place to regulate pet trade in South Africa, and the TPCP has brought this alarming situation to their attention. It is hoped that prompt action will be taken to address this and other illegal pet trade that poses a threat to South Africa's environment and economy. Our responsibility, as people who are aware of the dangers of illegal pet trade, is to inform those who may not be aware of the risks, and where necessary, to report such incidences.



Ganoderma is a genus of wood-degrading fungi that grow on coniferous and hardwood trees. They are frequently observed on trees in natural forests, plantation forests and gardens as large, perennial, woody brackets, also called "conks". The genus includes more than 80 species, many of them occurring in the tropical regions of the world.

Several Ganoderma species are important to a number of diverse industries. Some species occur as saprophytes, utilising dead wood as nutrient source; these species are important to biopulping by virtue of their ability to produce enzymes that degrade lignin and cellulose. Some species are also valued for their medicinal properties and have been used in traditional Asian medicine for thousands of years. They are believed to have therapeutic effects on cancer, cholesterol, hypoglycaemia, fungal infections and many other diseases or disorders. A number of Ganoderma species survive as phytopathogens, causing wood rot and eventually the death of infected trees. Pathogenic species may cause extensive infection centres, leading to great financial losses to forestry industries due to the death of commercially grown trees. For example, in Malaysia mortality of Acacia mangium due to G. phillippii has been estimated at up to 40% in severely infected plots.



Street tree killed by a Ganoderma sp.

On account of their importance to different industries, the taxonomy, distribution and host range of *Ganoderma* species have received much attention by mycologists and plant pathologists world-wide. In South Africa, a number of *Ganoderma* species had been reported from various indigenous and introduced tree species, where they give rise to Ganoderma root rot disease. The *Ganoderma* species most frequently linked with this disease in South Africa are *G. lucidum* and *G. applinatum*. It is known, however, that these two

species actually represent species complexes and may include several species not previously identified. Another gap in scientific knowledge of *Ganoderma* relates to the fact that very little attention has been afforded to elucidating the species occurring in the rest of Africa. As a result, the taxonomic status and the distribution of *Ganoderma* species on this continent are currently uncertain.



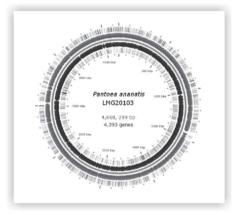
Typical fruiting body (bracket) of a Ganoderma sp.

Because of the uncertainties described above, we have embarked on a research programme to investigate the taxonomy of Ganoderma in South Africa. This programme has led to a number of interesting discoveries to date. Results of a recent investigation undertaken by Ms Vuledzani Muthelo showed that a Ganoderma species very similar to G. lucidum is causing root rot on Jacaranda trees in the Brooklyn suburb of Pretoria. A number of these trees have died as a result of this disease, and many more have been infected by the fungus since this research project commenced. A large number of Ganoderma samples were also collected from various hosts and localities in South Africa by researchers in the CTHB. Using DNA sequences from these samples, preliminary sequence comparisons by Dr. Martin Coetzee has revealed the presence of Ganoderma species not previously reported from South Africa. It is clear that much is yet to be discovered about the fungus in this country.

Future work on *Ganoderma* will focus on the species diversity of this fungus in selected indigenous forests. Earlier this year, Prof. Jolanda Roux was awarded a research grant from the South African Biodiversity Initiative (SABI) to investigate the systematics of a number of fungal genera, including *Ganoderma*, from natural forests in South Africa. While the flora and fauna in these forests are well documented, little is known about their fungal diversity. We believe that information gleaned from research projects funded by this grant will make a significant contribution to our understanding of the taxonomy and distribution of *Ganoderma* species in these forests.



Pantoea ananatis is the causal agent of bacterial blight and die-back of *Eucalyptus* in South Africa. GN hybrids are particularly susceptible and the disease is mainly a problem in nurseries where the pathogen not only infects seedlings but also ramets. *P. ananatis* is a ubiquitous bacterium and is known not only to infect eucalypts but also numerous agricultural crops including, for example, maize and onion. *P. ananatis* is also known to infect humans, where it causes bacteremia, and insects. In the latter case, it has been used as a biocontrol agent.



In 2006, the genome of *P. ananatis* was sequenced by Inqaba Biotech. The numerous contigs (DNA segments) were assembled and annotated (attaching biological information to sequences). Once this was completed and verified, the announcement of the genome sequence of *P*. *ananatis* was submitted for publication. It will now be published in the June volume of the Journal of Bacteriology. This is the first genome sequence to be completed and published of a plant pathogen in Africa. This sequence has allowed team members of the TPCP to discover how this pathogen is able to cross kingdoms (it infects plants, insects and humans), what factors allow it to function as a plant pathogen and how it survives in the absence of its host.



Prof Teresa Coutinho, Me Annie Chan, Prof. Fanus Venter and Mr. Pieter de Maayer, the driving forces behind the publishing of the first plant pathogen genome in Africa.



Most people think of fungi only as those things that mold our bread, grow between some people's toes or kill our trees. Some realize that they also give us bread, wine, cheese and medicine.

Fungi range from the microscopic, such as the pine pitch canker fungus, *Fusarium circinatum*, to macroscopic such as the puffballs commonly growing in Eucalypt plantations. Some fungi can reach enormous proportions. A species of *Armillaria* in the United States was, for example said to more than 15 hectares in size!. More recently, publications appeared of an *Armillaria* spp. of more than 990ha in size. This is based on measurements of its underground mycelium (http://botit.botany.wisc.edu/toms fungi/apr2002.html).

In 2009 we received photos from people in Limpopo of our own humongous fungus, possibly *Phaeogyroporus sudanicus*, also known as the bushveld bolete. This fungus is a common inhabitant of South African savannas and one can regularly find mushrooms of up to 30cm in size. The specimen from Timbavati is, however, the biggest one that most of us have seen!



Jacques Brits, warden of the Timbavati Private Nature Reserve, with the fungus he found in December 2009.

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By: Darryl Herron

FABI EXCELLENCE

Recently the International journal, Mycologia, published its list of the 50 most cited articles published by the journal in the past ten years. This list included 3 papers where current members of the TPCP/CTHB are authors! A past student in the group is co-author or author of 5 of the publications on the list. This list, amongst many other criteria, again emphasizes the quality and impact of the research being done by the programme. None of this would be possible without the incredible team work between industry/farmers and academia.

FEATURED RESEARCH PUBLICATION

Researchers in the TPCP and CTHB teams produce numerous scientific publications each year. The published research gets communicated to stakeholders in forestry and conservation at field days, the annual TPCP/CTHB meeting, on our website and at informal/formal discussions. To make some of this research even more accessible we will be featuring summaries of these research articles in our newsletter. We start with a recent publication of Brett Hurley and co-workers on the interaction between insects and pathogens in a nursery system.

Hurley BP, Slippers B, Wingfield BD, Govender P, Smith JE, Wingfield MJ. 2009. Genetic diversity of *Bradysia difformis* (Sciaridae: Diptera) populations reflects movement of an invasive insect between forestry nurseries. Biological Invasions DOI 10.1007/s10530-009-9509-1.

INVASIVE INSECTS POTENTIAL VECTORS OF PLANT PATHOGENS

Besides the direct damage that insects cause to plants by their feeding, some insects also pose a serious indirect threat to the plant's health by acting as vectors of plant pathogens. In fact, numerous plant pathogens require insects for their dispersal. This includes the well known example of Dutch Elm Disease caused by *Ophiostoma ulm*i, and spread by Elm Bark Beetles.

Various insects have been recorded to transmit plant pathogens in nurseries. Of these, fungus gnats (flies in the family Sciaridae) are often prevalent in nurseries of various crops and have been recorded to transmit *Botrytis cinerea*, *Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The most serious pathogen in pine nurseries of South Africa is the pitch canker fungus, *Fusarium circinatum*. Insects are known to be associated with the spread of this fungus on pine trees in forests, but no association between insects and this fungus has been recorded on pine seedlings in nurseries.

Our study was initiated to examine the presence, species composition and genetic diversity of fungus gnats present in South African pine nurseries and to examine their possible association with *F. circinatum*. Only one fungus gnat species, *Bradysia difformis*, was detected, and this species was detected in all the main pine nurseries. Examination of a 395bp portion of the COI gene from nursery populations in South Africa and Europe indicated a historical connection between the two regions. South African populations showed a high genetic diversity and low genetic differentiation, reflecting multiple and /or relatively large introductions of *B. difformis* into South Africa, with frequent movement between nurseries (probably with the movement of plants). These results indicate the ease at which invasive insects can enter into and spread within the country. Fortunately, investigations using standard isolation techniques and sensitive DNA markers have shown that adult fungus gnats do not have a major role in the transmission of F. circinatum. The role of the larvae requires further examination.

Related publications:

Hurley BP, Govender P, Coutinho TA, Wingfield BD, Wingfield MJ. 2007. Fungus gnats and other Diptera and their possible association with the pitch canker fungus. South African Journal of Science 103:43-46.

Hurley BP, Slippers B, Coutinho TA, Wingfield BD, Govender P, Wingfield MJ. 2007. Molecular detection of fungi carried by Bradysia diffomris (Sciaridae: Diptera) in forestry nurseries of South Africa. Southern Hemisphere Forestry Journal.

SCREENING OF PINE SPECIES AND HYBRIDS FOR TOLERANCE TO THE PITCH CANKER FUNGUS

In 2005 a collaborative project was launched by Sappi Forests, Mondi and Komatiland Forestry to have their pine breeding stock screened for tolerance to the pitch canker fungus, Fusarium circinatum. Twice a year, in February and November, between 15 000 and 20 000 seedlings and/or cuttings are inoculated with the fungus. Research was undertaken previously to determine the best inoculation technique to use as well as which isolates to select for optimal screening. A 10µl droplet of a spore suspension, comprised of three virulent isolates, is added to the cut surface of each pine stem with a micropipette. Six weeks later lesion length is determined. Our research has subsequently shown that inoculating with three isolates is not ideal as competition between them occurs. In further screenings only one isolate, the most virulent, will be used.



Lesion caused by F. circinatum on 10-year-old pine tree

The screening facility is managed by a committee composed of Andre Nel (chair), Kitt Payne, Nico Oliver and Teresa Coutinho. At a recent meeting it was decided that we would invite other companies to participate in the screening service offered by the TPCP. Three others have expressed an interest.



Fusarium circinatum infection of nursery plants

WORKSHOP

On Monday, 23rd of August 2010, a workshop will be held in FABI. The purpose of the workshop will be to critically review the current screening protocol as practised in the screening facility. Some of the screening results already obtained by the different forestry companies during the last couple of years will be presented. Bill Dvorak and Gary Hodge from Camcore, as well as South African forestry company representatives will attend. If you require additional information on the workshop, please contact Andre Nel (Andre.Nel@sappi.com).



Evaluating *P. patula* trees for susceptibility to the pitch canker fungus



The Battle Continues

The bronze bug *Thaumastocoris peregrinus* is one of the most serious pests affecting *Eucalyptus* plantations in Southern Africa and South America. For this reason the TPCP maintains a focused research project on this insect. Amongst other aspects we have aimed to characterize the spread of the pest and key elements of its biology. We are also particularly working towards developing biological control options for it.



Adult bronze bugs on a Eucalypt leaf

An important pattern that is being recognized in the spread of invasive species is that outbreaks in one part of the world leads to increased possibilities of further invasions by that species in other regions. This was evident with the movement of T. peregrinus. Using mitochondrial COI sequence data Ryan Nadel of the TPCP program has now documented this movement in detail. The data show that South Africa has probably had two independent introductions of the insect, and South America a separate (third) introduction. Populations further north in Africa appear to be of the same genotype as South Africa and most likely represent natural northward spread of the insect. Interestingly, the genotypes in Africa and South America are identical to those found in outbreak populations in Sydney, from where it most likely originated.

A long term monitoring project, spanning 2-3 years at six sites (Pretoria, two sites near Tzaneen, Sabie, Richardsbay and Pietermaritzburg), and done in close collaboration with industry partners, has now been concluded. The data shows highly consistent population fluctuations at various sites, linked to temperature and humidity fluctuations. Fluctuations at each site are unique compared to other sites, but consistent within the site over time. Consistent high humidity and temperatures, however, seem to inhibit the populations, explaining the lower levels of populations in some parts of the country. These data, and the tools developed during the course of it, will be invaluable to direct future control efforts.



Eggs masses on Eucalypt leaf

Biological control remains the most promising control strategy for *T. peregrinus*. Following collection trips to Australia by TPCP researchers, and collections by collaborators, there have been successful imports of an egg parasitoid, *Cleruchoides noackii*, into quarantine facilities in South Africa. The diversity of these control agents have also been characterized and shows extensively distributed diversity in the native environment that needs to be taken into account during collection. The program is ongoing and will build on these experiences to study the life history of the parasitoid that is needed to assess its biocontrol potential, as well as its potential threat to the environment (non-target hosts), before it can be released in the field. This work is continuing with great urgency.



Close-up of bronze bug egg mass

Diagnostic Clinic News

TPCP and CTHB Diagnostic Clinic 2009

The TPCP/CTHB diagnostic clinic is a service provided to the industry to assist in the identification and management of disease and pest problems of trees. This is a free service, available to all member companies of the TPCP/CTHB Programmes. The Clinic also serves as a surveillance tool in monitoring the occurrence and spread of known pests and pathogens, as well as in the detection of new pests and pathogens.

Pine samples comprised 73 % of the total number of samples received, with the majority of these samples received for *Fusarium* screening. *Eucalyptus* samples made up 6 % and *Acacia mearnsii* (Wattle) samples only comprised about 1.3 % of the total amount of samples. Soil samples received comprised 3.1% of all samples received. Seed samples, received for *Fusarium* screening comprised 14% while samples from nonforestry and indigenous trees as well as water samples, categorized as "other", comprised 2% of received samples.



2010 Diagnostic Clinic Members: From left to right: Jean Hakizimana, Johan van der Linde, Gabrielle Carstensen and Tondani Kone



Clinic members with Mark Holmes at the Mondi Kwambonambi nursery

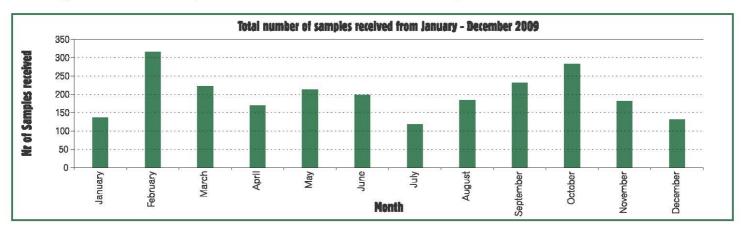
Diagnostic Team 2010

The clinic can not function without the help of student members who process the samples, do analyses and make diagnoses. In return they gain experience and knowledge in working with and identifying different pests and pathogens. Every year we appoint a few new members to the team, enbaling us to expose more students to the clinic and practical tree health.

The new student members of the clinic are Jean Hakizimana, a MSc student from Congo, Gabrielle Carstensen and Tondani Kone, both MSc students from South Africa. They will be joining Johan van der Linde who will be staying on in the clinic from last year. Bernice Porter will be doing the molecular screening for *F. circinatum* as well as helping out in the clinic.

As part of the training for the new (and not so new) students, clinic members go a fieldtrip to gain more knowledge on the workings of various aspects of the forestry industry as well as pests and diseases in

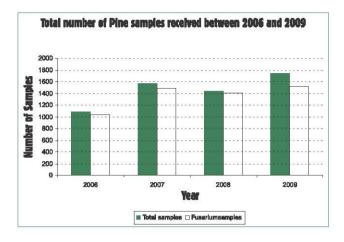
field. This year we started our trip in Zululand, travelled through the Midland's and ended at Ugie and Maclear. On the way we saw Cossid moth, *Gonipterus*, *Thaumastocoris*, *Leptocybe*, Psyllids, Thrips, a *Eucalyptus* nursery, a Pine nursery, got an insight into Pine breeding research, saw *Sirex*, *Diplodia*, Pine Emperor Moth, Wattle chafers, Wattle bagworms, Ceratocystis and of course Pitch canker. We would like to thank all foresters, nurserymen and researchers for taking time out of their busy schedule to show us around the various operations.

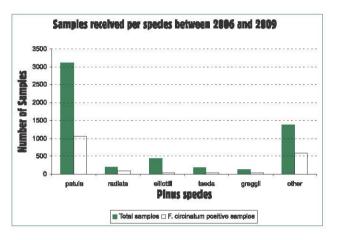


The Diagnostic Clinic & Fusarium Circinatum

Pine trees and *Fusarium circinatum* form a large part of the samples that the clinic deals with on a day to day basis. For the period between January 2006 and December 2009 a total of about 6000 pine seedling or tree samples were analysed. 93 % of all pine seedling and or tree samples were received for either *Fusarium* screening or were sent with *F. circinatum* being the main suspected cause of mortality. In total for the 4 year period *F. circinatum* was detected on 34% of the pine plant material analysed. The percentage positive samples went down from 27 % in 2006 to 21 % in 2007, increased in 2008 to 37% and increased even further in 2009 to 48%.

The most samples received are *P. patula* samples followed by *P. elliottii*, a very distant second. Research material as well as hybrid samples e.g. Pat x Tec and Ell x Car are classified under other and comprise the second largest sample group. We found *F. circinatum* on 34% of all *P. patula* samples received for testing. 83 % of all samples came from nurseries, 50% of which were asymptomatic and showed no obvious disease symptoms. *F. circinatum* was found on 31% of all nursery samples received, with 18 % detection of *F. circinatum* on the asymptomatic nursery plants received for testing.





Field Extension Activities

Field extension work for 2009 comprised various monitoring activities, pest and/or disease consultations as well as attendance and/or presentations at various field days organised by the industry. The group spent more than 600 person days in the field. Members of the TPCP presented and/or attended 6 field days and industry organised courses including ICFR regional field days, SAIF/ICFR *Fusarium* awareness days and the CAMCORE tree breeding course. Monitoring activities included surveying the spread of *Leptocybe invasa* in Zululand as well as assessing damage caused by *Gonipterus* sp. We are also continually on the look out for new pests or diseases or the reappearance of old foes that may be causing new problems.



If you have any questions on pests or diseases, need us to come and look at problems in field or want to send samples to the clinic, contact izette Greyiing (izette.greyiing@fabi.up.ac.za), Jolanda Roux (jolanda.roux@fabi.up.ac.za) or Brett Hurley (brett.hurley@fabi.up.ac.za).

Welcome to the TPCP & CTHB

Stephen Taerum joined the programme for his Phd. He comes from Canada, and has a BSc in Environmental Biology from the University of Alberta and a masters in Ecology and Evolutionary Biology from the University of Kansas. For his PhD Stephen will be studying Ophiostomatoid fungi, their bark beetle and mite associates on pine trees in China.

Dylan Chapman is currently busy with his B.Sc honours degree in Microbiology and Plant Pathology. He obtained his B.Sc degree at Wits. He will be working on the wood rot genus *Phellinus* in South Africa for his honours project.

Bertha Lucia Castro works for Cenicafe in Colombia, dealing with diseases of coffee in that country. She recently joined FABI for her PhD, working on species of *Ceratocystis* and *Rosellinia*.

Silvia Mausse Sitoe is from Mozambique and joined the TPCP for her M.Sc degree. She has a degree in forestry and has been working at the Eduardo Mondlane University. Silvia will be working on Eucalypt diseases in Mozambique, with a specific focus on Cryphonectria cankers.

Angelica Marsberg joined the programme for her MSc degree. She will be looking at the distribution and diversity of endophytic fungi in *Syzygium cordatum*, a tree native to South Africa, using DNA barcoding and pyrosequencing. Angelica completed a BScMedScHons at the

Cornell Kortenhoeven joined the programme for a B.Sc. honours degree in Genetcis and will be working on tree pathogens in the genus *Armillaria*.

Phia van Coller joined the TPCP for her B.Sc honours degree and will be working on Fusarium circinatum.

University of Stellenbosch, before coming to FABI.

Lerato Maubane comes from Limpopo and completed her B.Sc honours at the University of Limpopo on the Antibacterial activity of five selected medicinal plants in 2009. She joined the TPCP for her MSc studies and will be working on the fungal endophytes of *Pinus radiata* from California and South Africa.











SIREX: THE THREAT CONTINUES

Fifteen years after the Sirex wood wasp was first detected in South Africa, it continues to spread north in to more of South Africa's pine resource, and is still the most serious pest of pine in the country.

In 2009, Sirex was detected for the first time in the Mpumalanga province where approximately half of the pine plantations are located. In 2010, Sirex has already been detected in numerous sites in Mpumalanga. In a matter of a few years, Sirex is likely to have spread throughout the province and probably in to Limpopo. It will also soon spread to our neighbors Swaziland and Zimbabwe, if it has not already arrived there undetected!

It is not known how serious a pest Sirex will be as it spreads to these new areas. Well managed saw timber stands may decrease the impact of Sirex, but the many areas of heavily stressed, overstocked and baboon damaged trees could easily serve as a source for an epidemic population of the wasp as occurred in KwaZulu-Natal. Of concern is that while parasitism levels by the parasitic nematode Deladenus siricidicola are increasing over time due to the natural spread of the nematode population in the field, the success with inoculating the nematodes is still very low. This means that we do not yet have a tool to immediately control Sirex as it moves to new areas. The TPCP has continued to investigate the causes for this low inoculation success and to examine Sirex, its symbiotic fungus Amylostereum areolatum, and the nematode D. siricidicola more closely, in an effort to better understand this biological system and investigate potential approaches for improving the management strategy.

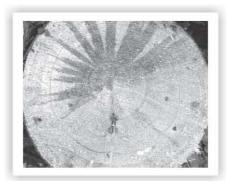
Although much research on the Sirex-Amylostereum complex and the biological control agents has been done, there is still much missing in our knowledge which could have serious consequences to both the short and long term management of this pest.

Factors currently being investigated by the TPCP include the influence of other fungi in the tree on the survival of the nematode and a possible incompatibility between the nematode and fungal strain used. Efforts have also been made to better understand the functioning of *A. areolatum*, the fungus that actually kills the tree. Further, molecular markers have been developed for *D. siricidicola* which are crucial for the process of screening different nematode strains for their efficacy as biological control agents.

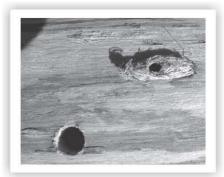
In addition to research efforts of the TPCP, the TPCP is also assisting the forestry industry to import and rear additional biological control agents. This includes parasitic wasps and other strains of the parasitic nematode. The TPCP has established a quarantine and rearing facility for biological control agents, to enable us to secure their availability and increase the numbers available for release. In 2009, hundreds of the parasitic wasp *Ibalia leucospoides* were imported from Canada in to the facility where they need to acclimatize to the southern hemisphere over one or more generations. Other strains of the nematode *D. siricidicola* were also obtained from Canada and some of these strains will be tested in the field within the next few months.



Sirex noctilio female wasp



Sirex infested pine log with blue stain



Tunnel caused by *S. noctilio* larvae (bottom) and frass cocoon of the pine weevil (top)

Gene to 'ome: Scientific Excellence at Genetics Congress

South Africans face many challenges, but our scientists excel at turning those challenges into opportunities. This was one of the golden threads that ran through the SAGS 2010 (South African Genetics Society) meeting held at the University of the Free State in Bloemfontein in April.

Both students and academia from around the country attended the meeting. However, there was a high representation from the University of Pretoria (UP), the University of the Free State (UFS) and the University of Stellenbosch (US). This provided excellent opportunities for networking to all that attended.

Most of the oral and poster presentations were by students from the various research groups, and covered subjects such as gene organization, genome sequencing, population genetics and cytogenetics. Disease problems of plants, animals and humans, and problems relating to the quantity and quality of natural resources make up the bulk of genetic research undertaken at South African institutions. This was also reflected in the subjects of most of the presentations. The society awarded prizes and honorable mentions to students who delivered the best presentations.

The prize for best poster presentation was awarded to Janine Silberbauer from the FMG group at UP (Forest Molecular Genetics, FABI), for her poster describing the analysis of digital expression profiling data from *Eucalyptus* tension wood forming tissues. The best MSc oral presentation was awarded to Simon Martin, from the TPCP-CTHB group (Tree Protection cooperative Programme / DST/NRF Center of Excellence in Tree Health Biotechnology, FABI), for his presentation on mating pheromones of *Fusarium* species. Nicky Creux, also from the FMG group, pocketed the best PhD oral presentation for her work on the characterization of regulatory elements of cellulose synthase genes of *Eucalyptus*. One of the criteria for these awards was the ability of the student to explain complex concepts to those who do not work in the same field. These awards thus underline the excellent standard of research undertaken and training provided by institutes such as FABI, and imprints confidence in the next generation of scientists to lead research in South Africa.

Although the South African Genetics Society has seen a steady decline in member numbers over the past few years, the executive committee believes that it is only temporary. Efforts have been made to improve the society's online presence with a new website (http://www.sagene.co.za), and the society has also b e e n a f f i I i a t e d w i t h S A C N A S P (http://www.sacnasp.org.za), the counsel representing professional natural scientists in South Africa. Anyone working in genetics or a related field was invited to become a member of SAGS. Membership costs R200 biannually, and can be obtained through the website. Membership to SAGS also grants affiliation to SACNASP, which in itself is a huge benefit.

As a newly elected member of the executive committee of SAGS, Albe van der Merwe urges anyone working in a genetics related field to join the society. Any field such as plant or animal breeding, phylogenetics and phylogeography, human genetics, or population genetics of any organism is welcome. There is especially a huge gap in representation of bioinformatics fields, which we hope to rectify. We invite all to consider attending the next meeting to be held at the University of Stellenbosch in 2012. It promises to be just as informative and productive as the meeting we had at UFS, and it will no doubt aid just as much in turning those national challenges into national opportunities.



Students who received awards and honourable mention at the 2010 SAGS congress.

FLTR: Daria Rutkowska (Dept Genetics, UP, honourable mention poster presentation); Steven Hussey (Dept Genetics & FABI, UP, honourable mention MSc oral presentation); Hesmari van der Westhuizen (Dept Genetics, UFS, honourable mention poster presentation); Tyren Dodgen (Dept Pharmacology, UP, honourable mention PhD oral presentation); Simon Martin (Dept Genetics & FABI, UP, best MSc oral presentation); Janine Silberbauer (Dept Genetics & FABI, UP, best poster presentation); Juliet Mentoor (Dept Genetics, UP, honourable mention MSc oral presentation); Nicky Creux (Dept Genetics & FABI, UP, best PhD oral presentation); Ritesh Mewalal (Dept Genetics, UP, honourable mention poster presentation).

PHYTOPHTHORA ROOT ROT

A Focus on the plant (tree) destroyer

The latin name *Phytophthora* means 'plant destroyer'. This is a fitting name for these obligate pathogens of plants. On trees, they are best known to cause root and collar rots, but are increasingly recognized as foliar pathogens. Black butt of *Acacia meamsii*, establishment failure of cold tolerant *Eucalyptus* and DFP on *Pinus radiata* in Chile are some of the well known examples to the forestry community in South Africa.



The TPCP makes a point of maintaining capacity to deal with most of the important pathogen groups that affect, or could be expected to cause health problems to trees in the South African plantation industry. *Phytophthora* is one of those that is important given its history of devastation in forests and plantations around the world. The devastation of whole plant communities in Western Australia and other parts of the world caused by *Phytophthora cinnamomi* is a well known example, as is the recent outbreak of Sudden Oak Death (causing diseases of many different tree species) caused by *P. ramorum* on the American west coast and Europe. The devastating outbreaks of DFP on *P. radiata* in Chile, caused by *P. pinifolia* (which was discovered and described by Alvaro Duran of the TPCP and Arauco in Chile), is a stark reminder of how quickly a devastating new pathogen can emerge. This work will continue in collaboration with Arauco.

Very little is known regarding the native *Phytophthoras* that occur in water and soil in South Africa and which might affect trees in the country. A recent project has been launched to address this knowledge gap, and to continue to build the capacity of the TPCP group to deal with these pathogens. The projects are led by Dr. Eunsung Ho (a postdoctoral fellow from the USA/Korea) and Mr. Jan Nagel (MSc student), together with Marieka Gryzenhout, Bernard Slippers and Mike

Slippers and Mike Wingfield. Early results have already revealed numerous previously unknown s p e c i e s a n d promise to open a whole new field of endeavour.

Three scientists from the TPCP recently attended



the fifth IUFRO meeting on *Phytophthoras* in forests in Rotorua, New Zealand. They shared their results on the work done in the group on *P. pinifolia*, as well as in native ecosystems. Bernard Slippers gave an overview of the published work that exist on *Phytophthoras* in Africa, based on a review prepared by Jan Nagel. This is the first time that such a regional overview is given for Africa at this meeting, and we believe marks the beginning of an exiting journey of discovery in this domain in future. Continuing on this topic, Eunsung presented her work that has already revealed three new *Phytophthora* species from rivers in South Africa. A number of other species are also currently being characterized. There was a lot of interest in the work on *P. pinifolia* done by Alvaro Duran in collaboration with researchers from the TPCP and Arauco in Chile. Alvaro recently completed his PhD on this topic in the TPCP group.

Alvaro had the opportunity to present two talks on this topic, of which one was unplanned.

We were not only there to discuss our work, but importantly also to learn from the leading researchers in the world on this topic. There are a n u m b e r of devastating emerging diseases caused by *Phytophthora* species in many parts of the world. This follows a general trend of increasing numbers of invasive pathogens in for est. Understanding origins of *Phytophthora*, and importantly their pathways of spread is clearly a topic of



Black butt disease of Acacia mearnsii in South Africa, caused by Phytophthora nicotianeae

clearly a topic of international importance and urgency. For South Africa (and Africa) there is a warning that we are lagging behind the world in terms of baseline knowledge and quarantine measures to deal with this threat. Vast programs of millions of dollars are being spent in various parts of the world to eradicate or contain these devastating pathogens. Many more such introductions might well become overwhelming and should be avoided at all possible costs.

The influence of new technologies, in particular in the field of molecular genetics, were evident throughout the meeting. These tools are informing everything from the origin of the invasions to the origins of the species. From the occurrence and fluctuations in occurrence, to the infection process and resistance. But clearly these tools are of very little value if they can not be combined with well informed basic biology, from which meaningful questions can be defined. It was also very informative to learn some of the most advanced tools from the world experts on how to collect, handle, store and study these special organisms that are so devastating to plants.

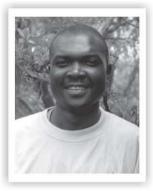
GRADUATIONS

Ph.D degrees

• Didier Aime Begoude Title:

> Supervisors: External Examiners:

Characterization of Botryosphaeriaceae and Cryphonectriaceae associated with *Terminalia* spp. in Africa. Profs. Jolanda Roux, Mike Wingfield and Bernard Slippers Dr. Gary Samuels (USDA Forest Service), Prof. Gerry Adams (Michigan State University)





M.Sc degrees

ar and and	
Donald Chungu (With	distinction)
Title:	Pathogens associated with plantation tree diseases in Zambia
Supervisors:	Prof. Jolanda Roux, Dr. Muimba A. Kankolongo, Prof. Mike Wingfield
External Examiners:	Dr. Angus Carnegie (Department of Primary Industries, Forest Health Research, Australia), Dr. Pat Caldwell (University of KZN)

B.Sc honours

- Angelica Marsberg:
- Gabrielle Carstensen: The d
- Zander Human:
- : The mat 1 idiomorph of *Ophiostoma quercus*.
- le Carstensen: The diversity of bacterial endophytes in the Shepherds tree (Boscia albitrunca).
- ler Human: Survey of bacteria associated with the cossid moth (Coryphodema tristis).



The Tree Protection Co-operative Programme (TPCP) was formally inaugurated in 1990. The programme is a cooperative venture between the University and all the major players involved in commercial forestry in South Africa and provides research support focused on understanding and reducing the impact of diseases and insect pests that damage plantation- grown forest trees. Each year, since 1991 the programme has provided a research symposium for the members and stakeholders. During the week of May 10th, the TPCP celebrated its 21st year of operation and this milestone was highlighted at the 20th annual research symposium of the Co-operative, attend by approximately 200 forest managers, researchers and students. Since 2005, the annual research symposium has also included the activities of the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) also housed in FABI and that was synergized by the TPCP.

During the course of the past 21 years, the TPCP has grown from a relatively modest initiative to the single largest research team working on pests and diseases of forest and plantation trees in the world. Collectively, the TPCP and the

CTHB attract more than R12 million Rand of external research funding to the University of Pretoria. Funding to the programme has facilitated the education of approximately 50 M.Sc. and 50 Ph.D. students and it has supported the publication of in excess of 500 publications in internationally recognised science journals. Currently the TPCP and CTHB include some 50 M.Sc., Ph.D. and Post Doctoral fellows a group of some 13 academics as well as a team of administrative and technical staff. The 20th annual meeting of the TPCP, spanned two days, included nine overseas guest scientists and the three previous staff members of Mondi, Sappi and H. L & H. Timber Products that were involved in initiating the Co-operative Programme 21 years ago.



Neville Denison Mike Shaw Peter Roberts John Tew & Mike Wingfield



Wubethu Bihon



PhD Student from Ethiopia

Research / Expertise: The fungus Diplodia pinea is one of the most known pathogens of pines and other coniferous trees worldwide. It causes tip dieback, lesions, stem canker, collar rot, blue-stain, root diseases and complete tree death. It results in extensive losses in South African plantation forestry. especially where susceptible species are planted and when trees are subjected to physiological stresses. The aim of my project is to study the gene and genotypic diversity and population structure of this fungus at different spatial levels using microsatellite markers and DNA sequences, in order to address the possible mode of reproduction. The mechanisms of dissemination and the infection process of the pathogen in South African pine plantations is another focus of my study. The information gained from this study will assist the forest industry to devise protection strategies, assist breeders to select resistant pine species, and assist guarantine officials to identify new isolates of this invasive fungus.

Hobbies / Interests: I like to watch soccer, especially the European Championship and the English and Spanish Premier Leagues.

Johan van der Linde



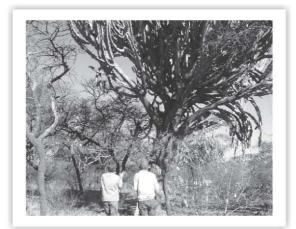
Msc student South African

Research / Expertise: My MSc project is an investigation into the possible factors that are involved in the decline of Euphorbia ingens (Naboom) in the Limpopo province of South Africa. Euphorbia ingens is indigenous to South Africa and occurs in the northern provinces and our northern neighbouring countries. In the last ten to twenty years symptoms of disease have been noticed on these trees, mainly as a grey type of discoloration, ultimately leading to a dramatic decline of these trees especially in the Limpopo province. The objective of the proposed project is to gain a better understanding of the factors associated with the decline and death of E. ingens in the Limpopo Province. My study includes the identification of both abiotic and biotic factors that possibly play a role in the decline of E. ingens.

Hobbies / Interests: I enjoy sport, movies and have a deep passion for music. I play squash, the drums and guitar.



Wubethu collecting samples from Pinus patula trees for his project



Johan discussing the death of *E. ingens* trees with nature conservator, Rentia Malan in Limpopo.

DIVERSE COMMUNITIES OF THE BOTRYOS PHAERACEAE INFECT NATIVE TREES SOUTHERN AFRICA

Poster at annual conference of the South African Association for Botanists, January 2010

Slippers, Roux, Begoude, Mehl, van der Linde, Pavlic, van der Walt, Maleme, Hinze, Marais, Wingfield and Wingfield

The Botryosphaeriaceae is a well-known fungal family that has been studied for more than 150 years. Many species in this group are important pathogens of trees globally. In Southern Africa only ten species were known a decade ago, mostly on non-native hosts. During the past decade surveys on native trees in this region, using modern molecular tools combined with traditional morphological species characterization, have revealed a staggering diversity of species in this fungal family. Studies on *Acacia* spp., *Terminalia* spp., *Pterocarpus angolensis, Sclerocarya birrea, Syzygium cordatum* and a number of species in the Proteaceae revealed the presence of at least 40 species in the Botryosphaeriaceae, more than 25 of which is thought to be new to science. Many of these species overlap in host distribution on the native tree genera, as well as on non-native agricultural and forestry trees. These results illustrate just how under-explored the fungal diversity of Southern Africa is, even in important pathogen groups. This is especially concerning given increasing reports of native tree die-back in southern Africa, and the frequency with which Botryosphaeriaceae are linked to these diseases. This is not entirely unexpected, because the impact of pathogens in the Botryosphaeriaceae has been predicted to increase due to stress on trees caused by climate change.

INCREASING REPORTS OF MATIVE TREES DYING SOUTH AFRICA

Presented at annual conference of the South African Association for Botanists, January 2010



Roux, van der Merwe, Malan, van Rooven and Wingfield

In recent years increasing numbers of reports have been received of unexplained mortality of native trees in South Africa and Namibia. In some cases the cause of mortality has been shown to be changes in rainfall patterns and amounts, such as in the Outeniquas, and other climate driven factors such as the deaths of Aloe dichotoma in Nambia. The death of some plants, such as Leucospermum spp. in the Western Cape, has been shown to be caused by microbial infection. However, a number of reports, such as those of dying Acacia erioloba and Euphorbia ingens, remain as yet unexplained. It is well recognized that interactions between plants, humans, the environment, insects and micro-organisms are complex and difficult to characterize. Pilot studies of declining *A. erioloba* and E. ingens has revealed a number of undescribed fungal genera, emphasizing the lack of information regarding fungi in Africa. Surprisingly, a number of insect species, possibly representing undescribed species,

were also collected. Without a better understanding of the reasons behind the large-scale mortality of native South African trees observed at the moment, we run the risk of possibly loosing some of our trees completely. Finding answers to these questions, as well as possible solutions to the decline currently observed, will require crossdisciplinary research initiatives and an adjusted strategy in future research studies.



Poster presented at Fifth IUFRO working Party 7.02.09 meeting on Phytophthora Diseases in Forests and Natural Ecosystems held in Rotorua, New Zealand in 7-12 March, 2010.

Oh, Wingfield, Wingfield and Roux

Quercus cerris (Turkey Oak) is native to the Orient and South east Europe and is a well known component of native forests of Turkey. Phytophthora spp. have been recognized as being involved in decline of Quercus spp., including Q. cerris, in eastern and north-central U.S., Europe, and the Iberian Peninsula. continue Those species isolated from soil associated with declining Q. cerris include P. cryptogea and P. guercina,







while *P. syringae*, *P. ramorum* and *P. cinnamomi* have been isolated directly from sapwood of symptomatic trees showing bleeding canker in Europe. Recently, bleeding cankers very typical of *Phytophthora* infection were found on *Q. cerris* trees growing on the Vergelegen Estate near Somerset West in the South Western Cape Province of South Africa. Isolations were made from diseased tissue and a *Phytophthora* sp. was recovered from all diseased trees sampled. Isolates were identified based on morphology and comparisons of sequence data for the ITS gene regions as being *P. cinnamomi*. Four isolates were tested for mating type by pairing with known *P. cinnamomi* mating tester strains. One isolates was of the A1 and the remaining three of the A2 mating type. *Phytophthora cinnamomi* is a well-known pathogen of native and introduced plants in the South Western Cape Province and it has been shown to have been introduced into the country. This study represents the first report of *P. cinnamomi* on *Q. cerris* in South Africa and it is most likely also the cause of the disease on these trees.

ALLOPATRIC SPECIATION IN *CHRYSOPORTHE* IS NOT LINKED TO CONTINENTAL DRIFT

Presentation at annual conference of the South African Genetics Society, 2010

Van der Merwe, Steenkamp, Coetzee, Gryzenhout, Wingfield and Wingfield

The genus Chrysoporthe (Cryphonectriaceae) includes important pathogens of trees in the Myrtales. While C. austroafricana is a native fungus on the African continent, C. cubensis occurs in South and Central America and C. deuterocubensis attacks trees in Southeast Asia. Although these species are very closely related when phylogenies based on multiple gene sequences are considered, they appear to have a continental distribution. The aim of this study was, therefore, to investigate whether allopatric speciation in *Chrysoporthe* is linked to post-Gondwana continental drift. Fossil evidence was used to calibrate a phylogeny of the fungi based on the 18S rrl ribosomal gene. This phylogeny included the order Diaporthales and the family Cryphonectriaceae. Secondary calibration points were recovered from the rrl chronogram and applied to a phylogeny of the Diaporthales based on ITS (internal transcribed spacer ribosomal DNA) and β -tubulin gene sequences. Divergence estimates were recovered from the ITS+ β -tubulin phylogeny and contextualized with continental drift and the movement of host plants around the world. The divergence time of Chrysoporthe spp. was estimated at 7.07 Mya (2.34 – 13.07 Mya, 95% HPD). This estimate was well after the breakup of Gondwana, and implied that allopatric speciation in Chrysoporthe was not a result of continental drift. The oldest lineage within Chrysoporthe was that of C. deuterocubensis, the Southeast Asian fungus. Interestingly, the Myrtales evolved in this region and subsequently spread around the world. Therefore, the speciation pattern of Chrysoporthe spp. mirrored the movement of the Myrtales around the world. We are consequently of the opinion that allopatric speciation in *Chrysoporthe* exists as a result of host-derived geographic separation, rather than continental drift.

ALLOPATRIC DIVERSITY AND SPECIES-SPECIFICITY IN PEPTIDE MATING PHEROMONES OF ASCOMYCETES

Presentation at the meeting of the South African Genetics Society, 2010

Martin, Steenkamp, Wingfield & Wingfield

In fungi, like many other organisms, reproductive genes are thought to be more divergent than house-keeping genes. This phenomenon could be associated with the formation of reproductive barriers and speciation. In heterothallic ascomycetes, the primary signals between mates are peptide pheromones and their receptors. Two classes of pheromone peptides, homologous to the a-factor and α -factor of *Saccharomyces cerevisiae*, have been characterised in several yeast and filamentous species. Little is known regarding the diversity and the extent of species-specificity in these peptides among ascomycetes. In this study we compared the putative protein-coding DNA sequences for the two pheromone classes from 72 ascomycete species. Our dataset included previously described pheromones and putative pheromones identified from genomic sequences. In addition, we amplified and sequenced pheromone genes from twelve species in the *Gibberella fujikuroi* species complex. Over half the species studied had some level of species-specificity in their pheromone peptide sequences. However, pheromones were largely conserved among species in the *Gibberella fujikuroi* species cannot alone account for the reproductive barriers observed between these species. Our results show that pheromone peptides have diversified rapidly among many other ascomycetes, occasionally under positive selection but usually due to lack of selective constraint. Furthermore, we found that repeats of the α -factor-like pheromone, which occur in tandem arrays, can differ vastly in copy number between and even within species. We hypothesise that frequent duplication and loss of tandem-repeats can lead to birth-and-death evolution, which can contribute to the rapid diversification of these peptides.

New Research on the Eucalyptus Snout Beetle in South Africa

No stranger to Eucalyptus growers in many parts of the world, the Eucalvotus snout beetle (Gonipterus scutellatus) remains a pest in South Africa nearly a century after its introduction from Australia in 1916. Larvae and adults of this insect feed primarily on new leaf tissue from a variety of Eucalyptus species and clones, and can seriously defoliate trees leading to significantly reduced growth. For many years, beetle populations remained low, at least in part due to the success of a specialist egg parasite (Anaphes nitens), introduced as a biological control agent for Gonipterus in 1925. Control by this tiny wasp has been widely cited as an example of a successful biocontrol Recently however, Gonipterus has programme. again been causing considerable damage, particularly in areas of Zululand and KZN. In response, researchers at FABI are actively pursuing several new and ongoing projects aimed at understanding the causes underlying the resurgence of this pest, with the goal of improving the effectiveness of control.



Damage to the crown of a eucalypt clone caused by feeding of adult Gonipterus

Our work to date has three major components. First, we are in the process of establishing a long term monitoring effort where semi-permanent plots along

two broad transects within Eucalyptus growing areas in KZN and Zululand which will be repeatedly sampled for *Gonipterus* and *Anaphes*. This will allow us to measure rates of parasitism as they vary seasonally and geographically, and to better understand why these patterns appear to have changed in recent years. This project will be carried out by Izette Greyling in partnership with the forestry industry and will continue for a minimum of 2-3 years.

Second, in a project driven by PhD student Donald Chungu, we are studying the genetics of *Gonipterus* and *Anaphes* with the goal of understanding patterns of diversity and the potential for adaptation to variable climate conditions, tree hosts, and management practices, including the use of pesticides. Donald has recently confirmed the existence of unusually high genetic diversity in *Gonipterus* populations and has uncovered geographic patterns that strongly suggest multiple introductions of the pest, perhaps even of sister species. In contrast, *Anaphes* is highly uniform genetically throughout South Africa. Donald will also explore what these emerging patterns might mean for the long-term effectiveness of biological control.



Gonipterus larvae

Finally, Marc Bouwer has just completed a MSc characterizing the chemical components in *Eucalyptus* foliage that in part determine tree attractiveness to *Gonipterus* in the hopes of improving our understanding of host preference and beetle feeding and egg-laying behavior. Mark is now working on a PhD degree at FABI following up on some of this work.



Gonipterus adult