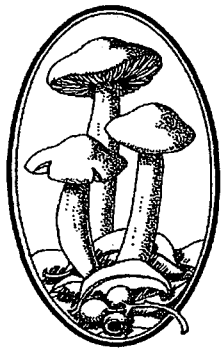

PROGRAM

Australasian Mycological Society Meeting

Saturday, 21 August 1999
Westmead Hospital
Education Block, Lecture Theatre 4

TALKS

10.00	Patron's lecture: Mycological warfare, *G. Weste.*
10.30	Molecular epidemiology of clinical and environmental isolates of *Cryptococcus neoformans*, *S. Kidd*, D. Carter & W. Meyer.*
10.45	The development of molecular probes able to detect *Cryptococcus neoformans* var. *gattii* VGI and VGII in the environment, *G. Scandurra* & D. Carter.*
11.00	Microevolution in *Cryptococcus neoformans* var. *gattii* as a consequence of antifungal drug therapy, *S. Lim*, R. Malik & D. Carter.*
11.15	**MORNING TEA**
11.30	*Cryptococcus neoformans* var. *gattii* as a model system to study differential gene expression in experimental infection, *D. Wood*, P. Zuccolotto, M. Lorenz, R. Malik, T. Sorrell, S. Dowd & W. Meyer.*
11.45	Characterisation of genes that may be important in the virulence of *Cryptococcus neoformans* var. *gattii*, *P. Ruma Haynes*, T. Sorrell & A. Brownlee.*
12.00	The balance between symbiosis and pathogenesis in *Rhizoctonia solani*, *E. Pope*, P. McGee & D. Carter.*
12.15	Transformation of *Aspergillus carbonarius* based on a hygromycin B resistance marker, *Z. Ilic*, J. Pitt & D. Carter.*
12.30	Biological control of aflatoxigenic *Aspergillus* spp. using molecular approaches, *N. Tran-dinh*, J. Pitt & D. Carter.*
12.45	The identification and quantification of airborne allergenic fungal spores by quantitative PCR, *G. Gaskell*, D. Carter, W. Britton, E. Tovey, F. Benyon & U. Løvberg.*
1.00	**LUNCH**
2.00	POSTER SESSION
2.45	Adaptive insensitivity to arsenate in populations of *Hymenoscyphus ericae*, *J.Sharples*, A. Meharg, S. Chambers & J. Cairney.*
3.00	A preliminary investigation of the phylogeny of *Gymnopilus* and related genera using sequence data from the internal transcribed spacer (ITS) region of ribosomal DNA, *B. Rees, D. Orlovich* & G. Zuccarello.*
3.15	*Phialophora, W. Gams*
3.30	Deterrence of herbivory by endophytes of cotton, *P. McGee.*
3.45	Value and reliability of records of Australian fungi from the Fungimap scheme, *T. May* & P. Grey.*
4.00	**AFTERNOON TEA**
4.15	Australian boletes, *R. Watling*
4.30	Taxonomy and population biology of south-eastern Australian *Pisolithus* species, *I. Anderson*, S. Chambers & J. Cairney.*
4.45	Generic limits of the sequestrate relatives of *Russula, T. Lebel.*
5.00	The effect of wildfire on the fruiting of macrofungi in Karri regrowth forests in the south-west of Western Australia, *R. Robinson.*
5.15	Investigation of survey methods and macrofungi communities after fire and logging in *Eucalyptus regnans* forest, *S. McMullan-Fisher.*

* Presenting author.

POSTERS

1 Molecular determination of diversity in ericoid mycorrhizal endophytes from *Woollsia pungens* (Cav.) F.Muell. (Epacridaceae), *D. Midgley*, S. Chambers, G. Liu, A. Williams & J. Cairney.*

2 Genet distribution and genetic variation of *Cortinarius rotundisporus* (Clel. & Cheel) Horak & Wood in eastern Australian sclerophyll forests, *N. Sawyer*, S. Chambers & J. Cairney.*

3 *Cryptococcus neoformans* var.*gattii* as model system to study differential gene expression in experimental infection, *P. Zuccolotto*, M. Lorenz, R. Malik, D. Wood, T. Sorrell, S. Dowd & W. Meyer.*

4 Characteristics of extracellular cryptococcal phospholipases, *R. Santangelo*, S. Chen, T. Sorrell & L. Wright.*

5 Development of an ELISA for detection of cryptococcal phospholipase antibodies, *R. Santangelo, J. Hook, R. Weinberger, T. Sorrell & L. Wright.*

6 Investigation of a 'cryptic species' within *Aspergillus flavus* using molecular techniques, *T. Bui, J. Pitt & D. Carter.*

7 Smut fungi of New Zealand, *E. McKenzie* & K. Vánky.*

8 The *Armillaria* Species of New Zealand (Fungi: Agaricales), *G. Ridley.*

(People giving posters need to be next to their poster during the poster session.)

5.45	AMS Annual General Meeting.
8.00	Dinner. Dees Carter's home, 3 Colbourne Avenue, Glebe, NSW 2037. Phone: 9660 0302.

AMS CONFERENCE DINNER

How to get to the barbeque (starts 8 pm)

3 Colbourne Avenue
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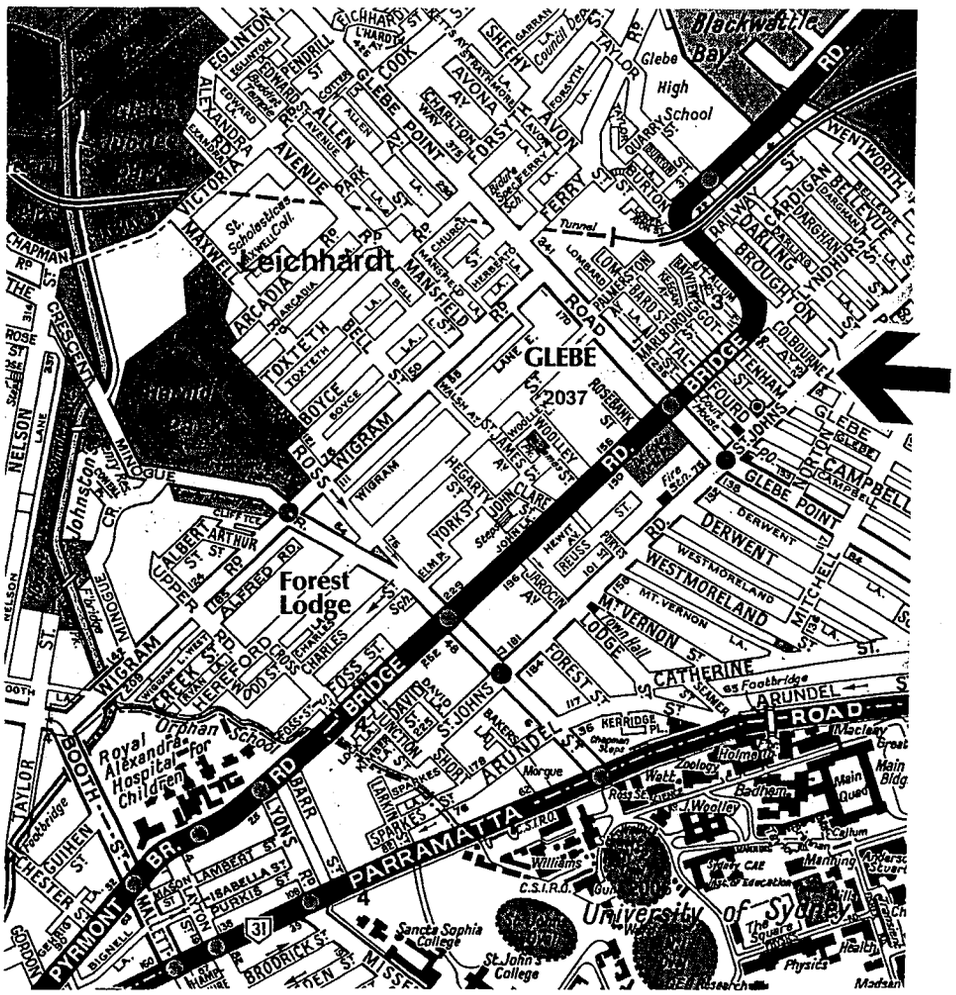

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WESTMEAD HOSPITAL

How to get to Westmead Hospital

Westmead Hospital is situated on the corner of Darcy and Hawkesbury Roads, Westmead, two kilometres west of Parramatta.

Train services

Westmead Hospital is approximately 300 metres north of the Westmead railway station. There are frequent train services from all areas from the mountains to the city centre.

Bus services

Return bus services are provided from the Parramatta Central Business District (CBD) to Westmead Hospital. Westbus Route 710 includes stops at Market Street, Parramatta via Westfield shopping complex (Church & Argyle Sts), Wentworthville, Old Toongabbie and Toongabbie.

A shuttle bus operates daily between 7 am and 8 pm for passengers from the Parramatta Rivercat service, CBD and the Westmead Hospital campus. Route 711 includes stops at Parramatta Station (Argyle and Darcy Sts), Riverside Theatres, Charles Street ferry wharf, Westfield Shopping Centre, Westmead Railway Station, the New Children's Hospital and Westmead Hospital.

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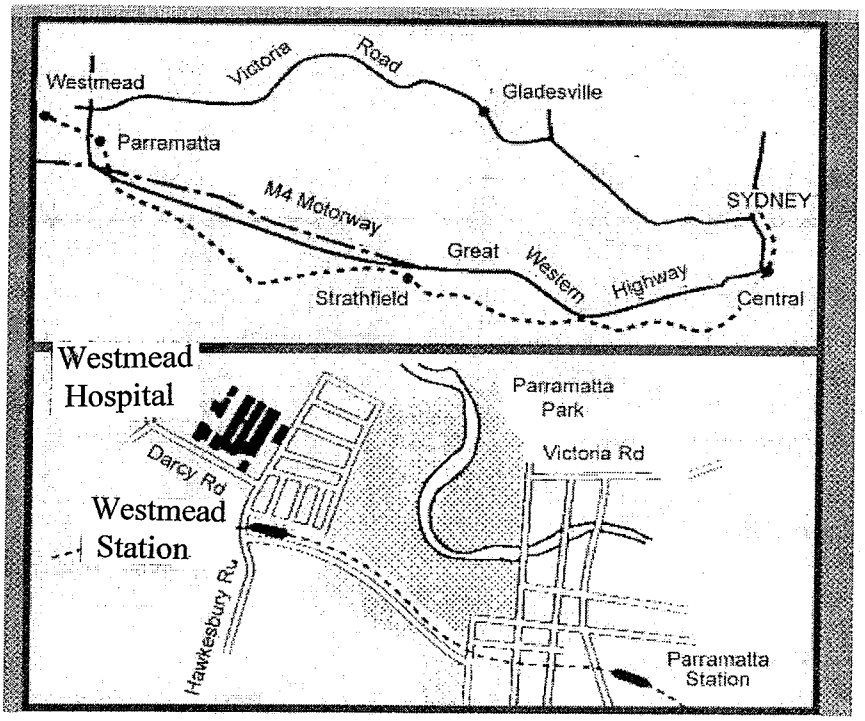
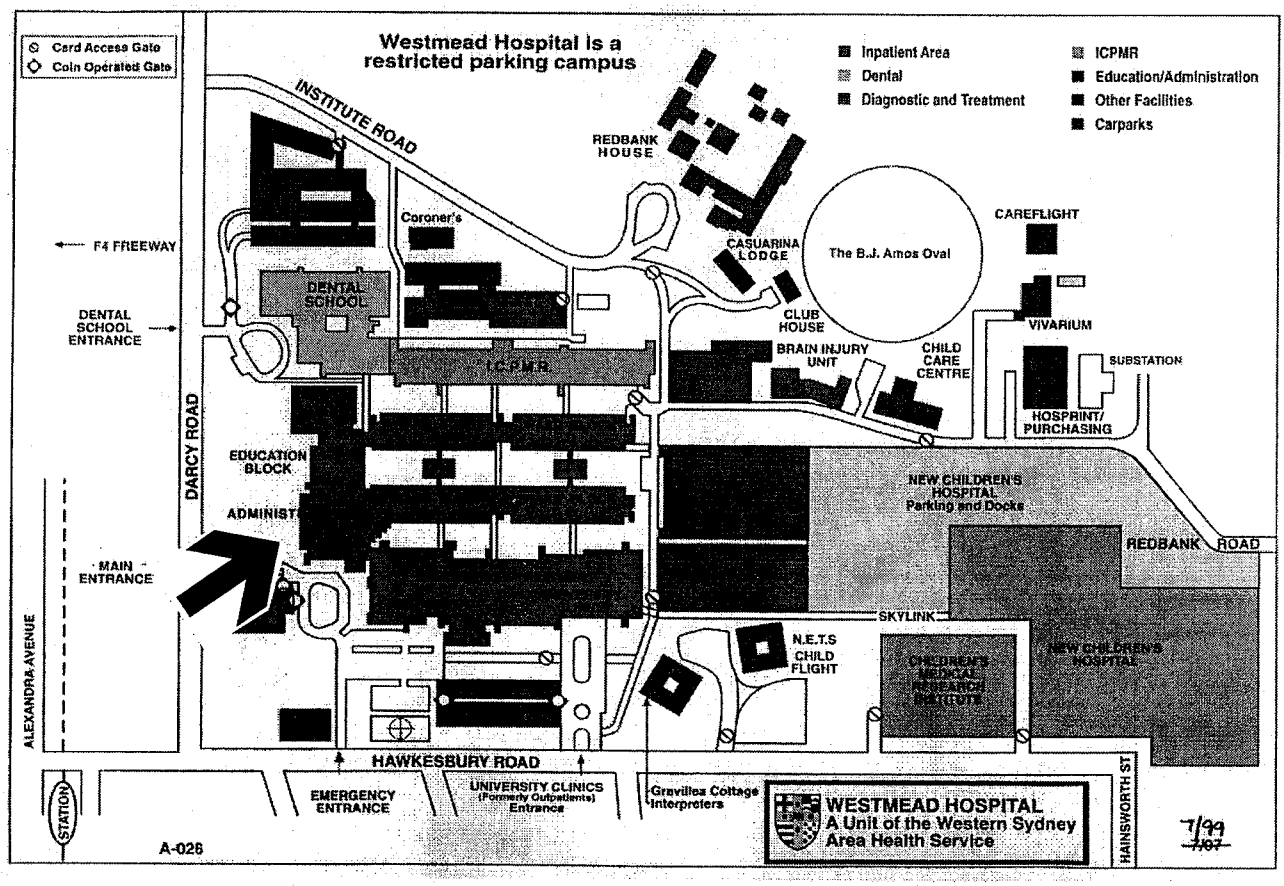
Limited car parking is available on site for a nominal fee.

Taxi services

Direct taxi telephones are provided at each entrance to the Hospital (*i.e.* Emergency, Outpatients, Main Entrance and the Dental School).





➔ Lecture theatre 3 is in the administration block of the hospital (near the main entrance) just above the hospital cafeteria.

ABSTRACTS

TALKS

MYCOLOGICAL WARFARE

G. Weste

Botany School, University of Melbourne, Parkville, Vic. 3052.

Microscopic parasites belonging to the *Phytophthora* genus cause fatal diseases such as the potato blight, which attacks only two hosts, the potato and the tomato and yet caused a major European famine in 1845, aided by the rapid spread of its sporangia in a wet summer. *Phytophthora palmivora* attacks tropical crops such as cocoa and durian, and is spread by both sporangia and swimming spores which infect all parts of the tree. The cinnamon fungus, *Phytophthora cinnamoni*, has at least 2,000 hosts and is dispersed by swimming spores which infect susceptible roots. Aggressive attack by the cinnamon fungus caused epidemic disease and death of many susceptible shrubs in some areas of open forests and heathlands. Reductions in both numbers and diversity of plants and animals three years after infection in the Brisbane Ranges and the Grampians is tabled. Endangered susceptible species are at risk. The susceptible shrubs were replaced by resistant sedges, rushes and teatree. The cinnamon fungus was attacked by soil micro-organisms and declined after about 10 years because few living susceptible roots remained. Tables show the decline of the parasite and the regeneration of susceptible seedlings in the 1980s. About 30 years after infection the ultimate victory belongs to the soil microbes. The cinnamon fungus is confined to scattered pockets, and in 1998 a population explosion of the susceptible, but dominating grass tree, *Xanthorrhoea australis* was measured in some areas of forest and heathland of the Brisbane Ranges, Wilsons Promontory and Anglesea. The weather is a major factor in *Phytophthora* warfare. The potato famine occurred after three weeks of summer rain. The cinnamon fungus epidemics followed years of excessive summer rain. The recent grass tree regeneration has survived Victoria's driest three years. Production, dispersal and infection by swimming spores are all water dependent.

MOLECULAR EPIDEMIOLOGY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF *CRYPTOCOCCUS NEOFORMANS*

*S. Kidd**[1], *D. Carter*[1] *& W. Meyer*[2]

[1] *Department of Microbiology, University of Sydney, NSW, Australia.*
[2] *Molecular Mycology Laboratory, Department of Infectious Diseases, Westmead Hospital, NSW, Australia.*

Cryptococcus neoformans is an encapsulated basidiomycetous yeast which causes disease in both immunocompromised and immunocompetent hosts. Disease typically manifests primarily in the lungs via inhalation, later disseminating to the skin, bones and the CNS (Central Nervous System). *Cryptococcosis* is the leading mycological cause of mortality in persons with AIDS. *Cryptococcus neoformans* exists as two genetically distinct varieties, var. *neoformans* (serotypes A, D and AD) and var. *gattii* (serotypes B and C). As a result of an international epidemiological study there exists an extensive collection of clinical and environmental isolates of *C. neoformans* obtained from around the world. While many of these isolates have been used in a variety of different studies, there have been no standardised molecular analyses of the entire collection. It has been found that PCR-fingerprinting using single primers specific for minisatellite or microsatellite DNA, and RAPD analysis with two combinations of three 20–22-mer primers can separate *Cryptococcus neoformans* strains into eight major molecular groups. VNI and VNII correspond to serotype A, VNIII to serotype AD, VNIV to serotype D, and VGI, VGII, VGIII and VGIV to serotypes B and C. In this study we are employing a range of techniques, including PCR-fingerprinting and RAPD analysis, in order to gain an understanding of the genetic diversity and relatedness of these isolates, variety, mating type and geographical strain distribution. Here, preliminary results from this study will be presented and discussed.

THE DEVELOPMENT OF MOLECULAR PROBES ABLE TO DETECT *CRYPTOCOCCUS NEOFORMANS* VAR. *GATTII* VGI AND VGII IN THE ENVIRONMENT

G. Scandurra & D. Carter*

Department of Microbiology, University of Sydney, NSW, Australia.

Cryptococcus neoformans is a pathogenic yeast which can cause life threatening meningitis in humans and animals. Two varieties of *Cryptococcus* exist and have distinct ecological distributions. *Cryptococcus neoformans* var. *neoformans* occurs worldwide and is commonly found in soil and pigeon excreta, and *C. neoformans* var. *gattii* is found in tropical and subtropical regions in association with certain *Eucalyptus* species. Within *C. neoformans* var. *gattii*, two genetically distinct subtypes have been found and are designated VGI and VGII. The majority of clinical isolates and almost all environmental isolates of *C. neoformans* var. *gattii* are of the VGI subtype. The VGII subtype is much less common and has not yet been isolated from *Eucalytpus* or related tree species. We are interested in analysing the presence and distribution of both subtypes of *C. neoformans* var. *gattii* in the environment and in finding out more about the yeast/*Eucalyptus* association. The traditional means of detecting *C. neoformans* cells relies on plate culture, which is time consuming and complicated by the presence of many other yeasts and moulds in environmental samples. We are therefore developing molecular probes based on anonymous repetitive DNA that is specific to *C. neoformans* var. *gattii* VGI and VGII. Probes have been constructed by cloning 0.5–1kb fragments of genomic DNA into a plasmid vector and hybridising these with total genomic DNA from the same strain. Several candidate probes have been isolated which are specific to *C. neoformans* var. *gattii* and do not cross hybridise with DNA from other yeasts that may be present in environmental samples. We are currently testing these probes for their sensitivity and specificity to each VG type. The probes will be used to assess the levels of the VG subtypes in debris from candidate host trees and the surrounding soil.

MICROEVOLUTION IN *CRYPTOCOCCUS NEOFORMANS* VAR. *GATTII* AS A CONSEQUENCE OF ANTIFUNGAL DRUG THERAPY

S. Lim[1], R. Malik[2] & D. Carter[1]*

[1] *Department of Microbiology, The University of Sydney, NSW 2006, Australia.*
[2] *Department of Veterinary Clinical Sciences, The University of Sydney, NSW 2006, Australia.*

Cryptococcus neoformans var. *gattii* is an important fungal pathogen of both humans and animals. The lung is usually the initial site of infection, from where the disease may become systemic if the immune response is poor. Patient therapy depends on the type of infection; a cocktail of antifungal agents is necessary to aggressively treat cases of cryptococcal meningitis. Typically, the 'gold standard' antifungal drug, amphotericin B, is used in conjunction with another agent such as 5-fluorocytosine or fluconazole. Not only do these drugs act synergistically, but the use of combination therapy reduces the chance of developing antifungal resistance. Recently, there have been several reports of recurrent var. *gattii* infection. One suggested reason for this is that the infecting strain undergoes rapid genetic change in response to the presence of the antifungal agents in a process referred to as microevolution, and this results in the development of resistance. In this study, isolates of var. *gattii* were treated with amphotericin B, fluconazole and/or 5-flucytosine, beginning at a level equalling the minimum inhibitory concentration (MIC) of the strain. Each successive generation was then subjected to sequentially higher levels of antifungal agents until the strain could grow at a concentration 100 times the MIC. This highly stressful environment was expected to promote the occurrence of microevolution. These isolates are now being analysed using three different techniques: 1) Random Amplification of Polymorphic DNA (RAPD) that may reveal small genomic changes in the form of variable banding patterns, 2) microsatellite analysis as microsatellites are inherently unstable and known to become more variable when mutation control breaks down, and 3) Pulsed-Field Gel Electrophoresis (PFGE) as gross changes to entire chromosomes are sometimes seen.

Keywords: *Cryptococcus neoformans* var. *gattii*, microevolution, drug resistance.

CRYPTOCOCCUS NEOFORMANS VAR. *GATTII* AS A MODEL SYSTEM TO STUDY DIFFERENTIAL GENE EXPRESSION IN EXPERIMENTAL INFECTION

*D. Wood**[1,3], *P. Zuccolotto*[1], *M. Lorenz*[1], *R. Malik*[4], *T. Sorrell*[1,2], *S. Dowd*[5] & *W. Meyer*[1,2]

[1] *Centre for Infectious Diseases and Microbiology, Molecular Mycology Laboratory, The University of Sydney at Westmead Hospital,* [2] *Department of Medicine,* [3] *Department of Microbiology,* [4] *Department of Veterinary Clinical Sciences,* [5] *Department of Cancer Medicine, The University of Sydney, Sydney, Australia.*

Cryptococcus neoformans is an encapsulated yeast which can cause pneumonia and meningitis. Putative virulence factors include encapsulation, growth at 37°C, melanin production, and extracellular products including phospholipase. The rat lung was used as a model system for infection, since primary infection is pulmonary. Differential display was chosen to identify upregulated or newly expressed genes, which may be important in *in vivo* survival and pulmonary pathogenesis. Rats were infected by intratracheal inoculation of 10^7 *C. neoformans* var. *gattii* cells. Lungs were harvested after six weeks, digested with collagenase, and cryptococci separated from the digested tissue by Ficoll gradient. RNA was extracted from these cells and differential display performed, identifying several differentially expressed gene fragments. These fragments were partially sequenced, and a GenBank homology search performed. Eleven of these fragments have been selected for further study. Six of them (based on GenBank homology) have been completely sequenced. These 11 fragments have been transformed into *E. coli* cells, and hybridisation studies will be performed to confirm their fungal origin. Full length genes will then be identified from a cDNA and/or genomic library, and their functions assessed. This study of differentially expressed genes during pulmonary infection will enhance the understanding of the pathogenesis of pulmonary cryptococcosis. The findings may be applicable to other fungal diseases and may serve as the basis for novel antifungal therapies targeting primary cryptococcosis.

Keywords: differential display, *Cryptococcus neoformans* variety *gattii*, pathogenesis.

CHARACTERISATION OF GENES THAT MAY BE IMPORTANT IN THE VIRULENCE OF *CRYPTOCOCCUS NEOFORMANS* VAR. *GATTII*

*P. Ruma Haynes**[1,2], *T. Sorrell*[1] & *A. Brownlee*[2]

[1] *Centre for Infectious Diseases and Microbiology, University of Sydney, Westmead Hospital, Westmead, NSW.*
[2] *CSIRO-Animal Production, Prospect, NSW.*

Cryptococcus neoformans is a pathogenic yeast-like fungus that causes a number of pulmonary and neurological diseases. Three varieties of *C. neoformans* exist, variety *neoformans* (Serotype D), variety *grubii* (Serotype A) and variety *gattii* (Serotype B and C) which differ in host preference, presentation of disease, morbidity and mortality of the infected host. These differences may be attributable to different virulence factors produced by the organism. Genes for several putative factors have already been characterised by other researchers but the search for other genes possibly involved in virulence was undertaken. These include two heat-shock protein genes (HSP-70 and HSP-90) which may regulate temperature tolerance, and a proteinase gene (Subtilisin-like serine proteinase) which may be involved in tissue disruption and dissemination of the organism. Complete sequences for the HSP genes and partial sequence for the Subtilase gene were obtained from variety *gattii* genomic DNA. Gene segments were amplified by PCR using degenerate primers designed from consensus protein sequences from other fungi, cloned and then sequenced. The remainder of each gene was obtained by PCR screening of an 'ARRAYED' genomic library of *C. neoformans* var. *gattii*. The genomic library yielded up to 7 kb of novel sequence for each gene of which approximately 4 kb was sequenced. Some comparative sequence and Southern hybridisation patterns between the three varieties were also obtained for the heat-shock protein genes. *In vitro* expression of the three genes were examined using reverse transcriptase PCR.

Keywords: *Cryptococcus neoformans*, characterisation, heat shock proteins, proteinases.

THE BALANCE BETWEEN SYMBIOSIS AND PATHOGENESIS IN *RHIZOCTONIA SOLANI*

E. Pope[1], P. McGee[2] & D. Carter[1]*

Department of Microbiology[1] & School of Biological Sciences[2], The University of Sydney, NSW 2006.

Rhizoctonia solani is an asexual, soilborne basidiomycetous fungus which is commonly recognised as a plant pathogen and saprophyte. In Australia, isolates belonging to anastomosis groups 6 and 12 also form a mycorrhizal relationship with the native orchid *Pterostylis acuminata*. In phylogenetic studies of the internal transcribed spacer (ITS) region of the rDNA unit in this fungus, the mycorrhizal isolates were distinct but positioned relatively closely to their pathogenic and saprophytic counterparts, suggesting that there are only minor differences between otherwise similar organisms. Additionally, virulence tests found the mycorrhizal isolates to cause varying levels of damage to a range of seedlings including lettuce, cauliflower and radish. This implies a fine balance between symbiosis and pathogenesis in these plant-fungus interactions, which offers us a unique opportunity to study differential gene expression in a single fungus undergoing a range of different associations. This study uses the PCR-based differential display technique to examine gene expression in *R. solani* when undergoing a symbiotic, saprophytic or pathogenic lifestyle.

Keywords: *Rhizoctonia solani*, plant interactions, gene expression.

TRANSFORMATION OF *ASPERGILLUS CARBONARIUS* BASED ON A HYGROMYCIN B RESISTANCE MARKER

Z. Ilic[1], J. Pitt[2] & D. Carter[1]*

[1] *Department of Microbiology, University of Sydney, NSW 2006.*
[2] *Food Science Australia, North Ryde, NSW 2113.*

Transformation systems have been recently developed for a number of filamentous fungi. The establishment of a transformation system in *Aspergillus carbonarius* will predominantly rely on the methods developed in the 1980s for *A. nidulans* and *A. niger* (a close relative of *A. carbonarius*). *Aspergillus carbonarius*, belonging to the family of black aspergilli, is a common producer of ochratoxin A (OA). Similarly to other mycotoxins, OA is very toxic and can be carcinogenic if ingested. It is therefore important to develop a transformation system that could be useful in studying the genetic and biochemical processes in this species of *Aspergillus*. Protoplasts prepared with lytic enzymes have been previously favoured as recipients in transformation experiments that use PEG/CaCL2 to promote entry of DNA into cells (Punt & van den Hondel 1992). A more recent technique has been developed whereby introduction of DNA into germinating conidiospores is aided by electroporation (Sanchez & Aguirre 1996; Weidner *et al.* 1998). Results show that the OA producing ability of *A. carbonarius* is not disrupted through the electroporation process, suggesting potential for this method in the investigation of genes responsible for OA production and regulation. Both techniques utilise the dominant selectable marker, hygromycin B resistance. Most filamentous fungi are sensitive to hygromicin B, thus viable transformants can easily be identified on hygromycin selective media. The relative transformation efficiencies of both techniques in *A. carbonarius* will be investigated.

Keywords: *Aspergillus carbonarius*, ochratoxin A, transformation systems, hygromycin B.

BIOLOGICAL CONTROL OF AFLATOXIGENIC *ASPERGILLUS* SPP. USING MOLECULAR APPROACHES

N. Tran-dinh[1], J. Pitt*[2] & D. Carter*[1]

[1] *Department of Microbiology, University of Sydney, NSW 2006.*
[2] *Food Science Australia, North Ryde, NSW 2113.*

Aspergillus flavus and *Aspergillus parasiticus* are perennial contaminants of Australian peanuts and are both able to produce a secondary metabolite known as aflatoxin. Aflatoxin is among the most potent hepatocarcinogenic natural substances and causes considerable loss for the Australian peanut industry, as well as posing a serious health risk. At present the techniques used to control aflatoxin contamination are expensive and unreliable. A possible solution is to use nontoxigenic strains as biological control agents to prevent aflatoxin contamination during crop growth. The introduction of biological control strains requires an understanding of the relationship between toxigenic and nontoxigenic strains and a means of identifying the introduced isolates. Randomly Amplified Polymorphic DNA (RAPD) has been used to investigate the former. Microsatellite markers can be used to identify strains to the individual level. Microsatellites are short, tandemly repeated simple sequences, that are widely dispersed in eukaryotic genomes. They are highly polymorphic due to variability in the number of tandem repeats and are useful molecular markers because they are PCR-based and can be unambiguously scored. Three variable microsatellite markers have been found and will be used to 1) type individual isolates, 2) provide information on the genetic diversity of field isolates and, 3) study the plant/fungus relationship.

Keywords: biological control, microsatellites, *Aspergillus flavus*, *Aspergillus parasiticus*.

THE IDENTIFICATION AND QUANTIFICATION OF AIRBORNE ALLERGENIC FUNGAL SPORES BY QUANTITATIVE PCR

G. Gaskell[1,3], D. Carter*[2], W. Britton*[3], E. Tovey*[1,3], F. Benyon*[4] & U. Løvborg*[1]

[1] *Institute of Respiratory Medicine, University of Sydney,* [2] *Department of Microbiology, University of Sydney,* [3] *Department of Medicine, University of Sydney,* [4] *Department of Crop Sciences, University of Sydney, NSW 2006.*

As part of a study designed to identify and quantify allergen sources present in the environment of allergy sufferers, we developed genus- and species-specific oligonucleotide probes to use as tools in a spore identification and quantification assay for the common aeroallergenic fungal genera: *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*. These probes were designed from the internal transcribed spacer (ITS) regions of the rDNA repeat unit. A total of 83 isolates representing common airborne species from these genera were obtained. DNA from these isolates was extracted and amplified using the rDNA primers ITS1 and ITS4. Dot-blot hybridisations of the amplified DNA with the oligonucleotide probes found the probes to be highly specific with no or negligible cross-hybridisation. Using these probes we are developing a quantitative PCR assay for the fungi: *Alternaria alternata*, *Aspergillus flavus*, *Cladosporium herbarum* and *Penicillium chrysogenum*. In this assay, genomic DNA is extracted from samples of fungal spores, the ITS regions are amplified with primers ITS1 and ITS4, aliquots of the PCR reactions are removed at successive cycles and then dot-blotted onto a nylon membrane for subsequent oligonucleotide probe hybridisation. A set of external standards comprising known numbers of ITS molecules is concurrently amplified with aliquots removed and dot-blotted along with the samples. By comparing the appearance of hybridisation signals between samples and external standards, the amount of DNA and thus the number of spores in the original samples can be estimated. Using this assay, we have been able to detect as little as 10 spores of *Alternaria alternata*, *C. herbarum* and *P. chrysogenum*. The final goal will be to perform this technique on fungal spores trapped in an adhesive support system, which is part of environmental air sampling systems.

ADAPTIVE INSENSITIVITY TO ARSENATE IN POPULATIONS OF *HYMENOSCYPHUS ERICAE*

J. Sharples[*1,2], *A. Meharg*[2], *S. Chambers*[1] *& J. Cairney*[1]

[1] *Mycorrhiza Research Group, School of Science, University of Western Sydney (Nepean), PO Box 10, Kingswood, NSW 2747, Australia.*
[2] *Institute of Terrestrial Ecology, Monkswood, Huntingdon, Cambridgeshire PE17 2LS, UK.*

Calluna vulgaris is found growing on arsenic mines in the south-west of England in association with its ericoid mycorrhizal symbiont *Hymenoscyphus ericae. Hymenoscyphus ericae* mycelia (identified by ITS-RFLP analysis) were isolated from arsenic-contaminated mines and screened for tolerance to arsenate (the dominant form of soil arsenic). Results indicated an adapted tolerance to arsenate in the mine site population when compared to *H. ericae* isolated from uncontaminated areas. Arsenate is a phosphate analogue and is taken up by the high affinity phosphate uptake system. We hypothesized that arsenate tolerance was due to suppression of the high affinity phosphate uptake system, as has previously been shown in higher plants. Michaelis menten kinetics, however, showed that there was no difference in arsenate uptake between tolerant and non-tolerant *H. ericae* isolates. An extremely efficient arsenite efflux mechanism was found to be operating in the arsenate-tolerant mine populations of *H. ericae*. The diversity of ericoid mycorrhizal endophytes of *C. vulgaris* at the mine sites has also been investigated using ITS-RFLP and ITS sequence analyses. These data will also be discussed in relation to differential arsenate tolerance.

Keywords: arsenate, insensitivity, adaptation, uptake, *Hymenoscyphus ericae*.

A PRELIMINARY INVESTIGATION OF THE PHYLOGENY OF *GYMNOPILUS* AND RELATED GENERA USING SEQUENCE DATA FROM THE INTERNAL TRANSCRIBED SPACER (ITS) REGION OF RIBOSOMAL DNA

B. Rees[1], *D. Orlovich*[*2] *& G. Zuccarello*[1]

[1] *School of Biological Science, University of New South Wales, Sydney, NSW 2052, Australia.*
[2] *Department of Botany, University of Otago, Dunedin, New Zealand.*

A recent study of *Gymnopilus* has identified 40 species in Australia, including 26 new taxa. Most Australian *Gymnopilus* are separate and distinct from northern hemisphere species. However, several are thought to be closely related on morphological grounds. In some cases, these species may be conspecific with northern hemisphere species. Argument regarding the inclusion of *Gymnopilus* and related saprotrophic genera in the Cortinariaceae or Strophariaceae on the basis of morphological or chemical grounds has long remained unresolved. To discover the phylogenetic relationships between species of *Gymnpilus* and related genera, we have sequenced the internal transcribed spacer (ITS) region of ribosomal DNA from a range of Australian *Gymnopilus* species and representative samples of related genera using fresh or dried fruit bodies. A phylogeny was determined for the species by parsimony analysis of aligned sequences. Outgroup taxa were selected from the Cortinariaceae, Strophariaceae and Crepidotaceae. Results obtained so far have shed light not only on the inter-relatedness of *Gymnopilus* species in Australia, but also on their affiliations with similar *Gymnopilus* species from northern Europe. Cladistic analysis of the ITS dataset has shown *Gymnopilus* to be monophyletic only when *Galerina eucalyptorum* is included. Within *Gymnopilus*, several clades were well-supported. *Gymnopilus junonius* and *Gymnopilus pampeanus* were found to be sister taxa as were *Gymnopilus picreus* and *Gymnopilus austropicreus*. By including representative species of several related genera and families, we have shown that a closer affinity exists between *Gymnopilus* and other members of the Cortinariaceae than with those genera of the Strophariaceae which produce the same pigments. Also *Galerina eucalyptorum* has greater affinity with small-statured *Gymnopilus* species than with other *Galerina* species. In a genus comprising over 150 species, for which few infrageneric groupings exist, the results of the phylogenetic analysis conform closely to groupings of species arrived at on morphologicala grounds.

Key words: *Gymnopilus*, Cortinariaceae, Strophariaceae, phylogeny, ITS region.

PHIALOPHORA

W. Gams

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Phialophora is a little-differentiated genus of more or less pigmented, phialidic hyphomycetes and, with the addition of numerous species, it has become highly polyphyletic. It comprises anamorphs of both discomycetes and pyrenomycetes. The core of the genus belongs to the Chaetothyriales, Herpotrichiellaceae and comprises anamorphs of *Capronia.* Some taxa have already been segregated from *Phialophora* into genera like *Lecythophora* (Coniochaetaceae) and *Phaeoacremonium* (Magnaporthaceae?). Ascomycete orders in which *Phialophora*-like fungi have been placed are reviewed, and some further segregations are proposed. For anamorphs of the discomycete family Dermateaceae the old generic name *Cadophora* is available which should be used for *Ph. fastigiata* and related species (-aggregates). For anamorphs of *Gaeumannomyces* and *Magnaporthe* (Magnaporthaceae) a new genus is being proposed. Criteria that allow a correlation with the suggested broad-scale subdivision are outlined.

DETERRENCE OF HERBIVORY BY ENDOPHYTES OF COTTON

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The role of endophytic fungi is debated widely. It is now accepted that most plants are colonised by endophytes, and that in their native habitats, the plants may house a suite of specific endophytes. Where plants are outside their native habitats, however, the colonists are not specific, yet exist without any apparent rejection by their host. The role of endophytic fungi in cotton in eastern NSW was examined. More than 2,000 fungi were isolated from cotton leaves of various ages collected from three sites in eastern Australia. Selected fungi were used in two tests to determine their potential influence on herbivory by the commercially important insect *Helicoverpa armigera.* Methanol extracts of some fungi were found to slow the rate of growth of second and third stage instars when the insects were grown on a medium normally used for culturing the insects. When inoculated into leaves of cotton, some fungi influenced the feeding behaviour of third stage instars. Taken together, the data support the view that endophytic fungi influence the behaviour of herbivorous insects.

Keywords: endophyte, herbivory, cotton, *Helicoverpa armigera.*

VALUE AND RELIABILITY OF RECORDS OF AUSTRALIAN FUNGI FROM THE FUNGIMAP SCHEME

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FUNGIMAP is a scheme to rapidly improve knowledge of the distribution of selected species of Australian fungi. Information is received from an Australia-wide network of volunteer recorders. To June 1999 some 4,145 records have been received of the 100 target species, with almost 400 people receiving the regular *FUNGIMAP Newsletter.* The *Newsletter* and information on target species are also provided on the FUNGIMAP web page (http://calcite.apana.org.au/fungimap/). The records received through FUNGIMAP significantly expand the distribution and habitat preferences of many of the target species in comparison to available information from herbarium material or published records. The 100 target species have been chosen so as to be readily recognisable in the field (such as *Mycena interrupta* and *Amanita muscaria*), and specimens are not collected. However, approximately 8% of the records are supported by photographs, and from this sample a very low error rate has been detected. Most incorrect identifications are flagged as doubtful by the recorders. Data on batches of records, and individual records are stored in a database. Recorders are encouraged to supply geographic coordinates (latitude/longitude or Australian Mapping Grid references) with their records, but processing and entry of this type of data remains the most time consuming part of maintaining the records database. In addition

to the recording itself, there is a significant contribution to the scheme by volunteers in databasing, administration and organisation. The scheme commenced with eight, and then 50 target species, which were mostly relatively common fungi, so as to ensure that recorders had a reasonable chance of encountering targets. The scheme has recently been expanded to cover 100 target species. Some of the additional targets are thought to be rare. When allowance is made for the visibility of the different species, the relative number of records is a valuable initial indication of the relative rarity of species.

Keywords: fungi distribution, mapping, rarity, community involvement.

AUSTRALIAN BOLETES

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The Australian bolete mycota is extremely rich with almost 250 tubulate and lamellate members now recorded; it will undoubtedly be in excess of 300 species as more work is carried out. Tropical, endemic and introduced species will be identified in the mycota and a discussion as to the origins or at least the distributions of some of the more important genera offered. Some distinctive species will be discussed in more detail.

TAXONOMY AND POPULATION BIOLOGY OF SOUTH-EASTERN AUSTRALIAN *PISOLITHUS* SPECIES

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We have investigated genetic variation of the ectomycorrhizal basidiomycete genus *Pisolithus* in south-eastern Australia. Internal transcribed spacer (ITS) restriction fragment length polymorphism (RFLP) and ITS sequence analyses indicate that at least three species exist in NSW, Australia. Population biology of one of the *Pisolithus* species (type I) has been investigated over a two year period at a field site in NSW using microsatellite-primed PCR with the three degenerate primers 5'BDB(ACA)5, 5'DDB(CCA)5 and 5'DHB(CGA)5 [where B = C, G or T; D = A, G or T; >H = A, C or T]. The population at the site comprises a number of mycelial genets of variable size. The largest of the 33 genets identified in the first year of the study had a diameter of at least 30 m, while most other genets were generally <4 m in diameter. A similar picture was obtained during the second year with 21 genets identified (including the largest genet from the first year of the study). The largest of these genets had a diameter of 8.5 m, while four other genets were 4–5 m in diameter. All other genets were <4 m in diameter. The data will be discussed in relation to the ecology of the *Pisolithus* genus.

Keywords: *Pisolithus*, genetic variation, population dynamics.

GENERIC LIMITS OF THE SEQUESTRATE RELATIVES OF *RUSSULA*

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Generic limits within the sequestrate relatives of *Russula* were for the most part based on northern hemisphere species. The limits between these genera are indistinct as they form a morphological gradient from sequestrate to mushroom-like. Investigation of abundant collections from Australia and New Zealand and a re-examination of holotype material, have enabled a re-evaluation of these boundaries using cladistic methods. Various morphological characters traditionally used to distinguish genera will be discussed and some new characters proposed. Proposed realignments include i) merging *Martellia* into *Gymnomyces*; ii) merging *Elasmomyces* into

Macowanites; iii) redefining *Gymnomyces* to exclude species with an epithelial peridiopellis; and iv) expanding *Cystangium* to include astipitate species.

Keywords: taxonomy, truffle-like fungi, *Russula*.

THE EFFECT OF WILDFIRE ON THE FRUITING OF MACROFUNGI IN KARRI REGROWTH FORESTS IN THE SOUTH-WEST OF WESTERN AUSTRALIA

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On Christmas Day 1997, a wildfire swept through a large tract of 20–25-year-old karri regrowth forest in the south-west of Western Australia. Immediately following this fire, plots were established in the burnt stands and in similarly aged unburnt stands. During 1998, the number and species of fungal fruitbodies occurring in the plots were recorded. Results showed a distinct mycoflora fruited on recently burnt sites. A total of 249 species were recorded in the first year of the study. Ninety-two species were recorded on the burnt and 147 on the unburnt plots. Nine species were recorded on both burnt and unburnt plots. Species diversity was at its maximum in the autumn, and fruitbody production peaked in both autumn and spring on the burnt plots and in the autumn on the unburnt plots. On the burnt sites, *Polyporus mylittae*, *P. tumulus* and *Neolentinus dactyloides*, responded by developing large fruitbodies from subterranean sclerotia within days following the fire. Ascomycetes then dominated the fruiting on the burnt plots. Several species of *Peziza* and several Discomycetes, including *Peziza tenacella* and *Anthrocobia muelleri*, fruited in large numbers in the autumn and spring. A mass fruiting of *Morchella elata* also occurred in the spring. The high species diversity recorded on the unburnt plots can be attributed to litter-decaying fungi. For example, 34 species of *Mycena* were (tentatively) recorded fruiting on leaf and twig litter. In addition, several common genera of mycorrhizal fungi, including ten species of *Cortinarius* and seven species of *Russula*, recorded on the unburnt plots were not present on the burnt plots.

INVESTIGATION OF SURVEY METHODS AND MACROFUNGI COMMUNITIES AFTER FIRE AND LOGGING IN *EUCALYPTUS REGNANS* FOREST

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Study sites in *Eucalyptus regnans*-dominated forests, in the Eastern Central Highlands of Victoria, were used to study the succession of macrofungi after forest activities and fire. Data from two survey methods were used for analysis; these were seasonal intensive surveys of marked plots and monthly short surveys of the sites. Different age classes were used to compare communities at different times since disturbance, the classes were 0, 2, 4, 7, 13 and 54 years since fire. Macrofungal surveys were carried out from April 1996 to January 1997, and 124 macrofungal taxa were identified. Use of 'field taxa' facilitated the macrofungal surveys. Of the macrofungi found, about 50% were named to species level; the rest were grouped as field taxa. Recommendations of methods for future macrofungal community studies are made. Pattern analysis through classification and ordination showed that there was a distinct change in the macrofungal community over time since disturbance. The change in the suite of macrofungi reflected the changes in macrofungal microhabitats in the forests of different ages. The macrofungi from the 1996 age class were the most distinctive from the other age classes.

Keywords: macrofungi, *Eucalyptus regnans*, fire disturbance, survey techniques, community ecology.

POSTERS

MOLECULAR DETERMINATION OF DIVERSITY IN ERICOID MYCORRHIZAL ENDOPHYTES FROM *WOOLLSIA PUNGENS* (CAV.) F. MUELL. (EPACRIDACEAE)

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One hundred and sixty-eight sterile endophytic mycelia were isolated from roots of four *Woollsia pungens* (Cav.) F. Muell (Epacridaceae) plants collected from a field site in New South Wales, Australia. All isolates formed typical ericoid mycorrhizal structures when inoculated onto roots of *Vaccinium macrocarpon* (Ericaceae). Microsatellite-primed PCR fingerprints generated using the primers (GTG)5 and (GACA)4 indicated that considerable genetic diversity exists within the endophyte population. It was estimated that a minimum of 43 genetically distinct mycelial genets were present in the root systems of the sampled *W. pungens* population, with most genets confined to individual plants. Two genets, however, were present within the root systems of two adjacent plants. While most genets were represented by less than eight isolates, three genets contained up to 41 isolates, suggesting that root system colonisation by some endophytic mycelia might be extensive. ITS-RFLP analysis and ITS sequencing were used to further characterise the mycorrhizal endophyte community. These data suggested that six distinct taxa were present in the root systems of the four *W. pungens* plants and that up to four of these were present in the root system of a single plant. One of the endophytes was identified by sequence comparison as an *Oidiodendron* species, while neighbour-joining and parsimony analyses indicated that most other isolates form a discrete clade in the order Leotiales. These data will be compared with recently obtained molecular data for endophytes from a second epacrid plant species *Epacris microphylla* R. Br.

Keywords: ericoid mycorrhizal fungi, *Oidiodendron*, internal transcribed (ITS) sequences, ITS-RFLP, microsatellite-primed PCR.

GENET DISTRIBUTION AND GENETIC VARIATION OF *CORTINARIUS ROTUNDISPORUS* (CLEL. & CHEEL) HORAK & WOOD IN EASTERN AUSTRALIAN SCLEROPHYLL FORESTS

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The size and distribution of the ectomycorrhizal basidiomycete *Cortinarius rotundisporus* (Clel. & Cheel) Horak & Wood genets at three sclerophyll forest field sites in New South Wales, Australia have been estimated using microsatellite-primed PCR (MS-PCR) of DNA extracted from sporocarp tissue. MS-PCR fingerprints generated using the primers (GTG)5 and (GACA)4 indicated that two to five genets were present at each site, with each site being characterised by a single large genet (9–30 m diam.). Analysis of ITS-RFLP patterns from individual sporocarps used in the study suggested that three distinct RFLP types were present in the sampled *C. rotundisporus* population. ITS sequence data indicate that the three RFLP types had <88.4% sequence identity to each other, strongly suggesting that *C. rotundisporus* (Clel. & Cheel) Horak &Wood represents a complex of three species. Results will be discussed in the context of *Cortinarius* ecology.

Keywords: *Cortinarius rotundisporus*, ectomycorrhizal fungi, microsatellite-primed PCR, internal transcribed (ITS) sequences, ITS-RFLP.

CRYPTOCOCCUS NEOFORMANS VAR. *GATTII* AS A MODEL SYSTEM TO STUDY DIFFERENTIAL GENE EXPRESSION IN EXPERIMENTAL INFECTION

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The basidiomycetous, encapsulated yeast *C. neoformans* was chosen as a model system to study virulence genes differentially expressed during rat lung infection because it causes one of the most prevalent life-threatening mycoses. Virulence factors putatively established for *C. neoformans* include: capsular formation, growth at 37°C, and melanin and phospholipase production. We chose differential display as an approach to identify potential virulence genes which are regulated by exposure to the *in vivo* environment of the rat lung, representing the conditions that would be encountered during primary infection of a host with *C. neoformans*. Rats were infected by intratracheal inoculation of 10^7 cryptococci. Lungs containing localised cryptococcomata or a diffuse infection were harvested after 6 weeks. Prior to RNA extraction, the infected lung tissue was digested with collagenase, fungal cells were separated from contaminating mammalian cells via Ficoll gradient centrifugation. Differential display of the lung-derived fungal RNA showed negligible contamination with mammalian RNA, when compared with control RNA from non-infected lung tissue. Several gene fragments expressed *in vivo* were identified by differential display, subsequently isolated and partially sequenced. Six of these gene fragments, which may represent potential virulence genes (selected after a homology search at GenBank), were completely sequenced. RT-PCR was carried out using primers based on these sequences to confirm their fungal origin. The identification of full length genes from a cDNA and/or genomic library is in progress and the importance of their functions will be assessed. The identification of virulence genes expressed during cryptococcal infection will provide an insight into the pathogenesis of cryptococcosis and other fungal diseases. It may also lead to the development of new antifungal therapies.

CHARACTERISTICS OF EXTRACELLULAR CRYPTOCOCCAL PHOSPHOLIPASES

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Cryptococcus neoformans var. *neoformans* is an important opportunistic pathogen in patients with AIDS and is the most common cause of fungal meningitis worldwide. Its ability to establish infection in the host has been attributed to its large polysaccharide capsule, melanin production and growth at 37°C. More recently in our laboratory, extracellular phospholipase activities [phospholipase B (PLB), lysophospholipase (LPL) and transacylase (LPTA)] have been implicated in the virulence of this organism. The aims of this study were to characterise the phospholipase activities in their extracellular milieu ('natural') with respect to pH, temperature stability and substrate specificity and compare these to the characterisics of purified enzyme(s). Natural phospholipase preparations were most active between 25–40°C in acidic pH environments (pH 3–5.5) and stable at temperatures up to 60°C. Bovine lung surfactant and its major lipid components, disaturated phosphatidylcholine and phosphatidylglycerol were the optimal substrates for PLB. While lysophosphatidylcholine was the favoured substrate for LPL and LPTA activities. Purified phospholipase(s) were slightly less heat stable, being active at temperatures ≤42°C and exhibited a narrower pH optima also in the acidic range. The effect of cations differed from that of the natural enzyme where activity is independent of cation presence. Extracellular phospholipase(s) were modified by Triton X-100, and palmitoyl carnitine was a potent inhibitor of all enzyme activities. Effects of other inhibitors examined were similar in both cases, and the only other apparent difference was the substrate concentration requirements for the natural and purified PLB. The ability of extracellular cryptococcal phospholipase(s) to be active at physiological temperatures, degrade lung surfactant and its major lipid species support their role as a virulence factor of *C. neoformans*.

DEVELOPMENT OF AN ELISA FOR DETECTION OF CRYPTOCOCCAL PHOSPHOLIPASE ANTIBODIES

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Extracellular phospholipase(s) have recently gained attention as a new virulence determinant of *Cryptococcus neoformans.* In order to investigate their role in disease development in detail we sort to produce a monoclonal antibody by producing an antibody secreting cell line. An integral part of monoclonal antibody production is the screening of hybridoma supernatants for clones producing the antibody of interest. The screening procedure used at this step must be reliable, sensitive, rapid, have minimal cross reactivity with other antigens and enable a large number of samples to be tested simultaneously. Enzyme-linked immunoabsorbent assay (ELISA) is a commonly used technique as it is versitile and can be adapted to individual needs. There are essentially six main steps involved in a direct ELISA and 50% of these require optimisation for indiviual purposes.

INVESTIGATION OF A 'CRYPTIC SPECIES' WITHIN *ASPERGILLUS FLAVUS* USING MOLECULAR TECHNIQUES

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Aspergillus flavus is a conidial fungus which belongs to the *Aspergillus* section *flavi. Aspergillus flavus* is the major producer of aflatoxins, which are potent carcinogens, and the fungus can grow on and contaminate food crops such as maize, groundnuts and treenuts. Tran-Dinh *et al.* (*Mycological Research,* in press) using RAPD profiles analysed by neighbour joining, and Geiser *et al.* (*Proceedings of the National Academy of the United States of America* 95: 393–399, 1998) using DNA sequence polymorphisms, found that a collection of *A. flavus* isolates divided into two distinct groups, or cryptic species. In both studies one group (Group 1) was larger and also contained *A. oryzae.* Further analysis of the smaller group (Group 2) found it to also differ from Group 1 in morphology and in the production of aflatoxin; all Group 2 isolates appeared capable of producing both blue (B) and green (G) aflatoxins and cyclopiazonic acid (CPA). Normally, *A. flavus* can only produce B aflatoxin and CPA. Although the analysis by Tran-Dinh *et al.* found the two groups appeared to be nearly as distinct from one another as *A. flavus* was from *A. parasiticus*, phylogenetic analysis based on sequencing of the Internal Transcribed Spacer (ITS) region of the rRNA gene could not distinguish between them. In *A. flavus*, the ITS region is very short and appears to be highly conserved, and we are therefore investigating additional genes which may be more polymorphic. The outcome of this investigation may allow the resolution of a new species of *Aspergillus.*

Keywords: *Aspergillus flavus*, *Aspergillus oryzae*, polymorphism, aflatoxins, cyclopiaxonic acid.

SMUT FUNGI OF NEW ZEALAND

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A mycoflora describing and illustrating smut fungi known from New Zealand is in preparation. It will include at least 82 species (in 19 genera), although critical examination and revision of some species, *e.g.*, the heterogeneous collective species *Anthracoidea caricis* and *Entyloma dactylidis* on numerous *Carex* and grass hosts, respectively, may increase this number. Approximately 59% of the smut species are considered to be introduced, 28% indigenous, and 13% endemic. Many of the indigenous species are also found in Australia, while a few also occur in South America, Europe, South Africa, or Asia. Five indigenous species are of cosmopolitan distribution. *Entorrhiza scirpicola* is found in Europe, and on both indigenous and introduced *Juncus* species throughout New Zealand, including the remote outlying Snares Islands. The first smuts from New Zealand were recorded by M.J. Berkeley in 1855 and included 2 new species—the Australasian smut, *Farysia endotricha* (as *Ustilago endotricha)*, and the cosmopolitan grass smut, *U. bullata*. Most of the smuts known from New Zealand have been recorded following surveys of plant diseases. The only taxonomic compilation of New Zealand smuts is that of G.H. Cunningham in 1924 (+ additions 1926–1945). However, of the 41 species that are included only 42% are recorded under the names that are accepted today. Two smut genera have been studied in some detail. Cunningham revised the New Zealand species of *Farysia* in 1945, but those species on *Carex* are in need of further revision. The root-inhabiting *Entorrhiza* species on sedges were revised and studied ultrastructurally by J.M. and B. Fineran in 1978 and 1992.

Keywords: mycoflora, New Zealand, smuts, Ustilaginales.

THE *ARMILLARIA* SPECIES OF NEW ZEALAND (FUNGI: AGARICALES)

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The genus *Armillaria* (Fr.: Fr.) Staude in New Zealand is represented by three distinct morphological species, *A. limonea* (G. Stev.) Boesew., *A. hinnulea* Kile & Watling, and *A. novaezelandiae* (G. Stev.) Herink. Records of *A. mellea* (Vahl: Fr.) P. Kumm., *Armillaria* sp. *sensu* Hood, and *Armillariella elegans* (R. Heim) J.B. Taylor, Hawkins & McLaren are rejected. *Armillaria novaezelandiae* is abundant all over New Zealand and the Chatham Islands, whereas *A. limonea* is abundant only in the North Island and northern South Island. *Armillaria hinnulea* is restricted to the nor-western South Island and may represent a recent introduction from Australia.