SCIENTIFIC MEETING OF THE AUSTRALASIAN MYCOLOGICAL SOCIETY

CANBERRA 14-16 JULY 2015

PROGRAM AND ABSTRACT BOOK

Scientific Meeting of the Australasian Mycological Society 14-16th July 2015

Dear conference delegate,

Welcome to Canberra and the **2015 Australasian Mycological Society Scientific Meeting, to be held jointly with the Australian Society for Microbiology Scientific Meeting**. The AMS scientific program includes two outstanding plenary speakers in Professor Judith Berman from the Department of Molecular Microbiology and Biotechnology, Tel Aviv University, and Professor Adrienne Hardham from the Plant Pathogen Laboratory, Australian National University. Symposiums cover a wide variety of areas including Animal/Plant-Fungal Interaction, Ecosystem Health, Omics and Systems Biology, Molecular Mycology, Fungal Ecology and Systematics, and Applied Mycology. We hope you find these sessions interesting, beneficial and enjoyable. Throughout the conference we also hope you find the opportunity to attend a free fungal skills workshop, reacquaint with colleagues and form new research partnerships. We also invite you to attend a pre-conference foray to the Li-Sun mushroom tunnel at Mittagong (http://www.li-sunexoticmushrooms.com.au/) on Tuesday 14th July, and the conference dinner at Boffin's restaurant, ANU, on Wednesday 15th July at 7.00 pm. We would particularly like to thank the ANU for providing an exceptional venue for Thursday of the meeting.

Julie Djordjevic (Conference Chair)
Leona Campbell
Dee Carter
John Dearnaley
Celeste Linde
Ana Traven

AMS 2015 conference committee

Cover design by John Dearnaley (*Marasmiellus candidus* growing on rainforest log)

id #25610

Proffered paper Wednesday

What will climate change mean for infectious disease? The soil perspective

Barbara Drigo [1] , Thomas Jeffries [1] , Brajesh Singh [1] , Anshu Garg [1], Catriona A Macdonald [1] , Yui Osanai [1] , Ian C Anderson [1]

[1]*Hawkesbury Institute for the Environment THE UNIVERSITY OF WESTERN SYDNEY - HAWKESBURY CAMPUS, Penrith, NSW, Australia*

Scientists have long predicted large-scale responses of infectious diseases to climate change, giving rise to a polarizing debate, especially concerning human pathogens for which socioeconomic drivers and control measures can limit the detection of climate-mediated changes. Climate change has already increased the occurrence of diseases in some natural and agricultural ecosystems, but in many cases, outcomes depend on the form of climate change and details of the host-pathogen system. Here, we describe how climate change will affect terrestrial ecosystems and their capacity to reduce infectious diseases. Rhizosphere and bulk soil was collected from grassland, forest and agricultural ecosystems at the Hawkesbury and EUC-FACE climate change field and greenhouse experiments in Western Sydney (Australia). Real-time PCR approaches targeting toxins-encoding genes revealed that elevated CO2 and rainfall patterns intensified the effect of warming by significantly increasing the virulence of soil-borne human pathogens associated with grassland and forest rhizosphere and bulk soils. As opposed to simply increasing the biomass of soil-borne pathogens at ambient CO2 under changes in rainfall patterns and temperature, elevated atmospheric CO2 strongly selected for virulent human pathogens and effected shifts in pathogens composition. 16S rRNA, 18S rRNA and ITS region sequencing with the Illumina Miseq platform revealed the dominance of several opportunistic and true human pathogens in the rhizosphere microbiome, including *E.coli 0157:H7, Enterobacteriacae, Chlamydia, Staphylococcus, Salmonella* and *Clostridium* species. The potential mechanisms involved in the interplay between human pathogens in the rhizosphere microbiome are presented in a bioclimatic model of relative microbial abundance that specifically incorporates interactions between biological units.

id #25592

Proffered paper Wednesday

Copper (II) Lead (II) and Zinc (II) inhibit the growth, reproduction and rate of attachment to organic substrates of four zoosporic fungi species from soils of NSW

Linda E Henderson[1], Osu Lilje[1], Frank H Gleason[1]

[1]*Biological Sciences, University of Sydney, Sydney, NSW, Australia*

Zoosporic true fungi (chytrids) are widely distributed in soils. They reproduce by motile spores (zoospores), which attach to, and grow saprotrophically on many substrates of plant and animal origin, such as pollen, keratin and chitin (Sparrow 1960). Here we investigate the *in vitro* effects of soluble Copper (II), Lead (II) and Zinc (II) on the zoosporic true fungi species: *Rhizophlyctis rosea* (A13), *Terramyces sp.* (A3) and *Chytriomyces hyalinus* (A14) from soils of the Sydney Basin and Central coast regions and *Gaertneriomyces* (Mar-CC2) from a soil of north-western NSW. The growth, zoospore production and attachment of all isolates showed toxicity to soluble metals in the following order; Cu>Zn>Pb. All isolates showed significant reduction in growth at 60 ppm (0.94 mmol m^{-3}) for Cu, three declined significantly at 60 ppm (0.92 mmol m^{-3}) Zn and two declined significantly at 100 ppm (0.48 mmol m^{-3}) Pb. All isolates showed reduced zoospore production when grown in solid PYG media with 60 ppm Cu, three isolates declined in zoospore production at 60 ppm Zn and three at 100 ppm Pb. Two isolates did not recover growth after incubation in 60 ppm Cu, while all isolates recovered growth after incubation in solid media with 60 ppm Zn or 100 ppm Pb. If these metals cause similar effects in the field, Cu, Pb and Zn contamination of NSW soils is likely to reduce the biomass of zoosporic true fungi and reduce attachment to organic materials, thereby reducing the rate of mineralisation of soil organic matter.

id #25656

Selected from Abstract: Host-pathogen Interaction Wednesday

Endophytic pathogens, water stress and dieback in an invasive tree (25656)

Tracey V Steinrucken [1 2] , Jeff Powell [1] , Andrew Bissett [3] , Anil K.H. Raghavendra [2] , Rieks D van Klinken [2]

[1]*University of Western Sydney, Penrith, NSW, Australia*
[2]*Biosecurity Flagship, CSIRO, Brisbane, Queensland, Australia*
[3]*Agriculture Flagship, CSIRO, Canberra, ACT, Australia*

Dieback is prevalent in many populations of invasive woody weeds globally. There are many preventative and contributing biotic and abiotic factors related to dieback occurrence[1]. Previous dieback studies have focused on specific potential causative biotic agents, but the majority remain unexplained. *Parkinsonia aculeata* L. (parkinsonia), an invasive tree in northern Australia. It has naturalised across a wide range of climatic zones in northern Australia[2], many of which experience long periods of extensive drought interrupted by extreme rain events. Parkinsonia dieback has been observed in both drought-affected and regions prone to flooding[3] but little is known about whether or not water availability has a role to play in dieback occurrence. In a glasshouse trial we tested the interactive effects of water availability and fungal inoculation on growth and pathogenesis of parkinsonia. We sampled roots, stems and stem tips from healthy and dieback-affected parkinsonia from northern Queensland. Fungal isolates from the samples were cultured and identified via ITS sequencing. A number were identified as known tree pathogens, some of which are associated with dieback in other host species. Eight of these were selected for a 10-week glasshouse pathogenicity trial on 1 year-old parkinsonia seedlings. Seedlings were also subjected to three different water treatments to simulate drought, normal water availability and inundation. We observed lesion formation and gummosis in parkinsonia inoculated with *Pestalotiopsis mangiferae*, *P. clavispora*, *Lasiodiplodia*

pseudotheobromae and *Botryosphaeria dothidea* and satisfied Koch's Postulates, however we did not observe systemic infection, typical of dieback. Normal and inundated treatments were associated with larger lesion formation and increased gummosis. Lower water levels were also correlated to less plant growth, as indicated by plant height, dry weight and stem girth. Parkinsonia dieback is a complex phenomenon, occasionally attributed to specific fungal pathogens[4]. We have shown, however, that water availability and subsequent plant stress contributes to overall plant health and therefore susceptibility to infection by pathogens.

id #25071

Selected from abstract: Host-Pathogen Interaction Wednesday

Fungal inositol pyrophosphate IP_7 is crucial for host-pathogen interaction and virulence

Sophia Lev[1], Cecilia Li[1], Desmarini Desmarini[1], Adolfo Saiardi[2], Tania C Sorrell[3], Julianne T Djordjevic[1]

[1]*Centre for Infectious Diseases and Microbiology, The Westmead Millennium Institute for Medical Research, Sydney Medical School, University of Sydney at Westmead Hospital, Westmead, NSW, Australia*

[2]*Medical Research Council Laboratory for Molecular Cell Biology, University College London, London, UK*

[3]*Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, NSW, Australia*

Inositol molecules phosphorylated with mono- and di-phosphates (IPs and PP-IPs respectively) perform multiple key functions in eukaryotes. However, their role in fungal pathogens has never been addressed. We investigate IP/PP-IP biosynthesis pathway in a model pathogenic fungus, *Cryptococcus neoformans,* which causes life-threatening meningoencephalitis. Phenotypic analysis of IP and PP-IP-deficient mutants combined with gene expression profiling identifies IP_7 ($PP\text{-}IP_5$) generated by the IP_6 kinase Kcs1 as a key signaling molecule in *C. neoformans*. Absence of this crucial metabolite affects cell wall integrity, melanization, mating and virulence in wax moth and murine models of cryptococcosis. IP_7-deficient (*Δkcs1* mutant) cells fail to be recognized and internalized by monocytic THP1 cells and blood-derived monocytes, as compared to WT *C. neoformans*. At the gene expression level, the absence of IP_7 leads to elevated expression of protein biosynthesis machinery and reduced expression of genes encoding enzymes of citric acid and glyoxylate cycles, fatty acid β-oxidation pathway and gluconeogenesis. Furthermore, expression of multiple genes encoding transmembranal proteins, particularly sugar transporters, and secreted proteolytic enzymes is lower in *Δkcs1*, as compared to wild type. Taken together, our findings establish Kcs1-generated IP_7 as a major regulator of cellular metabolism affecting nutrient acquisition and surface properties of the fungal cell, and therefore crucially important for host-fungus interaction and virulence.

id #25288

Proffered paper Wednesday

Phosphate acquisition strategies in *Cryptococcus neoformans*

Desmarini Desmarini[1], Sophia Lev[1], Yong-Sun Bahn[2], Julianne Djordjevic[1]

[1]Centre for Infectious Diseases and Microbiology, The Westmead Millennium Institute for Medical Research, Sydney Medical School, University of Sydney at Westmead 2145, New South Wales Australia;

[2]Dept. of Biotechnology College of Life Science and Biotechnology, Yonsei University, Seoul 120-749 Republic of Korea

Phosphorus in a form of phosphate (P_i) is an essential nutrient for all eukaryotes including fungi. For fungi to become successful pathogens, they must acquire P_i from the host environment via enzyme-mediated hydrolysis of P_i-containing molecules and/or by using their own P_i more efficiently. We previously demonstrated that the acid phosphatase, Aph1, is produced and secreted when P_i is deficient, and contributes to the virulence of the human fungal pathogen *Cryptococcus neoformans.* This suggests that the fungus encounters and responds to a P_i-limited host environment. By screening a *C. neoformans* transcription factor knockout library (strain H99 serotype A) for mutants defective in Aph1-secretion during P_i deprivation, we identified a basic helix-loop-helix transcription factor, Hlh3 (CNAG_06751), as the sole regulator of known (*APH1* and P_i-transporters) and novel (e.g. *APH3* and *BTA1*) P_i-responsive cryptococcal genes. By tagging Hlh3 with the mCherry fluorescent protein, we confirmed that Hlh3 is indeed P_i-responsive, translocating from the cytosol to nuclei during P_i deprivation. Our data indicate that Hlh3 is essential for the mobilization and import of free P_i from the environment since *APH1* and the phosphate transporter-encoding genes, PHO84, PHO840, PHO89, were not induced in the *HLH3* deletion mutant (*hlh3Δ*) during P_i starvation and because *hlh3Δ* growth was compromised when the Aph1 substrate, β-glycerol phosphate, was used as the sole source of P_i. Hlh3 also appears to be essential for mobilizing P_i from intracellular sources since *hlh3Δ* growth was compromised in the absence of an external source of P_i. The two novel Hlh3-dependent, P_i responsive genes identified in this study, *BTA1* and *APH3,* encode diacylglyceryl-N,N,N-trimethylhomoserine (DGTS) synthase predicted to produce a P_i-free phosphatidylcholine substitute, and a putative intracellular acid phosphatase, respectively. The roles of *BTA1* and *APH3* in P_i mobilization and/or virulence will be discussed. In summary, we demonstrate that the transcription factor, Hlh3, is responsible for the induction of multiple genes during P_i starvation, which are predicted to enable the mobilization of P_i from both intra- and extracellular sources, and thus allow fungal adaptation to a pathogenic lifestyle.

id #25424

Proffered paper Wednesday

Role of the inositol polyphosphate kinase Ipk1 in the pathogenesis of *Cryptococcus neoformans*

Cecilia Li[1,3], Sophie Lev[1,3], Adolfo Saiardi[2], Desmarini Desmarini[1,3], Tania Sorrell[3], Julianne Djordjevic[1, 3]

[1]*Centre for Infectious Diseases and Microbiology, The Westmead Millennium Institute for Medical Research, Sydney Medical School, University of Sydney at Westmead Hospital, Westmead, NSW, Australia*

[2]*Medical Research Council Laboratory for Molecular Cell Biology, University College London, London, UK*

[3]*Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, NSW, Australia*

Cryptococcus neoformans (*Cn*)is the leading cause of fungal meningitis worldwide. *Cn* utilises a number of signalling pathways to aid its survival within a human host and regulate the expression of key virulence-related traits. We have identified a novel signalling pathway in *Cn*, involving the phosphorylation of inositol trisphosphate (IP_3) to more complex inositol polyphosphates (IPs) and inositol pyrophosphates (PP-IPs) by a series of

inositol polyphosphate kinases (IPKs). To determine the role of Ipk1 (a putative IP_5 kinase) in IP homeostasis and its contribution to the virulence profile of *Cn*, an *IPK1* gene deletion mutant (*CnΔipk1*) was created using PCR and biolistic transformation. HPLC revealed that IP_5 accumulated in *CnΔipk1,* consistent with Ipk1 functioning as an IP_5 kinase.Phenotype characterisation of *Δipk1* showed an attenuated virulence composite: a cell wall defect, reduced laccase/urease activity, and reduced secretion of the phosphate (Pi)-repressible acid phosphatase (Aph1) activity in phosphate limiting conditions due to reduced induction of *APH1* gene expression. *Δipk1* was hypersusceptible to antifungals including amphotericin B and the azole drug family. *Δipk1* was hypovirulent in a mouse inhalation model of cryptococcosis over a 64 day infection period but established a persistent low-grade asymptomatic lung infection. *Δipk1* was not readily phagocytosed by THP-1 cells and failed to activate them to the same extent as the wild-type strain as assessed by flow cytometry and qPCR respectively. For qPCR, an RT^2 profiler array designed for analysis of the antifungal immune response was used. In conclusion, *Cn*Ipk1 is an IP_5 kinase required for the expression of virulence-related traits *in vitro,* thepromotion of drug toleranceand pathogenicity *in vivo*. These results suggest that IP species produced down-stream of Ipk1 are involved in regulating virulence and phosphate homeostasis, and studies into these mechanisms are ongoing.

id #25509

Proffered paper Wednesday

Drivers' Disconnect: Deterministic processes vary during community assembly for ecologically dissimilar taxa

Jeff R Powell[1], Senani Karunaratne[1], Colin D Campbell[2, 3], Huaiying Yao[4], Lucinda Robinson[2], Brajesh K Singh[1]
[1]*University of Western Sydney, Penrith, NSW, Australia*
[2]*The James Hutton Institute, Aberdeen, Scotland*
[3]*Swedish University of Agricultural Sciences, Uppsala, Sweden*
[4]*Institute of the Urban Environment, Chinese Academy of Sciences, Xiamen, China*

The continuum hypothesis states that both deterministic and stochastic processes contribute to the assembly of ecological communities. However, the contextual dependency of these processes remains an open question that imposes strong limitations on predictions of community responses to environmental change. We measured community and habitat turnover across multiple vertical soil horizons at 183 sites across Scotland for two microbial groups, both dominant and functionally vital components of all soils, that differ substantially in their growth habit and dispersal capability (bacteria and fungi). We found that habitat turnover was the primary driver of bacterial community turnover in general, although its importance decreased with increasing isolation and disturbance. Fungal communities, however, exhibited a highly stochastic assembly process, both neutral and non-neutral in nature, largely independent of disturbance. These findings suggest that increased focus on limitations to dispersal and biotic interactions within assemblages are necessary to manage and conserve the key ecosystem services provided by these assemblages.

Plenary Speaker Thursday

Molecular cell biology of *Phytophthora* pathogenicity

Adrienne R. Hardham
The Australian National University, Canberra, ACT, Australia.

Phytophthora is a genus of aggressive plant pathogens that cause extensive, often devastating, losses in agricultural crops, horticultural plants and natural ecosystems throughout the world. While some species have a narrow host range, others may infect thousands of plant species. *Phytophthora,* and other members of the class Oomycetes, are not fungi. They belong to the Kingdom Protista and a central feature of their life cycle and successful infection of host plants is the production of motile, biflagellate zoospores. *Phytophthora* zoospores use endogenous nutrient reserves to remain motile for many hours as they seek a suitable host. They are chemotactically and electrotactically attracted to favourable infection sites on the plant surface. There, the zoospores encyst and attach to the plant through the rapid secretion of adhesive material. Cysts quickly germinate and secrete a diverse range of cell wall degrading enzymes that facilitate plant invasion.

Early studies of the infection of plants by *Phytophthora* species used light and electron microscopy to describe the basic features of pathogen cells and disease development. These traditional approaches can now be extended by advanced microscopy techniques that use methods, such as immunocytochemical labelling and GFP-tagging, to visualise the distribution of pathogen molecules and cell components. This molecular cell biology not only enables identification of specific cell structures in fixed and sectioned material but also allows studies of dynamic processes in living cells. In addition, the generation of molecular probes can now draw on proteomic, transcriptomic and genomic data. As in other areas of biology, these developments mean that molecular cell biology is revolutionising our ability to elucidate the roles of selected proteins and cell components in *Phytophthora* pathogenicity.

Invited speaker Thursday

Sex and '-omics' a powerful combo for hunting down fungal effectors of scab pathogens

Jason Shiller[1], Cecilia H. Deng[2], Dan Jones[1,3], Carl H. Mesarich[2], Adam P. Taranto[1,5], Andrew J. Robinson[3,6,7], Patrick Kastner[1], Nathan E. Hall[3,6,7], Matthew D. Templeton[2], Joanna K. Bowen[2], **Kim M. Plummer**[1,3]
[1]Department of Plant, Animal and Soil Science, AgriBio, La Trobe University, VIC 3086.
[2]The New Zealand Institute for Plant & Food Research Limited (PFR), Auckland, NZ.
[3]Plant Biosecurity Cooperative Research Centre, LPO Box 5012 Bruce, ACT 2617, Australia.
[4]The School of Biological Sciences, University of Auckland, NZ. Life Sciences.
[5]Plant Sciences Division, Research School of Biology, ANU, Canberra, Australia.
[6]Computation Centre, Victorian Life Sciences Computation Initiative, VIC.
[7]La Trobe University, Melbourne, VIC.

Scab diseases of fruit trees are caused by closely related Dothideomycete fungi in the genus *Venturia*. Frequent fungicide applications are required for scab control, at considerable cost to the grower. Scab fungi reproduce sexually and asexually, and have a high potential for development of fungicide resistance, with chemical residues and resistance to fungicides an on-going issue. Disease resistant host germplasm exists, however selecting for durable disease resistance is critical as breeding is a slow process with these woody, outcrossing hosts. We are investigating non-host resistance as a possible durable resistance solution. The scab fungi display a range of host specificities and are thought to have coevolved with their various hosts in the family Rosaceae. The most widely researched of these is *V. inaequalis* that causes apple scab. Related *Venturia* species cause scab disease of other *Malus* relatives, for example, *V. pirina* infects European pear and *V. nashicola* infects Asian pears. Additional levels of complexity exist, within the Venturia species, for example certain isolates, classified as *V. inaequalis,* are unable to infect *Malus* but instead infect different hosts belonging to the subfamily Maloideae, such as loquat. Host-cultivar specificity is also demonstrated by isolates of *V. inaequalis* that infect *Malus*; 17 gene-for-gene pairings between effectors (a pathogen protein that enhances disease often via suppression of the defence reaction) and resistance (*R*) gene products have been identified to date. Thus the effector repertoire of *Venturia* isolates determines their cultivar specificity and most probably host specificity. Effectors have yet to be cloned from *Venturia* but whole genome sequences (WGSs) and transcriptome analyses in *V. inaequalis* races and *V. pirina* have identified candidates that share the characteristics of known fungal effectors (small and putatively secreted proteins). Candidates for effectors determining host specificities of *V. inaequalis* and *V. pirina* have been identified by comparative genomics between WGS of isolates with differing specificities. These candidates are currently being characterised with respect to functionality.

id #25727

Invited speaker Thursday

The potential ecological impacts of newly described true fungal and fungal-like parasites in wild vertebrate populations

Frank H Gleason[1], Osu Lilje[1], Jodi Rowley[1]
[1]*University of Sydney, Cromer, NSW, Australia*

Recently, many species of newly discovered fungal and fungal-like microbes have emerged as important contributors to aquatic food webs. Some of them are highly infective, have short generation times, can cause rapid declines in host population sizes and can significantly change the species composition of aquatic ecosystems. Frequently, several species of parasites often simultaneously infect populations of the same host species in the same or in different parts of their life cycles, and many parasites have broad host specificity. Despite increasingly sophisticated microbiological techniques, the basic knowledge to fully appreciate the ecological importance of microbial parasites is lacking. In this presentation, some examples of fungal and fungal-like parasites of some vertebrate hosts will be considered. In fish the lack of knowledge is likely due to the nature of their habitats as fishes suffer from living beneath turbid water away from easy recording. However, fishes represent key ecosystem services for millions of people around the world, and a functional ecological understanding of all parasites is necessary for conservation and food security. Fungal and fungal-like microbes that cause EIDs in wild and farmed fish include species of *Saprolegnia, Aphanomyces* and *Achyla* (Phylum Oomycota) and species of *Ichthyophonous,* and *Spaerothecum* (Phylum Mesomyetozoea). We also will discuss some examples of zoosporic parasites in the phyla Chytridiomycota, Mesomycetozoa, Perkinsozoa and Oomycota, all of which infect amphibians and the Ascomycete, *Ophidiomyces ophiodiicola*, which is the causative agent of snake fungal disease of wild and captive snakes in North America.The mode of transmission, environmental influences, and effective treatment options still need to be investigated for each EIDs. The pathosystem model provides an excellent basis for understanding host-parasite interactions. Chemotactic zoopores and several families of proteases facilitate infection. Introduction of non-native host may accelerate the dispersal of these parasites. Unlike *B. dendrobatidis* some of the other zoosporic parasites grow well at or slightly above 25 ˚C, and their growth rates are likely to increase with global warming.

id #25887

Invited speaker Thursday

Regulation of mitophagy in the yeast *Saccharomyces cerevisiae*

Karen Dawson[1], Alexander May[1], Rod Devenish[1], Yeliz Boglev[1], **Mark Prescott**[1]
[1]*Monash University, Melbourne, VIC, Australia*

Autophagy ('self-eating') is an evolutionarily conserved pathway that involves the sequestration and delivery of material into the acidic hydrolytic environment of the vacuole (yeast) or lysosome (mammals) for degradation and recycling. Long thought to be a non-selective process for bulk turnover, it is now accepted that autophagy selectively targets particular cellular components to the exclusion of others, and different forms of selective autophagy have been described. The selective turnover of mitochondria by autophagy is termed mitophagy. Autophagy is a complex process with many different targets each requiring distinct but overlapping regulatory networks and mechanisms. We are interested in how mitophagy in regulated.OTP1 is a novel open reading frame with no reported function but appears to be involved in the regulation of mitophagy. Yeast cells (Δ*OTP1*) lacking expression of OTP1 have a delayed mitophagy phenotype, similar to that of a strain lacking expression of UTH1, a gene shown to be required for correct formation of the cell wall. Growth of ΔUth1, but not ΔOtp1/ΔUth1 or ΔOtp1 cells are sensitive to caspofungin, an inhibitor of (1-3)-ß-D- glucan synthase, an enzyme required for cell wall formation. OTP1 is located in the late Golgi and sequence homology predicts it to be a cargo receptor. Results will be discussed in a model encompassing cell wall integrity signalling and the regulation of mitophagy.

id #25626

Invited speaker Thursday

Molecular identification of mycorrhizal Russulaceae fungi

Morwenna Boddington[1], John D Dearnaley[1], Teresa Lebel[2], Patrick Leonard
[1]*University Of Southern Queensland, Toowoomba, QLD, Australia*

[2]*Landcare Research, Auckland, New Zealand*

The Russulaceae is a family of basidiomycetous fungi found in a variety of habitats around the world. While reasonably common in Australian temperate forests, little work has been conducted on this group, with the result that many taxa are undescribed or poorly documented. The aim of this research project is to improve this situation. The identification of Russulaceae fruiting bodies to species level has many challenges and is predominantly based on characteristics that are highly subjective, changeable, require specialised chemicals, and specialised equipment. A certain amount of expertise is also required. However, even this is no guarantee of consistently obtaining a positive species identification.

Molecular analysis potentially offers a non-subjective method of identification. In particular, the internal transcribed spacer (ITS) region of fungal DNA has been proposed as the primary standard molecular benchmark for identification of fungi. Coupled with access to millions of sequences held in databases such as GenBank, it would seem that molecular identification offers a useful opportunity to streamline and improve the identification of the Russulaceae. However, this is not always the case, and in sequencing the ITS region of numerous specimens from this family a number of issues have arisen. This presentation will examine some of the difficulties experienced in the molecular identification of Australian Russulaceae species and discuss some of the potential solutions.

id #25628

Invited speaker Thursday

Modifications to the International Code of Nomenclature in support of modern fungal taxonomy

Tom W. May[1]

[1]*Royal Botanic Gardens Melbourne, South Yarra, VIC, Australia*

Discussions at Nomenclature Sessions during the 10th International Mycological Congress indicated strong support among mycologists for a number of proposals to amend the *International Code of Nomenclature for algae, fungi and plants* (ICN). Such proposals include: (1) changing the conditions for epitypification so that sequenced epitypes can be designated without having to establish that DNA is not recoverable from the holotype, (2) introducing a requirement to register later typification acts such as lectotypification, (3) changing the citation of sanctioned names, (4) prohibiting cross-kingdom homonyms, and (5) ending the priority of sexually typified names. Another change strongly supported by mycologists is to transfer governance so that matters in the ICN peculiar to fungi are dealt with by International Mycological Congresses. Beyond such changes, a proposal should be considered to include in the ICN an article or recommendation dealing with provision of DNA sequence data for new species of fungi, especially in the light of primary and secondary barcodes becoming well-established.

Mandatory registration of names of fungi was introduced in the Melbourne ICN. There is occasional mis-citation of identifiers by authors of fungi names, but otherwise registration has been well-accepted by the mycological community. Nevertheless, there are some issues. Firstly, synchronisation of the three approved registration databases remains problematic. Secondly, it would be ideal if registration databases output all data that is input on registration, in a searchable form, and as web services. This ability to freely recover the protologue and other key information was one of the main reasons for introducing registration in the first place. There is currently much duplication of nomenclatural information across global, national and institutional databases (within Australia, examples are AusFungi, APPD, and individual reference collection databases). Mechanisms to reduce duplication and increase the utility of fungi name databases will be discussed.

id #25605

Invited speaker Thursday

Hunting for antifungal-chelator drug synergy using biological networks

Chi Nam Ignatius Pang[1, 2], Yu-Wen Lai[3], Leona Campbell[3], Sharon Chen[4], Dee Carter[3], Marc Wilkins[1, 2]

[1]*School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia*

[2]*Systems Biology Initiative, The University of New South Wales, Sydney, New South Wales, Australia*

[3]*School of Molecular Bioscience, University of Sydney, Sydney, New South Wales, Australia*

[4]*Institute for Clinical Pathology and Medical Research, Westmead Hospital, Sydney, New South Wales, Australia*

Invasive fungal infections (IFIs) in humans are difficult to treat. The few effective antifungal drugs available have issues regarding toxicity and long-term effectiveness against drug-resistant strains. It is difficult to develop novel antifungal drugs, thus a promising direction of research is to identify synergistic agents for existing therapy. Iron chelators administered with certain antifungals have been found to improve the clearance of some fungal infections, but the mechanistic role of antifungal-chelator combinations is complex and poorly understood. Using checkerboard assays, we found the iron chelator lactoferrin (LF) was synergistic with amphotericin B (AMB) for *Saccharomyces cerevisiae*. We extracted mRNA from *S. cerevisiae* treated with i) AMB only, ii) a combination of AMB + LF or iii) corresponding matching controls. RNA-seq data were generated using Illumina HiSeq 2000 with biological triplicates multiplexed and randomized across two sequencing lanes. Differentially expressed genes were identified using EdgeR and the results were co-visualized with biological networks using Cytoscape, to find the mechanistic basis of drug synergy. AMB alone resulted in to the up-regulation of Aft1, a transcription factor that regulates iron uptake. AMB + LF co-treatment halted Aft1 up-regulation and down-regulated genes involved in iron transport. The zinc responsive transcription factor Zap1 and Zap1p target genes were also down-regulated. The analysis of Aft1p and Zap1p single gene deletion mutants and rescue assays confirmed the role of iron and zinc homeostasis in mediating the synergistic effect of AMB and LF, and suggests these proteins as synergistic drug targets.

id #25593

Invited speaker Thursday

Improving the recovery outcomes of the critically endangered Wollemi pine: is translocation success determined by soil microbes?

Jessica L Mowle[1], Heidi Zimmer[2], Cathy Offord[3], Ian Anderson [1], Brajesh Singh[1], Jeff Powell[1]
[1]*Hawkesbury Institue for the Environment , University of Western Sydney, RICHMOND, NSW, Australia*
[2]*Department of Forest and Ecosystem Science, University of Melbourne , RICHMOND, VIC, Australia*
[3]*Science and Conservation, Royal Botanic Gardens and Domain Trust, Mount Annan , NSW, Australia*

Wollemi pine (*Wollemia nobilis* W. Jones, K. Hill & J. Allen) is a monotypic species, of which fewer than 100 trees are known in the wild. The Wollemi pine Recovery Team has proposed translocation as a conservation strategy to establish 'back-ups' to the wild population; however, knowledge regarding the environmental/ biotic requirements of individuals planted in new environments is limited. One of the most important limitations to the introduction or reintroduction of tree species is the presence of suitable microbial partners. Plants in novel environments will encounter fewer coevolved mutualists. Wollemi pine grows on shallow soils of poor nutrient status and high acidity and is likely to be highly dependent on mycorrhizal fungi, which have been observed associated with the roots of Wollemi pine, and bacteria that contribute to nutrient cycling. An experimental translocation study was established in 2012 at a new location in the wild. At this location, 191 WPs were planted along an altitudinal gradient and in patches of low and high light availability and their survival and growth are being monitored. Wollemi pine performance will be related to nutrient dynamics, climate, light and associated microbial communities. This knowledge is essential to strategically target land appropriate for future Wollemi pine translocations, a critical next step identified in the Wollemi pine Recovery Plan.

id #25856

Invited speaker Thursday

Ecto- and endomycorrhizal dynamics in *Eucalyptus* species

Jennifer KM Walker[1], Ian C Anderson[1], Jeff R Powell[1]
[1]*Hawkesbury Institute for the Environment, UWS, Richmond, NSW, Australia*

Eucalyptus species are primarily colonised by ectomycorrhizal (EM) fungi, but arbuscular endomycorrhizal (AM) fungal colonisation has also been detected in varying proportions. The dynamics of this dual symbiosis may be a direct response of the mycorrhizal community to abiotic conditions, or may be driven indirectly, due to host plant requirements. Trees can drive AM-EM shifts by preferentially allocating resources to those mycorrhizal fungi that provide them with the greatest benefit. The requirements of the host may depend on its growth stage and may also vary with environmental conditions. Our objective was to identify potential drivers of the reported AM-EM shifts in roots of *Eucalyptus* species. We proposed two hypotheses: that the timing and extent of these shifts is a) constant, and linked to specific growth stages, or b) flexible, and dependent on environmental conditions. We used bioassay seedlings grown from sterilised canopy-collected seed and subsequently planted out in fertilization treatment plot soil and elevated CO_2 plot soil to test mycorrhizal community responses. The fertilisation plots and controls contained 8 yr.-old *E. saligna* in a reclaimed paddock, while the elevated CO_2 plots and controls contained naturally growing *E. tereticornis* on native Cumberland Plain woodland. At all plots (N = 3), root samples were collected from seedlings that were harvested 8 and 12 months after germination and planting, and these were examined for extent and type of colonisation. EM fungal morphotypes on fine root tips were counted, and the fungal ITS region was sequenced. The seedlings were on average 50 % colonised by EM fungi after only 8 months, and this increased to 100 % after one year. EM fungal diversity was lower in younger seedlings, and lowest in the fertilized plots. *Hydnangium*, *Tomentella*, *Inocybe*, *Sebacina*, Sordariomycetes, and Descomycetes (Cortinariaceae) were among the most frequently detected groups, and the EM fungal communities were distinct at each site (i.e. on each plant host), with only a few shared species.

Invited Speaker Thursday

Molecular phylogeny of the tropical lichen family Pyrenulaceae

Cécile Gueidan[1], André Aptroot[2], Marcela Eugenia da Silva Cáceres[3]
[1]Australian National Herbarium, National Research Collections Australia, National Facilities and Collections, CSIRO, P.O. Box 1600, Canberra, ACT, 2601, Australia.
[2]ABL Herbarium, Gerrit van der Veenstraat 107, NL-3762 XK Soest, The Netherlands.
[3]Departamento de Biociências, Universidade Federal de Sergipe, CEP: 49500-000, Itabaiana, Sergipe, Brazil.

The family Pyrenulaceae is one of the main components of the epiphytic lichen flora in tropical rainforests. This family mainly comprises corticolous species and *Pyrenula*, the most species-rich genus (about 200 species), is typically found on smooth, shaded bark. Although a world key is available for the currently accepted species of *Pyrenula*, recovering molecular data from these tropical taxa has proven to be challenging. As a result, generic and species concepts have not been tested and little is known about phylogenetic relationships between species of Pyrenulaceae. A recent attempt using material from Sri Lanka highlighted the presence of two main well-supported monophyletic groups. However, the number of taxa for which sequences were generated in this study was still low, mostly due to the difficulty to recover enough genomic DNA from dry herbarium specimens collected more than a year before DNA extraction. A new method of DNA collection and storage was therefore investigated in this study. Because the genomic DNA of corticolous crustose tropical lichens seems to degrade rapidly, often before to reach the laboratory, we investigated the use of FTA Classic Cards (Whatman) to collect and transport DNA samples of our lichen material. These chemically-treated matrix cards lyse cell membranes on contact, physically bind the DNA, protect it from UV damage and microbial degradation. In this study, several fieldtrips to South-East Asia (Vietnam, Thailand and Laos) and to Brazil allowed us to collect fresh specimens for various species of Pyrenulaceae and recover genomic DNA from both freshly collected material and FTA card samples. Sequences generated give us a new insight into generic and species concepts in Pyrenulaceae.

Invited Speaker Thursday

Understanding and overcoming antifungal drug resistance

Richard D Cannon[1], Kyoko Niimi[1], David RK Harding[2], Ann R Holmes[1], Masakzu Niimi[1], Brian C Monk[1] and Erwin Lamping[1]
[1]Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand.
[2]Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Fungi that infect humans can develop resistance to antifungal drugs. A significant antifungal drug resistance problem is azole resistance in *Candida* species. High-level azole resistance in *Candida albicans* clinical isolates is often due to over-expression of ATP-binding cassette (ABC) efflux pump Cdr1. The objective of this study was to identify an inhibitor of Cdr1. *C. albicans* Cdr1 was heterologously expressed in *Saccharomyces cerevisiae* AD1-8u$^-$. Screening of a ~1.9 x 10^6 member D-octapeptide combinatorial library that concentrates library members at the yeast cell surface identified RC21v3, a 4-methoxy-2,3,6-trimethylbenzenesulfonyl derivative of the D-octapeptide D-NH_2-FFKWQRRR-$CONH_2$, as a potent and stereospecific inhibitor of Cdr1. RC21v3 chemosensitized the *S. cerevisiae* strain over-expressing Cdr1 (AD/CaCDR1) but not strains over-expressing other fungal ABC transporters, the *C. albicans* MFS transporter Mdr1p or the azole target Erg11p, to FLC. RC21v3 also chemosensitized clinical *C. albicans* isolates over-expressing Cdr1p to FLC, even when Cdr2p was also over-expressed. Specific targeting of Cdr1p by RC21v3 was confirmed by analysis of spontaneous RC21v3 chemosensitization-resistant suppressor mutants of AD/CaCDR1. The suppressor mutations introduced a positive charge beside, or within, extracellular loops 1, 3, 4 and 6 of Cdr1 or an aromatic residue near the extracytoplasmic end of transmembrane segment 5. The mutations did not affect Cdr1 localization or Cdr1p ATPase activity but some increased susceptibility to the Cdr1 substrates FLC, rhodamine 6G, rhodamine 123 and cycloheximide. These results demonstrate that drugs targeting the extracellular loops of Cdr1 can overcome the FLC-resistance of *C. albicans* clinical isolates.

id #26213

Invited Speaker Thursday

Development of commercial formulations of a fungal bio-control agent for house fly control

Rosamond Godwin[1*]; Diana Leemon[2]; Steven Rice[2], Peter James[1]
[1]QAAFI; Univ. of Qld, Ecosciences Precinct GPO Box 267 Brisbane, Qld 4001
[2]Agri-Science Qld; Ecosciences Precinct GPO Box 267 Brisbane, Qld 4001

The house fly (*Musca domestica)* is an on-going problem for intensive animal holdings such as cattle feedlots. Uncontrolled fly populations can be a health hazard and may lead to reduced production, annoyance stress to feedlot workers and complaints from residents adjacent to cattle feedlots. Repeated use of insecticides for fly control can result in residues in produce and the environment and the development of resistance in flies. Previous studies conducted by Agri-Science Qld attained a proof of concept that fungal biopesticides based on the fungus *Metarhizium,* have potential as house fly control agents in cattle feedlots. The aim of a current project is to build on the previous research and develop commercially acceptable *Metarhizium* based bait and spray formulations for strategic management of house fly populations in cattle feedlots. Monitoring across a number of feedlots will provide data on temporal and spatial variation in fly populations to develop a regime for the efficacious deployment of the formulations. *Metarhizium* isolates will be selected on characteristics including high spore yield and pathogenicity to *M. domestica.* Formulations are being developed to maintain spore viability under ambient conditions, protect from UV damage, attract flies and provide stability for very low and ultra-low volume spraying.

Invited speaker Thursday

Fingerprinting Analysis Of Soil Fungal Communities Affected By Controlled Recurrent Fires In Temperate Grassy Ecosystems Of Victoria

Eleonora Egidi[1], JW Morgan[2], AE Franks[1]
[1] Department of PAM, La Trobe University
[2] Department of EEE, La Trobe University

Controlled recurrent fires are a popular land-management strategy in fire-prone landscapes of southern Australia. However, the effect of prescribed fires on structure and functioning of ecosystems, in particular below-ground fungal communities, has not been completely addressed yet. Understanding the consequences of fire management at microbial level represents a crucial step in order to predict short- and long-term consequences of controlled fires on soil ecological dynamics and, thus, ecosystem health.
In this study, we explored fungal community response to repeated prescribed burning in native grassy ecosystems of Victoria. Effects of fire frequency (1 to 2- years; 2 to 3 years; >3 years) and time since last fire (1 month; 3 to 6 months; 6 to 18 months; >18 months) have been investigated by Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprints. Our results provide evidence that a significant change in fungal assemblages composition occurs between frequently and unfrequently burnt plots (>3 years intervals). As the vegetation response to low-frequent fires is characterised by a shift from native, fire-adapted grasses to exotic plants, it may be inferred that soil fungal communities in these plots are composed by a less fire-adapted community as a consequence of the change in plant composition after burning. The strong relationship between plant and fungal dynamics is also suggested by the significant variability in soil fungal composition related to season of sampling.

Invited speaker Thursday

How to be a good pathogen: molecular interactions in the Fusarium-wheat pathosystem

Donald M. Gardiner[1], Ailisa Blum[1,2], Jason Carere[1], Aurelie H. Benfield[1], Andrew Kettle[1,2], Jacqui Batley[1,2,3], John Manners[4], Kemal Kazan[1]
[1] CSIRO Agriculture Flagship, Brisbane, Australia
[2] The University of Queensland, St Lucia, Australia
[3] The University of Western Australia, Crawley, Australia
[4] CSIRO Agriculture Flagship, Canberra, Australia

Fusarium species cause two main diseases of wheat in Australia; Fusarium crown rot and Fusarium head blight. The most important pathogens for these disease are F. pseudograminearum, F. culmorum and F. graminearum and the only available resistance sources to these pathogens are quantitative. Our group is interested in how these pathogens cause disease and have a particular focus on the regulation and biosynthesis of secondary metabolites by the pathogen and degradation of plant derived antimicrobials. We are also developing tools to monitor mutant strains of *F. graminearum* in mixed inoculations which should provide us for the first time with the ability to measure the contribution of individual virulence mechanisms to pathogen fitness when in competition with other strains.

id #25647

Invited speaker Thursday

Species delimitation and mycorrhizal specificity in *Caladenia* spider orchids

Michael R Whitehead [1 2] , Renee A Catullo [3] , Kingsley W Dixon [2] , Rod Peakall [1] , Monica P Ruibal [1] , Celeste C Linde [1]
1. *The Australian National University, Canberra, ACT, Australia*
2. *Kings Park Botanic Garden, Perth, WA, Australia*
3. *Ecosystem Sciences, CSIRO, Canberra, ACT, Australia*

Orchids depend on mycorrhizal partnerships in order to germinate and establish. The specificity of these relationships is known to vary among species, from highly specific to more generalist orchid-fungi interactions. Still unclear however, is how these partnerships influence orchid diversity and distributions. *Caladenia* is a hyperdiverse lineage of terrestrial orchids partnering with the widespread fungal genus *Sebacina.* Its immense diversity, wide distribution and importance in conservation make it an ideal system for exploring orchid-fungal interactions. Our aim was to assess taxonomic diversity in *Sebacina* associating with the breadth of diversity in *Caladenia*. Due to the paucity of non-genetic characters in these fungal taxa we used recently developed Bayesian species delimitation tools for molecular diagnosis of taxa based on loci ITS, ATP6, LSU and four newly developed nuclear and mitochondrial markers. Our results show *Caladenia* orchids partitioned on several distinct clades of *Sebacina* fungi. Specificity within*Caladenia* varies, with single sub-genera and in some cases single species of *Caladenia* found to associate with multiple *Sebacina*lineages. Other orchid taxa appear to host only a single specific fungal lineage. The delineation of *Sebacina* taxa in this study will aid downstream efforts to understand the role of fungi in the evolution, ecology and conservation of terrestrial orchid diversity.

Invited speaker Thursday

Insight into fungal pathogenicity using whole genome sequencing

Wieland Meyer[1], Kennio Paim[1,2], Carolina Firacative[1], Marcio L. Rodrigues[3] and David Engelthaler[4]

[1]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School - Westmead Hospital, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Millennium Institute for Medical Research, Sydney, NSW, Australia;
[2]Infectious Disease Department, Triangulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil;
[3]Fundação Oswaldo Cruz – Fiocruz, Centro de Desenvolvimento Tecnológico em Saúde (CDTS), Rio de Janeiro, Brazil;
[4]Translational Genomics Research Institute, Flagstaff, AZ, USA

C. gattii has historically been associated with neurological disease in immunocompetent patients in tropical and subtropical regions. Atypical *C. gattii* infections, first identified in 1999 during an outbreak on Vancouver Island, British Columbia appear to differ not only in their geographic niche, but also in virulence and clinical outcomes. Three major VGII subtypes VGIIa and VGIIc (high virulent) and VGIIb (low virulent) have been identified in the Pacific North West (PNW). The new VGIIa subtype is associated with a high frequency of respiratory disease and lower frequency of neurologic disease. MLST analysis followed by whole genome sequencing put the PNW strains in a phylogenetic context with the global population, and indicated multiple dispersal out of South America. Comparative genomics analyses identified distinct genomic differences among the PNW subtypes and between the PNW and other global VGII populations. For initial studies two genes presented only in the VGIIa (Aldo/keto reductase and collagen-binding domain of a collagenase) and one presented in the VGIIa/c genotypes (Endoribonuclease) were disrupted in the reference strain CDC-R265, and their impact on virulence was studied in the *Galleria mellonella* model, comparing the wild type (CDC-R265 WT) and the mutant (*Δaldo, Δcoll, Δendo*) strains. The median survival was 144 and 188 h for WT and Δ*coll* strain, respectively. Unlikely Δ*coll*, the median survival of Δ*aldo* and Δ*endo* mutants was 180 and 132 h, respectively. The mutant Δ*coll* showed slow growth in the presence of cell-wall perturbing agents Congo red and Calcofluor-white. The presence of a collagen-binding domain only in VGIIa in the PNW could be a highly significant

association with the fact that this subtype causes a severe pulmonary disease. Collagen binding has been shown as a likely cause of similar pathogenesis seen in other organisms. Further studies are currently been performed to understand its role in fungal virulence.

Invited speaker Thursday

Form and function: glycoproteomics of the *Saccharomyces cereviseae* cell wall

Benjamin L. Schulz.
School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia QLD 4072, Australia. E: b.schulz@uq.edu.au

The fungal cell wall is a highly complex and dynamic structure, critical for many aspects of fungal biology. A key constituent of the cell wall are diverse proteins, covalently linked to polysaccharide through GPI anchor remnants or cross-linked through alkali sensitive linkages. These proteins play central enzymatic roles in cell wall remodeling, affect the structural integrity and properties of the cell wall, and are critical in determining intercellular interactions. Most cell wall-associated proteins are highly modified by O- and N-linked glycosylation, which increases protein folding efficiency, stability and solubility. We have used these cell wall glycoproteins of *Saccharomyces cerevisiae* as model substrates to investigate the molecular mechanisms regulating N-glycosylation in eukaryotic cells. Glycoproteomics and yeast genetics have allowed us to characterize the roles of key steps in the pathway of N-glycosylation, including how site-specific glycosylation is regulated through direct physical interactions between substrate polypeptide and non-catalytic subunits of the oligosaccharyltransferase enzyme. We have also used these quantitative proteomics and glycoproteomics tools developed using lab yeast to investigate the cell wall proteins of diverse brewing yeast strains. This has shown a surprising diversity of modifications and proteins not present in standard lab-adapted yeast, and likely important in determining industrially relevant phenotypes.

Invited speaker Thursday

Arbuscular mycorrhizal fungi associated with New Zealand native plants used in traditional medicine (rongoā)

Wisnu Wicaksono[1], E. Eirian Jones[1], Jana Monk[2] and **Hayley J. Ridgway[1]**
[1]Faculty of Agriculture and Life Science, Lincoln University, Canterbury, New Zealand
[2]AgResearch Ltd, Lincoln, Canterbury New Zealand

In New Zealand there is a significant knowledge gap of functional diversity of the microbial endophytes of plants used in traditional medicine (rongoā). *Leptospermum scoparium* (manukā) and *Sophor*a spp. (kowhāi) are culturally important medicinal plants with well-recognized health effects. As part of a larger project examining the endomicrobiome of rongoā plants, the arbuscular mycorrhizal fungi (AMF) associated with these two species were identified. Roots of *L. scoparium* and *Sophora* spp. were obtained from mature plants at three and four diverse sites, respectively. The AMF community was recovered by both baiting in sterile pot culture and direct DNA sequencing of root DNA. For *L. scoparium* and *Sophora* spp. the results identified at least six and four different AMF species, respectively. They also showed that species diversity could be underestimated when only one methodological approach was applied. Further research will characterize the seed, leaf, stem and root endomicrobiome of *L. scoparium* using molecular techniques and standard microbial culturing. Key endophytes will be characterized *in vitro* for their bioactivity and *in vivo* for their ability to improve the growth of mānuka.

Invited speaker Thursday

Deciphering the role of pathogen effectors in flax rust disease

Ann-Maree Catanzariti[1], Claire Anderson[1], Jeff Ellis[2], Peter Dodds[2], David A. Jones[1], Adrienne Hardam[1]
[1] Division of Plant Sciences, Research School of Biology, ANU, Canberra, Australia.
[2] CSIRO Agriculture, Canberra, Australia.

During infection microbial plant pathogens secrete a suite of effector proteins. Many of these are known to enter host cells and some have been found to target subcellular organelles. Effectors are thought to have roles in pathogenicity, principally in the manipulation of hosts to avoid plant defences and acquire nutrients. ‘Effector discovery’ has been a significant area of research over recent years, and a large number of effectors have now been identified from a wide variety of plant pathogens. However, determining the precise roles they play during infection has been far more challenging, and few effectors have been assigned a function, particularly those from fungi. Flax rust (*Melampsora lini*) is a biotrophic fungus that causes disease on flax. This interaction is one of the best-characterised fungal pathosystems and has provided a strong basis for understanding rust resistance controlled by host resistance proteins in effector-triggered immunity. We have now isolated six effectors and all have been found to be small secreted proteins that are delivered into the host cytosol during infection. Unfortunately, the amino acid sequence of these proteins do not provide many clues to their function during infection of a susceptible host. We are therefore using a number of techniques to fill this gap in our knowledge, and gain a better understanding of the molecular mechanisms used by rust fungi to cause disease.

Invited speaker Thursday

Molecular dissection of the blackleg fungus *Leptosphaeria maculans* species complex interaction with its host *Brassica napus*

Alexander Idnurm
University of Melbourne, Melbourne, VIC, Australia

Blackleg disease of oilseed Brassicas is caused by two closely related species, *Leptosphaeria maculans* and *L. biglobosa*. Canola is an Australian crop worth more than $2 billion alone in exports, while employing canola as one of the crop rotations helps sustain the Australian wheat industry. However, a major threat to canola in Australia and world wide is blackleg disease. *L. maculans* and *L. biglobosa* have different disease properties in terms of their abilities to establish hemibiotrophic vs. necrotropic interactions with canola and their potential to cause the most serious stem canker aspects of the disease. Comparative analysis of the genomes, and the transcript profiles of both species in the early cotyledon and late stem cankering stages of disease reveal major differences between both stages of disease and between the two species. One experimental challenge to explore and exploit this genomic information is that gene disruption through homologous recombination is highly inefficient, with gene replacement frequencies below 1% of transformants. Hence, alternative approaches are being employed to identify and analyse the nature of the fungal components required for disease. These include the development of new constructs and strains for transformation, changing the regulation of fungal gene expression, and a mutant screens aimed to identify fungal gene targets suitable for a transgenic approach to prevent blackleg disease. Beyond the immediate practical applications, the research is revealing fundamental aspects about how fungal pathogens and plants interact.

Invited speaker Thursday

Characterising levels of fungicide resistance in the wheat pathogen *Zymoseptoria tritici* in Australia

Andrew Milgate, Dante Adorada, Merrin Spackman, Beverley Orchard and Melanie Renkin
NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Pine Gully Road, Wagga Wagga NSW 2650 Australia

Zymoseptoria tritici is an important pathogen of wheat globally and is capable of causing 50% yield loss in susceptible varieties. Control of the disease relies on the use of host resistance, cultural practices and fungicides. However the pathogen is capable of rapidly overcoming host resistance in cultivars and modern farming systems favour stubble retention creating ideal conditions for its survival. Thus, in many countries there has been an over reliance on fungicides to control the disease. This has led to the evolution of resistance to several classes of fungicides including the demethylation inhibitors (DMI) and quinine outside inhibitor (QOI). In Australia *Z. tritici* is a production constraint in the high rainfall zones of south eastern Australia. The use of DMI fungicides, in Australia, to control foliar diseases in wheat production has increased rapidly over the past decade. We have examined a historical set of isolates spanning from 1979 – 2013 and can confirm the evolution of mutations in the *Cyp*51 gene known to reduce DMI sensitivity occurring in Australia for the first time. Isolates carrying the L50S, Y137F and L50S-Y461S have been phenotyped and their resistance factors estimated to a number of DMI fungicides. The impact of the emergence of these mutations will be presented and discussed.

id #25728

AMS awardee Thursday

Quantifying keystone mammalian fungal dispersers

Susan Nuske[1], Karl Vernes[2], Tom May[3], Brad Congdon[1], Andrew Krockenberger[1], Sandra Abell-Davis[1]
[1]*James Cook University, Cairns*
[2]*New England University, Armidale*
[3]*Royal Botanic Gardens, Melbourne*

Dispersal is an important ecological process that drives community assembly and gene flow. When dispersal involves other organisms their ecologies are inevitably linked. Hypogeous sequestrate fungi rely primarily on mammalian consumption for long-distance dispersal. Most sequestrate fungi are important ectomycorrhizal plant-symbionts, which links their mammalian dispersers to the maintenance of plant-fungal relationships, fungal diversity and ecosystem functioning. Australia has the highest rate of mammalian extinctions of any country. Understanding how important different mammals are as dispersers helps understand how this loss in mammal diversity could affect plant-fungi interactions and fungal diversity. We reviewed the literature on the diversity, abundance and seasonality of fungi within mammalian diets. We also gathered data on the home range of mammal species as a proxy for dispersal distance. Combining these variables we ranked Australian mammals in their 'dispersal effectiveness' and defined potential keystone fungal dispersers. We defined an 'important mammal disperser' as one that dispersers a high diversity of fungi, throughout more times of the year at high abundance and over a longer distance. Potoroidae species, including *Bettongia tropica* and *B. gaimardi* were identified as potential keystone fungal dispersers as they fell outside the normal distribution of our index of 'dispersal effectiveness' for all mammals. This method also identified other mammal species, such as *Wallabia bicolor,* as important sequestrate fungal dispersers as they occur in areas were Potoroidae species have been lost and consume a high diversity of fungi and have large home ranges. These results illustrate the significance of mammals for maintaining diverse ectomycorrhizal fungal communities.

Poster

Dissecting the response to fluconazole in susceptible and resistant strains of *Cryptococcus gattii*

Aidan Kane[1], Hin Siong Chong[1,4] ,Leona Campbell [1], Chi Nam Ignatius Pang [2,3], Marc Wilkins [2,3] and Dee Carter [1]
[1]School of Molecular Bioscience, University of Sydney, Sydney, New South Wales, Australia
[2]School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia
[3]Systems Biology Initiative, The University of New South Wales, Sydney, New South Wales, Australia
[4]Current address: Department of Animal Sciences, School of Environmental and Biological Sciences Rutgers, the State University of New Jersey, USA

Cryptococcus gattii is a fungal pathogen capable of causing respiratory and systemic infection with potentially fatal sequelae. Infections are treated with antifungal induction therapy using amphotericin B and flucytosine, followed by maintenance therapy with fluconazole. Fluconazole prevents the biosynthesis of ergosterol and causes toxic sterol intermediates to accumulate, resulting in stress to the cell membrane. Strains of *C. gattii* exhibiting intrinsic resistance to fluconazole are emerging. Chong et al (PLOS ONE, 2010) used proteomic analysis to study the growth dynamics and protein expression profiles of intrinsically resistant strains of *C. gattii* in response to fluconazole and compared these with strains exhibiting typical fluconazole susceptibility. Differentially expressed proteins were represented as features in a protein interaction network. The aim of the current study is to further analyse and validate significant proteins in these networks. Using the latest gene annotation data and a new network analysis program, genes with a role in coordinating an effective cellular response to fluconazole, particularly in the resistant strain, were identified. These included a satellite network grouping ATP synthase subunits with high level of interconnectivity that had strain-dependent regulatory responses; HCR1, a highly connected hub protein that was highly down-regulated in the resistant strain. In addition, ontological sub-grouping of the network highlighted GRX5, which plays a role in oxidative stress response and was significantly up-regulated in the resistant strain. Deletion of these genes in *Saccharomyces cerevisiae* resulted in decreased resistance to fluconazole and various other stressors. Identifying how these cellular processes and pathways interact to coordinate an effective fluconazole response may allow new targets for novel antifungal treatments to be discovered.

id # 26227

Poster

Fungal-insect symbiosis: Exploring the relationship between the yeast, *Kodamaea ohmeri*, and its host, the small hive beetle, *Aethina tumida*.

Amos, B.[1, 2], Leemon, D.[1], Hayes, R.A.[3] and Furlong, M.[2]
[1]*Agri-Science QLD, Department of Agriculture and Fisheries, Brisbane, QLD.*
[2]*School of Biological Sciences, University of Queensland, Brisbane, QLD.*
[3]*University of the Sunshine Coast, Sippy Downs, QLD.*

The significance of symbiosis in fungi-plant systems is well recognised and represented in the scientific literature. However, fungal-insect symbioses are less well understood, except for a few well known examples, even though they appear to be present in almost every terrestrial ecosystem. Recent molecular investigations suggest that there is a great diversity of fungi associated with insects, potentially forming complex and varied symbioses. These relationships could be of great interest from both an evolutionary and ecological perspective. The close association between the yeast, *Kodamaea ohmeri* and the small hive beetle, *Aethina tumida* suggests a symbiotic relationship. *A. tumida* is a serious pest of European honeybees (*Apis mellifera*) in Australia and the U.S.A. The yeast *K. ohmeri* appears to be carried by the adult beetle and has been shown to be associated with the fermentation of hive products during development of *A. tumida* larvae. In order to explore this relationship, we will establish whether the yeast is an obligate or facultative symbiont of this host, the reciprocity of this relationship, and so determine the contribution of the relationship to beetle bionomics and ecology. This will allow qualitative and quantitative evaluation of the nature of their biological, evolutionary and ecological relationships. These investigations will be used as a model to better understand the role of symbiotic microbes in the evolution of insects and their roles in contributing to better exploitation of certain niches in ecological systems.

id #24276

Poster

Biocontrol and plant growth promoting potential of *Streptomyces hydrogenans* strain DH16

Talwinder Kaur[1], Rajesh K Manhas[1]
[1]*Guru Nanak Dev University, Amritsar Punjab, India, Amritsar, PUNJAB, India*

Increased public concern about environmental problems caused by the use of agrochemicals, used to control plant diseases and to increase crop yield, has provoked researchers to find out the alternate sources. Therefore, biological control of fungal phytopathogens, and plant growth promotion by use of microorganisms has received an increased emphasis as a safe, environment friendly, long lasting, inexpensive alternative to the chemical fungicides and fertilizers, respectively. In light of this a *Streptomyces* strain, possessing activity against different fungal phytopathogens viz. *Colletotrichum acutatum*, *Cladosporium herbarum*, *Alternaria brassicicola*, *Alternaria mali*, *Colletotrichum gleospoiroides*, *Alternaria alternata, Fusarium oxysporum* f.sp. *dianthi* and *Fusarium moniliformae,* was isolated from soil and identified as *Streptomyces hydrogenans* strain DH16. Application of culture supernatant (5%) and cells (10^7 cfu/ml) of *Streptomyces* DH16 as seed and foliar treatments on *Raphanus sativus* suppressed the disease incidence of black leaf spot caused by *A. brassicicola.* The metabolites in the extract of *S. hydrogenans* DH16 showed insecticidal potential with 70 % larval, 66.66 % prepupal and 100% pupal mortality at concentration of 1600 µg/ml.The metabolites also prolonged the larval developmental period along with morphological abnormalities. Additionaly,this strain also posseses various plant growth promoting activities *viz*. indole acetic acid production (IAA; 80 µg/ml), ACC deaminase activity and nitrogen fixation. The *in vivo* effect of *Streptomyces* DH16, and IAA produced by it on plant growth promotion was evaluated on pea seedlings (*Pisum sativum*). Both the treatments showed enhanced seed germination, root length, shoot length, fresh and dry weights, and number of lateral roots. These results demonstrates that *Streptomyces hydrogenans* DH16 strain as well as its extracellular metabolites (antifungal, insecticidal and plant growth promoting substances) can be exploited as soil amendment as biofungicide, insecticide and biofertilizer.

id #25607

Poster

Genome editing by CRISPR-Cas9 in pathogenic fungi

Pearl Dadd-Daigle[1], Leona Campbell, Dee Carter
[1]*The University of Sydney, Camperdown, NSW, Australia*

Fungal infections are very important to the medical community and are a cause of morbidity and mortality, particularly among the immunocompromised. *Candida glabrata* and *Cryptococcus neoformans* are two prominent fungal pathogens, causing candidiasis and cryptococcosis, respectively. Although there are now substantial genetic and genomic resources for the study of these organisms, creating and maintaining multiple knockouts in a single organism remains problematic. CRISPR-Cas9 is a novel system for genomic editing and has been adapted to many eukaryotic organisms, recently including the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Originally discovered as part of the bacterial immune system, DNA segments from invading viruses or plasmids are incorporated into the CRISPR locus. These are transcribed into RNA and direct CRISPR associated (Cas) endonuclease to complementary sequences in the invader's genome, where Cas introduces a double stranded break. This system has been adapted to create gene disruptions or DNA insertions, as subsequent repair must alter the target site to prevent further Cas cleavage. Guide RNA (gRNA), a ~20 nt sequence specific to the gene of interest, and cloned bacterial Cas protein are introduced into prokaryotic or eukaryotic cells where they allow editing of the target gene. CRISPR-Cas9 has not yet been applied to fungal pathogens, and the aims of this study are to introduce it into the fungus *Candida glabrata* and *Cryptococcus neoformans*. Bioinformatic analysis has identified numerous gRNA target sites in multiple genes of interest across both organisms, including genes with easily scored phenotypes where we can optimize the system. Successful application of the CRISPR-Cas9 will enable highly targeted editing unhindered by marker availability in these medically important organisms.

id #25600

Poster

Cytokine induction by *Cryptococcus* strains of varying pathogenicity

Kenya Fernandes, Adam Brockway, Leona Campbell, Markus Hofer, Dee Carter
School of Molecular Bioscience, University of Sydney

Cryptococcosis, a systematic fungal infection caused by pathogenic *Cryptococcus* species, has emerged as a devastating cause of morbidity and mortality worldwide. It presents with a range of clinical outcomes that result from complex interactions between the pathogen and the mammalian host. During infection, *Cryptococcus* cells lodge in the alveoli of the lung where alveolar macrophages release cytokines in the response to the presence of antigens. These cytokines modulate the development and expression of T-c ells stimulating a particular activation profile. A Th1 response is pro-inflammatory and leads to pathogen killing and clearance while a Th2 response is anti-inflammatory and leads to fungal growth.

In this study we are examining cytokine induction using two sets of *Cryptococcus* strains: a collection of 72 *C. neoformans* and *C. gattii* clinical isolates from two hospitals in Botswana, and a set of seven strains that have been derived from the virulent type strain *C. neoformans* H99 that vary in virulence despite being almost identical at the genotypic level. To assess host immune responses induced by different strains, J774.1 macrophages were infected with each isolate, the cells lysed, and purified RNA obtained. Cytokine expression was assessed by RNase Protection Assay (RPA) where radiolabelled anti-sense RNA probes are hybridised to purified macrophage RNA, non-hybridising RNA is degraded, and the protected RNA is electrophoresed, resulting in a profile of bands corresponding to the different cytokines.

The results to date have revealed substantial variation in the amounts of TNF-α, IL-1β, and IL-1α induced by the different clinical isolates. However, statistical analysis found no significant association between cytokine profile and genotype or clinical outcome. We are currently optimising this analysis to ensure that it is thoroughly standardised and reproducible across all infection assays, and extending it to the H99 derivative strains.

Poster

The Westmead Medical Mycology Collection - a reference collection for basic and clinical research and diagnosis of fungal diseases

Kennio Ferreira-Paim[1,2], Krystyna Maszewska[1], Mansura Khan[1] and Wieland Meyer[1]
[1]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School - Westmead Hospital, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Millennium Institute for Medical Research, Sydney, Australia;
[2]Infectious Disease Department, Triangulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil.

The Westmead Medical Mycology Collection is completing 20 years of existence. Currently there are 10,073 strains, representing 437 human and animal pathogenic fungal species, from 52 countries maintained in the collection. Established originally at the Molecular Mycology Research Laboratory, in the Centre for Infectious Disease and Microbiology at the Sydney Medical School-Westmead Hospital, The University of Sydney it recently moved to the new Westmead Millennium Institute for Medical Research, Westmead, Australia. Its primary aim is to preserve Australian and global pathogenic fungal biodiversity, providing reference and clinical strains associated with strain-specific metadata for the mycology community. The stored strains are identified phenotypically, biochemically and molecularly. They are storage either lyophilized, in glycerol at -80°C or as living culture at 14°C. The majority of the stored strains are the result of specific clinical, molecular epidemiological and basic science projects. As such,

the pathogenic yeasts *Cryptococcus neoformans* and *C. gattii* (5,465 strains) account for 50% of the specimens. To further characterise the maintained strains specific MultiLocus Sequence Typing schemes have been developed for *C. neoformans, C. gattii, Scedosporium apiospermum*, *S. aurantiacum*, *S. boydii* and *Pneumocystis jirovecii*, globally accessible at http://mlst.mycologylab.org or http://isham.org. Reference strains of all *C. neoformans* major molecular types/species (VNI, VNII, VNB, and VNIV) and 56 out of 324 Sequence Types (ST) and all *C. gattii* major molecular types/species (VGI, VGII, VGIII, and VGIV) and 118 of the 336 ST's descripted are stored. The collection also holds 119 cryptococcal strains for which whole genome sequencing was performed. The collection formed the basis for the development of the quality controlled ISHAM-ITS sequence database for human and animal pathogenic fungi accessible at http://its.mycologylab.org or http://isham.org. 900 strains included in the newly established ISHAM- ITS reference database are maintained. For further information please contact the curator at: wieland.meyer@sydney.edu.au.

Poster

Closing the gap - DNA barcoding of pathogenic fungi

Laszlo Irinyi[1], Mansura Khan[1], Wieland Meyer[1] and the global medical mycology ISHAM barcoding working group
[1]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School - Westmead Hospital, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Millennium Institute for Medical Research, Sydney, Australia

Human and animal fungal pathogens are a growing threat worldwide. They lead to emerging infections and create new risks for established ones. There is a growing need for the rapid and accurate identification of pathogens to enable early diagnosis and targeted antifungal therapy. One of the most encouraging approaches is molecular barcoding using the internal transcribed spacer (ITS) of the rDNA, which is rapid, easily achievable, accurate, and applicable directly from clinical specimens. However, the major drawback of the fungal barcoding initiatives and its broad-scale applications is the lack of quality-controlled reference databases. To address this issue, an ISHAM (International Society for Human and Animal Mycology) working group focusing on "DNA barcoding of human and animal pathogenic fungi" created a reference ITS database for human and animal pathogenic fungi. The database is freely accessible from http://www.isham.org/ or directly at http://its.mycologylab.org/). It currently contains 3200 complete ITS sequences covering 524 fungal species. The generated sequences were used to estimate the variation and overall utility of the ITS region as the barcode region. The overall intraspecies variation was found to be less than 1.5%, with most of the phylogenetic taxa presenting a barcoding gap. However, in certain species the ITS showed a high variability (up to 2.25%) or was insufficient to discriminate closely related/cryptic species. To overcome these limitations, alternative genetic loci have been identified using whole genome comparison, amongst them the translation elongation factor 1 α gene (*EF1*α) is a promising secondary barcode candidate for the identification of clinically relevant species. The amplified region proved to be less variable at species level but provided higher resolution separating numerous closely related taxa. The ISHAM-ITS sequences have also been submitted to GenBank and UNITE in order to extend the number of quality controlled sequences from human/animal pathogenic fungi in these databases.