8th International Mycological Congress

IMC8 2006 CAIRNS

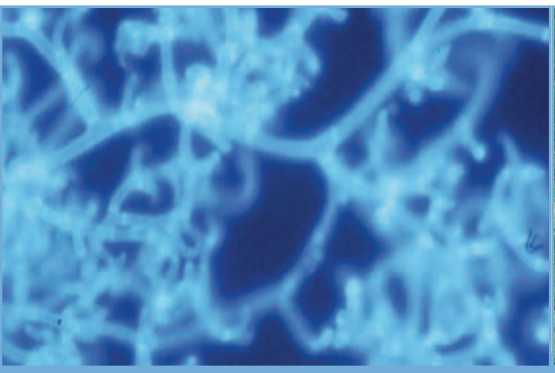
21 – 25 August, 2006

Cairns Convention Centre
Queensland, Australia

First in the Southern Hemisphere



Congress Handbook & Abstracts Book 1



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The Organising Committee of the 8th International Mycological Congress would like to sincerely thank the following sponsors and exhibitors for their generous support.

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MAF BIOSECURITY NEW ZEALAND

The Australian Society for Microbiology

MSA the Mycological society of america

bms British Mycological Society promoting fungal science

日本菌学会 The Mycological Society of Japan

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Cambridge University Press
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AUSTRALASIAN MYCOLOGICAL SOCIETY

CONTENTS PAGE

WELCOME TO IMC8 CAIRNS 2006

On behalf of the organising committee, it is our pleasure to welcome you to the first International Mycological Congress to be held in the southern hemisphere, IMC8 in Cairns, Australia.

It has been a challenge to design and bring to fruition a congress that can meet the high standards set by previous Congresses. We hope to provide you with a congress experience that is stimulating, rewarding and enjoyable.

We have developed an outstanding program, thanks to our national and international scientific program organising committee, and to your scientific input and participation. There are nine pre-congress workshops to choose from, and within the congress program, there is an invited internationally recognised plenary speaker each day. The daily program encompasses five concurrent sessions of scientific symposia that include a combination of invited speakers and presenters chosen from submitted abstracts, and an extensive range of scientific poster presentations for your perusal. We trust that there will be something of interest to all mycologists in our large and varied scientific program.

We invite you to attend the social events. On Monday evening, the Mycological Society of America and the British Mycological Society host a reception on the waterfront at the Cairns Hilton Hotel. Everyone is invited to attend.

Building on a previous successful IMC convention, please join us for a 'Wines of the World' evening on Tuesday night. You bring a bottle of wine to share with your friends and colleagues, and in an atmosphere of joviality, we challenge you to approach as many people as possible to taste and judge your wine. Prizes go to the best and worst wines of the world! This is a great networking experience/opportunity? and a great way to make new friends.

The Congress dinner will be held on Thursday evening at 'The Tanks'. This will be a unique Cairns dining experience, within the Botanic Gardens precinct.

During the Congress, 'The Clamp Connection Café/Bar' will be open to meet your 'between break' beverage and snack needs. Feel free to take advantage of this relaxed space to catch up with colleagues and friends, and to develop new research collaborations and partnerships.

A number of mycology-orientated tours and forays have been organised, and we encourage you to take the opportunity to explore Cairns and ints hinterland on these tours.

We promise you an outstanding scientific program and a memorable social experience in Australia. The Congress will be the biggest mycology meeting ever held in Australia. It will provide a forum for the sharing of information on all aspects of mycology and foster constructive interaction between participants from all over the world.

With its combined World Heritage-listed attractions of the Great Barrier Reef and the Wet Tropic Rainforests, Cairns has a lot to offer. We hope that you are able to take some time to visit the area and discover the natural wonders of tropical northern Australia.

Thank you for coming, we trust you find your visit enjoyable and rewarding.

Wieland Meyer
Chair
IMC8 Organizing Committee

Ceri Pearce
Vice-Chair

IMC8 2006 ORGANISING COMMITTEE

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Ceri Pearce, Vice-Chair
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GENERAL INFORMATION

The following information is offered to make your attendance at IMC8 2006 as pleasant and as trouble-free as possible. If you require help, please call at the registration Desk and we will do everything we can to assist you.

Registration and Information Desk
The Registration Desk will be located in the main foyer of the Cairns Convention Centre. It will be open at the following times:

Sunday 20th August	14:00-18:00
Monday 21st August	07:00-17:30
Tuesday 22nd August	07:30-17:30
Wednesday 23rd August	07:30-18:00
Thursday 24th August	07:30-17:30
Friday 25th August	07:30-18:00

Accommodation Contacts

Map ref		
B	Hilton Cairns	07 4050 2000
C	Sofitel Reef Casino Cairns	07 4060 8888
D	Holiday Inn Cairns	07 4050 6070
E	Oasis Resort Cairns	07 4080 1888
F	Pacific International Cairns	07 4051 7888
G	Tradewinds Esplanade Hotel	07 4053 0300
H	Heritage Cairns	07 4051 1211
	Rydges Esplanade Resort	07 4031 2211
I	Rydges Plaza Cairns	07 4046 0300
J	Club Crocodile Hides Hotel	07 4051 1266
K	Cairns Holiday Lodge	07 4051 4611
L	181 The Esplanade	07 4052 6888
M	Cairns Aquarius Apartments	07 4051 8444
O	Inn Cairns Boutique Apartments	07 4041 2350
	Mantra Trilogy Resort	07 4080 8000
P	Mid City Luxury Suites	07 4051 5050
	Oaks City Quays & Piermonde Cairns	07 4042 6400
Q	City Terraces Apartments	07 4051 8955
R	Il Centro Luxury Apartments	07 4031 6699
S	Il Palazzo Boutique Apartment	07 4041 2155
T	Southern Cross Atrium Apartments	07 4031 4000
U	Gilligan's Backpackers Hotel & Resort	07 4041 6566

Accreditation
A Certificate of Attendance will be provided indicating your attendance at the Congress. Individuals may then claim CPD points from their respective organisations.

Name Tags
For security purposes your name tag must be worn at all times. Entry to all sessions, exhibition and Welcome Reception is by name tag only.

Banking
Normal banking hours are Monday - Thursday 9:30am-4:00pm and Fridays 9:30-5:00pm, excluding public holidays. 24 Hour Automatic Teller Machines (ATMs) can be found throughout the City and Shopping Centres and in the Sofitel Reef Casino Cairns.

Coat Check/Bag Store
The Cairns Convention Centre does not have a facility. When checking out of your hotel for your return journey home, please make arrangements for your bags to be stored at the hotel.

Childcare
Please note, no official arrangements have been made for childcare during the congress. Your chosen accommodation may be able to assist you further with babysitting services during your stay.

Public Internet Facilities
Inbox Cafe- 119 Abbott St, Cairns
Reff Highspeed Netcafe- shp 2/31 Shields st, Cairns
The Call Station- 123 Abbott st, Cairns
Gilligan's - Grafton St
Global Gossip Cairns- 125 Abbott st, Cairns
Wireless facilities are available at the Convention Centre

Goods and Services Tax (GST) / Tourist Refund Scheme (TRS)
GST is included in all prices, unless otherwise stated. You can claim a refund of the GST and wine equalisation tax (WEX) that you pay on goods you buy in Australia. The refund only applies to goods you take with you as hand luggage or wear on to the aircraft when you leave the country. (the goods can be used in Australia before departure). To qualify for the TRS, you must: spend $300 or more in the one store and get a single tax invoice; buy goods no more than 30 days before departure; wear or carry the goods on board and present them along with your tax invoice, passport and boarding pass to a TRS facility. *Claims are only available up to 30 minutes prior to the scheduled departure of your flight.*

Medical Services
If you require medical assistance, please contact the Registration Desk staff.

Messages
Message sent to the secretariat will be placed on a notice board near the Registration Desk. The secretariat will not locate the individual delegate.

Mobile Phones
Please respect the presenter and other members of the audience by ensuring your mobile phone is switched off or to silent while you are in sessions.

Parking
Cairns Convention Centre has a public Car park below. Entry is via Sheridan Street. $3 coin operated boomgate. Carpark is open from 7am-until program concludes.
Parking metres are around the CBD area. You have to pay from approx 8.30am - 5.00pm.
In the pier marketplace carpark, which is located on Esplanade, you still have to pay until 10pm. Cairns Central Shopping Centre is Free of Charge + along wharf St, you can park your car for $2 per day or undercover at the Casino for $5 per day.

Shopping
There is a shopping facilities within a 5 min walk of the Cairns Convention Centre: either Cairns Central Shopping Centre on McLeod Street or the Mall located on Cnr Shields & Lake Street. General Shops i.e Cairns Central are open approx 9.30am - 5.30pm
Supermarkets are open to approx 9pm on weekdays and 5.30pm weekends.

Post Offices
Orchard Plaza - Abbott St
Cairns Central Shopping Centre
Grafton St (2 min walk)

Pharmacies
Cairns Central Chemart Pharmacy
Terry White Cairns Central Shopping Centre
Pharmacy - Shop 1/86 Lake St, Cairns
Chemist Warehouse - 50 McLeod St, Cairns
Esplanade Day & Night Pharmacy - Shop 10/85 The Esplanade

Refreshments
Morning tea and afternoon tea are included in your registration fee and are provided during the programmed breaks in the exhibition and outside patio areas.

Lunches: are not included in the registration. Tickets can be bought for lunches at AU$15.00 per person per day, order by 5pm the day prior. Pre purchase was required and your tickets will be in your registration pack.

Supper Meals: a light Meal will be available on a ticket basis AU$22.00 each, for Monday and Tuesday. Pre purchase was required and your tickets will be in your registration pack.

Clamp Connection Café/Bar:12 noon-close of conference each day! The Café offers you an opportunity to spend time talking with representatives from exhibition stands, viewing poster displays, and networking with colleagues and new friends. Refreshment, beer, wine, soft drinks, tea and coffee will be available on a cash basis.

Speakers Preparation Room – Meeting Room 9
All Presenters must check in to ensure your audio visual needs are confirmed, and also that you have arrived at the congress in time to present.

Special Dietary Requirements
Delegates who have specified their special dietary requirement on their registration form should identify themselves to the service staff at the Convention Centre.

Telephones
Public Telephones, coin operated, are located in the Sheridan St foyer.

Tipping
Tipping is not expected in Australia but is appreciated for particularly good service.

Telephone Directory

Emergency Services 000
(fire/police/ambulance)
Registration Desk
Tel: 07 4042 4300 Fax: 07 4042 4302
Taxi Services
Black and White Taxi - 13 10 08
Taxi phone is located outside the main entrance of the centre.
Airlines

Qantas	131 313
Virgin Blue	136 789
Jetstar	131 538
Air New Zealand	132476
Cathay Pacific	131747
Japan Airlines	(07)3229-9916

Travel and Touring
There will be a Visitor Information booth situated in Hall 2 to assist you with any tours and travel enquiries that you might have.

Posters
The Posters will be judged by a panel and prizes awarded.

Registration Cancellation & Refund Policy
Cancellations received on or before Friday 7 July 2006 received a refund of registration fees, less an administrative charge of AUD$100.00. Cancellations after this date are not refunded

SOCIAL PROGRAM

Welcome reception - Sunday 20 August -
Cairns Convention Centre - *1800–1930*
Renew old acquaintances and join fellow delegates for informal drinks and canapés in the exhibition area for IMC8.
Cost included in registration. Entry is via name tag or ticket.
Dress: Casual.

BMS/MSA joint Reception - Monday 21 August -
Cairns Hilton- *2000-2100*
The British Mycological Society and the Mycological Society of America are jointly hosting a reception following the Honorary Lecture.
Included in registration.
Dress: Casual. Delegates will make their own way to the venue.

Wines of the World - Wednesday 23 August -
Hall 2 – *1900-2200 hours*
Bring a bottle of wine along to the Convention Centre and share with your colleagues.
Tickets: AU$22 per person.
Dress: Casual. Delegates will make their own way to the venue.

Congress Dinner - Thursday 24 August – The Tanks -
1900–2400 hours
The premier social occasion of the congress will be an event not to be missed!. Not included in registration.
Tickets: AU$120 per person includes three course meal and beverages.
Dress: Smart casual. Delegates will be collected from their hotels from 1830 and shuttle busses will return guests from 2230.

Partners program
A Partners program has been developed as an introduction to Cairns and its attractions. Included in the program is the Welcome Reception on Sunday. On Monday the day will start with a morning tea at the Cairns Convention Centre at 0930 followed by a City Sights Tour and concluding with a lunch back at the Cairns Convention Centre.

Posters
The Posters will be judged by a panel and prizes awarded.

Registration Cancellation & Refund Policy
Cancellations received on or before Friday 7 July 2006 received a refund of registration fees, less an administrative charge of AUD$100.00. Cancellations after this date are not refunded.

Workshops and Tours

Wednesday - 16th – Friday 18th August

0900-1800 - Workshop 1 (by Invitation only): Ceratocystis and Ophistoma

Moreton Bay Research Station, Brisbane
Chair: Mike Wingfield (South Africa) / Keith Seifert (Canada)

Friday 18th August

0730-1800 - Cairns Hinterland Lichen Tour

Friday 18 August to Saturday 19 August 2006, and Sunday 20th August at James Cook University

0730-1800 - Daintree Rainforest Photo Tour

0900-1800 - Workshop 2: Filamentous Fungi in the Clinical Laboratory

James Cook University, Cairns
Chair: Richard Summerbell (The Netherlands)

0900-1800 - Workshop 3: Insect Pathogens in the Tropics

James Cook University
Chair: Nigel Hywel-Jones (Thailand)

Saturday 19th August

0730-1800 - Cairns Hinterland Lichen Tour cont…

Friday 18 August to Saturday 19 August 2006, and Sunday 20th August at James Cook University

0900-1800 - Workshop 4: Food Mycology and Mycotoxins

Cairns Convention Centre
Chairs: John Pitt (Australia) / Ailsa Hocking (Australia) / Brett Summerell (Australia)

0900-1800 - Workshop 5: Rust Taxonomy

James Cook University
Chair: Yoshitaka Ono (Japan)

0900-1800 - Workshop 9: Hypogeous Fungi

James Cook University
Chairs: Teresa Lebel (Australia) / Sandra Abell (Australia)

Sunday 20th August

0900-1700 - Cairns Hinterland Lichen Tour – details above

James Cook University
Pick up from your hotels and transfer to James Cook University.

0900-1800 - Workshop 4: Food Mycology and Mycotoxins cont…

Cairns Convention Centre
Chairs: John Pitt (Australia) / Ailsa Hocking (Australia) / Brett Summerell (Australia)

0900-1800 - Workshop 6: Smut Taxonomy Workshop

James Cook University
Chairs: Kálmán Vánky (Germany) / Roger Shivas (Australia)

0900-1800 - Workshop 7: AnaSat2: From Spore to Culture

James Cook University
Chairs: Pedro Crous (The Netherlands) / Keith Seifert (Canada)

0900-1800 - Workshop 8: Compendium of Rust Fungi

James Cook University
Chairs: Reinhard Berndt (Switzerland) / Yoshitaka Ono (Japan)

Monday 21st August 2006

Time	Activity				
07:00	**Registration** *Foyer*				
08:30	**Opening Ceremony** *Halls A & B*				
09:15	**Plenary 1:** Franz Oberwinkler (Germany) **Fungal Tree of Life** *Halls A & B*				
10:15	**Coffee Break** – *Hall 2*				
10:45	**Symposium 1** *Halls A & B* **Phylogenetic Biology of Fungal and Fungal-like Phyla** Rytas Vilgalys (USA) Joseph Spatafora (USA)	**Symposium 2** *Hall C* **Do Plant Pathogens have a specific Armory?** Barbara Howlett (Australia) Thierry Rouxel (France)	**Symposium 3** *MR 1 & 2* **Insect Associated Fungi** Diana Six (USA) Meredith Blackwell (USA)	**Symposium 4** *Hall D* **Fungal Cell Biology** Reinhard Fischer (Germany)	**Symposium 5** *MR 3 - 5* **Ecology and Diversity of *Penicillium* and *Aspergillus* in Australia** John Pitt (Australia) Ailsa Hocking (Australia)
12:45	**Lunch [pre purchase]** – *Hall 2*				
13:30	**Poster Session 1: Phylogeny, Systematics & Evolution** **Poster Session 9: Mycorrhizae**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 6** *MR 1 & 2* **Molecular Taxonomy of Yeast** Cletus Kurtzman (USA) Andre Lachance (Canada)	**Symposium 7** *Hall C* **Transcriptome Analyses of Fungal Pathogens during Infection** Yue Wang (Singapore) Marc-Henri Lebrun (France)	**Symposium 8** *MR 3 - 5* **Diversity of Microfungi in Neotropica** Jose Dianese (Brazil) José Hernandez (Argentina)	**Symposium 9** *Hall D* **The Fungal Septum and associated Organelles** Katsu Kitamoto (Japan) Gregory Jedd (Singapore)	**Symposium 10** *Halls A & B* **Population Genetics of Fungi** Jeremy Burdon (Australia) Linda Kohn (Canada)
17:30	**Poster Session / Supper [pre purchase] / "Clamp Connection Café/Bar"** – ***cash basis bar for drinks and coffee*** – *Hall 2*				
19:00	**Honorary Lecture:** David Hawksworth (Spain) **Mycology and Mycologists** *Halls A & B*				
20:00	**Joint Reception of the British Mycological Society and the Mycological Society of America** *Cairns Hilton*				

Tuesday 22nd August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**Symposium 11** *Hall D* **Ascomycete Phylogenetics** Kevin Hyde (Hong Kong) Joseph Spatafora (USA)	**Symposium 12** *Halls A & B* **Proteome Analysis** Jim Kronstad (Canada) Scott E. Baker (USA)	**Symposium 13** *Hall C* **Mycorrhizal Ecology** Tom Bruns (USA) John Kilronomos (Canada)	**Symposium 14** *MR 1 & 2* **Propagation Strategies of Fungi** Akira Suzuki (Japan) Felix Baerlocher (Canada)	**Symposium 15** *MR 3 - 5* **Emerging and Reemerging Pathogenic Fungi** Hester Vismer (South Africa) Sue Coloe (Australia)
10:00	**Coffee Break** – *Hall 2*				
10:30	**Plenary 2:** John Taylor (USA) **Species Concepts in Fungi** *Halls A & B*				
11:30	**Lunch [pre purchase]** – *Hall 2*				
12:00	**Poster Session 2: From Genomics to Proteomics** **Poster Session 6: Food Mycology and Mycotoxins**				
13:30	**Symposium 16** *Halls A & B* **Fungal Phylogenomics** Teun Boekhout (Netherlands) Bernard Dujon (France)	**Symposium 17** *Hall C* **Signal Transduction during Pathogenesis** Jinh-Rong Xu (USA) Marty Dickman (USA)	**Symposium 18** *MR 3 - 5* **Veterinary Mycology** Kishio Hatai (Japan) Mark Krockenberger (Australia)	**Symposium 19** *Hall D* **Substrate Colonisation and Succession in Wood Inhabiting Fungi** Jan Stenlid (Sweden) Lynne Boddy (UK)	**Symposium 20** *MR 1 & 2* **Fungi of Monsoon Asia: - Linking Diversity and Ecosystem Function** Morten Christensen (Denmark) Seiji Tokumasu (Japan)
15:30	**Coffee Break** – *Hall 2*				
16.00	**Symposium 21** *Halls A & B* **DNA Barcoding for Fungi** Richard Summerbell (The Netherlands) Andre Levesque (Canada)	**Symposium 22** *MR 1 & 2* **Polyketides, Non-Ribosomal Peptides, and Terpenes as fungal signal molecules** Jens Frisvard (Denmark) Barry Scott (New Zealand)	**Symposium 23** *Hall C* **Adhesion of Fungi to Plant or Animal Hosts** Nick Talbot (UK) Ester Segal (Israel)	**Symposium 24** *MR 3 - 5* **Protein Secretion in Fungal Biotechnology** David Archer (UK) Merja Penttila (Finland)	**Symposium 25** *Hall D* **Evolution of Symbioses** Dominik Begerow (Germany) Martin Grube (Austria)
18:00	**Supper [pre purchase] / "Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
18:30	**Poster Session** *Hall 2*			**Roundtable 1** *Halls A & B* **Is it Time for a Mycological Code of Nomenclature?** Pedro Crous, David Minter, Amy Rossman, Franz Oberwinkler, John Taylor, Tsuyoshi Hosoya	
20:00	**MSJ Editorial Meeting** *MR8*				
20:30	**Evening Free**				

Wednesday 23rd August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**Discussion Group** *MR 1 & 2* **Lichen-Fungal Genome Sequencing Project** Paul Dyer (UK)	**Australasian Mycological Society AGM** *Hall C*	**Discussion Group** *MR 3 - 5* **Fungal Genome Sequencing Programs at the DOE Joint Genome Institute** Scott Baker (USA)		
09:30	**Break**				
10.00	**Plenary 3:** James Galagan (USA) **Comparative Fungal Genomics** *Halls A & B*				
11:00	**Coffee Break** – *Hall 2*				
11:30	**Symposium 26** *Halls A & B* **Lichen Symbiosis: Extraterrestrial Life, Evolution and Penguin Rookery** Francois Lutzoni (USA) Magdalena Pavlich (Peru)	**Symposium 57** *MR 3 - 5* **Chytridiomycete Fungi** Gordon Beakes (UK) Peter McGee (Australia)	**Symposium 28** *MR 1 & 2* **Enzymes and Infection Mechanisms** Michel Monod (Switzerland) Matt Templeton (New Zealand)	**Symposium 29** *Hall D* **Fungal Physiology** Helena Nevalainen (Australia) Ken Hammel (USA)	**Symposium 30** *Hal* **Asia-Pacific Fungal Biodiversity** Gareth Jones (Thailand) Sitti Aisyah Alias (Thailand)
13.30	**Lunch [pre purchase]** – *Hall 2*				
14.00	**Poster Session 3: Plant and Fungal Pathogens** **Poster Session 10: Animal Pathogens**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 31** *MR 3 - 5* **Systematics and Ecology of Dimorphic Basidiomycetes** Alvaro Fonseca (Portugal) Jose Paulo Sampaio (Portugal)	**Symposium 32** *MR 1 & 2* **Bioinformatics and Databases** Vincent Robert (Netherlands) Peter Dawyndt (Belgium)	**Symposium 33** *Hall D* **Antifungal Resistance** Richard Cannon (New Zealand) Dominique Sanglard (Switzerland)	**Symposium 34** *Hall C* **Importance of Small Non-Coding RNAs in Fungi** Carlo Cogoni (Italy) Rodolfo Aramayo (USA)	**Symposium 35** *Halls A* **Gondwanan Fungi** Tom May (Australia) Peter Buchanan (New Zealand)
17:30	**"Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
18:00	**Poster Session** *Hall 2*	**Roundtable 2** *Hall C* **Access and benefit sharing in relation to the Biodiversity Convention** Lene Lange (Denmark)	17:45-19:00 **Meeting of the International Association Lichenology** *MR1&2*		
20:00	**Wines of the World** *Hall 2*				

Thursday 24th August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**General Meeting of the International Commission for the Taxonomy of Fungi** *MR 1 & 2* Keith Seifert (Canada)	**Symposium 42** *Hall C* **Genomes and Fitness** Gareth Griffith (UK) Simon Avery (UK)	**Proffered Session 1** *MR 3 - 5* **Phylogeny 1** Heide-Marie Daniel (Belgium)	**Proffered Session 2** *Hall D* **Medical Mycology** Sharon Chen (Australia)	
10:00	**Break**				
10:15	**Plenary 4:** Regine Kahmann (Germany) **Mating in Fungi** *Halls A & B*				
11:15	**Coffee Break** – *Hall 2*				
11:45	**Symposium 36** *MR 1 & 2* **Straminopiles: Why and How?** Daiske Honda (Japan) Gordon Beakes (UK)	**Symposium 37** *MR 3 - 5* **Advanced Cellular Imaging and Micromanipulation** Nick Read (UK) Rosa Morinho-Perez (Mexico)	**Symposium 38** *Hall C* **Fungal Pigments and Virulence** Josh Nosanchuck (USA) Beatriz L. Gomez (UK)	**Symposium 39** *Halls A & B* **Biosynthetic Gene Clusters for Fungal Secondary Metabolites** Nancy Keller (USA) Marc Stadler (Germany)	**Symposium 40** *Hall D* **Biosecurity** Geoff Ridley (New Zealand) Ceri Pearce (Australia)
13:45	**Lunch [pre purchase]** – *Hall 2*				
14:30	**Poster Session 4: Cell Biology and Physiology** **Poster Session 8: Population Genetics**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 41** *MR 1 & 2* **Evolution, Ecology and Systematics of Endophytic Fungi - Horozontally Transmitted Endophytes** Elizabeth Arnold (USA) Gerard Verkley (The Netherlands)	**Symposium 56** *MR 3 - 5* **Phylogeography** Greg Mueller (USA) Thorsten Lumbsch (USA	**Symposium 43** *Hall D* **Biocontrol** Augusto Schrank (Brazil) Naresh Magan (UK)	**Symposium 44** *Hall C* **Industrial Mycology** Cees van den Hondel (Netherlands) Lene Lange (Denmark)	**Symposium 45** *Halls A & B* **Worldwide Movement of Fungal Forest Pathogens** Brenda Wingfield (South Africa) Matteo Garbelotto (USA)
17:30	**Poster Session / "Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
19:00-23:30	**Conference Dinner** *The Tanks*				

Friday 25th August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	*Conference Room 1* **International Mycological Association (IMA) Board Meeting**	**Proffered Session 3** *Hall C* **Phylogeny 2** Clement Tsui (Australia)		**Proffered Session 4** *Hall D* **From Mycological Diversity to Phylogeny** Kálmán Vánky (Germany)	
10:00	**Coffee Break** – *Hall 2*				
10:30	**Symposium 46** *Hall C* **Anything Specific about Human Pathogens?** Alex Adrianopoulos (Australia) James Fraser (Australia)	**Symposium 47** *Halls A & B* **Biodiversity of Microfungi - A Phylogenetic Approach** Andrew Miller (USA) Amy Rossman (USA)	**Symposium 48** *Hall D* **Molecular Plant Mycorrhizal Interaction** Mark Tibbett (Australia) Paola Bonfante (Italy)	**Symposium 49** *MR 1 & 2* **Marine Fungi** Ka-Lai Pang (Hong Kong) Mohamed Abdel-Wahab (Egypt)	**Symposium 50** *MR 3 - 5* **Mycetozoan Biodiversity** Steven Stephenson (USA) David Orlovich (New Zealand)
12:30	**Lunch [pre purchase]** – *Hall 2*				
13:30	**Poster Session 5: Biodiversity and Conservation** **Poster Session 7: Industrial Mycology**				
14:30	**Symposium 51** *Hall D* **Finding the Missing Taxa: the Search for Fungi in Under-explored Habitats** Wendy Untereiner (Canada) James Scott (Canada)	**Symposium 52** *Hall C* **Fungi and Eucalypts** Eric McKenzie (New Zealand) David Ellis (Australia)	**Symposium 53** *MR 3 - 5* **Epidemiology of Fungal Pathogens** Wieland Meyer (Australia) Rosely Zancope-Olivera (Brazil)	**Symposium 54** *Halls A & B* **Fusarium - New Advances in Taxonomy, Biology and Detection** Brett Summerell (Australia) Keith Seifert (Canada)	**Symposium 55** *MR 1 & 2* **Conservation and Utilization of Fungal Biodiversity through Genetic Resource Centres** Pedro Crous (Netherlands) Akira Nakagiri (Japan)
16:30	**Coffee Break / "Clamp Connection" closes** – *Hall 2*				
17:00	**Plenary 5:** Mike Wingfield (South Africa) **Forest Fungi in a Changing World** *Halls A & B*				
18:00	**Closing Ceremony** *Halls A & B*				

Monday 21st August 2006

Time	Activity				
07:00	**Registration** *Foyer*				
08:30	**Opening Ceremony** *Halls A & B*				
09:15	**Plenary 1:** Franz Oberwinkler (Germany) **Fungal Tree of Life** *Halls A & B*				
10:15	**Coffee Break** – *Hall 2*				
10:45	**Symposium 1** *Halls A & B* **Phylogenetic Biology of Fungal and Fungal-like Phyla** Rytas Vilgalys (USA) Joseph Spatafora (USA)	**Symposium 2** *Hall C* **Do Plant Pathogens have a specific Armory?** Barbara Howlett (Australia) Thierry Rouxel (France)	**Symposium 3** *MR 1 & 2* **Insect Associated Fungi** Diana Six (USA) Meredith Blackwell (USA)	**Symposium 4** *Hall D* **Fungal Cell Biology** Reinhard Fischer (Germany)	**Symposium 5** *MR 3 - 5* **Ecology and Diversity of *Penicillium* and *Aspergillus* in Australia** John Pitt (Australia) Ailsa Hocking (Australia)
12:45	**Lunch [pre purchase]** – *Hall 2*				
13:30	**Poster Session 1: Phylogeny, Systematics & Evolution** **Poster Session 9: Mycorrhizae**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 6** *MR 1 & 2* **Molecular Taxonomy of Yeast** Cletus Kurtzman (USA) Andre Lachance (Canada)	**Symposium 7** *Hall C* **Transcriptome Analyses of Fungal Pathogens during Infection** Yue Wang (Singapore) Marc-Henri Lebrun (France)	**Symposium 8** *MR 3 - 5* **Diversity of Microfungi in Neotropica** Jose Dianese (Brazil) José Hernandez (Argentina)	**Symposium 9** *Hall D* **The Fungal Septum and associated Organelles** Katsu Kitamoto (Japan) Gregory Jedd (Singapore)	**Symposium 10** *Halls A & B* **Population Genetics of Fungi** Jeremy Burdon (Australia) Linda Kohn (Canada)
17:30	**Poster Session / Supper [pre purchase] / "Clamp Connection Café/Bar"** – ***cash basis bar for drinks and coffee*** – *Hall 2*				
19:00	**Honorary Lecture:** David Hawksworth (Spain) **Mycology and Mycologists** *Halls A & B*				
20:00	**Joint Reception of the British Mycological Society and the Mycological Society of America** *Cairns Hilton*				

Monday 21st August Program

0830-0915 **Halls A&B**

Opening Ceremony

0915-1015 - 0995 **Halls A&B**

Plenary 1

Fungal Tree of Life

Franz Oberwinkler (Germany)

1045-1245

Symposium 1: Phylogenetic Biology of Fungal and Fungal-like Phyla

Chairs: Rytas Vilgalys (USA) / Joseph W. Spatafora (USA)

This symposium summarizes just a few recent advances in phylogenetic studies across fungal (and fungal-like) phyla. The talks here represent current research on diverse lineages including Ascomycota, Basidiomycota, basal fungal lineages, and also Myxomycota and Oomycota. For each phylum, speakers will present new results, and discuss the transformative effects of phylogenetic studies on the field of mycology.

1045-1105 IS1 - 0678

The Saprolegniaceae — new species concepts

David E. Padgett (USA)

1105-1125 IS2 - 0178

Trichomycetes: major taxonomic revisions based on molelcular phylogenies

Robert Lichtwardt (USA)

1125-1145 IS3 - 0293

An up to date assessment of the place of the Mycetozoans among the Eukaryotes

Frederick W. Spiegel (USA)

1145-1205 IS4 - 0296

Molecular systematics and evolution of Boletales

Andrew Wilson (USA) *Author: M Binder*

1205-1225 IS5 - 0433

Assembling the fungal tree of life: evolution of the Ascomycota

Joseph W. Spatafora (USA)

1225-1245 IS6 - 0748

Evolution of basal lineages in Fungi: deconstructing Chytridiomycota and Zygomycota

Rytas. Vilgalys (USA)

1045-1245 **Hall C**

Symposium 2: Do Plant Pathogens have a Specific Armory?

Chairs: Barbara Howlett (Australia) / Thierry Rouxel (France)

Fungi use a diverse range of strategies to invade and colonize plants. The five speakers in this symposium describe some of these strategies, focusing on the initial stages of the interaction between fungus and plant.

1045-1115 IS1 - 0682

The establishment of biotrophy in the Ustilago maydis/maize pathosystem"

Regine Kahmann (Germany)

1115-1145 IS2 – 0999

A secondary metabolite is involved in recognition of the blast fungus Magnaporthe grisea by resistant rice cultivars

Marc-Henri Lebrun (France)

1145-1215 IS3 - 0705

Are A+T-rich isochores niches for pathogenicity genes in the genome of Leptosphaeria maculans ?

Marie-Hélène Balesdent (France)

1215-1230 PS1 - 0459

Early onset of toxin biosynthesis in a forest pathogen"

Arne Schwelm (New Zealand)

1230-1245 PS2 - 0519

Defining the role of the Avr3a avirulence gene in Phytophthora infestans – potato interactions"

Steve Whisson (UK)

1045-1245 **Meeting Room 1&2**

Symposium 3: Insect Associated Fungi

Chairs: Diana Six (USA) / Meredith Blackwell (USA)

Fungi and insects live together in many habitats, and this closeness fosters a variety of intimate interactions. The speakers in this symposium will discuss the systematics and community and population level aspects of associations between fungi and insects in ligno-cellulosic substrates.

1045-1115 IS1 - 1005

Microbial communities in the gut of wood-ingesting beetles

Meredith Blackwell (USA)

1115-1145 IS2 - 0659

Temperature driven symbiont shifting in a bark beetle-fungus ectosymbiosis: a mechanism of stability?

Diana Six (USA)

1145-1215 IS3 - 0665

Comparing molecular ecological patterns in populations of different fungal mutualists of woodwasps

Bernard Slippers (South Africa)

1215-1230 PS1 - 0582

A new phylogenetic lineage of Ophiostoma spp., discovered on termites and termite combs in South Africa

Wilhelm de Beer (South Africa)

1230-1245 PS2 - 0018

Interactions of fungi and tree killing bark beetles: Geographic variation and interspecific competition

Kier Klepzig (USA)

1045-1245 **Hall D**

Symposium 4: Fungal Cell Biology

Chairs: Reinhard Fischer (Germany)

Fungal cells have a long history as model organisms to understand how eukaryotic cells function. The filamentous fungus *Aspergillus nidulans* in particular has been used to investigate the regulation of development, cell cycle control, nuclear division, the organization and dynamics of the cytoskeleton and nuclear migration. The session on Fungal Cell Biology will be introduced by three invited talks on the role of nuclear pore complexes in *A. nidulans*, the regulation of sexual and asexual development in *A. nidulans,* and the control of dimorphic switching in *Penicillium marneffei.*

1045-1115 IS1 - 0677

New insights into the mitotic regulation of the nuclear pore complex during the partially open mitosis of Aspergillus nidulans

Stephen Osmani (USA)

1115-1145 IS2 - 0752

The COP9 signalosome links protein turnover and oxidative stress response during fungal development

Gerhard Braus (Germany)

1145-1215 IS3 - 0997

Transcriptional regulation of morphogenesis in the human fungal pathogen Penicillium marneffei

Alex Andrianopoulos (Australia)

1215-1230 PS1 - 0189

Fission yeast cytokinesis: two paths to one destination

Volker Wachtler (Singapore)

1230-1245 PS2 - 0740

Localization and traffic of secretory vesicles in living hyphae of Neurospora crassa by laser scanning confocal microscopy

Meritxell Riquelme (Mexico)

1045-1245 **Meeting Room 3/5**

Symposium 5: Ecology and Diversity of Penicillium and Aspergillus in Australia

Chairs: John Pitt (Australia) / Ailsa Hocking (Australia)

Australia is known as a megadiverse continent because of its great diversity in fauna and fauna, much of it unique. High diversity in macrofungi, with many endemic genera and species, was established in the 20th Century. However only in the past few years have we realised that great diversity also exists in some microfungi. This symposium focuses on the *Trichocomaceae*, looking at diversity in geography, ecology and secondary metabolite production, especially in *Aspergillus* and *Penicillium*.

1045-1115 IS1 - 0872

Biogeography and ecology of Aspergillus in Australia

Ailsa Hocking (Australia)

1115-1145 IS2 - 0883

The astonishing biodiversity of Penicillium in Australia

John Pitt (Australia)

1145-1215 IS3 - 1012

Is the novelty of morphological species in Trichocomaceae reflected in metabolic diversity?

Ernest Lacey (Australia)

1215-1230 PS1 - 0291

Using GCPSR to resolve synonymies in Penicillium toxicarium

Stephen Peterson (USA)

1230-1245 PS2 - 0418

Aspergillus and Penicillium Teleomorphs from Thailand and Application of Talaromyces flavus against Plant Pathogenic Fungi in vitro

Leka Manoch (Thailand)

1330-1500

Poster Session 1: Phylogeny, Systematics & Evolution

Poster Session 9: Mycorrhizae

1530-1730 Meeting Room 1&2

Symposium 6: Molecular Taxonomy of Yeast

Chairs: Cletus Kurtzman (USA) / Andre Lachance (Canada)

Speakers will discuss use of molecular phylogenetic studies for determining species relationships among ascomycetous and basidiomycetous yeasts, and the application of this information for advancing ecological research.

1530-1600 IS1 - 0179

Phylogeny and Molecular Systematics of the Pichia Species Complex

Cletus Kurtzman (USA)

1600-1630 IS2 - 0066

Sex, endemism, and gene flow in natural yeast populations

Andre Lachance (Canada)

1630-1700 IS3 - 0771

Molecular Systematics and Ecology of Trichosporon and Malassezia

Takashi Sugita (Japan)

1700-1715 PS1 - 0407

Multi-locus sequence typing of the Cryptococcus neoformans – Cryptococcus gattii species complex

Marjan Bovers (The Netherlands)

1715-1730 PS2 - 0506

Diversity of yeasts from gastropods in Gunung Halimun National Park, West Java, Indonesia

Wellyzar Sjamsuridzal (Indonesia)

1530-1730 Hall C

Symposium 7: Transcriptome Analyses of Fungal Pathogens during Infection

Chairs: Yue Wang (Singapore) / Marc-Henri Lebrun (France)

Genome wide transcriptome analysis is powerful approach to gain both qualitative and quantitative data on fungal transcripts under defined physiological or environmental conditions, including infection of hosts. This symposium is focused on transcriptome analysis of fungal infection in both plants and animals fungal. Theses expression studies reveal which cellular functions are expressed during infection and should highlight mechanisms involved in pathogenicity.

1530-1555 IS1

DNA microarray analysis of signal interference of the dimorphic transition of Candida albicans

Haibao Zhang (Singapore)

1555-1620 IS2 - 1013

Transcriptome study of C. albicans genes important for infection and virulence?

Yue Wang (Singapore)

1620-1645 IS3 - 0862

Transcriptome dynamics in barley powdery mildew: insights into development and pathogenicity of an obligate biotrophic fungus

Pietro Spanu (UK)

1645-1710 IS4 - 0763

Microarrays meet pathogenicity: gene regulation during the early infection phase of Ustilago maydis

Regine Kahmann (Germany)

1710-1730 PS1 - 0523

Transient gene silencing in the oomycete, Phytophthora infestans, for determination of gene function

Anna Avrova (UK)

1530-1730 Meeting Room 3-5

Symposium 8: Diversity of Microfungi in Neotropica

Chairs: José Carmine Dianese (Brasil) / José Hernandez (Argentina)

Taxonomists working with microfungi in the Neotropics will be discussing the diversity of different groups of fungi including anamorphic fungi, ascomycetes, rust and smut fungi. The presentations and discussions are supposed to reveal the recent advances in Latin American Mycology in what concerns taxonomy and fungal diversity. It is also expected that the discussions will lead to further cooperation among mycologists from different countries.

1530-1600 IS1 - 0898

Diversity of microfungi of the Brazilan semi-arid Northeast region.

Luiz Pascholati Gusmão (Brasil)

1600-1630 IS2 - 0983

Rust fungi from Northern Argentina

José Hernandez (Argentina)

1630-1700 IS3 - 0992

Diversity of Discomycetes in Venezuela

Teresa Iturriaga (Venezuela)

1700-1730 IS4 - 0757

Microfungi of the Brazilian Cerrado: example of Neotropical mycodiversity and need for detailed study

José Carmine Dianese (Brasil)

1530-1730 Hall D

Symposium 9: The Fungal Septum and Associated Organelles

Chairs: Katsu Kitamoto (Japan) / Gregory Jedd (Singapore)

The majority of filamentous fungi produce hyphae that are partitioned by perforate septa and large groups of related fungi have evolved distinct septal pore-associated organelles. Two of these are the Woronin body of filamentous ascomycetes and the septal pore cap, which is found in certain basidiomycetes. Mycologist have long-recognized these organelles and their utility for taxonomic classification, however, only recently has their molecular composition and function been scrutinized. This symposium will highlight recent work focusing on the structure, function and genesis of these uniquely fungal organelles.

1530-1600 IS1- 0729

Woronin bodies: Crystalline peroxisomes close the door at the septal pore

Gregory Jedd (Singapore)

1600-1630 IS2- 0684

Structural and biochemical characterization of septal pore caps in basidiomycetes

Kenneth van Driel (The Netherlands)

1630-1700 IS3 - 0753

The MTOC associated protein ApsB interacts with the peroxisomal Woronin body protein HexA in Aspergillus nidulans

Reinhard Fischer (Germany)

1700-1715 PS1 - 0324

Septal seal: certified and secure host invasion

Naweed Naqvi (Singapore)

1715-1730 PS2 - 0513

Polarized localization of chitin synthases in Aspergillus nidulans

Hiroyuki Horiuchi (Japan)

1530-1730 Halls A&B

Symposium 10: Population Genetics of Fungi

Chairs: Jeremy Burdon (Australia) / Linda Kohn (Canada)

Drift, recombination, migration, selection, and extinction and recolonization are the major forces contributing to the structure of pathogen populations. In each host-pathogen association, the impact of each of these forces is tempered by the spatial context of the interaction and the particular combination of life-history features shown by both host and pathogen. Here we explore these interactions and the way they shape the evolutionary trajectories of host-pathogen interactions. The origins of crop pathogens, the comparison between pathogen populations in agricultural versus natural systems, and the influence of plant population dynamics on pathogen populations will be addressed.

1530-1600 IS1 - 0366

Genetic structure of fungal plant pathogens on wild and cultivated host populations at the host center of origin

Bruce McDonald (Switzerland)

1600-1630 IS2 - 0332

The impact of the domestication and cultivation of maize on the origin and evolution of the corn smut fungus, Ustilago maydis

Andrew B. Munkacsi (USA)

1630-1700 IS3 - 0214

The impact of plant population structure on disease epidemiology and pathogen evolution in the Linum marginale – Melampsora lini interaction

Peter H Thrall (Australia)

1700-1715 PS1 - 0156

Within and between bean field phenotypic variation of a fungal biotroph – estimation of populations

James Steadman (USA)

1715-1730 PS - 0558

Speciation and Gene Flow In The Botryosphaeria parva-B. ribis Complex On Native And Introduced Hosts In South Africa

Draginja Pavlic (South Africa)

1730-1900

Poster Session

1900-2000 - 0899 **Halls A&B**

Honorary Lecture

MYCOLOGY and Mycologists

David Hawksworth (Spain)

Invite to attend the Joint reception of the British Mycological Society and the Mycological Society of America

Geoff Gadd, British Mycological Society

Greg Mueller, Mycological Society of America

2000-2100 **Cairns Hilton**

Joint reception of the British Mycological Society and the Mycological Society of America

PLENARY 1

0915-1015 – 0995

Fungal tree of life

Franz Oberwinkler (Germany)

The monophylum Mycota is separated from Mycetozoa and Oophyta (Oomycetes). Glomeromycota are documented with vesicles similar to recent ones as the oldest so far known fossils of fungi from the Ordovician. Geosiphon will be discussed in a phylogenetic hypothesis. Maybe we know within one year that Microsporidia are true fungi and fall within Chytrids and/or Zygomycetes. Main phylogenetic lines in the Ascomycetes incl. of Ascolichens are discussed, including also the fossil records. At the moment we accept three major taxa in Basidiomycetes. These will be explained in some detail. Their evolution is not understandable without interpreting coevolutionary developments with their hosts. I conclude that mycotropism was a prerequisite for plants to conquer land habitats, and that the evolution of land plants is obligately bound to the evolution of fungi.

1045-1245

SYMPOSIUM 1- *Phylogenetic Biology of Fungal and Fungal-like Phyla*

S1IS1 - 0678

The Saprolegniaceae - new species concepts

David E. Padgett, J. Craig Bailey
Univ. of North Carolina Wilmington, Wilmington, NC, Australia

Species of the Saprolegniaceae are difficult to identify because of the plasticity of the morphological features used to characterize them. This has resulted in significant overlap of described taxa to the extent that unknowns often can be identified only to a 'species cluster'. Our attempt to resolve this problem focused on the genus *Saprolegnia* and was based on the proposition that the most valid criterion for circumscribing both genera and species is gene sequence comparisons. Our phylogram for 55 randomly-selected *Saprolegnia* isolates revealed 10 robustly supported clades that probably represent distinct species. Morphological identifications of individuals within each clade, however, yielded multiple names in most instances; thus confirming the need for comprehensive systematic revision of the genus. We found that all ten *Saprolegnia* clades were distinguishable from each other using unique combinations of morphological features but not without including some features that had not heretofore been used at the species level. Preliminary work on several other saprolegniaceous genera has shown morphological plasticity similar to or greater than in *Saprolegnia* and suggests that our approach to resolving species overlap may well be broadly applicable within the family.

S1IS2 - 0178

Trichomycetes: major taxonomic revisions based on molecular phylogenies

M.M. White, R.W. Lichtwardt
University of Kansas, Lawrence, Kansas, United States
Merlin M. White and Robert W. Lichtwardt

Department of Ecology & Evolutionary Biology, University of Kansas, Lawrence, KS, USA
It is now evident that two of the four traditional trichomycete orders associated with arthropods, Eccrinales and Amoebidiales, are not fungi, but rather protozoans falling within the basal Mesomycetozoa clade in the tree of life. Molecular studies now also make it clear that the fungal class Trichomycetes requires some other major taxonomic modifications. The genus *Orphella*, once considered to belong to the otherwise monophyletic Harpellales, fits into neither the Harpellales nor the sister order Kickxellales based on DNA sequence data as well as asexual and sexual reproductive structures. Within Harpellales there are currently two families; molecular data, however, justify only one family. Large harpellid genera such as *Smittium* and *Stachylina* are polyphyletic and need revisions. The other Trichomycetes order, Asellariales, currently consists of two genera, *Asellaria* and *Orchesellaria*, sufficiently distinct that they may need to be in separate orders. Thus, major revisions within Trichomycetes are called for at all taxonomic levels.

S1IS3 - 0293

An up to date assessment of the place of the mycetozoans among the Eukaryotes

F.W. Spiegel, L.A. Lindley, J.D. Silberman
Department of Biologcial Sciences, University of Arkansas, Fayetteville, AR, United States

Because of their history as "mycological property", amoeboid protists that fruit were classically treated as a single taxon, Mycetozoa. Since the publication of L.S. Olive's The Mycetozoans in 1975, considerable evidence has been gathered to demonstrate that the taxon Mycetozoa, as Olive predicted, is not a monophyletic group. We will concentrate on examples in the monophyletic Eumycetozoa (myxomycetes, dictyostelids, and protostelids) and the polyphyletic Acrasea to support this conclusion. We will: 1) review the morphological, developmental, and molecular data that argue for a need to revise the taxonomy of the myxomycetes, dictyostelids, and protostelids within Eumycetozoa: 2) cover the evidence testing the monophyly of Eumycetozoa and its placement in the more inclusive taxon Amoebozoa; 3) describe the differences among the taxa within Acrasea, sensu Olive; and 4) suggest further work to clarify the phylogenetic position of the guttulinopsid and copromyxid acrasids. Clarification of the phylogeny of the various slime molds will allow meaningful approaches to be made toward the questions concerning how various amoebae "learned" to fruit and why so many of them became cellular slime molds.

S1IS4 - 0296

Molecular systematics and evolution of Boletales.

Manfred Binder, David S. Hibbett
Clark University, Worcester, Massachusetts, United States

Historical patterns of morphological evolution and ecology in the Boletales appear to involve extensive convergence. The Boletales includes conspicuous stipitate-pileate forms that mainly have tubular and sometimes lamellate hymenophores, gasteromycetes (puffball-like forms), resupinate or crust-like fungi, and a single polypore-like species. Species in Boletales pursue diverse lifestyles, but unlike in their sister clades (Agaricales and Atheliales), white-rot saprotrophy is absent in the group. Instead, saprotrophs among Boletales have developed a special mode of brown-rot called "Coniophoraceae-rot". Mycorrhizal associations are established by the majority of Boletales and some Boletales are mycoparasites, a deviation of either the saprotrophic or ectomycorrhizal mode. We studied phylogenetic relationships of Boletales based on two data sets to resolve sister relationships between Boletales, Agaricales, and Atheliales, and to estimate ancestral states of morphology and nutritional mode of Boletales and their major groups. The multi-gene data set (nuc-ssu, nuc-lsu, 5.8S, mt-lsu, *atp6*) sampled 42 key species of Boletales in a framework of 14 representative homobasidiomycetes. Analyses on the multi-gene data set confirm sister group relationships between Boletales, Agaricales, and Atheliales – the Agaricomycetidae. The Boletales are strongly supported as monophyletic in our analyses using parsimony, maximum likelihood, and Bayesian approaches. Six major lineages of Boletales that are currently recognized, Boletineae, Paxillineae, Sclerodermatineae, Suillineae, Tapinellineae, Coniophorineae, received varied support. Most brown-rot producing forms were placed as a paraphyletic grade at the base of the Boletales. Boletineae and Suillineae received the highest support values, Paxillineae and Coniophorineae were not as monophyletic groups and need taxonomical revision. The Tapinellineae consisting of morphologically diverse brown-rotting fungi forms the basal group in the Boletales. The nuc-lsu data set consisting of 485 terminals was broadly sampled and included roughly 30% of the described species of Boletales and 51 outgroup taxa across the homobasidiomycetes. We performed most recent common ancestor (MRCA) reconstructions on the nuc-lsu data set using BayesMultiState, which suggested that the ancestor of the Boletales was a resupinate or polyporoid saprotrophic fungus, producing a brown-rot. The results of the MRCA analyses show that the diversification of brown-rotting fungi poses critical events in the evolution of early Boletales, including multiple transformations from resupinate to stipitate-pileate fungi. Mycoparasites in the Boletales represent transitions from ectomycorrhizal lifestyles.

S1IS5 - 0433

Assembling the fungal tree of life: evolution of the ascomycota

J.W. Spatafora, C.L. Schoch, AFTOL Consortium
Oregon State University, Corvallis, OR, United States

Ascomycota is the largest phylum of Kingdom Fungi and includes more than 33,000 described species. They are found in all ecosystems from terrestrial to aquatic and participate in numerous symbioses (e.g., lichens, mycorrhizae) and ecological processes (e.g., decomposition, disease). They also impact human society in beneficial and detrimental ways by serving as both the source of life saving pharmaceuticals and the cause of life threatening diseases. A robust understanding of the major evolutionary relationships of the phylum will advance research in all of these areas by providing a predictive and phylogenetic basis for research.

Data were sampled both to provide a broad and inclusive taxon sampling of Ascomycota and to include the maximum number of genes with the minimum amount of missing data. Sequence data from five nuclear genes (SSU rDNA, LSU rDNA, EF 1-alpha, RPB1 and RPB2) were obtained from the Assembling the Fungal Tree of Life (AFTOL) and Genbank sequence databases. Taxon sampling included representatives from 14 of the 15 Ascomycota classes currently recognized in the Outline of Ascomycota sensu Eriksson. The resulting multigene sequence data were concatenated into a single alignment and used in weighted parsimony, maximum likelihood and Bayesian analyses. Nodal support was assessed by nonparametric bootstrapping and posterior probabilities.

These data strongly supported the monophyly of Saccharomycotina, Pezizomycotina, Arthoniomycetes, Eurotiomycetes, Orbiliomycetes, and Sordariomycetes. Taphrinomycotina and Pezizomycetes were resolved as monophyletic but not strongly supported by the data. Lecanoromycetes and Dothideomycetes were each resolved as paraphyletic in parsimony analyses but monophyletic in Bayesian analyses, although neither resolution was strongly supported. Leotiomycetes were polyphyletic due to exclusion of Geoglossaceae. The two most basal classes of Pezizomycotina were Orbiliomycetes and Pezizomycetes, both of which comprise species that produce apothecial ascomata. The seven remaining classes formed a monophyletic group that corresponds to Leotiomyceta. Within Leotiomyceta, the supraclass clades of (Leotiomycetes s. s., Sordariomycetes) and (Arthoniomycetes, Dothideomycetes) were resolved in both parsimony and Bayesian analyses, but with moderate support. These results are consistent with the apothecium as the most ancestral character state of ascomatal morphology with multiple derivations of perithecial and cleistothecial ascomata. Ascus complexity arose early during the evolution of the subphylum with inoperculate, excluding Orbiliomycetes, and bitunicate taxa forming a crown monophyletic group Leotiomyceta. Thick-walled bitunicate asci likely arose once with multiple modifications and reductions of the thick, separable ascus wall layers.

S1IS6 - 0748

Evolution of basal lineages in Fungi: deconstructing Chytridiomycota and Zygomycota

T. Y. James, F. Kauff, C. Schoch, P. B. Matheny, V. Hofstetter, C. Cox, G. Celio, J. E. Longcore, D. S. Hibbett, F. Lutzoni, D. Mclaughlin, J. Spatafora, R. Vilgalys, many others
Biology Dept. Duke University, Durham, NC, United States

Most theories on the origin of the Fungi agree that the earliest lineages arose from a simple aquatic ancestor with a single flagellated zoospore, similar to modern unicellular chytrids. Uncertainty exists, however, about the timing and frequency of key events associated with diversification of major fungal phyla, including times of divergence, numbers of losses of flagellae, and even how many fundamental units (phyla) might exist. Phylogenetic analyses using data from six gene regions (and nearly 200 species) reveals a paraphyletic basal grade that includes several lineages including Microsporidia, chytrids (4 lineages), zygomycetes (2 lineages), and Dikarya (including Glomeromycota, Ascomycota, and Basidiomycota). During the course of early fungal evolution, flagellae were lost at least three, and possibly as many as eight times, and loss of the motile spore appears to be coincident with novel innovations of aerial dispersal and Microsporidian polar tube eversion. In spite of combined evidence from six gene regions, support for most basal nodes is weak or lacking, suggesting a radiation of basal lineages during the early evolution of Fungi. Our results also suggest that the Microsporidia may belong to the basalmost fungal lineage, which was derived from an endoparasitic chytridiomycete ancestor similar to *Rozella allomycis*.

1045-1245

SYMPOSIUM 2- *Do Plant Pathogens have a Specific Armory?*

S2IS1 - 0682

The establishment of biotrophy in the *Ustilago maydis*/maize pathosystem.

R. Kahmann, T. Brefort, K. Schipper, O. Mueller, B. Macek, M. Mann
Max Planck Instititute for terrestrial Microbiology, Marburg, Hessen, Germany

Ustilago maydis is a dimorphic fungus that switches from a yeast-like haploid stage to a filamentous dikaryon after mating. In nature it is the dikaryon that is able to differentiate infection structures and cause disease on corn plants. While many crucial determinants for cell fusion and the switch to filamentous growth have been identified in recent years, insights into those stages of fungal development that rely on the plant host (biotrophy) have remained a black box. To establish the biotrophic phase the fungus has to sense the plant surface, has to avoid or suppress plant defense reactions and has to acquire nutrients while being located in the apoplast. I will provide evidence that *U. maydis* uses a set of novel secreted proteins for establishing biotrophy.

S2IS2 - 0999

A secondary metabolite is involved in recognition of the blast fungus *Magnaporthe grisea* by resistant rice cultivars

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Recognition of the fungal plant pathogen *Magnaporthe grisea* by resistant rice cultivars is controlled by interactions between fungal avirulence genes (*AVR*) and their corresponding plant resistance genes (*R*). Most fungal *AVR* genes encode small peptides secreted into host tissues during infection. *ACE1* from *M. grisea* differs from other *AVR* genes as it encodes a polyketide synthase fused to a non-ribosomal peptide synthetase, an enzyme involved in the biosynthesis of a secondary metabolite. This *AVR* gene controls the production of a signal recognized by rice cultivars carrying *Pi33* resistance gene. *ACE1* is specifically expressed in mature appressoria during penetration of the fungus into rice leaves. The protein Ace1 is only detected in the cytoplasm of appressoria and not in infectious hyphae differentiated inside infected epidermal cells. *Ace1-ks0*, a non-functional *ACE1* allele obtained by site-directed mutagenesis of an amino acid from polyketide synthase KS domain essential for its enzymatic activity, is unable to confer avirulence. This result suggests that the avirulence signal recognized by *Pi33* is not the Ace1 protein, but the secondary metabolite synthesized by Ace1. In order to characterize this metabolite, *ACE1* was expressed in *M. grisea* under the control of a constitutive promoter. *ACE1* was also expressed under the control of an inducible promoter in *Aspergillus oryzae* and in *Fusarium venenatum*. Secondary metabolites produced by these transgenic strains are currently analyzed by LC-MS-MS (coll. Certon, Metcalf and Drivon, Bayer CropScience, France). At the *ACE1* locus, we identified 14 genes predicted to encode enzymes involved in secondary metabolism, including two enoyl-reductases and a binuclear zinc-finger transcription factor. These genes have the same expression pattern as *ACE1* defining a cluster of co-expressed genes, suggesting that they are involved in the same biosynthetic pathway. The inactivation of these genes in an avirulent isolate is underway to assess their role in the biosynthesis of the metabolite recognized by *Pi33* resistant rice cultivars.

Böhnert, H. U. *et al.* (2004). A Putative Polyketide Synthase/Peptide Synthetase from *Magnaporthe grisea* signals pathogen attack to resistant rice. *The Plant Cell*, 16:2499-2513.

S2IS3 - 0705

Are A+T-rich isochores niches for pathogenicity genes in the genome of *Leptosphaeria maculans* ?

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Leptosphaeria maculans is a phytopathogenic ascomycete that belongs to a species complex mainly composed of two species, *L. maculans* and *L. biglobosa*. These can be further divided into seven sub-species, depending on their host plant specificity and geographic distribution. The sub-species *L. maculans* 'brassicae' is responsible for severe yield losses on oilseed rape (*Brassica napus*) and develops very specific "gene-for-gene" interactions with its host plant where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes.
A 1.1 Mb region around the avirulence genes *AvrLm1* and *AvrLm6* was recently sequenced. This genomic region displayed a particular organization, with 5 small (20 – 70kb) G+C-equilibrated, ORF-rich isochores intermingled with 3 large (170-450 kb) A+T-rich isochores mainly composed of degenerated and truncated retrotransposons.
These A+T-rich regions contain only seven isolated genes (one every 90 kb on average), of which four are predicted to be small-secreted proteins. These include the two avirulence genes *AvrLm1* (205 amino acids, one cysteine) and *AvrLm6* (144 amino acids, 6 cysteines), along with two single-copy genes encoding for two cysteine-rich proteins termed LmCys1 (233 amino acids, 8 cysteines) and LmCys2 (247 amino acids, 8 cysteines). Except for *LmCys1*, all these genes have a low G+C content, which is similar to that of their A+T-rich genomic environment and clearly different from the average estimated 53% G+C for genes of *L. maculans*.
Both Southern blot analysis and PCR amplifications showed that, contrasting with genes occurring in GC-equilibrated regions of the genome, *AvrLm1*, *AvrLm6*, *LmCys1* and *LmCys2* are specific of *L. maculans* 'brassicae', and absent from other species or sub-species of the *L. maculans-L. biglobosa* species complex, or from the closely related species, *Stagonospora nodorum*.
Quantitative RT-PCR studies further showed that the four genes are up regulated upon plant infection, with *AvrLm1*, *AvrLm6* and *LmCys2* being expressed constitutively at a very low level, but over-expressed up to 800-x in the first stages of plant infection.
These data, along with information on other genomic regions and particular meiotic and evolutionary behaviour suggest that rapidly divergent highly specific genes are used by *L. maculans* for pathogenicity and/or avirulence, and that A+T-rich isochores are shelters for such genes in the genome.
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S2PS1 - 0459

Early Onset of Toxin Biosynthesis In A Forest Pathogen

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The pine pathogen *Dothistroma septosporum* produces a red polyketide toxin, dothistromin, which is the hallmark of red band needle blight disease1. Dothistromin is non-host specific, being toxic to a broad range of organisms, and its specific role in needle blight is unknown. Since the toxin is a potential target for disease control we aim to understand its role in the disease process. In this presentation we focus on factors affecting dothistromin biosynthesis that could reveal clues to function.
Biosynthesis of toxin under different cultural conditions was assessed using ELISA and by real-time RT-PCR of dothistromin (*dot)* genes 2,3. *D. septosporum* strains containing a green fluorescent protein (*gfp*) gene driven by a *dot* gene promoter were also used to monitor expression.
Both the *dot* gene expression and dothistromin biosynthesis are switched on during the early exponential phase of growth in culture, although some differences were seen with different media. The early onset of gene expression is also seen with a *dot* driven *gfp* transformant which shows highest expression in the younger mycelium at the margin of colonies. Toxin biosynthesis also appeared to be induced in response to challenge with some other fungi and in response to a yeast elicitor.
The early growth-stage onset of dothistromin biosynthesis suggests the toxin is required at an early stage of infection of pine needles. This is an unusual pattern of regulation compared to aflatoxins, related secondary metabolites that are synthesised in the stationary phase of growth. If *dot* genes are switched on in response to other fungi this would suggest a role for the toxin in competition against other, faster-growing organisms on the needle surface. Studies with toxin-deficient mutants are in progress to investigate the role further.
1 For. Path. 34: 163-185
2 (2002) Appl. Environ. Microbiol. 68: 2885-2892
3 (2006) Mycopathologia 161 (5): 283-294

S2PS2 - 0519

Defining the role of the *Avr*3a avirulence gene in *Phytophthora infestans* – potato interactions

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The oomycete *Phytophthora infestans* causes late blight, the potato disease that precipitated the Irish famines in 1846 and 1847. It represents a re-emerging threat to potato production and is one of over 70 species which are arguably the most devastating pathogens of dicotyledonous plants. Until recently, little was known about the molecular bases of oomycete pathogenicity, especially the avirulence molecules that are perceived by host defenses. The recent cloning of the *Avr3a* avirulence gene offers an opportunity to study pathogenicity at the molecular level with the twin aims of: 1) understanding the process of recognition and defence response initiation; and 2) gaining an insight into the pathogenicity function of an avirulence gene.

To determine how AVR3a is perceived by host cells, we have screened a plant yeast 2 hybrid (Y2H) library for proteins which interact with AVR3a. To determine the pathogenicity function of AVR3a, we have used gene silencing to generate stably transformed lines of *P. infestans* that are silenced for *Avr*3a. Heterologous expression and delivery of AVR3a from bacterial typeIII and typeII secretion systems was used to demonstrate recognition of AVR3a in the host cell cytoplasm. Transformants, with targeted mutations in the *Avr*3a gene, have been generated to determine how AVR3a enters host cells. Additional transformants, expressing *Avr*3a fused to fluorescent proteins, have also been generated to determine which *P. infestans* infection structures secrete AVR3a.

Fusion of AVR3a to monomeric red fluorescent protein shows that AVR3a is secreted from biotrophic feeding structures called haustoria. AVR3a has previously been shown to be recognized inside potato cells expressing the *R*3a resistance gene and this was confirmed using heterologous expression and delivery from a bacterial plant pathogen. Two protein motifs thought to be involved in transport of AVR3a into host plant cells (RXLR and EER) have been identified that are common to all four oomycete avirulence proteins and other oomycete secreted proteins. Analysis of transformants mutated for these two motifs is in progress. Similarly, testing of *Avr*3a-silenced transformants to determine the role of AVR3a in pathogenicity is in progress. Y2H screening has identified an AVR3a-interacting potato protein of unknown function that is a member of a small gene family.

Our preliminary results show that AVR3a, and possibly other RXLR-EER pathogenicity factors, are secreted from haustoria into host cells where it interacts with its virulence target(s) to trigger resistance or modulate host defenses.

1045-1245

SYMPOSIUM 3 - *Insect Associated Fungi*

1045-1245 – 1005

Microbial communities in the gut of wood-ingesting Beetles

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Examination of the gut of fungus-feeding beetles has revealed the presence of many novel species of yeasts in many different clades of yeasts, although it is usual to find one yeast taxon per beetle individual. When one compares the yeast mycota of the gut of fungus-feeding beetles to those of wood-boring beetles, the wood-boring beetles differ in several ways. The location of yeasts in the gut of wood-boring beetles is the hindgut rather than the midgut as in fungus-feeding beetles and yeasts associated with wood-boring beetles often have the rare yeast traits of fermentation and assimilation of xylose. We isolated such a yeast, *Pichia stipitis*, from the gut of about 400 individuals of the wood-boring beetle *Odontotaenius disjunctus* (Passalidae). In addition to the yeast in the posterior half of the hindgut, other microbes are restricted to different parts of the highly compartmentalized hindgut. The microbial gut inhabitants include bacteria, parabasallids, and amoebae, many of which may contribute to the degradation of wood, whether for their own benefit or that of the host beetle.

S3IS2 - 0659

TEMPERATURE driven symbiont shifting in a bark beetle-fungus ectosymbiosis: a mechanism of stability?

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Mutualisms are predicted to be inherently unstable and prone to erosion due to cheating by established symbionts or invasion by parasites. Obligate mutualisms are also viewed as risky given that if one associate is lost, the other cannot survive. However, despite these predictions, many mutualisms, including many obligate associations, appear to have existed in a relatively stable state over long evolutionary periods. Long-term stability has been suggested to require interspecific population regulation or complex feedback among associates including sanctions, asymmetric competition or partner choice. However, for at least one multipartite mutualism, the mechanism behind stability and longevity may be much simpler and involve environmentally-driven symbiont shifts. We have recently discovered evidence that temperature plays a key role in determining the relative abundance of two mutualistic fungi, one coevolved with the host, the other a more recent invader, associated with an economically and ecologically important bark beetle. The two fungi possess different temperature tolerances that determine which fungus is vectored by dispersing host beetles as temperatures fluctuate over a season. Different optimal growth temperatures may facilitate the stable coexistence of the two fungi by supporting growth of each fungus at different times minimizing direct competition. Furthermore, the beetle may reduce its risk of being "left alone" by exploiting not one, but two symbionts, whose combined growth optima span a wide range of environmental conditions.. Such temperature-driven symbiont shifts are likely to have major consequences for host and fungal fitness and population dynamics under current climate conditions, as well as those predicted to occur due to climate change.

S3IS3 - 0665

Comparing molecular ecological patterns in populations of different fungal mutualists of woodwasps

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There are two common factors in many mutualistic relationships, which appear to stabilise them. These are vertical transmission of symbionts between host generations and genotype uniformity in the symbiont. The same factors can also have detrimental effects, if the symbiont population looses its adaptive ability or where deleterious mutations arise. The consequences of symbiont transmission and reproductive modes have not been extensively explored in the well known mutualism between *Amylostereum* fungi (Basidiomycotina) and various wood wasps in the family Siricidae (Hymenoptera). Female wasps carry asexual *Amylostereum* spores in internal mycangia and inoculate the fungus into softwoods when they oviposit. Sexual fruiting structures of these fungi, however, occur frequently in some areas. It has traditionally been thought that the transmission of these fungi between wasp generations is only vertical, from mother to female offspring. Our studies support this hypothesis in some populations. However, the fact that various wasp species share the same fungal symbiont, implies that horizontal transmission occurs, at least on evolutionary time scales. Recent experimental studies and field observations have, furthermore, suggested ways in which horizontal transmission might occur on ecological time scales. In this study we combine data generated using various molecular markers, including RAPDs, microsatellites, nuclear and mitochondrial sequence data, and PCR-RFLPs, to determine patterns of spatial distribution and ecological transmission in populations of *A. areolatum* and *A. chailettii,* associated with various wood wasp species. These data suggest that there are discrete differences in the molecular ecological patterns of populations of the *Amylostereum* spp. Overall *A. areolatum* appears to be more genotypically uniform with greater geographical and host associated structuring of populations, reminiscent of a strong influence of vertical transmission and asexual reproduction. In contrast, *A. chailletii* appears to be more genotypically diverse, with less structured populations, suggesting greater levels of horizontal transmission and sexual reproduction at ecological time scales. However, in both species, some local population patterns differ from the overall patterns We thus explore the differences in molecular ecological patterns of populations of *Amylostereum* spp. associated with Siricidae and the possible factors that affect them.

S3PS1 - 0582

A new phylogenetic lineage of *Ophiostoma* spp., discovered on termites and termite combs in South Africa

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The symbiotic relationship between *Termitomyces* spp. and fungus-growing termites (Macrotermitinae) has been known, although not completely understood, for a very long time. It is also known that other fungi are found in termite nests but almost nothing is known regarding their interactions with fungus-growing termites and *Termitomyces* spp. A number of preliminary surveys have recently been undertaken to collect fungi occurring together with *Termitomyces* spp. in the fungal combs of termite nests in South Africa. Surprisingly, ascomata resembling those of *Ophiostoma* spp., or mycelial growth resembling the *Sporothrix* anamorphs of *Ophiostoma*, were found in several of the combs. Subsequent to this initial discovery, several *Sporothrix* isolates were also obtained directly from living termites. A total of 75 isolates were obtained from 14 different termite mounds representing three termite species (*Odontotermes badius, O. latericius* and *Macrotermes natalensis*). These isolates were identified based on morphology and comparisons of DNA sequences for parts of the beta-tubulin, ITS and LSU gene regions. Few of the isolates produced teleomorph structures, making it difficult to distinguish between them. ITS and beta-tubulin sequence comparisons, however revealed that isolates reside in five distinct phylogenetic groups, only one of which produced teleomorph structures. Comparisons with known *Ophiostoma* and *Sporothrix* spp. revealed that all five groups represented undescribed taxa. Three of these, representing the majority (69) of the isolates, constituted a strongly resolved monophylum in the genus *Ophiostoma*, positioned between the *O. stenoceras–S. schenckii* and *O. piliferum–O. piceae* complexes. The discovery of these new *Ophiostoma* spp., as well as their consistent association with and abundance in termite mounds (found in 14 out of 15 mounds sampled), raise many intriguing questions. The Ophiostomatales (*Ophiostoma*, *Grosmannia*, and *Ceratocystiopsis*) are generally associated with Scolytine bark beetles, where the symbioses vary from strict mutualism to opportunistic. The phylogeny of the *Ophiostoma* spp. from termite mounds suggests that the three dominant species evolved with termites and/or *Termitomyces*, independently of the species associated with bark beetles. The ecological role of *Ophiostoma* spp. within the termite ecosystems, however, remains a mystery that deserves further study.

S3PS2 - 0018

Interactions Of Fungi and Tree Killing Bark Beetles: Geographic Variation And Interspecific Competition

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Bark beetles have well documented symbioses with fungi. Fungi are carried phoretically (stain fungi such as *Ophiostoma minus*) and internally in specialized mycangia (*Entomocorticium spp.* and *Ophiostoma spp.*). We have described in detail the complex interactions of *Dendroctonus frontalis* with its fungi in the southern US. Much less is known about how this beetle interacts with its fungi in other areas in which it occurs, such as the western US and Mexico. In addition, we recently described the first occurrence of *Dendroctonus mexicanus* in the US. Very little is known about the identity of the fungi associated with this insect in the US, or in its native Mexico.

We collected numerous beetles from field infestations in southern Mexico, southern Arizona and Mississippi in the US. We isolated phoretic and mycangial fungi from beetles. We identified individual isolates of fungi using morphological and sequencing approaches. We compared growth rates, competitive abilities and tolerances of host defensive compounds among isolates – examining trends based on geography and insect associates.

We have identified several new fungi associated with both of these tree killing bark beetles in various geographic regions. Ophiostomatoid stain fungi appear to be less prevalent in association with *D. mexicanus* than with *D. frontalis.* Fungi associated with *D. mexicanus* are capable of effectively competing with *D. frontalis* associated fungi. Fungi associated with both insects show variability in growth, competitive abilities and allelochemical tolerance.

These two tree killing bark beetles have overlapping geographic and host ranges and may even colonize the same trees. Despite this closeness of association, there are significant differences in the composition and function of their fungal associates. Informed biological, and ecological studies, and effective management approaches, will depend on the accurate characterization of the similarities and differences between these closely related symbiotic systems.

1045-1245

SYMPOSIUM 4 - *Fungal Cell Biology*

S4IS1 - 0677

New insights into the mitotic regulation of the nuclear pore complex during the partially open mitosis of *Aspergillus nidulans*.

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Mitotic hallmarks, such as chromosome condensation and spindle formation, are common to all eukaryotes whereas mitotic disassembly of the nuclear pore complex (NPC) was thought to occur only during the open mitosis of higher eukaryotes. However, we have recently determined that partial disassembly of the nuclear pore complex occurs during mitosis in *Aspergillus nidulans* under control of the NimA and Cdk1 mitotic kinases. This make *A. nidulans* an evolutionary intermediate between open and closed mitosis and a good system to study mitotic regulation of the disassembly and reassembly of the NPC.

To understand the magnitude of the mitotic modification of the *A. nidulans* NPC we have used bioinformatics in combination with affinity purification and mass spectrometry to identify nuclear transport proteins. Deletion analysis has shown that 18 of these genes are essential and 14 are non-essential. Somewhat surprisingly, phenotypic analysis using heterokaryon rescue suggests there is not a mechanism by which the composition of the NPC can be monitored to prevent mitosis when NPC proteins are limiting.

Endogenous GFP tagging and live cell spinning disc confocal microscopy indicates that karyopherins and RanGAP disperse throughout the cell during mitosis but An-Rcc1 remains with chromatin. This indicates RanGTP levels are likely higher in the vicinity of chromatin during *A. nidulans* mitosis as occurs during open mitosis of higher eukaryotes.

Regarding the NPC proteins (nucleoporins), they fall into two major categories. At least 12 nucleoporins remain at the nuclear periphery throughout mitosis and presumably generate a minimal NPC structure to provide a conduit between the cytoplasm and nucleoplasm during mitosis. Conversely 12 of the more peripheral components of the NPC reversibly disperse throughout the cell during mitosis but return to the NPC at various times during anaphase and telophase. Of particular interest is the mitotic behaviour of the essential An-Nup2 protein. Throughout interphase An-Nup2 remains at the NPC but during prophase it locates exclusively to chromatin before returning back to the NPC during telophase. Thus An-Nup2 is suitably located to have dual functions, one at the NPC during interphase and a second function at chromatin during mitosis.

In conclusion, our analysis demonstrates that the *A. nidulans* NPC undergoes massive changes in its composition during mitosis. The data further suggests that An-Nup2 may help coordinate NPC function with entry into and out of mitosis.

S4IS2 - 0752

Signalosome and development in the filamentous fungus *Aspergillus nidulans*

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The homothallic filamentous ascomycete *A. nidulans* is able to form fruitbodies (cleistothecia) either by mating of two strains or by selfing in the absence of a partner. The three-dimensional *A. nidulans* cleistothecium is the most complicated structure this fungus is able to form. This includes an energy and material consuming process where parts of hyphae have to be dissolved and locally rearranged. The COP9 signalosome which is involved in the control of SCF E3 ubiquitin ligase activities has been identified as a key regulator of fungal sexual development. The ubiquitinylation activity of SCF can be modulated by neddylation, a reversible conjugation of the ubiquitin-related protein NEDD8/Rub1 on the cullin subunit. Among the ubiquitin-like protein family, NEDD8 is most homologous to ubiquitin. Fungal *csn* mutant strains accumulate neddylated cullins during vegetative growth and therefore the intrinsic COP9 signalosome deneddylase activity seems to be critical for fungal development. SCF E3 ubiquitin ligases have to be neddylated and deneddylated at their cullin subunit to perform their function *in vivo*. Pror to the visible mutant phenotypes, the proteome of a csn deletion strain suggests a failure to induce an appropriate oxidative stress response. Since higher eukaryotes are not able to survive the embryonic state without a functional COP9 signalosome, we use *A. nidulans* as model system to understand principal mechanisms of the regulation of protein degradation.

S4IS3 - 0997

Transcriptional regulation of morphaogensis in the human fungal pathogen *Penicillum marneffei*

Alex Andrianopoulos

Fungal pathogens represent an increasing threat to human health as a consequence of the increasing population of immunocompromised individuals. Many of these fungi have the capacity to alternate between growth forms which are closely associated with infective and pathogenic states. Understanding the biology of these fungal pathogens is central to understanding how these fungi cause disease.
Penicillium marneffei is an emerging fungal pathogen endemic to South-east Asia. In response to an extrinsic stimulus (temperature), *P. marneffei* is capable of alternating between a hyphal and a yeast growth form, a process known as dimorphic switching. *P. marneffei* grows in the filamentous form at 25ºC and in the yeast form at 37ºC. At 25ºC the free-living saprophyte grows vegetatively as multinucleate filamentous hyphae and can undergo asexual development (conidiation). Conidiation proceeds with the formation of an aerial stalk from a hyphal foot cell. The stalk which is produced by apical growth switches to a budding mode of division to produce uninucleate metula cells. These in turn bud at their distal tip to produce phialide cells which then bud repeatedly at their distal tip to produce asexual spores (conidia). Conidia are likely to be the infectious agent. At 37ºC growth occurs as uninucleate yeast cells which divide by fission and which represent the pathogenic growth form. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host. The transition from the hyphal to the yeast growth form occurs by a process known as arthroconidiation where nuclear and cellular division become tightly coupled, junctions between hyphal cells break down and uninucleate arthroconidia are liberated which grow and divide by fission. Little is known about the molecular events involved in the establishment and maintenance of the developmental states in *P. marneffei* and the control of the dimorphic switching process.
The abaA gene is a member of the ATTS class of transcriptional regulators which control developmental processes in eukaryotes. In both Saccharomyces cerevisiae and *Candida albicans*, the abaA homologue TEC1 regulates filamentation during pseudohyphal growth and hyphal morphogenesis, respectively. In *P. marneffei* and the monomorphic fungus *Aspergillus nidulans*, abaA is a key transcriptional regulator of asexual development and in particular phialide differentiation. In addition to defects in the conidiation program, abaA mutants in *P. marneffei* affect yeast cell morphogenesis during the dimorphic switch, producing aberrant multinucleate yeast cells. The brlA gene encodes a C2H2 zinc finger transcriptional regulator which is known to regulate abaA expression in *A. nidulans.* To understand the regulation of the abaA gene in *P. marneffei*, the brlA gene was cloned and characterised. The data show that brlA regulates abaA expression during asexual development but not during yeast morphogenesis. Furthermore, ectopic expression of brlA can drive the asexual development program in vegetative hyphae as well as in yeast cells.

S4PS1 - 0189

Fission yeast cytokinesis: two paths to one destination

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Cell division in the fission yeast *Schizosaccharomyces pombe* requires the formation and constriction of an actomyosin ring at the division site. The actomyosin ring is assembled in metaphase, maintained throughout mitosis and constricts after completion of anaphase. Maintenance of the actomyosin ring during late stages of mitosis depends on the septation initiation network (SIN), a signalling cascade that also regulates the deposition of the division septum. However, SIN is not active in metaphase and is not required for the initial assembly of the actomyosin ring early in mitosis. The FER/CIP4-homology (FCH) domain protein Cdc15p is a component of the actomyosin ring. Mutations in *cdc15* lead to failure in cytokinesis and result in the formation of elongated, multinucleate cells without a division septum. Here we present evidence that the requirement of Cdc15p for actomyosin ring formation is dependent on the stage of mitosis. While *cdc15* mutants are competent to assemble actomyosin rings in metaphase, they are unable to maintain actomyosin rings late in mitosis when SIN is active. In the absence of functional Cdc15p, ring formation upon metaphase arrest depends on the anillin-like Mid1p. Interestingly, when cytokinesis is delayed due to perturbations to the division machinery, Cdc15p is maintained in a hypophosphorylated form. The dephosphorylation of Cdc15p, which occurs transiently in unperturbed cytokinesis, is partially dependent on the phosphatase Clp1p/Flp1p. This suggests a mechanism where both SIN and Clp1p/Flp1p contribute to maintenance of the actomyosin ring in late mitosis through Cdc15p, possibly by regulating its phosphorylation status.

S4PS2 - 0740

Localization and traffic of secretory vesicles in living hyphae of *Neurospora crassa* by laser scanning confocal microscopy.

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One of the most intriguing questions in fungal biology is how secretory vesicles move along hyphae. It has long been known that apical growth in fungal hyphae is determined by the way the cell wall is assembled and synthesized. This synthesis includes the addition of N-acetylglucosamine (GlcNAc) molecules to the growing chitin chain and is confined largely to the apex of growing cells and septa. Therefore a good starting point towards understanding vesicle dynamics appeared to be the analysis of the microvesicles that carry chitin synthase (CHS), the enzyme responsible of the addition of GlcNAc subunits during chitin biosynthesis. Seven classes of CHS genes have been described in fungi. While potential roles have been assigned to various CHS classes, we lack information on the cellular localization and trafficking of these proteins to their sites of action on the cell surface in regions of active cell wall growth.
Bioinformatic analysis of the genomic sequence of *Neurospora crassa* revealed seven open reading frames with homology to described *chs* genes. We used high-resolution laser scanning confocal microscopy to analyze trafficking of two GFP-labeled CHS (CHS-3 and CHS-6) from synthesis sites to the cell surface in growing hyphae of *N. crassa* and observed similar distribution patterns of both protein fusions. In region III (45 µm from the tip), CHS-GFP is found mainly in a highly stained network of large endomembranous compartments and in septa. Closer to the hyphal tip, the label is found in numerous vesicles or groups of vesicles that move predominantly forward until reaching region II (15-20 µm from the tip). At the tip (region I), the fluorescence congregates into a conspicuous single body corresponding to the known location of the Spitzenkörper (Spk). By using FM4-64 to label the Spk, we observed that CHS-GFP was localized in the inner core of the Spk, the same region in which microvesicles are detected by transmission electron microscopy. We used fluorescence recovery after photobleaching (FRAP) to monitor the movement of vesicles towards the Spk. Seemingly, these morphologically diverse fluorescent compartments constitute an unconventional secretory path traveled by CHS in the hyphal cell. The nature and dynamics of other endomembranous compartments involved in the conventional secretory route is being investigated with fluorescent dyes.

1045-1245
SYMPOSIUM 5 - ***Ecology and Diversity of Penicillium and Aspergillus in Australia***

S5IS1 - 0872
Biogeography and ecology of *Aspergillus* in Australia
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Aspergillus species are generally regarded as being more common in tropical and sub-tropical climates than in temperate or cool-temperate areas, however the biogeography of *Aspergillus* in Australia's natural environment has received little attention. Most research on this genus in Australia has focused on *Aspergillus* as a food spoilage agent, a producer of mycotoxins, or as a human, animal or plant pathogen. Limited sampling of Australian soils has revealed that *Aspergillus* is indeed more common in soils and other natural substrates collected in the warmer and drier areas of the continent, than the cooler southern areas.
Some common species of *Aspergillus* appear to be ubiquitous: molecular studies on some of the black Aspergilli (*A. niger* and *A. carbonarius*) and *A. flavus* indicate that Australian isolates of these species are indistinguishable from Northern Hemisphere isolates. Most of the common food-borne Aspergillus species are no doubt cosmopolitan, as world trade in food commodities has spread these species around the globe. The Australia natural environment, however, has revealed some species that appear to be unique to this continent, and others that are common in Australia but rarely encountered elsewhere. Australian isolates of some relatively common species may, on closer examination, be found to differ significantly from type species originally found in the northern hemisphere.

S5IS2 - 0883
The Astonishing Biodiversity of *Penicillium* in Australia
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Australia is known to be a megadiverse continent, with an exceptional range of unique animal and plant life. It would not be totally surprising, then, to find a wide range of unique fungi. Macrofungi and the plant pathogens introduced with various economic crops have been relatively well explored in recent years but remarkably little is known of the endemic micro mycoflora. A small grant from a government agency has provided us with the opportunity to look at the family *Trichocomaceae* from local sources around Sydney and selected other spot locations around the country. In the Sydney Basin, standard techniques of dilution and direct plating were used to isolate *Penicillium* species from soils, decaying vegetation, soil detritus and fresh leaves. Soils only were examined from other locations. All together about 550 samples were studied. Not unexpectedly, a wide range of known species was recovered, together with some undescribed species. What was unexpected was first, the number of undescribed species, and second, the specificity of habitat. Isolates from samples from Sydney had little in common with samples from Queensland, Victoria or Western Australia. Even within the Sydney Basin, most samples that were not true duplicates yielded one or more putative new species. The most astonishing results were obtained from fresh leaves. As part of a study of the effect of disturbance on biodiversity, fresh leaf samples were taken from a single type of common native small tree, *Banksia integrifolia*, from a range of natural and garden sources within three areas of an 80 km coastal strip of the Sydney Basin. Two hundred and seventy leaves were sampled, and 35 putative new species were found – one from each eight leaves sampled. Interestingly, only three of those 35 unknowns were isolated from adjacent soils. Moreover, 32 of these new species came from only a single site.

S5IS3 - 1012
Is the Novelty of Morphological Species in Trichocomacceae reflected in metabolic diversity?
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During Microbial Screening Technologies' investigations into fungal diversity as a source of novel therapeutics we have discovered hundreds of Trichocomaceae exhibiting potent activity in a range of bioassays. Phenotypic examination places many of these cultures into known and well documented species and species groups, yet analysis of the secondary metabolite profiles suggests that many of the metabolites are novel. This contradiction places pressure on our current understanding of taxonomy as a tool in drug discovery. On the one hand, researchers seeking novel species ignore vast areas of taxonomic space in the belief that these are well trodden paths, and on the other taxonomists fail to meet the needs of perhaps their strongest allies, the users of biodiversity.

The use of secondary metabolites in aiding the taxonomy of Trichocomaceae is well developed, thanks to the efforts of Frisvad and colleagues. These efforts have refined our understanding of the typical metabolite profiles that can be expected for many of the common species. But, what of the exceptions: those cultures fitting the phenotypic sense of the species but not the metabolic profile? Do we accept the need for a "chemotype" as an extra layer of descriptive detail to handle this diversity. Or, do we accept that our current phenotypic trees describe a clade of species rather than a unique species? In our work on Trichocomaceae we have been able to tap into the expertise and culture collections of Pitt and Hocking to highlight the complexities in the structure of a number of species by comparison of Australian with international cultures.

S5PS1 - 0291

Using GCPSR to resolve synonymies in *Penicillium toxicarium*

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The utility of phenotypic analysis of *Penicillium* for resolution of species is shown to be questionable by the application of GCPSR (genealogical concordance phylogenetic species recognition) to this problem. Members of the *P. citreonigrum* complex produce different secondary metabolites and it was of interest to establish the relationship between metabolite production and species identity. Species that are considered synonymous with *P. citreonigrum* on the basis of phenotype include *P. albocinerascens, P. syriacum, P. cinerascens, P. katangense, P. hirayamae, P. citreoviride, P. subcinereum, P. gallaicum, P. aeneum* and *P. lilacinoechinulatum*. Single locus phylogenetic analysis of the ITS region suggested that some of these species are distinct, but is equivocal on the relationship of more closely related species. DNA sequences were determined for the ITS and lsu-rDNA, beta tubulin, calmodulin, EF1-alpha, and RPB loci of type isolates of these species. The data were analyzed using the GCPSR method and the distinct species *P. cinerascens, P. citreonigrum, P. toxicarium, E. katangense, P. lilacinoechinulatum, P. syriacum* and *E. hirayamae* were observed. The species *P. citreonigrum, P. cinerascens* and *P. toxicarium* are quite closely related along with an undescribed species, referred to as *Penicillium* taxonX. The other species considered synonymous with *P. citreonigrum* are disparate and widely spread across the *Eupenicillium* lineage. *P. gallaicum* was not included in the present study. While not surprising that sibling species might be placed in synonymy using phenotypic analysis, it is unexpected that phylogenetically distinct organisms would be considered synonymous. *Penicillium* species are divided on the basis of penicillus complexity, and the highly complex penicillus associated with subgenus *Penicillium* species appears to be uniquely derived. The somewhat simpler penicilli of subgenera *Aspergilloides* and *Furcatum* are not good phylogenetic indicators of lineages since it is often a matter of judgement whether a penicillus is monoverticillate or furcate, as exemplified by Raper & Thom's series Ramigena, and it has been shown that penicillus complexity in the simpler types does not reflect the phylogeny of the species (Peterson 1999). There currently is insufficient resolution of the older branching points in phylogenetic analyses to determine the ancestral penicillus form in *Penicillium*, but both monoverticillate and furcate penicilli are widespread in the lineage suggesting convergence in these phenotypically simple organisms.

S5PS2 - 0418

Aspergillus* and *Penicillium* Teleomorphs from Thailand and Application of *Talaromyces flavus* against Plant Pathogenic Fungi *in vitro

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Some species of *Aspergillus* and *Penicillium* telemorphs are very important for their secondary metabolites. The purpose of this study was to isolate these fungi from various substrates, such as soil from termite mounds, forest and agriculture field, and food commodities. The soil plate method, alcohol and heat treatments and Gochenaur's glucose ammonium nitrate agar were used to isolate soil fungi. Culture were grown on potato dextrose agar, Czapek's, malt extract and cornmeal agars and incubated at 28 oC for 7 to 21 days. Microscopic characters were examined under a light microscope with Normaski Interference Contrast and scanning electron microscope. For the second investigation, *Talaromyces flavus* was used to test for the antagonistic activity against nine species plant pathogenic fungi *in vitro*.

Aspergillus and *Penicillium* telemorphs reported in this study were *Emericella nidulans, Emericella variecolor, Eupenicillium javanicum var. lineolata, Eupenicillium parvum, Eupenicillium stolkiae, Eurotium amstelodami, Eurotium cristatum, Fennellia niveus, Hamigera avellanea, Neosartorya delicata, Neosartorya fischeri, Neosartorya glaber, Neosartorya multiplicata, Neosartorya quadricincta, Neosartorya spinosa, Neosartorya takakii, Talaromyces bacillisporus, Talaromyces helicus* var. *helicus*, *Talaromyces flavus, Talaromyces stipitatus, Talaromyces trachyspermus Talaromyces luteus* and *Talaromyces wartmanii.* Most of *Aspergillus* and *Penicillium* teleomophs found in this study represent the new records for Thailand. Pure cultures of fungal species are maintained in a Culture Collection at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand .

Among 170 strains of *T. flavus*, 20 strains were used for antagonistic tests to 9 plant pathogenic fungi *in vitro* and in the green house. The antagonistic test indicated that 18 strains of *T. flavus* could effectively control *Phytophthora palmivora in vitro*. All strains of *T. flavus* produced moderate control of *Phytophthora parasitica*, *Fusarium oxysporum*, *F. semitectum*, *Colletotrichum capsici*, and *C. gloeosporioides*, but did not control *Lasiodiplodia theobromae*, *Rhizoctonia oryzae* and *Sclerotium rolfsii in vitro*. In green house experiment, *T. flavus* could effectively control *Sclerotium rolfsii*.

1330-1500
POSTER SESSION 1 - PHYLOGENY, SYSTEMATICS & EVOLUTION

PS1-1-0022
Detection of fungal endophyte DNA contamination using ITS primers in two grass species
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PCR is one of the modern molecular biology techniques useful in several molecular detection studies. DNA templates of two grass species Cynodon dactylis and Cyprus rotrandus (Poaceae) were extracted and tested for the presence of fungal contamination using ITS Pimer-5'GCATCGATGAAGAACGCAGC3'. Out of the two grass species studied, one was contaminated with fungal endophyte DNA. This is the simple and rapid technique for detecting fungal DNA in angiosperms

PS1-2-0028
Conidial morphology: homology or homoplasy? The analysis of molecular data shows that marine *Dendryphiella* species do not belong to the genus *Scolecobasidium*
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The marine *Dendryphiella* species, *D. arenaria* and *D. salina*, are mainly identified based on their conidial morphology, but more recently also on the basis of their metabolic profiles (paper submitted). The assumed high taxonomic value of this parameter led to the recent inclusion of *Dendryphiella* in the genus *Scolecobasidium*. Thus, according to the currently valid taxonomy, the species are named *S. arenarium* and *S. salinum* with *D. arenaria* and *D. salina* as the respective synonyms. However, due to the significant dissimilarity in the physioecological and other morphological characters, there is still disagreement on the placement of these species within *Scolecobasidium*. We were interested, therefore, in determining the position of the marine *Dendryphiella* species with respect to the modern molecular phylogeny of ascomycetes. *Dendryphiella* strains were isolated along coastal areas in subtropical (Gulf of Mexico) and temperate (North Sea, Baltic Sea, Mediterranean Sea, English Channel) waters and genomic DNA was extracted from 36 *Dendryphiella* strains and 10 representative strains of 8 *Scolecobasidium* species. RAPD profiles were initially considered in grouping the isolates for subsequent gene sequencing. Analysis of partial rpb2 sequences was used to find the next closest taxonomic relatives, while the infragenic structure of marine *Dendryphiella* was detected by the variable ITS1 and 2 of the rDNA repeat and the introns of partial tef1 gene. We found that the *Dendryphiella* strains form two sister clades, which correspond to *D. arenaria* and *D. salina*. Both species belong to the Pleosporaceae family, with *Pleospora herbarum (Stemphyllium botryosum)* as the next very close taxonomic relative. All *Scolecobasidium* species sequenced form a distinct genetically isolated phylogenetic group outside of the class Loculoascomycetes. Though the exact phylogenetic position of *Scolecobasidium* remains unclear, we have shown that the resemblance of conidial morphology of *D. arenaria* and *D. salina* to the species of *Scolecobasidium* is likely due to convergent evolution and not a result of a close genetic relationship. The need, therefore, for the revision of the taxonomy of both groups is warranted.

PS1-3-0030
Molecular Characterization of Thermophilic Fungi Using Internal Transcribed Spacer (ITS) Region Primers
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Thermophilic molds are included in a unique physiological group of fungi that have an ability to thrive at temperatures around 50-55°C. These fungi are of immense commercial importance and have been used as source of industrially important enzymes (Lipases, xylanases, acid proteases, cellulases, phytases, etc) with application in detergent, food & feed, textile and paper industry. This study reports molecular characterization of indigenous thermophilic fungi isolated from the composting soils and decomposing waste of region in and around Amritsar (Punjab) India. The characterization was based upon DNA sequence analysis (Clustal X) of the PCR amplified ITS region of rDNA as well as restriction mapping of 18s rDNA and ITS amplified regions using Amplified rDNA Restriction Analysis (ARDRA). The generated dendrogram revealed that *Mucor indicus,* formed an out group and was related distantly to another thermophilic zygomycete *Rhizomucor pusillus*. These zygomycetes were of distinct phylogenetic origin. *Melanocarus* sp., a rare thermophilic fungus, was distantly related to *Chaetomium thermophilium, Myceliophthora* sp., *Corynascus* sp., as evident from long branch length. Similarly, *Malbranchea flava*, showed distinct origin on phylogenetic tree and was related to *Thermoascus aurantiacus*, *Penicilium sp*., clade. *Thermomyces lanuginosus* belonging to class deuteromycete and an important source of thermostable industrial enzymes, showed low boot strap values amongst some of its strains that were compared indicating the presence of cryptic sexual process or faster molecular clock in this fungus.

PS1-4-0039

The dilemma of Georgefischeriales systematics

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The systematic position of the *genus Georgefischeria* is still under consideration because of some debatable morphological and physiological variations. Bauer et al studied hyphal septation, cellular interactions, teliospores, basidia, cultures and nucleotide sequencing in the order Georgefischeriales. Analysis of both morphological and molecular characters yields similar phylogenetic conclusions. Accordingly, the order Georgefischeriales is divided into three groups, Eballistraceae, Georgefischeriaceae and Tilletiariaceae. The basal dichotomy is between the Eballistraceae, and the branch uniting the Georgefischeriaceae and Tilletiariaceae. The Tilletiariaceae is phragmobasidiate, whereas the Eballistraceae, *Georgefischeriaceae* are holobasidiate. The Eballistraceae differ from Georgefischeriaceae and Tilletiariaceae in the lack of the ballistospore mechanism. However, the species of Georgefischeria so far established showed remarkable differences in the symptoms and germination patterns. The germination products are also differing in different species such as sterigmate basidiospores, ballistospores and blastospores. No conjugation of the germination products was observed in situ or after their separation from the basidia. Both monokaryotic and dikaryotic germination products were observed. The basidia also frequently develop septation-showing tendency towards Ustilaginaceae. Dikaryotic phase was observed in the sterigmate basidiospores, ballistospores and blastospores. The present paper deals with the different species of *Georgefischeria*, their morphological and physiological variations and their consequences in the systematic position of *Georgefischeria*.
KEY WORDS: *Georgefischeria*, symptoms, germination products, teliospore germination, systematic position.

PS1-5-0043

Morphometric taxonomy in the diversity of some South Indian species of *Phyllosticta*

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Phyllosticta is an unique and interesting *Coelomycetous fungus* (Mitosporic fungus). The conidia in this fungus are borne within some specialized cavities called pycnidial conidiomata. They are cosmopolitan in occurrence, causing leaf spot/lesions/blight/blotches diseases in ornamental plants, economically important agricultural crops/horticultural crops/ forest trees/ cereals and pulses. Species of *Phyllosticta* occurs under extreme climatic conditions ranging from tropics to arctic regions. Presence of mucilaginous sheath around the conidia and apical appendage of the conidia is considered to be an important taxonomic character in the species of *Phyllosticta*. It is a dimorphic fungus having both perfect and imperfect states in their life cycles. In the present study, various species of *Phyllosticta* were collected from South India has been taken with a view to build up a genetic resources collection in our centre. For this purpose, fungal collections were made from various places in and around Tamilnadu, South India. More than 50 different species of *Phyllosticta* were collected and 38 of them were successfully grown and characterized in cultural studies. In the case study the identification of the species is mainly based on host specificity in addition to the morphometrical characters including the dimensions of pycnidia, conidiogenous cells and conidia. Statistical analysis of the morphometrical data was made and hierarchical cluster were generated in order to study the (genetic relatedness/similarity) similarity/interrelationship with in 38 species of *Phyllosticta* described in the present study. Of the 38 species described, 35 were pathogenic and 3 were endophytes. In the results, 18 species were found to be new host record to science and 10 were found to be new species record to India. The results and significance of the findings are discussed in detail.

PS1-6-0050

The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA

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The Pleosporaceae is an important loculoascomycete family. There has however, been disagreement regarding the taxonomic placement of many genera within this family. This study investigates phylogenetic relationships among the genera *Cochliobolus, Kirschsteiniothelia, Leptosphaerulina, Macroventuria, Pleospora, Pyrenophora*, and *Wettsteinina*. Partial 28S rDNA sequences from taxa within these genera were analysed using Maximum Parsimony, Likelihood and Bayesian methods. *Cochliobolus* can broadly be segregated into two groups as previously proposed. Pleospora is polyphyletic in its current sense. Taxa with *Stemphylium* anamorphs are closely related to *Cochliobolus* and fit within the Pleosporaceae, whereas the affinities of *Pleospora herbarum* and *P. ambigua* are still ambiguous. *Pyrenophora* constitute a monophyletic group within the Pleosporaceae whereas Leptosphaerulina and Macroventuria appear to share phylogenetic affinities with the Leptosphaeriaceae and Phaeosphaeriaceae. Phylogenies indicate that Wettsteinina should be excluded from the Pleosporaceae. Similar findings are reported for Kirschsteiniothelia which is probably polyphyletic. Anamorphic characters appear to be significant (especially in *Cochliobolus*) while ascospore morphologies, such as shape and color and substrate occurrence are poor predictors of phylogenetic relationships among these loculoascomycetes.

PS1-7-0063

Phylogenetic positioning of *Ravenelia esculenta* using 18S rDNA sequence

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Ravenelia esculenta Naras. and Thium. is a rust fungus, which infects mostly thorns, flowers and fruits of *Acacia eburnea* Willd. The dominant stage is aeciospores which forms hypertrophy on host plant. The present study was undertaken to sequence 18S rDNA of Ravenelia esculenta and to establish its phylogeny. Aeciospores were scraped and DNA was isolated by 'freeze thaw' method. 18S rDNA was amplified and sequenced by automated DNA sequencer. BLAST of the sequence at NCBI retrieved 94 rust sequences. 18S rDNA sequences of *Pileolaria toxcodendri* and *Cronartium ribicola* were shown to be near duplicate sequences (97% and 96% similarity respectively). Multiple sequence alignment was done by ClustalW. These 18S rDNA sequences of rust fungi were useful for showing phylogenetic relationships. Phylogenetic analysis was done by using MEGA. UPGMA Minimum Evolution tree with bootstrap value of 1000 replicates was constructed using these sequences. Although BLAST showed *Pileolaria toxcodendri* and *Cronartium ribicola* as near duplicate sequences, these were far separated in phylogenetic tree. From phylogenetic tree it is observed that *Ravenelia esculenta* and the genus *Gymnosporangium* share a common ancestry, though *Ravenelia esculenta* is autoecious on angiosperm and the genus *Gymnosporangium* is heteroecious with *pycnia, aecia* on angiosperm and *uredia, telia* on gymnosperm. Two major clades are recognized which are based on the nature of aecial host (gymnosperm or angiosperm). It can be said that the nature of aecial host is more important from evolutionary point of view rather than life cycle pattern of the rust. These studies determine the phylogenetic position of *Ravenelia esculenta* among other rust fungi. This is a first report of DNA sequencing and phylogenetic positioning in genus *Ravenelia*.

PS1-8-0074

Caloplaca soralifera, a new sorediate species of *Caloplaca* (Lichenized Fungi, Teloschistaceae) lacking anthraquinones in its thallus

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A new lichen species, *Caloplaca soralifera*, belonging to the group of sorediate *Caloplaca* species lacking anthraquinones in their thalli and soralia is described. The species is described mainly from Czech Republic material deposited in the herbarium CBFS. For delimitation of the new species, an investigation of morphological and anatomical characters was used and the anthraquinones were analysed by HPLC-MS. *C. soralifera* is characterized mainly by the apothecia, which are very similar to *C. xerica*, and by the presence of soralia at the thallus margin, which occasionally cover the whole thallial surface. The soralia and cortex contain the pigment Sedifolia-grey and the apothecia contain the anthraquinone parietin as a dominant compound. *C. soralifera*, currently known form five European countries, occurs mainly on man-made substrata (concrete, asphalt), and more rarely on nutrient-rich siliceous rocks and on bark. During three years of observation, the abundance of *C. soralifera* has clearly increased in some localities; it is probably a neophytic and rapidly spreading species. An overview of European and North American species of Caloplaca lacking anthraquinones in their thalli is provided, in which *C. soralifera* is compared with similar species, *C. chlorina*, *C. furax, C. spalatensis* (syn. *C. areolata*) and *C. xerica*.

PS1-9-0080

Phylogenetics of *Phylloporus (Boletales)* species based on the large subunit rDNA

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Phylloporus is somewhat of a morphological oddity in that its species produce a lamellate rather than poroid hymenophore although other basidiome characters, spore morphology, and chemical and molecular data support placement in the Boletales (Bresinsky & Besl 2003). Despite several broad-scale phylogenetic studies in the *Boletaceae* the phylogenetic relationships of *Phylloporus* remain unclear. Previous phylogenies of this group include only two species from Europe and North America. The results of Binder (1999) suggest that *Phylloporus* is the sister group to the *Xerocomus subtomentosus* group, species of which produce poroid hymenophores. While the majority of *Phylloporus* species have a pantropical distribution, no studies on this group have included the majority of known tropical species in addition to the few north temperate taxa. In this study, we present preliminary results toward clarifying infrageneric phylogenetic relationships in *Phylloporus*; our analysis includes the largest selection of *Phylloporus* species represented in a phylogenetic study to-date. Phylogenetic relationships of selected species of *Phylloporus*, *Xerocomus*, *Aureoboletus*, and *Chalciporus* were estimated by maximum parsimony analysis of the rDNA large subunit; *Chalciporus piperatus* was used as an outgroup. The results reveal that specimens from Costa Rica show an unexpected phylogenetic diversity and include several undescribed species. Our study establishes preliminary hypotheses for species distributions and morphological evolution in this important ectomycorrhizal genus, and establishes the groundwork for further analyses that will incorporate additional taxa and characters.

PS1-10-0092

Generic trends in the Gnomoniaceae

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The Gnomoniaceae is a common but inconspicuous diaporthalean family of fungi associated with plants. Most occur[Amy Rossman] ing as symptomless endophytes of hardwood trees, some can be pathogenic. There are also some species associated with herbaceous plants and conifers. Usually they form ascomata on recently dead leaves or twigs. In earlier works based on morphological data, the family was defined as having single perithecia growing on non-woody plant tissues. The existence of the family as a separate entity in the order was confirmed by LSU rDNA sequence data by Castlebury et al. (2002). The main characters previously used for generic delimitation were presence/absence of stroma, position of the ostiole on the perithecia, ascospore shape and pattern of ascospore septation. The LSU dataset for the order Diaporthales has been significantly updated since 2002 and a dataset of over 200 ITS sequences representing ca. 100 gnomoniaceous species has been produced. Analysis of the sequence data has conflicted with the previous morphological studies. It has been revealed that some genera with perithecia formed in clusters on woody tissues definitely belonged in the Gnomoniaceae *(Cryptodiaporthe, Cryptosporella)*. Some gnomoniaceous species have been found outside of the family-level clade. Classical generic concepts show limited correlation with the clades of the phylogenetic trees. Some clades show correlation with the plant host although species on several unrelated hosts also occur. For example, there are two well defined clades: one includes parasites of conifers so far known only as anamorphs *(Sirococcus)* while a second group on Fagales has clustered perithecia on woody substrata *(Cryptosporella)*. ITS sequence data provide an initial overview of the generic trends. However resolution and support for clades that potentially represent genera are poor. Analysis of datasets of other genes (EF1, calmodulin, actin, RPB2) have clarified several genera within the family to date and the dataset is being expanded to representatives of the entire family based on the ITS and LSU results.

PS1-12-0095

PCR-RFLP Patterns Of 5.8 S And ITS2 Genes In The Taxonomy Of Neotyphodium Endophytic Fungi

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Endophytic fungi have mutualistic relationship with the plant family Poaceae. These fungi confer characteristics such as yield increase and biotic and abiotic stress resistance to host plants. Endophytes are classified in the class Ascomycetes, order Hypocreales and the family Clavicipitaceae. The endophytes spend all their life cycle in the aerial parts of plant hosts and live intercellularly. In the present investigation, endophytic fungi were isolated from seed and leaf sheath of *Festuca arundinaceae, Festuca ovina, Festuca pratensis, Bromus tomentellus, Melica persica* and *Lolium prenne*. Genomic DNA was extracted and three sets of primers: ITS1/ ITS4, IS1/IS3 and 111/112 were used to detect and identify endophytes. The results of PCR with three paires of primers indicated that most of isolates used in research were endophytic fungi belonging to *Neotyphodium* and isolates of *F. arundinacea* were *N. coenophialum*. For amplification of ITS1/ ITS2 and 5.8 S gene, primers ITS1 / ITS4 were used. PCR products were digested by *Sau3AI* and *CfoI*. The results of PCR- RFLP showed that restriction analysis is concordant with the morphological studies and PCR specific primers.

PS1-13-0098

Ribosomal DNA phylogenies of *Cyathus*: Is the current infrageneric classification appropriate?

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Phylogenetic relationships within the genus *Cyathus* (bird's nest fungi) and among other gasteromycetes were investigated using neighbor joining, maximum likelihood, weighted maximum parsimony and MrBayes analyses of ITS and LSU ribosomal DNA sequences datasets. Twenty-two taxa of *Cyathus* were used in the analyses based primarily on type and authentic specimens. The current infrageneric classification system of Brodie recognizes seven infrageneric groups based on morphological characters, including peridium plications, and variations in peridium hair anatomy, peridiole structure and fruitbody colour. These seven groups are not supported by molecular data. Instead, the ITS and LSU datasets support recognition of three infrageneric groups herein named the ollum, pallidum and striatum groups. Morphological characters useful in distinguishing these groups include basidiospore size, fruitbody coloration, and peridium anatomy. The inclusion of 22 *Cyathus* taxa in a molecular phylogenetic analysis of a broad sampling of gasteromycetes supports recognition of *Cyathus* as a monophyletic lineage within the euagarics. *Cyathus africanus* var. *latisporus* is considered a synonym of *Cyathus jiayuguanensis*, and a new combination *Cyathus lanatus* (Brodie) R.L. Zhao is proposed based on morphological and molecular data.

PS1-14-0107

The Internal Transcribed Spacer (ITS) Based Molecular Identification of Endophytic Fungi from *Garcinia* spp. which Produce Antimicrobial Substances

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In our preliminary screening for endophytic fungi isolated from five *Garcinia* species from Thailand; *Garcinia atroviridis*, *G. dulcis, G. mangostana, G. nigrolineata* and *G. scortechinii* which produce antimicrobial substances, 22 out of 377 isolates exhibited potential antimicrobial activity with interesting NMR profiles. Molecular identification of these potential fungal endophytes was carried out with sequence analysis of the Internal Transcribed Spacers (ITS1, 5.8S, ITS2) regions of rDNA. The phylogenetic trees generated from maximum parsimony showed that our 22 selected endophytes belonged to six orders; Diaporthales, Dothideomycetes et Chaetothyriomycetes incertae sedis, Eurotiales, Hypocreales, Pleosporales and Xylariales. Two most potential endophytes; D15 isolated from G. dulcis and M76 isolated from *G. mangostana* were identified as *Phomopsis* sp. and *Botryosphaeria* sp., respectively. D15 strongly inhibited *Staphylococcus aureus* ATCC25923 and methicillin-resistant *S. aureus* (MRSA) SK1, while M76 strongly inhibited both strains of *S. aureus* and *Microsporum gypseum*. Furthermore, another most potential endophyte A1 isolated from *G. atroviridis* which inhibited *Cryptococcus neoformans* ATCC90012 was identified as *Fusarium* sp. Other endophytes with moderate activity were closely related to several fungal genera; Aspergillus aculeatus, Curvularia sp., Guignardia *mangiferae, Penicillium paxilli* and *Xylaria* sp. This result suggested that the endophytic fungi isolated from *Garcinia* plants are diverse and can be used as potential sources of antimicrobial agents. Bioactive compounds and other interesting biological activities will be further studied.

PS1-15-0108

Utility of the Macro-Micromorphological Characteristics used in Classifying the Species of Termitomyces

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Conventional taxonomy of mushroom use micro-macromorphological features characteristics in taxa delimitation. General rules of observing the macroscopic features from the field to microscopic features in the laboratories are followed although not all the features observed are useful in delimiting the species of different genera. This bailiwick provides the review on the utility of the micro-macromorphological characteristics used in delimiting twenty five species of Termitomyces.

The distinguishing characteristics were analysed from; 4 species of our own collection and 5 holotype from the mycological herbarium of the "Museum National d' Histoire Naturelle Cryptogamie" (PC). Others were noted from the literatures of Heim (1977), 17 species; Pegler and Van haecke (1994), 9 species; Van der Westhuisen and Eicker (1990), 7 species; Buyck (1994), 5 species and Härkönen et al. (1995), 5 species. The characters were compiled in tables and analysed graphically where their usefulness in demarcating species of the genus were assessed.

Three groups based on cap sizes were established and colour of the cap was found to be very useful identification criterion although it varies enormously as well as the pigment responsible for the suites of a certain colour is not yet determined. While the pinkish colour of the spore print circumscribed the genus it self and not the species within it, pseudorrhiza absence showed to be useful to only two species *T. medius* and *T. microcarpus* in which it is missing. However, its colour, size and morphometry were apparently of no use in distinguishing the species of the genus as well as the annulus because of its fugacious nature.

The tetra and smooth basidiospore characters that circumscribed the genus were found to be discrepant as 1-2 young sterigmata and basidiospore were noted in one of the species *(T. umkowanii)* and fine tufts covering the basidiospore of *T. aurantiacus* and *T. striatus* as shown Plate. 1 and 2 respectively.

Morphometry of the microscopic features (basidia, cystidia and basidiospores) varies insignificantly and their sizes overlapped such that their usefulness in delimiting closely related species is refutable. However, *T. citriophylus* diverged in the size of basidiospore and basidia from other member of the genus requiring more justification for its taxon status as a member of this genus.

Plate 1: 1-2 young stergmata of T. umkowanii

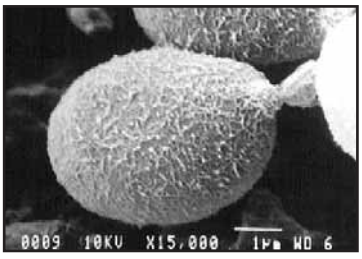

Plate 2: Unsmooth basidiospore of T. aurantiacus

PS1-16-0112

New records of Termitomyces (Basidiomycetes) from Cameroon and Central Africa : taxonomy, ecology and phylogeny

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Species of the genus *Termitomyces* Heim which are generally very good edible and tasty mushrooms belong to the Tricholomataceae and are today estimated to about 30 taxa worldwide. They grow as symbionts only in tropical regions of Africa and Asia in a more or less specific association with termites within termites nests. Many species particularly from tropical regions of Africa are still undescribed.
In a poster for IMC7(2002a) and later on in a publication (BSMF (118(3), 2002b), Mossebo et al. (2002a/b) described 14 species of *Termitomyces* among which 4 new species (*Termitomyces subclypeatus, T. grisumbo, T. mboudaeina* and *T. subumkowaanii)* and 4 new forms (*Termitomyces subclypeatus*, forma t*etrasporus; T. striatus, f. grisumboïdes; T. striatus, f. subumbonatus ; T. striatus, f. bibasidiatus)*.
Meanwhile, several collections were obtained in Cameroon and from the sub-region of central Africa among which we described 2 further new species *(Termitomyces camerunensis* sp. nov. and *T. infundibuliformis* sp. nov.*)* and 2 new forms (*Termitomyces striatus,* f. *pileatus* f. nov. and *T. subclypeatus,* f. *sterigmatus* f. nov.). During our investigations, their macroscopic and microscopic features didn't match those of any existing species and forms known in the literature. The preliminary results of the phylogenetic studies carried out on the 18 species and forms so far known in Cameroon and central Africa and based on 120 sequences of nLSU rDNA and the Bayesian analysis generally confirm our taxonomic and geographic sampling and testify that *Termitomyces* is a monophyletic group. However, many other collections are actually still under investigation with other primers in order to refine the above mentioned results.

PS1-17-0113

Some smut fungi (Ustilaginomycetes) from Thailand

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Several new and rare smut fungi (Ustilaginomycetes) were collected in Thailand during a survey by the authors in Dec 2005. About half of Thailand's 76 provinces were visited in the North, North-East, East and Central regions. New species of smut fungi were found in the genera *Macalpinomyces* (1 species), *Sporisorium* (1), *Tilletia* (4), *Ustilago* (1) and *Yelsemia* (1). Most of these new species were found on native or naturalised grasses with the exception of a new *Yelsemia* on the dicotyledonous *Drosera*. The host genera of grasses of new species of smut fungi were *Aristida, Arundinella, Coelorachis, Eragrostis, Hyparrhenia, Pennisetum* and *Setaria*. Three of the species of smut fungi that were collected were previously known only from the type material, namely *Franzpetrakia microstegii* (type from India), *Sporisorium arthraxonis* (type from Vietnam) and *Tilletia ischaemi* (type from India). Descriptions, scanned images of host symptoms, as well as images of spores taken by light microscopy and scanning electron microscopy have been prepared for an interactive identification and information guide (Lucid™) to the smut fungi of Thailand.

PS1-18-0126

Phylogenetic data and morphological characteristics provide evidence for *Pythium kunmingense* to be a synonym to *P. spinosum*

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The genus *Pythium* is a member of the phylum Oomycota within the Stramenopile Kingdom and contains a large number of different species. Many species within this genus are soil borne plant pathogenic organisms which typically have a wide host range and show few distinguishing features. Identification of the species is based to a large degree on morphology which may show considerable variability further confusing species identification. In an attempt to identify 21 isolates of *Pythium* associated with damping-off of cucumber in Oman, ITS-1 and ITS-4 primers were utilized to amplify the ITS region of the rDNA. Generated sequences of the ITS region for the 21 isolates were found to be identical. Comparisons to sequences in GenBank using blast search revealed high similarity to the ITS sequences of the ex-type strain of *P. kunmingense* (AY598700) and a representative strain of *P. spinosum* (AY598701). Bootstrap analysis showed *P. kunmingense* to fall within the variation of *P. spinosum* and only a single base pair mismatch was found in the ITS sequence between *P. kunmingense* (CBS 550.88) and *P. spinosum* (CBS 290.31). Morphological characterization of two isolates from Oman (P006 and P017) showed that they possess some characters in common with *P. spinosum*, e.g. the size of hypha swellings, and others in common with *P. kunmingense*, e.g. hypha main diameter. However, isolate P017 resembled *P. kunmingense* in the size of oogonia, while isolate P006 was found to have a similar oogonia size to *P. spinosum*. Surprisingly, isolates from Oman were distinct from *P. kunmingense* and *P. spinosum* in having mostly smooth, intercalary oogonia with aplerotic (to plerotic) oospores. The identical ITS sequence between isolates P006 and P017, which showed some variability in morphological characteristics, together with the lack of distinct differences in morphology and phylogeny between *P. spinosum* and *P. kunmingense* may be sufficient to consider the two species synonyms and the morphological differences as widening the range of infraspecific variation within this species. More data and facts will be presented on the origin of the two species and their characteristics.

PS1-19-0130

To Revision of the Teloschistaceae (Ascomycota) of Australia

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The most part of taxonomical papers on the Teloschistaceae hitherto belongs to the Northern Hemisphere. Only about 200 lichen species (or 25% of the total species number of the Teloschistaceae) were described from the Southern Hemisphere. 63 lichen species of the Teloschistaceae are recorded for Australia till now (McCarthy 2003).
The main aim of our study is a revision of the species diversity of the Teloschistaceae in connection of preparation of the next issue of "Flora of Australia". The following tasks within this study were planned: to carry out revision of specimens kept in the following herbaria BM, BRI, CANB, E, HO, LD, MEL, PERTH etc.; to collect fresh material for comparative morphological, biochemical and molecular studies; to prepare full descriptions, illustrations and maps of distribution in Australia for each species, as well as keys to representatives of each genus of the Teloschistaceae for the "Flora".
From our data species diversity of the representatives of the Teloschistaceae of Australia includes preliminarily 120 species belonging to 7 genera. About a half of species composition of the Teloschistaceae of Australia is recorded for the first time, and one third of them are new for science. Generic status of two groups of xanthorioid lichens (namely *X. ligulata* and *X. marchantii* ad int. groups) is in need of revision on molecular level.
Special revision of the representatives with "European" or "Northern Hemisphere" names shows that a number of such names were used incorrectly for Australian material. So specimens previously reported as Fulgensia subbracteata belong mainly to widely distributed *F. cranfieldii* ad int. as well as to rarer species *F. isidiosa* ad int. The former records of *Caloplaca citrina* belong mainly to *C. erythrosticta* as well as to one more still un-described species of *Caloplaca. C. hanneshertelii* represents main part of *Caloplaca cerina* records. Two recently described Australian taxa – *Xanthoria elixii* and *X. streimannii* cover almost equal potions of the biggest part of *Xanthoria parietina* localities, while status of the third taxon, specimens of which were included in '*Xanthoria parietina* s.l.", is under special revision at the moment.

PS1-20-0134

Phylogeny of *Nemania eleiodoxae* based on 18S and 28S rDNA sequence analyses

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In our study of palm fungi from a peat swamp in southern Thailand, a wide range of new taxa have been encountered and described: 11 anamorphic taxa, 5 ascomycetes. A new *Nemania* species was collected on *Eleiodoxa conferta*, a common peat swamp palm at Sirnidhorn Forest, Narathiwat, Thailand. The genus *Nemania* is placed in the *Xylariaceae, Xylariales* (Kirk et al., 2001) with 41 species. A molecular study was initiated to determine its relationship with other xylariaceous ascomycetes, especially those from aquatic habitats: *Nemania maritima* and *Halorosellinia oceanica*. Other xylariaceous species collected on peat swamp palms are: *Anthostomella bruneiensis*, *A. lunispora*, *A. palmaria, A, zongluensis, Astrocystis rachidis*, and *Stilbohypoxylon moelleri*. Sequence data from 18S and 28S rDNA were analysed phylogenetically using Maximum Parsimony (MP) and Markov Chain Monte Carlo (MCMC) analysis. *Nemania eleiodoxae* was basal to other members of the Xylariaceae (e.g. *Nemania maritima, Rosellinia necatrix*, *Xylaria acuta, X. hypoxylon*) based on 18S rDNA sequences, while the 28S analysis positions *Nemania eleiodoxae* in a Xylariaceae clade with *Astrocystis cocoës, Nemania maritima* and *Rosellinia necatrix*. *Nemania eleiodoxae* shows no affinity with *Halorosellinia oceanica*, which is positioned in a clade with *Xylaria* species. Morphologically *N. eleiodoxae* most closely resembles *N. maritima*, and *N. confluens*. It differs from *N. maritima* in having longer asci and ascospores with a mucilaginous sheath and its host and freshwater habitat. It differs from *N. confluens* with its narrower asci and longer and narrower ascospores. Ju and Rogers (2002) suggest that *N. maritima* should be excluded from *Nemania* and a similar case can be made for *N. eleiodoxae*.

PS1-21-0135

New fungal taxa described from peat swamps, mangroves, freshwater and on seeds, fruits and forest leaf litter in Thailand

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Thailand is one of the mega rich countries when it comes to its biodiversity. Although the plants, birds and mammals of Thailand are well documented, fungal diversity has only been studied in any depth over the past decade (Jones and Hyde, 2004). Thailand supports a rich diversity of habitats, from coral reefs, with some 275 offshore islands; significant mangrove stands, limestone outcrops, tropical rainforests, peat swamps and pine forests in the northern cooler provinces. These rich habitats and plant diversity has yielded over 400 new taxa (holotype from Thailand) and in this poster we illustrate some new taxa from mangroves, freshwater and peat swamp habitats and those collected on seeds, fruits and leaves in lowland forests. Four palms in a peat swamp at Narathiwat, southern Thailand yielded 315 species of which 16 new species have been described e.g. 3 *Craspedodymum* spp., 3 *Dactylaria* spp., *Chalara siamense* (anamorphic), *Flammispora bioteca, Phruensis brunniespora, Unisetosphaeria penguinoides* and *Jahnula appendiculata* (ascomycetes). 152 marine fungi have been documented for Thailand and include the ascomycetes *Pseudolignincola siamense* and *Thalespora appendiculata*, and recently the unique ascomycete *Manglicola guatamalensis* has been collected on the brackish water palm *Nypa fruticans*. A unique coelomycete, with a cupulate sporophore, has been described from leaf litter (*Infundibulomyces cupulata*) along with other anamorphic fungi: *Berkleasmium typhae, Cirrenalia nigropsora, Digitoramisoira lageniformis* and *Pseudoacrodictys dimorphospora*. Freshwater habitats have yielded some 600 taxa for the country and new species of *Biflagellospora* species (3) along with the ascomycetes *Jahnula siamense*, *Melanochaeta garethjonesii*, *Micropeltopsis quinquecladiopsis* and the *basidiomycete staurella aquatica*. Many of these taxa, especially the new genera, are supported by both morphological and sequence data. Type material of all new taxa are deposited in Bangkok BIOTEC Herbarium (BBH) and cultures in BIOTEC Culture Collection (BCC).

PS1-23-0147

Phylogenetic relationship of *Pestalotiopsis* species based on parsimony analysis of rDNA ITS sequences

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Pestalotiopsis Steyaert is the anamorphs of *Pestalosphaeria* Barr belonging to Amphisphaeriaceae (Barr, 1975; Sutton, 1980). At present, inter-specific delineation of this genus is based mostly on morphology of conidia (Guba, 1961; Sutton, 1980; Nag Rag, 1993). The taxonomic affinities of *Pestalotiopsis* species have been confused and equivocal. It is necessary to evaluate the traditional taxonomy of the genus by phylogenetic analysis. A molecular phylogenetic tree was generated by maximum parsimony method in PAUP*4.0b10 with the rDNA ITS sequences of 108 *Pestalotiopsis* strains belonging to 48 species (Guba, 1961) isolated from host plants in Southern China. The tree was firstly divided into two branches: A branch with brownish concolorous median cells of conidia and B branch with umber to fuliginous median cells of conidia. A branch was further divided into 7 subbranches (A1?A7) by the characters of apical and basal appendage. B branch was further divided into versicolorous subbranch (B1) and concolorous subbranch (B2). Based on the molecular phylogenetic tree, the following morphological characters were confirmed to be of significance for the taxonomy: 1) type of colored median cells of conidia; 2) the characters of apical appendages: knobbed or unknobbed terminal, single branched or multiple apical appendages, quantity of apical appendages and locations on apical cell of conidia; 3) absence or presence of basal appendage of conidia; 4) The length and width of conidia. Approximately 220 species of *Pestalotiopsis* were described (CABI Bioscience database, 2005), and many of them have morphological characters that overlap in many aspects and should be redefined. According to the phylogenetic tree, 41 strains in A7 subbranch identified as 11 species of *Pestalotiopsis* based on Guba (1961) were considered as only a species, *Pestalotiopsis microspora*; also 32 strains of *Pestalotiopsis* in B1 subbranch identified as 16 species based on Guba (1961) were considered as just one species, *P. versicolor* and 7 strains in B2 subbranch identified as two species *(P. aeruginea* and *P. theae)* were also considered as only a species, *Pestalotiopsis theae*. It is strongly proposed that when a new *Pestalotiopsis* species is described, morphological characters should be taken into account rather than host association and molecular phylogenetic information is also necessary to prove that unique from other known species. A parsimony analysis of multilocus DNA sequences including ITS region and beta-tubulin 2 gene (tub2) of *Pestalotiopsis* is undertaken.

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PS1-24-0162

Two new species of *Marasmius* (Basidiomycota, Marasmiaceae) from Brazil

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In the course of making collections of *Marasmius* for the revision of this genus in the Parque Estadual das Fontes do Ipiranga, São Paulo, SP, Brazil, two new species, *Marasmius* sp. 1 and *Marasmius* sp. 2 have been collected. They are here designated as species 1 and 2 by recommendation of the International Code of Botanical Nomenclature (recommendation 30A, and also article 29). Tentatively both are classified in *Marasmius* sect. Sicci Singer, subsect. Sicccini Singer, series Spinulosi (Clémençon) Desjardin. This group of *Marasmius* is characterized by the hymeniform pileal surface composed by smooth or Siccus-type broom cells with well-developed pileosetae. Until now, only three species of this group have been reported by Singer for Brazil: *M. echinatulus* Singer for Rio Grande do Sul State, M. flammans Berk. and *M. spiculosus* Singer for Amazonas State. The mention of *M. cohaerens* (Alb. & Schwein.: Fr.) Cooke & Quél. previously reported for São Paulo State was excluded by Pegler. *Marasmius* sp. 1 is characterized by the hygrophanous, light orange, reticulated pileus and the close lamellae with lamellullae. Microscopically by the small and ellipsoid basidiospores, absence of pleurocystidia and cheilocystidia, and by the presence of scattered setiform structures mixed with the broom cells in the pileipelis. The majority of the setae have the same shape of the broom cells; however they are easily recognized by the larger size, the thickness of the wall and the deep colour. *Maramius* sp. 2 is characterized by the pileus colour, which ranges from beige to light brown, with darker center; when fresh, some are pinkish brown, and after drying they become vinaceous brown, and distant cream gills. The structures called pileosetae in this species have a very distinct shape, with few or many ramifications in the apical portion, larger and more abundant near the centre and smaller and less present near the margin. These structures may occasionally be considered as transitional elements between the broom cells and setae, therefore they may differ by the color, the thickness of the wall and by the absence of a visible delimitation between the basal body and the setulae. These characteristics distinguish this interesting species from all other described species of *Marasmius*.

PS1-26-0174

REAPPRAISAL of *Chaetomium ampullare* Chivers and *Coniochaeta emodensis* Udagawa & Y. Hori from soil.

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In a first attempt to gain knowledge of the soil mycobiota of the Great Smoky Mountains National Park (an UNESCO Reserve, located in the eastern USA), soil samples were collected throughout this protected area using auto-sealing, sterilized plastic bags. Using a cellulose bait technique, which consisted of placing small pieces of sterilized balsa wood on 20-30 g of soil contained in 10 cm diam Petri dishes, two rarely collected fungi were isolated in pure culture: *Chaetomium ampullare* Chivers and *Coniochaeta emodensis* Udagawa & Y. Hori. *Chaetomium ampullare* is characterized by pyriform to ampulliform, beaked and ostiolate ascomata with long, septate, stiff, setae-like hairs surrounding the ostiolar pore and covering the ascomal wall, 8-spored, clavate, fasciculate, evanescent asci, and 1-celled, brown, limoniform and bilaterally flattened ascospores, umbonated at both ends and with a germ pore; anamorph is not produced. *Coniochaeta emodensis* produces nearly black, almost glabrous, subglobose, ostiolate ascomata, 8-spored, cylindrical asci with a small apical ring-like structure, and 1-celled, opaque, olive-brown to dark brown ascospores that, while variable in shape, are usually inequilateral-ellipsoidal to concavo-convex, with a longitudinal germ slit extending almost the entire equatorial length. Our strain did not produce the Geniculosporium-like anamorph, but the reverse of the colonies growing on potato-carrot agar (PCA) produced the typical dark olive-green colour, similar to that of the holotype. Living strains derived from the holotypes are not available in any culture collection and later isolations have not been reported. Thus, our isolations are noteworthy since they represent the first report of these species for the America's, and also provide new material for further molecular studies.

PS1-27-0175

Speciation and taxonomy in *Peniophora*

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Species of the genus *Peniophora* are found on dead branches or wood in a primary stage of decay. Each species is usually confined to a narrow range of hosts and host preference is often part of the species delimitation. There are many characters available in their basidiomata, which are useful in taxonomic delimitations, and distinct differences have also been found in their life cycles. This may give the impression of clearcut limits between species, but some of the morphological characters used are strikingly variable and may be the result of ecological adaptations.
In the study presented here, a global sample of specimens from selected Peniophora species was investigated. A phylogenetic analysis based on ITS sequences was performed, and taxonomical conclusions made from an evolutionary context:
Peniophora pseudoversicolor has a basal position in *Peniophora*.
Peniophora incarnata is a well characterized taxon with a world-wide distribution.
P. aurantiaca is a paraphyletic taxon, widely distributed on the northern hemisphere.
P. erikssonii and *P. laurentii* are ecologically specialized derivates from *P. aurantiaca*.
P. pseudonuda is synonymized with *P. laeta* despite clear morphological differences, because of sequence similarities and intercompatibility.
An undescribed species with morphological characters similar to *P. incarnata* appeared as a derivate from *P cinerea*. The latter species appears to be paraphyletic.
The study shows the importance of accepting character overlaps when closely related species are compared, in particular when considering taxonomy on a global scale.

PS1-28-0177

A Phylogenetic Re-evaluation Of The Sections Within The Genus *Phoma*

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The anamorph genus *Phoma* represents more than 220 specific and infra-specific taxa, and consists of both ubiquitous saprobes as well as destructive plant pathogenic species. The genus and species concepts of *Phoma* are under continuous discussion. Presently the genus has been subdivided into nine sections: *Phoma, Heterospora*, *Paraphoma, Peyronellaea, Phyllostictoides, Sclerophomella, Plenodomus, Macrospora* and *Pilosa*. This subdivision is based on morphological characters for which molecular support has thus far been lacking.
To judge the validity of various sections, a phylogenetic study was performed using more than 180 strains, including the type strains of the nine sections. Therefore, ITS1, 5.8S, ITS2 and (partial) 28S rDNA-sequences were obtained and compared with data derived from GenBank.
Although most clades contain strains classified in different sections, several monophyletic groups show correlation with the morphological classification system. Strong support is found for a large group of *P. exigua*-like strains belonging to the section, and for a clade resembling the section Plenodomus. The latter clade shows less similarity with the other strains tested and the recently proposed re-establishment of the genus Plenodomus is also supported by our results.
Although analysis of our dataset does not support the other sections as being monophyletic, some clustering of species was found to occur in well-characterized sections such as Peyronellaea and Heterospora.

PS1-29-0183

Raffaelea Species in the Gallery of Ambrosia Beetle, Platypus quercivorus

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An ambrosia beetle, *Platypus quercivorus Murayama*, causes mass mortality of Japanese oak (*Quercus mongolica* var. *grosseserrata*) in Japan. This beetle carries several fungi in its body surface and mycangia, but there is little information available about taxonomy, pathogenicity and function of associated fungi. We tried to characterize the fungi associated with *P. quercivorus*, in particular, ambrosia fungi, which are considered to serve as feeds for the insect in its breeding galleries.
Fungal isolates were obtained from the galleries in *P. quercivora*-infested logs and from the pronotum including mycangia to characterize them based on morphology and 18S rDNA sequence data.
Twenty fungal species were found in the galleries of *P. quercivorus*. Among them, *R. quercivora* was a species, previously known to be associated with the insect. Other fungi consisted of *Fusarium, Ambrosyozima*, and *Ophiostoma*, as well as unidentified fungi phylogenetically placed in the Raffaelea clade. A total five species were included in the *Raffaelea* clade, including *R. quercivora*. Three of them were isolated also from mycangia of *P. quercivorus*.
In this study, we found five species of *Raffaelea* from *P. quercivorus*. Four are undescribed. About 2000 species of *Platypodinae ambrosia* beeltes have been described as possible carriers of *Raffaela* spp., indicating that diversity of *Raffaelea* species in the world still remains unclarified. In general, each *Raffaelea* species is thought to have a specific relationship with each ambrosia beetle, as is the case with *P. quercivorus* having *R. quercivora* as a specific counterpart. However the present study yielded five *Raffaelea* species from *P. quercivorus*. This implies that the ambrosia beetle-fungus relationship is not always species-specific.

PS1-30-0186

A taxonomic revision of the Australian plant-infecting clavicipitalean fungi

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Based on current taxonomy, 23 plant-infecting teleomorphic genera have been assigned to the Clavicipitaceae, and only seven of these genera (*Balansia, Claviceps, Epichloë, Parepichloë, Nigrocornus, Myriogenospora* and *Ustilaginoidea*) have been recorded in Australia. *Ephelis* and *Neotyphodium*, *anamorphic* genera of clavicipitalean fungi, have also been recorded in Australia. There is some evidence that *Corallocytostr*oma ornicopreoides, described from northern Australia in the mid 1990's, is an anamorph of *Claviceps*. The indigenous Australian genus *Cepsiclava*, with *C. phalaridis* as the type and only species, was tentatively assigned to the Clavicipitaceae, despite it having some characteristics that could preclude it from inclusion.

The clavicipitalean genera include many important plant pathogens of grasses, some of which produce metabolites that are toxic to animals. The *Claviceps* species on sorghum, paspalum, winter cereals, buffel grass, and other grazing grasses produce alkaloids that are toxic to livestock. *Claviceps africana*, the causal agent of sorghum ergot, has had a significant impact on all sectors of the Australian sorghum industry by increasing costs of hybrid seed and commercial grain production, and by causing toxicity in animals. Overseas, species of *Balansia* also produce toxic metabolites, but the significance of *Balansia* poisoning in Australia is unknown. The *Neotyphodium* anamorphs of *Epichloë* species produce ergot alkaloids, and are known to cause stock poisoning in Australia, particularly on ryegrass (ryegrass toxicity). Black soil blindness in northern Australia has been shown to be associated with infection of Mitchell grass by *Corallocytostroma ornicopreoides*, but the metabolites responsible have not been identified. Similarly, there have been reports of toxicity in cattle grazing on grasses infected by *Cepsiclava*, but no toxicity or chemical studies have been conducted. Overseas evidence suggests that Myriogenospora may be involved in toxicity of livestock grazing on infected pastures.

In the 1940's and 1950's, R.F.N. Langdon undertook a study of the Australian species of *Claviceps*, describing many new species, eight of which are still recognised. Since then, taxonomic work on the Australian clavicipitalean fungi has been intermittent and restricted to individual genera. In a project funded by the Australian Biological Resources Study we aim to conduct a taxonomic revision of the Clavicipitaceae in Australia, and where possible identify the alkaloids produced by the fungi. Apart from the taxonomic revision, distribution maps of all species will be generated.

PS1-31-0188

Systematics and evolution of the soybean sudden death syndrome and dry bean root-rot fusaria

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Soybean sudden death syndrome (SDS) is a serious constraint to the commercial production of this crop in North and South America. The etiological agent of the disease has been reported as *F. solani* or its forma specialis, f. sp. glycines since its discovery. Our recent molecular phylogenetic analyses of multilocus DNA sequence data have revealed that the *F. solani*-species complex, or section *Martiella*, includes a large number of phylogenetically distinct species, many of which are undescribed. The objective of this study was to investigate North and South American isolates of the soybean SDS pathogens and their allies morphologically and molecularly to understand their genetic diversity and species limits. Soybean SDS pathogens isolated within the US, Argentina and Brazil, dry bean root-rot pathogens from the US and Japan, and mung bean root-rot pathogens from Canada were investigated by conducting a detailed phenotypic comparison together with a multilocus molecular-phylogenetic analysis (13 loci, >24 kb/strain). Detailed phenotypic comparisons of macro- and microscopic features including their colony colors and growth rates on PDA, and phylogenetic analyses of multilocus DNA sequence data indicated that the soybean SDS and Phaseoli root-rot pathogens comprised six morphologically and phylogenetically distinct species. Soybean SDS in North and South America (US, Argentina and Brazil) was found to be caused by four distinct species: *Fusarium virguliforme*, *F. tucumaniae, F.brasiliense* and an undescribed species of *Fusarium*. By way of contrast, dry or mung bean root-rot in North America (US and Canada) and Japan is caused by two closely related species, *F. phaseoli* and *F. cuneirostrum*. The SDS species do not form an exclusive group within the molecular phylogeny, indicating they may not have a monophyletic origin. The multilocus phylogeny further indicates that the single SDS pathogen within the US, *F. virguliforme*, is also present in Argentina and it appears to represent a highly clonal lineage. Artificial inoculation tests on a susceptible variety of soybean, using the isolates from soybeans, dry beans and mung beans, revealed that all six species can induce typical soybean SDS symptoms. These results add to a growing number of molecular phylogenetic studies that indicate the "forma specialis" naming system should be abandoned within *Fusarium* because it obscures the genetic diversity of these pathogens.

PS1-32-0190

Lecanicillium, Simplicillium, Pochonia, and *Verticillium* species isolated from arthropod and soil samples collected in Indonesia

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Lecanicillium, Simplicillium, and *Pochonia* are the genera formerly classified in *Verticillium* sect. Prostrata (Zare et al. 2000, Gams and Zare 2001, Sung et al. 2001, Zare and Gams 2001, Zare et al. 2001). They have been known as parasites of insects, other fungi, or nematode cysts and eggs (Gams and Zare 2001). During our survey of Indonesian filamentous fungi in 2005, we isolated sixteen cultures of the species of these genera from epiphytic soil arthropods, leaf-inhabiting arthropods, and soil samples. These cultures were identified based on their morphology and sequences of the 18S rDNA, the ITS 1 and 2 regions including 5.8S rDNA, and the D1/D2 domains of 28S rDNA. As a result, seven species were recognized, namely *Lecanicillium psalliotae*, *Pochonia chlamydosporia* var. *chlamydosporia*, *Lecanicillium* sp.1, *Simplicillium* sp.1 and sp. 2, and *Verticillium* sp.1. The last four of them were found to be undescribed species, and taxonomic characteristics of these species will therefore be described.

Among these species, *Lecanicillium psalliotae*, *Simplicillium* sp.2, and *Verticillium* sp.1 were isolated from soil arthropods inhabiting epiphytic plants (epiphytic soil arthropods). *Lecanicillium psalliotae* and V. sp.1 were isolated from collembolans collected in the West Java, and S. sp. 2 was isolated from a spider collected in the West Timor. Quite recently, the ecological importance of epiphytic soil has become known because of a huge and unique invertebrate biomass within it (Wardle et al. 2003, Ellwood and Foster 2004). The method with which these fungi were successfully isolated from epiphytic soil arthropods will be introduced.

PS1-33-0191

The family Umbilicariaceae (lichenized Ascomycota) in Russia: systematic, phylogeny and geography

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The aim of this study was to determine the species composition of the family Umbilicariaceae in Russia, and to investigate phylogenetic relationships among Russian species of that lichen family by using morphological, chemical, and ribosomal DNA data.

The work is based on the material collected by the first author as well as herbarium collections in H, H-ACH, KAZ, KPABG, LE, LECB, M, MW, TK, TU, and VLA. About one hundred double-stranded 5.8S/ITS nrDNA sequences were obtained from 41 described species of *Umbilicaria* and 8 of *Lasallia* representing *Umbilicariaceae* of Russia and adjacent area.

The *Umbilicariaceae* are represented in Russia by 33 species of *Umbilicaria* and 5 of *Lasallia*. *U. altaiensis* (Altai Mtns., Caucasus Mtns.), *U. nepalensis* (Altai Mtns.), and *U. dendrophora* (Kola Peninsula) were reported for the first time for Russia. *U. microphylla, U. grisea, U. trabeculata*, and *L. papulosa* need to be excluded from the lichen biota of Russia. The geographical distribution of the species is discussed.

The distribution of different morphological and anatomical characters used in traditional classifications is discussed against the background of ITS nrDNA phylogenetic trees from maximum parsimony and maximum likelihood analyses. To gain a clearer understanding of the morphological delimitations of monophyletic *Lasallia* from the non-monophyletic *Umbilicaria*, the major diagnostically important characters were analyzed and re-evaluated. Despite the considerable number of phenotypic characters examined during the study, only one – the ascospore type – proved to fully separate the two genera. Species of *Lasallia* have big-sized (25 µm and larger), multi-septate, eumuriform ascospores with relatively many septa and more then (40-) 100 cells in the optical section. The *Umbilicaria* species usually have uni-, rarely bicellular or submuriform spores rarely exceed 12 (-20) cells.

Comparison of morphological and molecular data resulted in maintaining 12 groups in the genus Umbilicaria and 4 in *Lasallia*. There are "*U. hyperborea*" (5), "*U. cylindrica*" (5), "*U. decussata*" (2), "*U. aprina*" (4), "*U. thamnodes*" (2), "*U. leiocarpa*" (5), "*U. torrefacta*" (1), "*U. vellea*" (8), "*U. muehlenbergii*" (1), "*U. esculenta*" (3), "*U. deusta*" (1), "*U. polyrrhiza*" (1), "*L. caroliniana*" (1), "*L. pensylvanica*" (1), "*L. pertusa*" (1), "*L. pustulata*" (5) [number of species is in parentheses]. Monophyletic groups as suggested in the study are not in congruency with any of the traditional classifications.

PS1-34-0194

Genetic Variation within ITS1 Region of *Hypoxylon* Species Found in Thailand

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Hypoxylon is a large and complex genus of the family Xylariaceae. The genus has high species diversity especially in tropics and subtropics, and high variation in morphological characteristics. Several species are difficult to identify and some are uncultured. The aim of this study was to investigate genetic variation within the internal transcribed spacer (ITS) region of *Hypoxylon* species found in Thailand. These species were identified according to their morphological and cultural characteristics.
Hypoxylon specimens were collected from different localities in Thailand and they were identified into species based on their morphological and cultural characteristics. The ITS1 and ITS2 regions including 5.8S rRNA gene were amplified and sequenced. The alignment of these sequences was performed. The phylogenetic trees were then constructed.
One hundred and fifteen *Hypoxylon* specimens were collected and identified into nineteen species including four different species, which were likely to be new. Approximately 445 to 906 bp of ITS1-5.8S-ITS2 region were achieved. The alignment of the whole ITS sequences showed the highest variation in ITS1 region ranging from 202 to 588 bp whereas 5.8S region was constant at 155 bp. The ITS2 region ranged from 160 to 170 bp. The extremely long sequences (477 to 588 bp) of the the ITS1 region were found in *H. atroroseum, H. stygium, H.* cf. *stygium* SUT231, *H. urceolatum*, and new species of *Hypoxylon*, which belonged to section Annulata. While the ITS1 sequences of other species in section, H. bovei var. microspora, *H. moriforme, H. nitens, H. purpureonitens*, and *H. leptascum var. macrospora*, and all species in section Hypoxylon found, ranged from 202 to 248 bp. Additionally, the results of phylogenetic tree construction of *Hypoxylon* based on ITS1-5.8S-ITS2 sequences and only ITS2 sequences revealed clearly separation among different species. These phylogenetic trees did not support the separation between sections Annulata and Hypoxylon.
Hypoxylon sp. SUT069, *Hypoxylon* sp. SUT250, *Hypoxylon* sp. SUT182, and *Hypoxylon* sp. SUT085 were likely to be new species found in Thailand, and showed their distinguishing characteristics in each species. The size variation of ITS1 sequences within *Hypoxylon* species examined might be due to the repeated sequences, which affects the reliability of phylogenetic analysis. The nucleotide sequence results could be used to differentiate *Hypoxylon* species, which were difficult to identify by morphological and cultural characteristics, and supported the finding of new species.

PS1-35-0195

A phylogenetic study of *Lecania* and closely related genera

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The crustose lichen genus *Lecania* is estimated to comprise some forty species worldwide which mainly occur on bark, wood, and rocks in temperate areas. Lecania belongs to Lecanorales and has been placed in several different families since the genus was first described in 1853. It is currently assigned to Ramalinaceae. Previous classifications have been based on one or a few morphological features, and the genus has only been represented by 1-2 species in a few molecular studies of the family and the order. Nevertheless, these molecular studies revealed *Lecania* to be a polyphyletic genus. The genus is thus in critical need of revision.
An investigation of the phylogeny of the species so far included in *Lecania* and species in closely related genera has been conducted. The study was based on three genes, the nuclear internal transcribed spacers (ITS), the small subunit of the mitochondrial ribosomal RNA gene (mtSSU), and the first part of the RNA polymerase II subunit (RPB2). Approximately 25 Lecania species and 15 species from other genera were included.
The results of the phylogenetic analyses are presented here. Also in these analyses *Lecania* are clearly polyphyletic, and major modifications of the generic delimitation are suggested.

PS1-36-0201

A new bluing species of *Gymnopilus* (Cortinariaceae, Agaricales) from Mexico

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Thirty-one species of *Gymnopilus* (Cortinariaceae, Agaricales) are known from Mexico. From them, *G. subpurpuratus* and *G. subearlei* stain blue or green when bruised, so it can be expected that they have hallucinogenic properties. Within *Gymnopilus*, psilocybin and baeocystin have been detected only in the non-bluing *G. purpuratus*. Here, a new species, *G. cyanopalmicola,* is described from tropical Mexico. In addition, its phylogenetic relationships, based on ITS sequence data, are presented. This species is of special interest because it is probably hallucinogenic, because it stains blue when bruised. Micromorphology observations were made from sections of the basidioma or gill fragments mounted in 3% KOH, Melzer's reagent, cotton blue and cresyl blue. Measurements of microscopic structures were made in KOH at 100X with a calibrated optical micrometer in a Zeiss K-7 optical microscope. DNA isolation, amplification and sequencing of the nuclear ribosomal ITS were made following Guzmán-Dávalos et al. (2003). *Gymnopilus cyanopalmicola* is distinguished by a large basidiomata, size of basidiospores, which have big warts, the pileus trama, the subhymenium and the basidioma (stipe) changing to blue when bruised. It is closely related to *G. palmicola, G. subearlei* and *G. subpurpuratus*. All these species have reddish-brown to purplish scales on a yellowish background. However, *G. palmicola* has smaller basidioma, its basidiospores have very large tubercles with an obtuse to truncate apex, and its cheilocystidia have obtuse to subcapitate apex. Lastly, *G. palmicola* is not bluing. *Gymnopilus subearlei* and *G. subpurpuratus* have bluing basidiomata, but smaller basidiospores with small to medium warts.

PS1-37-0202

New records of *Gymnopilus* from America and Africa

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The genus *Gymnopilus* has been studied mainly from Europe and North America; there are few studies from South America and less from Africa. Following Singer (1986), *Gymnopilus* belongs to the Cortinariaceae, mainly by its ferruginous spore print, and basidiospores with a compound wall, ornamented with warts. However, some authors have followed Kühner (1980) and considered it in Strophariaceae, by the lignicolous habitat (not mycorrhizic as many Cortinariaceae) and by the presence of styrilpyrones, as in Pholiota. Here six species are recorded, one from Cameroon and six from Colombia.
Micromorphology observations were made from sections of the basidioma or gill fragments mounted in 3% KOH, Melzer's reagent, cotton blue and cresyl blue. Basidiospores shape was determined according to the Q coefficient (length-wide ratio) of at least 20 randomly selected but mature basidiospores. Measurements of structures were made in KOH at 100X with a calibrated optical micrometer in a Zeiss K-7 or in an Olympus BX 50 optical microscopes.
Gymnopilus bryophilus was described from Jamaica and recently was recorded from India. Here, it is cited from other locality in America (Antioquia, Colombia), and from Dja Biosphere Reserve in Cameroon, Africa. It seems that it has a wide tropical distribution.
Besides *G. bryophilus*, other four species are recorded from Colombia: *G. hispidellus, G. luteofolius, G. subpurpuratus* and *G. tomentulosus*. Also, a new locality from *G. lepidotus* is indicated; this species was previously cited from Caquetá region, Colombia. *Gymnopilus hispidellus* was known from Brazil, Cuba and France; the Yanomamo in Brazil considers it as edible. *Gymnopilus luteofolius* has been cited from Argentina, Italy and USA, and *G. subpurpuratus* from Mexico. It is interesting that *G. tomentulosus* was previously only known from Australia.

PS1-38-0203

A taxonomic review of Ganoderma resinaceum (Ganodermatales, Ganodermataceae) complex

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In *Ganoderma*, some mycologist have discussed on the little utility of morphological data in species circumscription. Often times, few characters and species have been systematically studied and for this reason many different species were grouped under the same name. One example is the *G. resinaceum* complex; for this group, Steyaert (1980) considered the size of the basidiospores as the most important feature. So he synonymized *G. argillaceum, G. chaffangeonii, G. sessile, G. polychromum, G. praelongum* and *G. subperforatum* under *G. resinaceum*. This wide species concept was followed by many authors and subsequently other synonyms were included: *G. areolatum, G. nitidum, G. perturbatum, G. pulverulentum, G. sessiliforme, G. subincrustatum, G. subfornicatum, G. subtuberculosum* and *G. triviale*. In this work, ten type materials of the complex were macro and micromorphogically studied using traditional mycological methods. The materials studied were different regarding consistency of the basidiomata, presence of resinous deposit in the context, colour and consistency of the context, basidiospore apex, thickness and disposition of the pillars in the basidiospores, and shape of the cuticle cells. The results show that the colour context is the more visible feature splitting the complex in two groups. The first group includes species with a brown context (*G.* resinaceum, *G. nitidum, G. perturbatum, G. pulverulentum*, and *G. subfornicatum*); however *G. perturbatum* has subacute basidiospores and cuticle cells wider and shorter, *G. pulverulentum* has entire cuticle cells but partially anastomosed pillars, *G. nitidum* and *G. subfornicatum* have diverticulate cuticle cells. In contrast, the second group has species with a pale or duplex context (*G. argillaceum, G. praelongum, G. sessile* and *G. sessiliforme*), but cuticle cells entire as in *G. resinaceum*. Because of its basidiospores, *G. areolatum* does not belong to the genus. We found that there are enough features for the identification of the studied species and for this reason we consider them as independent species from *G. resinaceum*.

PS1-39-0212

A new thermophilic species of Paecilomyces from Xinjiang, China

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The genus *Paecilomyces* Bainier established in 1907, is typified by *Paecilomyces variotii* Bainier. Samson (1974) monographed and redefined the genus, in which he accepted 31 species and grouped the species into two sections: section and section Isarioidea. Section Paecilomyces comprises mesophilic, thermotolerant and thermophilic species, with teleomorphs in the genera *Byssochlamys* Westling, *Talaromyces* Benjamin, and *Thermoascus* Miehe (Stolk & Samson, 1972, Samson, 1974). Section Isarioidea includes mainly mesophilic, entomogenous species. Today, more than 40 species have been accepted in *Paecilomyces*.
Paecilomyces thermophilus sp. nov. isolated from soil samples under decaying tree fibers layer at Tianchi Lake (Heavenly Lake), Tianshan Mountains, Xinjiang Uygur Autonomous Region, China is described and illustrated. This thermophilic species shows mycelial growth between 37^0 and 55^0, with optimum growth at 50^0. No growth occurred at 25^0. P. thermophilus is also characterized by its floccose appearance and the draft-gray colour of the colonies on MEA, the verticillate or irregularly branched conidiophores (56-62^{0}2.2-3.8 µm), the relatively short cylindrical phialides (6-11^{0}0.8-2.4 µm) which are tapering and somewhat bent away from the main axis, and its divergent or tangled conidial chains with conidia ellipsoidal to fusiform, hyaline to yellow brown, smooth at first, becoming spinulose, 3.5-5.2^0 2.2-3.0 µm or 2.5-3.5 µm in diam.
Phylogenetic analysis of the 18S rDNA demonstrated that the new species has affinities with other thermophilic species in the order Eurotiales.
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PS1-40-0213

STIPITATE hydnoid fungi of Southern Australia

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Stipitate hydnaceous Basidiomycetes have gained little attention in Australia. Several species are renowned for their extractable dyes, but little is known of their distribution and ecology. In southern Australia, this informal group includes species of *Auriscalpium, Beenakia, Hydnum, Hydnellum, Phellodon* and *Sarcodon* but generally their taxonomy and nomenclature is either incomplete or uncertain. Recent systematic fungal survey in southwestern Australia has resulted in collections, accompanied with detailed morphological descriptions, which have assisted in better defining the limits of known species. The survey also turned up several taxa that were previously unknown. *Sarcodon* species form conspicuous fruit-bodies, but are rarely collected, and may merit listing on conservation schedules.

PS1-41-0216

Phylogenetic relationships and evolution of cyphelloid homo-basidiomycetes

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The presented results are focussing on the phylogenetic placements and putative relationships of cyphelloid fungi within the homobasidiomycetes.
Besides macrofungi with conspicuous fruiting bodies the morphologically extremely diverse homobasidiomycetes also includes the socalled cyphelloid fungi. Cyphelloid fungi produce minute (typically not exceeding 1 mm in length or diameter), cup, barrel or tube-shaped fruiting bodies with smooth hymenium and the sterile external surface formed by specialized hyphae. Most of their relatively few taxonomically informative characters are derived from spore morphology and anatomy of the surface hyphae. Cyphelloid fungi include roughly 120 species that have been accommodated in ca. 40 widely accepted genera while there is an estimated actual diversity of about 500 species worldwide. Due to their derived morphology the taxonomic placement and the closest relatives of many cyphelloid forms are dubious to date.
So far, sequence data for about 75 cyphelloid collections representing ca. 45 species in 26 anatomically defined genera have been created. They include data from multiple loci (nuc-SSU, nuc-LSU, mt-SSU, mt-lsu, ITS). Phylogenetic analyses using maximum parsimony and Bayesian inferences were performed on most inclusive nuc-LSU data sets. The taxon sampling was focussing on cyphelloid taxa and potentially related *non-cyphelloid* forms that had been identified by preliminary analyses such as BLAST searches.
Consistent with anatomical evidence that suggests putative relationships to various stipitate-pileate and resupinate forms the results indicate that cyphelloid fungi represent a polyphyletic group of taxa that have been derived multiple times from within the euagarics clade. Unconstrained tree topologies indicate that there have been at least 17 independent origins of cyphelloid forms among both *white-spored* and *dark-spored* agarics. Within the homobasidiomycetes the relationships of cyphelloid forms seem to be limited to the euagarics clade according to the current state of knowledge. For many cyphelloid taxa the putatively closest related non-cyphelloid forms could be identified, none of them being ectomycorrhizal.
Whatever the actual number of independent origins, the repeated conver-gent evolution of groups with cyphelloid fruiting bodies suggests that the morphological reduction via "cyphellization" represents a major theme in the evolution of euagarics. Besides, the formation of reduced cyhelloid fruiting bodies may be connected with the transition from terrestrial to marine habitats as is indicated by the Nia clade that, so far, represents the main concentration of cyphelloid *homobasidiomycetes*.

PS1-42-0219

Ribosomal DNA and ITS Sequence Heterogeneity of *Astrocystis* and *Rosellinia* from Thailand

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The genera *Astrocystis* and *Rosellinia* of the family Xylariaceae (Ascomycota), are very similar in their morphological characters. There is controversy over the status of both genera. *Astrocystis* could be *Rosellinia*-like fungi. In this study, the 18S ribosomal DNA and nuclear ribosomal internal transcribed spacer (ITS) sequence heterogeneity as well as morphological and cultural characteristics of *Astrocystis* and *Rosellinia* found in Thailand, were investigated to support their taxonomic study.
Astrocystis and *Rosellinia* specimens collected from forest areas in Thailand, were classified by their host specificity and morphological characters, then identified into species using their morphological and cultural characteristics. The 18S rDNA and ITS1-5.8S-ITS2 regions were amplified, sequenced, and analyzed. The comparison of their nucleotide sequences to sequences available from GenBank database was performed.
Specimens of *Astrocystis* and *Rosellinia* were collected and classified. *Astrocystis* has been separated from *Rosellinia* on the basis of host specificity on bamboo and by features of the stromata splitting the host surface or containing a carbonaceous extension at the base. From nine specimens collected, they could be identified by their morphological and cultural characteristics as belonging to three species, *Astrocystis mirabilis, Rosellinia procera*, and *Rosellinia* sp. ST2301. From the nucleotide sequence analysis, approximately 2,000-2,200 bp and 500 bp of 18S rDNA and ITS1-5.8S-ITS2 fragments were achieved respectively. The alignment of 18S rDNA sequences between genera *Astrocystis* and *Rosellinia*, which obtained from this study and from GenBank database, indicated 68-70% identity. Both genera showed the most conserved region of about 1,000 bp at the 5'-end of 18S rDNA, whilst the rest of the sequences, especially the middle of sequences, was highly variable among genera as well as species. For the comparison of ITS1-5.8S-ITS2 sequences of both genera, the high variation in ITS1 region was found. In addition, the phylogenetic tree analysis result showed that *Astrocystis* was separated from *Rosellinia*.
The nucleotide sequences from both 18S rDNA and ITS regions demonstrated the dissimilarity between genera *Astrocystis* and *Rosellinia*, and could be used to support their taxonomic study. This is in agreement with the concept that *Astrocystis* can not be *Rosellinia*-like fungi.

PS1-43-0220

Barcoding the genus *Phoma*: The next phase for *Phoma* taxonomic research in The Netherlands

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The publication of the *Phoma* Identification Manual in 2004 represents a comprehensive work spanning 40 years of taxonomic research on the anamorphic genus *Phoma* in the Netherlands. A total of 223 *Phoma* taxa, including over 1100 synonyms in various coelomycetous genera, have been described in nine sections based on morphological characters. Where teleomorphs are known, they reside in genera such as *Didymella, Leptosphaeria, Mycosphaerella* and *Pleospora*. The current morphological identification system for Phoma species by study of isolates in pure culture is very laborious and requires a high level of expertise.
Presently all studied *Phoma* reference strains in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) and the Dutch Plant Protection Service (PD) represent approximately 1100 isolates that are available for DNA phylogenetic studies. It is hoped that molecular data may answer questions relating to the delimitation of the Phoma sections, and differences with related genera like *Pyrenochaeta*, *Deuterophoma* and *Ascochyta*. Specific species complexes, such as the *Phoma exigua* complex, will be studied in more detail. Furthermore, molecular data will lead to a better understanding of anamorph - teleomorph relations within the genus. The PD initiated a four years *Phoma* study as part of the CBS barcoding project. All *Phoma* strains will be typified by sequence data of potentially informative loci such as ITS, tubulin, actin, and EF 1-alpha. The isolates represent all known important plant pathogenic *Phoma* species, as well as ubiquitous saprophytes. A *Phoma* barcoding system will be developed for identification purposes. The obtained data will generate the development of new, fast molecular methods for detection of important (quarantine) plant pathogens. Most of the isolates studied thus far originate from Europe. For a worldwide approach of *Phoma* taxonomy, isolates from other continents are, however, urgently required.

PS1-44-0228

Some New Species of *Amanita, Boletus* and *Cantharellus* (Agaricales) from Korea

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Many higher fungi were collected from at Korea from 2002 to 2005. They were identified. As the result, one *Amanita*, three *Boletus* and one *Cantharellus* are new to the world. Among them, *Amanita lateritius-farinosa* D.H.Cho is pileus deep reddish farinaceous based on white, edge furrow, annulus white, volva none. *Boletus tabicinus* D.H.Cho is pileus light yellow, stipe yellowish rough furrow-net, none exchanged color when burised. *B. alboporus* D.H.Cho is pileus darkish, rugolose, tubepores white. *B. chlorinus* D.H.Cho is pileus mixed green and yellowish, tubepores and stipe yellowish. *Cantharellus minor* f. *pallid* D.H Cho is pallid color with white at edge.
Key words : *New Species, Amanita lateritius-farinacea, Boletus tabicinus, B. alboporus, B. chlorinus, Cantharellus minor f. pallid*

PS1-45-0237

Phylogenetic relationships of the *Polyporus* sensu lato and allied genera.

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The genus *Polyporus* contains morphologically various species, and is often divided into six infrageneric groups based on morphological characters: *Polyporus, Polyporellus, Favolus, Melanopus, Admirabilis*, and *Dendropolyporus*. But morphological characters of polypores do not always represent phylogenetic relationships. The aim of the present study is to reveal the phylogenetic relationships within the genus *Polyporus* and between *Polyporus* and allied genera. We generated nLSUrDNA, atp6 and rpb2 sequences using direct sequence method from mainly Asian isolates of the Polyporus spp. and allied species. Phylogenetic analyses based on the combined dataset of nLSUrDNA and rpb2 showed that all the species showing the dimitic hyphal system with skeletal-binding hyphae were in a single clade and distinct from those with typical trimitic hyphal system. Within the clade of dimitic species, Group *Polyporellus*, Group *Melanopus* excepted for *P. varius* and tropical components of Group *Favolus* were monophyletic, respectively. On the other hand, two of the highly supported clades contained some *Polyporus* spp. and species of other genera. One clade included Group *Polyporellus* and *Lentinus* spp., another clade included two species of Group *Polyporus*, *P. varius* of Group *Melanopus*, *Pseudofavolus cucullatus* and *Datronia* spp. We suggest some morphological groups partly represent phylogenetic groups but the genus *Polyporus* in the present concept is not monophyletic.

PS1-46-0255

Oidiodendron species isolated from the roots of *Rhododendron* spp. growing the region near the tropic of Cancer in Taiwan

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Several species of *Oidiodendron* colonize on/in the roots of ericaceous plants as saprotrophic or mutualistic fungi. Recently, taxonomical or physiological studies of the genus have been extensively conducted and published. However, our information on the species distribution on a global scale is still insufficient, especially very fragment in subtropical and tropical regions. *Oidiodendron* spp. have been recorded very often from the roots of ericaceous plants in temperate regions and appear to have an affinity for the substrate. Currently, we have studied the distribution of *Oidiodendron* inhabiting ericaceous roots in Japan and confirmed that *Oidiodendron* species are distributed throughout Japan. The fungi were also recorded from Iriomote island, a southernmost islands of Japan where the subtropical climate dominates. Consequently, we also expect the distribution of *Oidiodendron* in Taiwan located in the west of Iriomote island, though *Oidiodendron* have not been recorded yet. We selected the roots of native *Rhododendron* spp. (Ericaceae) as the substrate with a high possibility that we can discover the fungi.
The roots of *Rhododendron* species were sampled in Yanminshan (25°18' N, 121°54 E, about 750m above sea level) located in northern Taiwan and Hui-san forest (24°09'N, 121°03' E, about 720-890m above sea level) located in central Taiwan in November 2005. We selected five trees (Yanminshan: *Rhododendron* sp., Hui-san forest: *R. lasiostylum*) on each site. The lateral roots were collected from the surface part of soil under each tree. The roots were washed serially, dried, and roughly divided into two diameter classes (class 1: < 0.25mm, class 2: 0.25-0.5mm). 40-60 segments (about 5mm long) were cut off from the class 1 roots and 60 segments from the class 2 at each tree. Segments were plated out on the corn meal agar and incubated at 15°C for about one month in the dark and observed with a microscope at proper intervals.
Oidiodendron spp. occurred at both sites. The frequencies of occurrence per site were 0% (class1) and 20% (class2) in Yanminshan. Those were 100% (class1) and 80% (class2) in Hui-san. The most common species was *O. mius* that is known as a common species to form mycorrhizas in roots of ericaceous plant in temperate regions. Our results show that *Oidiodendron* species are distributed more widely in Taiwan than we think, and probably the existence of ericaceous plants is one of the most important factors supporting the distribution of *Oidiodendron* in this subtropical region.

PS1-47-0258

Molecular and morphological description of *Pestalotiopsis hainanensis* sp. nov., a new endophyte from tropical region in China

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Pestalotiopsis is an important group of endophytic fungi. Approximately 220 species of *Pestalotiopsis* were described (CABI Bioscience database, 2005), among them at least 46 *Pestalotiopsis* species have been reported as endophytes, some of which produce secondary metabolites with a great potential for anti-microbial and anti-tumor medicinal application. The traditional taxonomy of this genus was based mostly on morphology of conidia (Guba, 1961; Sutton, 1980; Nag Rag, 1993) and affinities of *Pestalotiopsis* species have been confused and equivocal. During a survey of the diversity of species in the tropical region of China, a new endophytic fungus *Pestalotiopsis* hainanensis was isolated from the stem of *Podocarpus macrophyllus* at Xinglong Tropical Botanical Garden of Hainan Province which is morphologically distinguished from similar species *P. karstenii* in unbranched and short apical appendages, and from *P. heteroconis* in absence of basal appendages. Phylogenetic analysis based on ITS region (ITS1, 5.8S, ITS2) and beta-tubulin 2 gene (tub2) indicated that for 5.8S gene and ITS sequence *P. hainanensis* shared similarity of 97.4% with three P. karstenii strains and 96.1%-96.6% with three *P. heterocornis* strains; for tub2 gene sequence the new species shared similarities of 92.3%-93.8% with three *P. karstenii* strains and 94.1% with three *P. heterocornis* strains. Molecular results support that *P.hainanensis* is a new species which is distinguished from *P. karstenii, P. heterocornis* and other *Pestalotiopsis* species. It is suggested that when a new taxon of *Pestalotiopsis* is described, both of morphological characters and molecular phylogenetic information are necessary to prove the taxon unique from other known species.

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PS1-48-0259

Taxonomic studies on species of *Russula* in Taiwan

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Russula (Russulaceae) is one of the major mycorrhizal macrofungal genera that occur in broad-leaved, mixed and conifer forests in Taiwan. A taxonomic study of *Russula* (Russulaceae) has been undertaken in Taiwan during 2000-2002, based on specimens collected at 500-2,500 m altitude. Macromorphological characters were recorded from fresh condition of the specimens. Micromorphological characters of cuticle and lamellar were microscopically observed in a medium of 3% KOH. Spores size and shape are from optical sections in side view in Melzer's regent and exclude the ornamentation. A total number of 17 species were obtained from this study. They are: Subgenus Compactae: *Russula nigricans, R. delica. Subgenus Russula: R. cyanoxantha, R. virescens, R. amoena, R. violeipes, R. alboareolata* (Sect. Heterophyllae), *R. castanopsidis* (Sect. Pachycystides), *R. foetens, R. subfoetens, R. laurocerasi, R. senecis* (Sect. Ingratae), *R. emetica, R. betularum, R. sanguinea, R. fragilis* (Sect. Piperinae), *R. xempelina* (Sect. Polychromae). *R. violeipes, R. subfoetens, R. betularum* and *R. xempelina* are reported for the first time in Taiwan. *R. alboareolata, R. castanopsidis* and *R. senecis* are E Asia species, only distributed in Japan, Korea, China and Taiwan.

PS1-49-0260

Molecular phylogeny of *ophiostoma* spp. associated with *Protea* species

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Species of *Ophiostoma* and their asexual states include some of the world's best known tree pathogens, many of which are also economically important agents of sap stain in lumber. Most of these fungi are known from the Northern Hemisphere where the majority exist in a symbiotic relationship with bark beetles that infest trees, especially conifers. Intriguingly, a guild of species of these fungi also occurs in the flower heads (infructescences) of serotinous members of the African endemic plant *genus Protea*. Recent molecular phylogenetic studies on *Ophiostoma* s.l. have suggested that the three *Ophiostoma* spp. (*O. africanum, O. protearum and O. splendens*) specifically found in *Protea* infructescences, form a strongly supported monophyletic lineage within *Ophiostoma* s.s.

As part of an effort to better understand the association of *Ophiostoma* spp. within *Protea* infructescences, we have conducted new surveys including areas and species of *Protea* not previously considered. The *Ophiostoma* spp. residing in the infructescenses are all morphologically similar, thus DNA sequence comparisons provide an important tool to confirm their identity. At the time of their first discovery some three decades ago, this research tool was not available and cryptic species were most likely overlooked.

New collections of *Ophiostoma* spp. from *Protea* infructescences were subjected to molecular phylogenetic reconstructions based on large subunit, ITS and beta-tubulin sequence data. These revealed the presence of at least five undescribed species of *Ophiostoma* associated with these plants. Intriguingly, our results also suggest a polyphyletic origin for the *Protea*-associated *Ophiostoma* spp. This indicates multiple invasions of this unusual niche, by these fungi. Subsequent speciation events appear to have taken place in at least two lineages. Interestingly, our results also revealed the first case of an *Ophiostoma* sp. jumping hosts between a native *Protea* sp. and the non-native tree genus *Eucalyptus*.

PS1-50-0262

Basidiomycetes on palms and bamboo in Thailand, with special reference to the phylogeny of *Ganoderma colossus (Tomophagus)* and *G. tsunodae (Trachyderma)*

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A wide range of basidiomycetes are known as parasites and saprophytes of palms (119) and bamboo (110) but their occurrence in Thailand has not been documented in any detail. Rostrup (1902) was the first to describe the larger basidiomycetes of Thailand, with subsequent studies by Heim (1962), Hjortstam and Ryvarden (1982), and Bandoni (1998). More recent studies are reviewed by Desjardin et al. (2004), who estimate that some 300 species are now known for the principality which probably account for only 20% if its estimated basidiomycetes diversity. Our study of basidiomycetes on senescent palm and bamboo has yielded 31 and 40 on bamboo and palms, respectively. During our study *Ganoderma colossus* was collected on coconut palm in Morib mangrove, Malaysia. In culture this fungus produced an abundance of anamorphic spores, golden brown in colour and verrucose. Genomic DNA was isolated from the basidioma and the anamorphic culture and partial nuclear ITS region and mitochondrial SSU amplified and sequenced Nucleotide Blast search and phylogenetic analysis of both nuclear ITS and mitochondrial SSU revealed that the anamorphic culture observed is *G. colossus*. These sequences, together with *G. colossus* sequences in the GenBank, formed a well-supported group and are always basal to other *Ganoderma* species. Its relationship with *G. tsundae* is also under investigation. Both species are known to occur on woody dicotyledons, but not on palms (Moncalvo, 2000). Moncalvo (2000) showed that these two species nestle together in a separate clade to Ganoderma species with 100% bootstrap support. Our study supports the view that G. colossus is not monophyletic with other *Ganoderma* species. Therefore consideration should be given to the generic name Tomophagus for *G. colssus.*

PS1-51-0263

A new *Ceratocystis* sp. from *Phoracantha acanthocera* tunnels on *Eucalyptus* in Australia

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Ceratocystis spp. include some of the most important tree pathogens and many are species of quarantine significance. This provides strong justification to collect and identify these fungi in new environments. In this study, we consider the identity of a *Ceratocystis* sp. isolated from the tunnels of the serious *Eucalyptus* pest, *Phoracantha acanthocera* in Australia. The fungus was commonly found in tunnels of this insect in plantations west of Cairns, which has a tropical climate. The *Ceratocystis* sp. found in this niche was morphologically similar to *C. fimbriata*, an important tree pathogen that is not known to occur on *Eucalyptus* in Australia. The fungus was different to *C. fimbriata* in having a much darker mycelial growth, longer ascomatal necks shorter hat-shaped ascospores and no aleuroconidia. The species from Australia also has doliform (barrel-shaped) conidia while these are absent in *C. fimbriata*. Comparisons of combined sequence data for three gene regions (Internal Transcriber Spacer region 1 and 2 including the 5.8S rDNA, the, tubulin and Transcription Elongation Factor 1) confirmed that the Ceratocystis sp. from P. acanthocera represents a new taxon. The species has been provided with the provisional name Ceratocystis atrox prov. nom. It appears to have a close relationship with *P. acanthocera*, although its role in the biology of the insect is unknown and its potential pathogenicity has not been considered. Further surveys are planned to determine the distribution and biology of *C. atrox* prov. nom.

PS1-52-0269

A New *Leptographium* Species Associated With The Pine Root Collar Weevil *Hylobitelus chenkupdorjii* On Pinus *wallichiana* In Bhutan

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Leptographium spp. are commonly associated with bark beetles and weevils (Coleoptera: Curculionidae), and some are important plant pathogens. In the North America, *Leptographium procerum* is closely associated with various root infesting insects including the seriously damaging pine root collar weevil *Hylobius radicis*. In, Europe, *L. procerum* is more loosely associated with the large pine weevil, *H. abietis*, which is an important pest in conifer afforestations. In a recent survey of tree diseases and pests of conifers in Bhutan, a similar root collar weevil, *Hylobitelus chenkupdorjii* was found girdling young Himalayan blue pine trees, *Pinus wallichiana*, near Dhur in the administrative district Bumthang in Central Bhutan. Intensive blue-stain and a *Leptographium* sp. were associated with damage by this insect. The fungus was also consistently isolated from individuals of *H. chenkupdorjii*. Fungal isolates were initially identified based on morphology and they were thereafter compared using the combined DNA sequences of the ITS2 and part of the large subunit (28S gene) of the rDNA operon, partial ,tubulin gene, and partial elongation factor 1-a gene. Morphological characteristics showed that the fungus was similar to *L. procerum*, but it could be distinguished from this species. DNA sequence comparisons revealed that the isolates from Bhutan formed a distinct clade, with a bootstrap value of 100 %, and confirmed that they represent an undescribed taxon. This fungus has thus been provided with the tentative name *Leptographium bhutanense* nom. prov. It is clearly very closely associated with Hylobitelus chenkupdorjii but unknown whether it contributes to killing of *P. wallichiana* saplings in Bhutan.

PS1-53-0283

Molecular phylogeny of European *Trametes* (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences

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Phylogeny of all European and one American species of the genus *Trametes* in relation to the genera *Coriolopsis*, *Lenzites* and *Pycnoporus* was studied using the sequence of LSU and ITS regions of nuclear ribosomal DNA. Datasets of LSU and ITS were analyzed using the Fitch-Margoliash method with maximum likelihood distances, maximum parsimony, maximum likelihood and Bayesian method. All *Trametes* species except for *Trametes cervina* formed a clade, whereas *T. cervina* suprisingly grouped with *Ceriporiopsis aneirina*. In the genus *Lenzites, L. betulina* is more closely related to Trametes gibbosa than to *L. warnierii*. The earlier published synonym Lenzites gibbosa (Pers.) Hemmi was therefore adopted for *Trametes gibbosa*. Our study confirmed the monophyly of the genus Pycnoporus inside the paraphyletic *Trametes* clade. The genus *Coriolopsis*, sometimes considered as belonging to *Trametes*, was clearly delimited in a different clade. The work was supported by Grant agency of the Czech Republic, project no. 526/06/P017, and by Ministry of Education, Youth and Sports, project no. MSM 6215648902.

PS1-54-0286

A recently emerging green mold disease in Eurasian oyster mushroom farms is caused by new species of *Trichoderma*

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The mushroom green mold disease was first reported by champignon (Agaricus bisporus) cultivating farms in nineties. It has got wide spread in North America and Europe. The causative agent of the disease was recognized to be a new species *T. aggressivum* (*f. aggressivum* and *f. europeaum*, respectively), which interestingly has been never isolated from environmental soil samples. In recent years, a green mold disease was also detected in oyster mushroom (*Pleurotus ostreatus*) producing industry in, Italy, Hungary and Korea. Here we will provide evidence that the infection of *P. ostreatus* is caused by new *Trichoderma* species which is closely related to *T. aggressivum* and together with the later one belongs to the large "Harzianum Clade" of *Hypocrea/Trichoderma*. In order to recognize these fungi we have used an integrated phylogenetic and phenetic approach: the genetic uniqueness based on the concordance of multiple gDNA loci was powered by the distinctive carbon source utilization profile (Phenotype MicroArray analysis), wide geographic distribution and the specific ecological adaptation. The DNA oligonucleotide barcode for quick molecular identification on the species level has been also developed. In contrast to *T. aggressivum*, the species causing *Pleurotus* green mold is often isolated from soils, and seems to be widely distributed throughout the Northern hemisphere (North America, Europe, Iran, India, China). A formal taxonomic description will be presented.

PS1-55-0287

Chorioactidaceae: A New Family of Pezizales

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Previous molecular phylogenetic studies in the *Pezizales* suggested that several genera, alternatively placed in either the families Sarcoscyphaceae or Sarcosomataceae, formed a distinct group related to those two families. Using molecular phylogenetic and morphological methods we have studied these genera: *Chorioactis, Wolfina, Desmazierella* and *Neournula*. Most taxa in these genera are monotypic or have only two described species. The species have broadly disjunct distributions in temperate regions of the northern hemisphere. The disjunct distributional pattern has been investigated only in *Chorioactis geaster*, which is found in North America in the central Texas area and Japan. Species of *Desmazierella* form small apothecia but the other genera form large apothecia with brown hairs covering the receptacle and light-colored hymenia. Ascospores have cyanophylic ornamentations. These taxa are not often collected because of their limited distributions worldwide and have not often been studied in fresh condition.
Parsimony, likelihood, and bayseian methods were used to analyze SSU, LSU and RPB2 sequences. Light microscopic study of hymenial and excipular structures were employed for morphological study.
The results of our molecular analyses indicate that these taxa consistently form a monophyletic group with moderate supported. The group is resolved either as sister to the Sarcosomataceae or sister to a Sarcosomataceae/ Sarcoscyphaceae group. Distinctive, brown, ornamented hairs, cyanophylic markings and cytological characters of the paraphyses and spores unite the taxa in this newly described family.
This study is of significance in understanding the relationships among the groups of the so-called C-lineage in the *Pezizales*. This is one of the largest and least well studied of the lineages and in the case of the families under study here represent early divergences within the lineage. This group offers several opportunities to address biogeographic questions.

PS1-56-0289

TrichoMASTER: integrated multiloci database for *Hypocrea/Trichoderma* species identification powered by sequence diagnosis, oligonucleotide barcode and similarity search tools

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Hypocrea/Trichoderma is a genus of soil-borne or wood-decaying fungi, which contains members that are important to humankind. Today the identification of *Hypocrea/Trichoderma* chiefly relies on the use of DNA sequence data. The most common approach for molecular identification is that by local alignment comparison. A popular tool for this task is BLAST. Unfortunately, results from such large public databases like GenBank, which already contains about one million of only fungal nucleotide sequences, can be flawed for two main reasons: (i) most public databases are riddled with misidentified sequences (~40% for *Hypocrea/Trichoderma* in GenBank), and (ii) not all public databases fully cover the sequences and species of a given genus.
In order to eliminate the latter two difficulties with respect to *Hypocrea/Trichoderma* and to provide a possibility for the correct identification of *Hypocrea/Trichoderma* sequences we have developed TrichoMASTER: a publicly available tool for sequences diagnosis supported by (i) multiloci database of phylogenetic markers, (ii) DNA barcode and (iii) the similarity search tools. TrichoMASTER covers all genetically characterized species of the genus and contains almost complete sets of 5 most frequently used phylogenetic markers: the internal transcribed spacers 1 and 2 – ITS1 and 2; two introns (tef1_int4(large), tef1_int5(short)) and one exon tef1_exon6(large) of the gene encoding translation elongation factor 1-alpha and, a portion of the exon between the 5th and 7th eukaryotic conserved amino acid motives of subunit 2 of the RNA polymerase gene(rpb2_exon). TrichoMASTER integrates our previously developed tools such as TrichOKEY (ITS1 and 2 oligonucleotide barcode), TrichoBLAST (sequence similarity search tool) and TrichoMARK, a script specifically written for detection and retrieval of phylogenetic markers in query sequences, and for the subsequent individual submission of them to the similarity search. All tools are located on the website of the International Subcommission of taxonomy of *Trichoderma* and *Hypocrea* (ISTH; www.isth.info) of the International Commission of Fungal Taxonomy (ICFT). For uncertain cases (for instance, detection of new species) when the final identification may be achieved only based on phylogenetic analysis, we have finally developed an additional module of TrichoMASTER, i.e. a publicly available Multiloci Database of Phylogenetic Markers, a relational sequence database built on a MySQL platform. It is accessed through a web interface system written in PHP scripting languages. This also database serves as a reference source for the similarity search and allows retrieving any custom set of sequences in FASTA file format for phylogenetic analysis. A search option by any keyword is included to speed up the access to sequences from known strains, species or GenBank accession numbers.

PS1-57-0299

Secondary metabolite profiles and their usefulness in the taxonomy of *Ganoderma*

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Although there are many papers using profiles of secondary metabolites in the taxonomy of few groups of mitosporic fungi and Ascomycetes, there are much less in Basidiomycetes. Commonly, culture or recent collected specimens are used, but frequently they are difficult to obtain. In *Ganoderma* many metabolites of medicinal importance have been identified; however, they have not been used in taxonomy. The objective of this study is to evaluate the stability of the secondary metabolites profiles in *Ganoderma herbarium* specimens to be used in taxonomic work. About 70 specimens of *Ganoderma*, from seven species (*G. applanatum, G. colossus, G. curtisii, G. oerstedii, G. oregonense, G. tsugae, G. weberianum*) and two very close related species were included. Basidiomata of ancient and recently collected material, with different maturity states were selected. Approximately 0.5 to 5g of sample was macerated sequentially, two days in methanol and seven days in dichloromethane, filtrated and concentrated by vacuum rotatory. Samples in TLC were visualized in UV at 254 and 365 nm. GC was performed on a Chrompack CP9000 chromatograph with a SID detector. The analysis conditions were injection 265°C, detector 280°C, initial temperature 100°C, programming from 100 to 200°C at 7.5°C for min, in the polar column AT-WAS 30 m x 0.32 mm x 0.25 µm. The methanolic and dichloromethane extracts visualized at 365 nm showed at least 20 different compounds, two of which were presented at least in 95% of the samples for methanol (Rf = 0.96, Rf = 0.62) and dichloromethane (Rf = 0.97, Rf = 0.47). Green, red and purple spots were observed at 365 nm. In GC, two peaks with retention time 18.4 and 19.3 min were observed in the majority of the samples, and specific patterns were found for the species. In general, samples of different specimens from the same species were consistent. On the other hand, different species present distinctive patterns. We concluded that although some specimens are more than forty years old, many secondary metabolites remain stable and that through careful study it would be possible to use them in taxonomic and phylogenetic studies.

PS1-58-0302

A New Record of *Emericella* From A Mexican Coral Reef

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During a survey of tropical Ascomycetes from Mexico, an interesting fungus was isolated from *Pacifigorgia rutila* collected from the Isla Larga coral reef located in Banderas Bay on the Pacific Ocean seacoast. The fungus was isolated from a small square portion of the surface disinfected colony on artificial sea water corn meal agar. The fungus produces abundant globose cleistothecia surrounded by numerous Hülle cells and the typical ornamented, red, unicelled ascospores; in addition, an *Aspergillus* anamorph was observed on Czapek agar. These characteristics place the fungus in the ascomycetes, in the order Eurotiales. Comparison with published species descriptions indicate that it belongs to the genus *Emericella*. Based on morphological characters and phylogenetic analysis of 18S rDNA sequences, this fungus is close to *Emericella* variecolor and therefore, we are reporting it as new record form Mexico.The species placement, however, is complicated by the high genetic diversity of *Aspergillus*, which is discussed in the paper.

PS1-59-0313

A New Record Of *Aniptodera* From A Freshwater Habitat Of Mexico City

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An interesting fungal specimen was isolated from the Xochimilco ecological zone, located in the metropolitan region of Mexico City. The fungus was growing on a submerged wood panel bait (*Pinus* sp.) that was incubated for 1 mo in the laboratory in transparent plastic boxes containing moist paper towels. It has subglobose, superficial or semi-immersed, dark, long-necked ascomata; 8-spored, clavate, persistent asci with the plasmalemma behind the apical region retracted; and ellipsoid, hyaline, thin-walled, one-septate ascospores without bipolar appendages. The characteristics of the Mexican fungus agree with the description of *Aniptodera* (Halosphaeriales). Comparison with published species descriptions indicate that it is close to *Aniptodera lignicola*.

PS1-60-0314

Molecular characterization of Beauveria species from Thai forests

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The genus *Beauveria* is characterized by its basally inflated conidiogenous cells from which conidia are sympodially produced on a slender rachis that increases in length with age. The spores are usually white in color and small, produced from clustered conidiophores that aggregate in spore balls when seen under the microscope. About eight species are described and *Beauveria bassiana* is the most well-known and probably the most important species in terms of insect biocontrol. Species recognition could be difficult at times due to morphological plasticity. Species collected with globose conidia were always identified as *Beauveria bassiana*. It is worldwide in its distribution and has a broad range of insect hosts, suggesting the possibility of cryptic species. Significantly, there are *Cordyceps* species that produce a *Beauveria* state in culture and allow us to establish anamorph-teleomorph connections. To analyze the genetic diversity of the Thai strains and their connections to the teleomorphs we collected we sequenced the ITS rDNA region of 50 *Beauveria* spp. Analysis of ITS rDNA regions have shown that several phylogenetic species can be recognized from our *Beauveria collections* and what we call B. *bassiana* is a complex of several species.

PS1-61-0318

The Genus *Coemansia* from Taiwan

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Fungi of the genus *Coemansia* (Kickxellaceae, Kickxellales, Zygomycetes) are inhabitants of soil or dung of herbivores and rodents. *Coemansia* are frequently overlooked since they grow slowly and possess small, delicate, light colored sporangiophores, they tend to be overgrown or covered by other fungi that thrive in the sample. Also the pure cultures of *Coemansia* are difficult to be established and maintained in laboratory. Information on *Coemansia* in Taiwan is scarce. Only very few new *Coemansia* have been described from Taiwan so far. During an investigation of Taiwan Zygomycetes, we have got 9 isolates of *Coemansia* which were studied by light microscopy and scanning electron microscopy and were identified as *Coemansia .erecta, C. furcata, C. kamerunensis, C. interrupta, C. spiralis, C. sp1* and *C*. sp2. The differences between these species are presented and discussed.

PS1-62-0333

Phylogenetic relationships among Asian and Australasian ectomycorrhizal ammonia fungi in Hebeloma subgenus *Porphyrospora*

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The identification of fungal species belonging to *Hebeloma* subgenus *Porphyrospora* is controversial due to only subtle morphological differences between taxa. Occurrences of ectomycorrhizal ammonia fungi belonging to *Hebeloma* subgenus *Porphyrospora* have been recorded from Japan, China, New Zealand, and Australia. To elucidate phylogenetic relationships among Hebeloma spp. belonging to Porphyrospora, i.e., Hebeloma sp.1 and Hebeloma sp.2 from New Zealand, H. *aminophilum* from Australia, and H. *vinosophyllum* from Japan, we evaluated sequences of both nuclear ribosomal ITS and beta-tubulin genes (long and short sequences). The aligned sequences were analyzed by neighbor-joining and maximum parsimony methods. The phylogenetic trees resolved from three kinds of sequences indicate that specimens examined can be segregated into two groups, one comprising Hebeloma sp.1 from New Zealand and *H. aminophilum*, and another containing *H. vinosophyllum*. To clarify the taxonomic rank of the two groups, di-mon mating tests were undertaken between monokaryotic tester strains of H. *vinosophyllum* and dikaryotic strains of Hebeloma sp.1 from New Zealand and of H. *aminophilum*. We were unable to resolve phylogenetic relationships between *Hebeloma* sp.2 and the above two Hebeloma groups due to few available specimens of sp.2 from urea-treated forests in New Zealand.

PS1-63-0335

Evolution of cyanobacterial symbiosis in Ascomycota.

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About two-fifths of the ascomycete species are lichenized. Of the lichenized species about 10% contain a cyanobacterium as their primary photobiont, while in about 3% of the species cyanobacteria are included as a secondary photobiont. We have investigated the evolution of cyanobacterial symbiosis in Ascomycota. Phylogenetic relationships within the Ascomycota are reconstructed in order to infer the patterns of gains and losses of symbiotic state. The phylogenetic relationships were assessed by using direct optimization of DNA sequences from the genes encoding for ribosomal RNA small and large subunits (SSU and LSU). Our results indicate that the symbiosis between ascomycetes and cyanobacteria has evolved repeatedly, but also that the gain of cyanobacterial photobiont has subsequently been followed by the gain of a green algal photobiont.

PS1-64-0338

Chrysoporthe species on *Myrtales* in Southern Africa

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Chrysoporthe cubensis and Chr. austroafricana, previously collectively known as *Cryphonectria cubensis*, are serious canker pathogens of *Eucalyptus* trees grown in plantations, world-wide. These pathogens have in recent years also been reported from a number of other trees in the *Myrtales*, including genera such as *Syzygium* and *Tibouchina*. In South Africa, only Chr. *austroafricana* is known, where it causes a serious canker disease and death of E. *grandis* and other susceptible *Eucalyptus* hybrids and species. In this country, it also causes branch death on ornamental *Tibouchina* spp. and it has been collected from native *S. cordatum* and *S. guineense* trees. This has led to the view that *Chr. austroafricana* is native to Southern Africa, but population genetic data to support this view are incomplete. In contrast to the situation with *Chr. austroafricana*, it has been hypothesized that *Chr. cubensis* is native to either South America or Asia. Recent surveys of *Eucalyptus* and *Syzygium* spp. in southern and eastern Africa have yielded collections of Chr. *austroafricana* from Mozambique, Malawi and Zambia for the first time. In these countries the fungus occurred on both non-native E. grandis and native S. cordatum. These surveys also showed that Chr. cubensis occurs on *Eucalyptus* trees in Kenya, Malawi and Mozambique. Subsequent surveys in 2005 and 2006 have shown that *Chr. austroafricana* also infects *Corymbia henryi*, a species being tested for commercial deployment in southern Africa, and S. *apiculatum*, an Australian tree commonly grown as ornamental in South Africa. Infection on the *S. apiculatum* trees resulted in branch and tip die-back, clearly showing that the Australian trees are highly susceptible to this pathogen. Furthermore, extensive die-back and main-stem cankers were found associated with *Chr. austroafricana* on S. *cordatum* in South Africa. It is clear from the results of these surveys that *Chrysoporthe* spp. are much more widely spread in Africa than previously believed. They also appear to pose a serious threat to native African and Australian Myrtales.

PS1-65-0339

Sporendocladia bactrospora associated with wounds of Norwegian broad-leaved trees: A potential pathogen?

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Sporendocladia bactrospora is an ascomycete anamorph, previously known as *Phialocephala bactrospora*, and considered part of the *Leptographium* complex. The fungus produces dark mononematous conidiophores, terminating in phialides, from which conidia develop in false chains via ring wall building development. *Sporendocladia bactrospora* is known to occur in Europe, Japan and Canada, although reports have been sporadic and almost nothing is known regarding its ecology. Recent phylogenetic studies have shown that *S. bactrospora* resides in the Microascales, although no teleomorph association has been determined for it. In this study, wounds were made on various broad-leaved trees in Norway, specifically to identify *Ceratocystis* and *Ophiostoma* spp. that might infect these wounds. Some logs in a loading depot in southern Sweden were also included in the sample set. Isolations from the cambium of the wounded trees commonly yielded a fungus resembling a Leptographium sp. The same fungus was also commonly isolated from stumps of harvested trees as well as log-ends in sawmills and loading depots. Morphological observations and DNA sequence comparisons for ITS regions of the rDNA region showed that the fungus was *S. bactrospora*. Artificial inoculation studies on young *Populus tremulae* and *Betula pubescens* trees showed that *S. bactrospora* is capable of causing lesions and wood-stain on inoculated trees. To the best of our knowledge, *S. bactrospora* has not previously been recognised as an inhabitant of freshly made wounds on trees. In Norway it appears to be common in this niche and as such it behaves very similarly to *Ceratocystis* and *Ophiostoma* species on wounds. Further infection studies will help to determine whether S. *bactrospora* is of any relevance as a pathogen. Nonetheless, our results clearly show that it is common in Norway and southern Sweden and that it is capable of infecting wounds on species of *Populus, Betula* and *Quercus*.

PS1-66-0343

Weird sisters - affinities of some Australian truffle-like fungi

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Australia and New Zealand have an incredible diversity of truffle-like fungi, with many endemic genera, and we are still discovering new taxa. The affinities of many truffle-like fungi genera to agaricoid or boletoid taxa have been well supported based upon morphology, and some recently confirmed using molecular data. However, some taxa have such reduced sporocarp forms and distinct microscopic characters, that affinities have remained suggestions only. Evidence of affinities from ongoing taxonomic studies of several of these 'weird-sisters' is presented.

Sequencing of two regions of nuclear DNA, the LSU and ITS, was undertaken for several Australian truffle-like fungi genera. Both herbarium and fresh specimens were sequenced. After a Blast-search against Genbank to obtain general affinities, separate alignments were constructed for different families or orders of macrofungi from our own datasets and genbank sequences for analyses.

The genus *Cribbea* has, at various times, been suggested to have affinities to *Crepidotus, Fayodia* and *Oudiemansiella*. Our data shows it is most closely related to *Xerula/Oudiemansiella*, which is not so strange as the sporocarp has a radicating stipe. However, the spores are quite distinct. The truffle-like form is rare in this family.

Gigasperma appears to be a 'weird-sister' to the Agariceae, which includes species assigned to *Endoptychum, Barcheria, Longula* and *Gyrophragmium*. Spore morphology and sporocarp form, support this close affinity.

Australian species of *Chamonixia* appear in a different clade within the *Boletales* to the northern hemisphere type species, *C. caespitosa*. *Chamonixia caespitosa* appears to be related to *Leccinum*, a genus that is rare in Australia, while the Australian taxa are related to *Austroboletus*. The genus *Royoungia* is also confirmed to have affinities to the Boletaceae.

As further taxonomic study of other Australian truffle-like genera is completed, more surprising new affinities will probably be uncovered.

PS1-67-0347

Coelomycetes and their _teleomorphs_, with special reference to the phylogeny of the cupulate genera _Infundibulomyces_ and _Satchmopsis_

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Coelomycetes are primarily known as anamorphs with their teleomorphic links often not established. Of 142 *coelomycets* with appendaged conidia, 74% have not been linked to their teleomorph. Of these, the majority were unitunicate ascomycetes, 7 referred to bitunicate ascomycetes while one was considered to be a basidiomycetes (Naj Raj,1993). As part of out inventory of Thai fungal diversity, *Coelomycetes* have been studied and 200 species documented. Plant pathogens account for 160 species causing leaf spots, twig and leaf blight, anthracnose, leaf, stem and fruit rots (Giatong, 1980). Forty species have been collected on leaves, twigs and fruits in native forests and two new species described: *Infundibulomyces cupulata* and *Pseudorobillarda siamensis*. Sequence analysis of three genes of selected *Coelomycetes* were examined. *Robillarda sessilis* nestled within the *Xylariales* with high bootstrap *support in clade with Seridium species, Truncatella angustata and Discostroma species with Pestalotiopsis and Pestalotia species* forming a sister clade. *Xepiculopsis gramineae* grouped within the Hypocreales within a clade comprising *Myrothecium, Didymostilbe* and *Peethamabara*. I*nfundibulomyces* cupulata, a new Infundibulomyces species and Satchmopsis brasiliensis are characterized by nidulariaceous-like conidiomata, but differ in their conidiogenesis and conidial morphology. Infundibulomyces species are holoblastic with appendaged conidia while in *Satchmopsis* they are enteroblastic lacking appendages (Plaingam et al., 2003). Sequence data indicates that the two genera are not closely related, with *S. brasiliensis* positioned in the *Helotiales*, while the *Infundibulomyces* species nestle in the *Sordariales* in a clade with *Kionochaeta* species. The *Infundibulomyces* species are monophyletic and group with 100% bootstrap support, but differ in conidial morphology, I cupulata with longer narrower conidia (6-10 x 1-1.5µm), while the new taxon has shorter conidia (4-7 x 1-1.5µm).

PS1-68-0354

Brunneocorticium _pyriforme_, A New Corticioid Fungal Genus And Species

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In the recent years, evidence derived from molecular studies has revealed that the corticioid fungal genera are present in all major clades of homobasidiomycetes. *Brunneocorticium pyriforme*, a new corticioid fungal genus and species belonging to the euagarics clade, is proposed. This new taxon was found from subtropical-tropical Taiwan, and southern Yunnan (China). Specimens were quite often found on bark of living *Murraya* spp. (Rutaceae), while collections without good fertile parts were frequently encountered. Establishment of this new genus was based on morphological and molecular studies. *Brunneocorticium pyriforme* has resupinate basidiocarp and smooth hymenial surface. It has dimitic hyphal system, with nodose-septate generative hyphae and abundant yellowish brown skeletal hyphae. Leptocystidia are occasionally present. Its basidia are 2-sterigmate and basidiospores are pear-shaped. Phylogenetic analysis based on sequence data derived from LSU rDNA indicated that taxonomic placement of *Brunneocorticium* is in the euagarics clade of homobasidiomycetes, and is closely related with the agaric genera (*Marasmiellus* and *Campanella*). This molecular analysis also supports independent status of *Brunneocorticium*, from other corticioid genera with similar morphological features.

PS1-69-0356

Ophiostomatoid fungi of the Czech Republic

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Ophiostomatoid fungi (Ascomycota) belong to organisms occurring in the woody plants, usually in the association with bark beetles. In the Czech Republic *Ophiostoma ulmi* caused dieback of elms and *Ophiostoma* species were considered as the pathogens of oaks. Till now, in CR the ophiostomatoid fungi have been predominantly identified no more than on the genus level. The aim of the present study is survey of ophiostomatoid fungi in CR, identification and revision of the strains isolated during last decades.
Methods and material: Ophiostomatoid fungi were isolated from branches and stems of the trees and from bark beetles. So far of 14 species of trees and 10 species of the bark beetles (*Hylesinus crenatus, Ips typographus, I. amitimus, Pityogenes chalcographus, Scolytus intricatus, S. mali, S. ratzeburgii, S. scolytus, Xyleborus dispar, X. saxesens*) were studied in this respect. Samples of the bark and wood were incubated in moist chamber method. Sawdust particle from the galleries and body of bark beetles were placed on agar medium and incubated.
More than 24 species of the ophiostomatoid fungi have been observed in the Czech Republic, seventeen of them have been recorded in the present project. From the bark and wood of the trees were isolated six species (*Ophiostoma minus, O. grandicarpum, O. piceae, O. quercus, O. stenoceras, Ophiostoma cf. fusiforme, Graphium sp. 1 and Graphium sp. 2*). In connection with bark beetles have been observed these fungi: *Ceratocystis polonica, Ophiostoma bicolor, O. piceae, O. quercus, O. novo-ulmi, O. cf. brunneocilliatum, O. penicilliatum, O. piceaperdum., Graphium fimbriisporum, G. cf. pseudormiticumi and Leptographium sp. 1. Ophiostoma novo-ulmi* has not been recorded in the Czech Republic until now. The atypical very slow growth of strain of O. piceae from galleries of *Hylesinus crenatus* living on ash at 32°C was noticed. The study is supported by the GA CR (No. 206/05/P279).

PS1-70-0359

Endophytic fungi of branches and leaves of the apple trees from the Czech Republic

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The apple is economically important fruit tree in many countries of the world. Little attention has been paid to composition of endophytic mycobiota of this plant. The aim of this study is recognize composition of endophytic mycobiota in healthy branches and leaves of *Malus domesticus*.
Methods and material: The study was conducted in two apple orchards in North and Central Bohemia in the Czech Republic. In 2005, mycobiota of leaves and thin twigs (0.3-0.6 cm diam.) was studied. Samples of leaves and twigs were repeatedly taken from five and ten apple trees for each study site, respectively. Ten leaves divided in petiole and blade and five twigs were examined for each tree for each study site, respectively.
So far, 26 and 20 fungal species have been isolated from apple branches and leaves, respectively. The composition of mycobiota in branches was different from mycobiota in leaves. The dominant fungi in branches are *Pleurophoma cava, Alternaria alternata, Aureobasidium pullulans, Seimatosporium* cf. *lichenicola¸ Phomopsis* cf. *mali, coelomycet* sp. 1 and Microsphaeropsis sp. A similar composition of the dominant fungi in the branches at both study sites was recorded, but some differences were detected between them. Fungal community with higher diversity was recorded in the North Bohemia. *Cryptosporiopsis* sp. and *Seimatosporium* cf. *lichenicola* were frequently observed in branches from this study site, whereas frequencies of their occurrence in the Central Bohemia were very low. *Alternaria alternata, Aureobasidium pullulans, Cladosporium herbarum* and *Spilocea sp*. of *Venturia inequalis* were most frequently occurring species in leaves. The interesting is occurrence of *Seimatosporium* cf. *lichenicola*, *Cryptosporiopsis* sp. and *Phomopsis* cf. *mali* in healthy branches. These species are known as potential pathogens of apple trees. The molecular-genetic methods will be used to identification of the nonsporulating strains and critical taxa.
The study is supported by the GA of Min. Agr. CR (No. QF 4074) and Min. Agr. CR (MZe No. 000270603).

PS1-71-0360

Endophytes of branches and leaves of grapevine (*Vitis vinifera*) in the Czech Republic

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Grapevine is frequently planted in many countries of the world, including the Czech Republic. Many species of the pathogenic fungi are known from this plant, but only a few studies concerning of the endophytes of vine have been conducted. The spatial and temporal diversity of the endophytic communities inhabiting healthy one year old branches and leaves of grapevine has been studied during two seasons.
Methods and material: Endophytes were researched in two vineyards in Central Bohemia and South Moravia in the CR. During 2004-2005, the samples were repeatedly taken from ten bushes for each locality. Mycobiota of three nodes, three internodes, 3 leaves (divided in blade and petioles) from one branch were investigated for each bush.
During the study was detected about 60 species of fungi. They were recorded approximately in 20% to 70% of the leaves and branches. Any significant differences in composition of the mycobiota of leaves and branches have not been found out. The dominant species (relative frequencies more than 1%) were *Alternaria alternata, Aureobasidium pullulans, Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, Epicoccum nigrum, Phoma sp. 1 and Phomopsis viticola*. A similar composition of the fungal community in the studied leaves and branches was observed from the vineyard in Central Bohemia and South Moravia. The other interesting micromycetes were isolated during this study: phytopathogenic fungi (e.g. *Botryosphaeria* cf. *obtusa*) and plant colonizing fungi (e.g. *Geniculosporium serpens, Sordaria fimicola, Nodulisporium* sp.). Mycotoxinogenic *Aspegillus, Penicillium* and *Fusarium* species were recorded at low frequencies. Some isolated fungi are known as the epiphytic saprophytic fungi, quiescent pathogens or coprophilic fungi. Occurrence of arthrosporic hyphomycete and Spiniger sp. is surprising. These fungi and selected nonsporulating strains will be studied.
The study is supported by the GA of Min. Agr. CR (MZe No. 000270603).

PS1-72-0368

An old forgotten genus for the "glomerellalean" anamorphs *Verticillium nigrescens* and *Acremonium furcatum*

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The polyphyletic genus *Verticillium* is being split up over several monophyletic genera. According to ITS and LSU sequences, the conserved type, *V. dahliae*, and the equally plant-pathogenic *V. albo-atrum* are phylogenetically related to *Plectosphaerella* with its *Plectosporium* anamorphs. The original type species, *V. tenerum*, has been excluded and transferred as *Acrostalagmus luteoalbus*. Plectosphaerella is so far the only teleomorph genus in a family that is sister to the Glomerellaceae. A number of anamorphic species with more or less verticillate conidiophores and varying degrees of formation of pigmented structures are closely related to but generically distinct from *Verticillium*, a genus reserved for plant-pathogenic species. Verticillium nigrescens is not congeneric with *Verticillium* s. str.; it is close to an isolate described from fish in Brazil, based on a number of genomic regions. Morphological features are confusing, however, not always conveniently sorting with the phylogenetic genus-level clades. An example is the presence of pigmented chlamydospores in both V. nubilum, which phylogenetically falls in *Verticillium* s. str., and in V. *nigrescens*, which is generically distinct together with a few other species, including *Acremonium furcatum. Verticillium theobromae*, causal agent of cigar end-rot of banana, is unrelated to both *Verticillium* s. str. and V. *nigrescens*.

PS1-73-0369

Phylogenetic trends in the Calosphaeriales

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Traditionally, the order comprised several small ascomycetes with nonstromatic perithecia, filiform beaks, solitary or in circinate groups; typically with clavate asci on elongating ascogenous hyphae, a thickened ascal apex without any visible discharge mechanism; long persistent paraphyses, and allantoid to suballantoid hyaline ascospores. The order accommodated seven genera, which occupy specialized woody substrata. These taxa have been poorly studied in the past. In our study, LSU and SSU nuclear rDNA sequences were generated from type species of five presumed calosphaeriaceous genera. The inferred phylogenies, comparative morphological studies and the respective phialidic hyphomycetous anamorphs obtained in vitro suggest that the Calosphaeriales as presently perceived are a polyphyletic group of phenotypically similar taxa. Several phylogenetic lineages could be distinguished: 1) the diatrypaceous (xylarialean) (Graphostromataceae: *Graphostroma*), 2) the diaporthalean (Togniniaceae: *Togninia* incl. *Romellia* as its generic synonym), and 3) the calosphaerialean lineage (Calosphaeriaceae: Calosphaeria s. str., *Togniniella*, and *Pleurostomataceae*: *Pleurostoma*). The conspicuous similarity in their phenotypes is likely a result of convergent evolution rather than of common ancestry. Within the Calosphaeriales, the Pleurostomataceae, the holomorph genus *Togniniella*, and the three anamorph genera, *Phaeocrella* (teleomorph *Togniniella*), *Calosphaeriophora* (teleomorph *Calosphaeria*) and *Pleurostomophora* (teleomorph *Pleurostoma*), were recently described. The closest relatives of the Calosphaeriales lie among the members of the Diaporthales: the Gnomoniaceae and the Togniniaceae. The major progress in understanding the phylogeny and taxonomy of the group was achieved by a detailed study of the ascogenous system and the arrangements of the asci (four patterns of ascus formation could be discerned) and cultivation studies. Current generic concepts in the Calosphaeriales are therefore based on holomorphic characters, i.e. arrangement of ascomata, centrum structures, and morphology of conidia and conidiophores of the anamorph. The taxonomic value of these characters at the generic level is confirmed by the results of molecular analyses based on LSU and SSU nrDNA sequence data. The phylogenetic position of *Enchnoa*, *Jattaea, Pachytrype* and *Wegelina*, traditionally accommodated in the Calosphaeriales, remains unknown.

PS1-74-0373

Morphology and Molecular Phylogeny of a New Synnematous, Dimorphic *Bloxamia* Species from Indonesia

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A new synnematous hyphomycete was isolated from a soil sample collected at a banana field in Cibinong, Indonesia during our survey of Indonesian filamentous fungi in a Joint Research Project conducted by five Indonesian government research organizations and NITE-DOB. This fungus was identified based on the morphological characteristics, and its phylogenetic position was determined by comparing its sequence of the D1/D2 domains of 28S rDNA with the known sequences of *Chalara* and allied species (Paulin and Harrington 2000).
Morphologically, this fungus has chalara–like phialidic mode of conidium ontogeny, but its phialophores are densely aggregated into synnemata, and the collarette is not visibly differentiated from the venter of the phialide. Its phialoconidia are rectangle or almost square, unicellular, hyaline or subhyaline, and formed at the apex of cylindrical, brown conidiophores aggregating into a synnema. Therefore, it was identified as the genus *Bloxamia*. The novel *Bloxamia* species forms another type of phialoconidia, which is different from other *Bloxamia* species. This type of phialoconidia is subglobose or pyriform with a truncate base, unicellular, brown to dark brown, and formed on sympodially branching, hyaline or pale brown conidiophores.
Phylogenetically, this fungus was placed in the same cluster with the *Ceratocystis* sensu stricto group. Some *Chalara* species in *Ceratocystis* sensu stricto group are also pleomorphic similar to this fungus.

PS1-76-0375

The Genus *Leptographium*: A Multigene Approach to Molecular Characterization

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Species of *Leptographium* are anamorphs of *Grosmannia* that can generally be recognized by their long, slender conidiophores, which terminate in brush-like conidiogenous apparatuses comprised of a series of branches. The typically hyaline ameroconidia are formed via percurrent proliferation of the conidiogenous cells. Most *Leptographium* spp. are associated with coniferous trees and most species have well-defined vectors, especially bark beetles. A small number of species cause serious tree diseases but the majority are best-known as agents of sap stain in lumber or as saprophytes. About 50 species of *Leptographium* are recognized and subsequent to the publication of the first monograph on the genus, new species have been added continuously. The increasing numbers of *Leptographium* spp. present a substantial taxonomic challenge because many species are morphologically similar and thus difficult to identify. The aim of this study was thus, to characterize *Leptographium* spp. using a multiple gene approach. For this purpose, sequences derived from the ITS2 and 28S region of the ribosomal DNA gene region as well the ,-tubulin and Elongation Factor 1-· gene regions were used in the comparisons. Results showed that sequences for the ITS region, previously employed to characterize *Leptographium* spp. do not distinguish between closely related species. Differences between well recognized species were more apparent when comparing sequences for the protein-coding regions. Sequences for the three gene regions have now been generated for multiple ex-type isolates of all *Leptographium* spp. for which cultures are available. The resulting data will substantially enhance the ability to identify isolates of known species, to recognize new species and to develop systems for rapid diagnostics.

PS1-77-0377

Further insights gained into *Togninia* (teleomorphs of *Phaeoacremonium*)

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The genus *Togninia* (T.) is characterized by small, brown to black ascomata with elongated necks, and oblong, unitunicate asci with truncate bases and thickened apices; the asci arise in a spicate arrangement from the ascogenous hyphae, and the paraphyses are hyaline and septate; ascospores are aseptate, hyaline, varying in shape from allantoid to ellipsoidal to oblong-ellipsoidal. Anamorphs of *Togninia* have been classified in Phaeoacremonium. To investigate possible teleomorph associations, isolates of various Phaeoacremonium spp. were mated in vitro on sterile grapevine canes. Eight *Togninia* teleomorphs formed in culture, including *T. argentinensis, T. austroafricana, T. krajdenii, T. minima, T. novae-zealandiae, T. parasitica, T. rubrigena* and *T. viticola*, of which seven were newly described during the course of this study. Single-conidial and ascospore strains of T. argentinensis and T. novae-zealandiae formed fertile perithecia, indicating that the taxa have a homothallic mating system. The remaining species are heterothallic.Morphological structures that can be used to distinguish Togninia species include perithecial size, neck length, shape of paraphyses, ascus and ascospore morphology. Furthermore, type specimens of *T. cornicola, T. inconspicua, T. rhododendri, T. minima var. timidula, T. vasculosa*, and *T. villosa* were examined. Most of these were found not to belong to Togninia, implying the following new combinations: *Calosphaeria (*C.) *cornicola, C. rhododendri, C. transversa, C. tumidula, C. vasculosa*, and *Jattaea villosa*. Togninia inconspicua is distinct from other species because of its small perithecia, short necks, absence of remnant bases on the ascogenous hyphae and large asci and ascospores. Besides the eight Togninia holomorphs established by crossing, *Togninia fraxinopennsylvanica* was linked to *Phaeoacremonium mortoniae* with DNA phylogeny of the partial b-tubulin and actin genes.

PS1-78-0378

A new gall-forming facultative mycoparasite of the Mucorales from Japan

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On the course of the floristic survey of the Japanese Zygomycota, a new Mucoraceous fungus was isolated as a facultative mycoparasite of *Mucor piriformis* on the fallen oranges *(Citrus unshui)* at the orchards, in central Honshu, Japan. In the field, the fungus sporulates only in the winter season (Dec. to Mar.) with host *M. piriformis.* In vitro, it can grow on the usual media without host, but rather slowly as a Mucoraceous fungus and prefers the lower temperature (5-15C). The fungus is characterized by the following features; 1) Its facultative mycoparasitism resembles that of the genera *Parasitella* and *Chaetocladium* in producing sikyospore-like gall at the contacting point to the host fungus (on the sporangiophores and vegetative mycelia). The parasite can attack both of the mating types of *M. piriformis* and several other species of the Mucoraceous hosts. 2) It has a typical asexual apparatus of the Mucoraceae, especially resembles that of the genus *Rhizomucor* in having the rhizoids and stolons developed in the later stage. 3) The fungus is heterothallic, producing black coloured, ornamented, subglobose to cylindrical zygospores in the aerial mycelia. Its sexual morphology is similar to that of the families Choanephoraceae and Pilobolaceae in producing the strongly twisted progametangia and the mature zygospores with tong-shaped apposed suspensors. This paradoxical situation of the combined characteristics of these various genera requires a proposal of a new genus for this new fungus in the Mucorales.

PS1-80-0385

Phylogeny of the *Postia – Oligoporus* complex of poroid Basidiomycetes

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The overall similarity and the high number of species cause problems in identification and generic subdivision of the annual monomitic polypores. While a few are well separated from the main group — e.g. *Amylocystis lapponica, Climacocystis borealis, Leptoporus mollis*, and *Sarcoporia polyspora (=Parmastomyces mollissimus)* — the remaining white-coloured, brown-rot-causing taxa needed a closer scrutiny. The so-called Postia – Oligoporus complex belongs to the phlebioid clade of the Basidiomycetes, including both polypore-like and corticoid brown-rot fungi. This study is based on recently collected and personally identified specimens mostly from North Europe. A maximum parsimony analysis based on rRNA sequences revealed a tree illustrating that the *Postia – Oligoporus* complex is monophyletic, but heterogeneous.
Postia sensu stricto (*P. alni, P. caesia, P. lactea* (type of the genus), *P. leucomallella, P. lowei, P. luteocaesia, P. tephroleuca*) and *Oligoporus (O. balsameus, O. balsaminus, O. cerifluus, O. floriformis, O. fragilis, O. guttulatus, O. hibernicus, O. immitis, O. lateritius, O. mappa, O. parvus, O. perdelicatus, O. persicinus, O. ptychogaster, O. rennyi* (type of the genus), *O. sericeomollis, O. stipticus*) still deserve further analysis of their subgroup relationships. Outside the core, a few independent species groups were determined, including *Fibroporia* (F. *gossypium,* F. *norrlandica*, F. radiculosa, F. vaillantii), the Antrodia-related *Oligoporus mappa* group, and two smaller genera: *Spongiporus (S. undosus)* and *Rhodonia (R. placenta*). We are continuing this study, which eventually will be presented as a generic revision of the whole *Postia – Oligoporus* complex.

PS1-81-0386

Molecular phylogenies suggest polyphyly in morphotaxa of brown-spored agarics

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The brown-spored agarics include saprotrophic and mycorrhizal fungi belonging to the families Bolbitiaceae, Cortinariaceae, Crepidotaceae, and Strophariaceae. The current classification of the brown-spored agarics is largely based on morphological characters and to less extent on molecular phylogenies. In the described project, the main goal is to analyse multi-locus sequence data by parsimony and Bayesian maximum likelihood methods in order to evaluate the current classification and to define monophyletic groups. A special emphasis is put on the genus *Galerina*, which includes saprotrophic and bryophilous species producing small, mycenoid and yellow to brownish basidiocarps. Preliminary results, based on analyses of partial LSU nrDNA sequences, strongly suggest that *Galerina* is polyphyletic and includes at least four independent lineages, in the phylogenies embedded with other genera of brown-spored agarics. These four groups largely reflect already recognized morphotaxa (subgenera or sections) within *Galerina*. Our findings also affirm that morphological characters, e.g. fruit body morphology and cystidial shape, often are highly homoplastic. Furthermore, the preliminary results indicate that the *Galerina* lineages, as well as the genus *Gymnopilus*, could be referred to a strongly emendated family Strophariaceae, which corresponds largely to the family as circumscribed by Kühner (1980).

PS1-82-0387

Further Saccardo's omissions of non-lichenized fungi: the forgotten Sydow's lists for 1895-1918 and Petrak's real first "List" for 1919

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Modern taxonomic fungal databases still are incomplete or contain a lot of citation gaps. Older fungal names in the Index fungorum data base of CABI, CBS and Landcare Research mostly were taken from Saccardo's Sylloge fungorum and from the collection of names by P.M. Kirk in his Index of Fungi Supplement with Saccardo's omissions.
As precursors of the printed edition of Index of Fungi today only Petrak's Lists No. 1-8 for 1920-1939 are well known, which mostly were published in the German series of Just's Botanischer Jahresbericht, but this tradition of thorough compiling all new published names in reality was founded by Paul Sydow in the year 1895. He wrote 24 lists of new names ("Verzeichnis der neuen Arten") for 1895-1918 in vols. 23-46 of this "Just's" bibliographical series. After Sydow's death Franz Petrak continued this compilation job with the list for 1919 in vol. 47, which also fell into oblivion in modern times.
The incompleteness of the Sylloge fungorum, which at last was edited by A. Trotter, is in part due to the fact, that collecting of names of new combinations was not the aim of the compilation strategy of the Sylloge and for the other part the editors omitted a lot of sources of different regional provenances like from Central and Eastern Europe etc. - in combination with literature availability problems during World War I.
About 10 genus names, like *Onygenopsis* P. Henn. (1910), and about further 1000 species names, which cannot be found in the Sylloge fungorum and in Kirk's Saccardo's Omissions - and which therefore are lacking in the Index fungorum/ Index of Fungi -, now can be transmitted from the 25 forgotten "Lists" into modern databases. We propose to call the most important of all these lists, containing 264 not yet indexed Saccardo omissions, "Petrak's List zero for 1919".
The rediscovery of the mentioned 25 lists in Just's Botanischer Jahresbericht will help to remove gaps and errors from modern databases. These and other older Central European literature sources should be introduced into the internet to make them usable for the scientific community.

PS1-83-0388

Accelerated evolution and profound AT bias among lineages of the medicinal fungus *Cordyceps sinensis*

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Cordyceps sinensis (Berk.) Sacc. grows parasitically on buried larvae of ghost moths *(Thitarodes/Hepialus spp.)* in Asian high-altitude grasslands and is known as a highly reputed medicinal fungus. In this study, the intraspecific variation in *C. sinensis* was analyzed employing 71 nrDNA ITS sequences accessioned in GenBank. Bayesian maximum likelihood analyses distributed the *C. sinensis* ITS sequences into five groups, referred to as A-E. Two groups (D-E), including a total of nine *C. sinensis* sequences as well as other Hypocreales/Clavicipitales taxa, were interpreted as erroneously accessioned sequences. The remaining sequences (62) constituted three highly supported clades (groups A, B and C), that may represent cryptic (phylogenetic) species currently ascribed to C. *sinensis*. Two sequences, accessioned as C. *multiaxialis* and *C. nepalensis,* also merged with the C. *sinensis* sequences of clades A-C. Sequences of groups B and C showed accelerated substitution rates and high AT nucleotide bias throughout the entire ITS region. A remarkably high sequence variation occurred in the 5.8S gene, which far exceeds what is normally observed in fungi. Compelling evidence from other studies exists for that reduced recombination frequencies can lead to AT bias due to lack of GC biased gene conversion, a process coupled to the meiosis. We hypothesize that a transition in life history attributes or ecology has happened among the putative cryptic species of C. *sinensis*, leading to accelerated evolution and AT bias. We also suggest that observed differences in medicinal effects among C. *sinensis* populations may be attributed to the existence of cryptic species in this morphotaxon.

PS1-84-0395

Classification of pathogenic *Aspergillus* section *Fumigati*

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Aspergillosis is a clinically important mycosis and the most significant causative agent is *Aspergillus fumigatus*. Recently it has also been reported that cases of invasive infections were caused by species of a related teleomorphic genus, Neosartorya. However, clinical isolates of the species are not necessarily morphologically uniform, and it is essential to clarify intra- and interspecies diversity in *A. fumigatus* and closely related species. This time, we analyzed about 30 clinical isolates and related species on the tubulin and hydrophobin genes and the ITS region, and discussed the correlation between molecular phylogeny and morphology in the section Fumigati.
The strains isolated from clinical specimens and soils, identified by light microscopy as *A. fumigatus* and preserved at Chiba University were used. Other species of the section Fumigati and Neosartorya species were also examined as references.
The beta-tubulin and hydrophobin genes, and the ITS region were sequenced directly from the PCR products by using primers pair Bt2a and Bt2b, primers pair rodA1 and rodA2, and primers pair ITS1 and ITS4, respectively. The PCR products were sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).
The sequences were aligned by using Clustal X software and phylogenetic trees were made by the neighbor-joining analysis.
The phylogenetic trees based on the sequences on the beta-tubulin and hydrophobin genes and the ITS region approximately matched. The species of the section Fumigati were divided into five clades; clade I, typical species of *A. fumigatus*; clade II, species including *A. lentulus* and *A. fumisynnematus*; clade III, atypical species of *A. fumigatus*; clade IV, species including *A. viridinutans*; and clade V, species including *A. brevipes, A. duricaulis* and *A. unilateralis*. Most of the examined strains from clinical specimens in Japan clustered together in clade I and exhibited globose conidia with lobate-reticulate ornamentations. *A. fumigatus* var. elliputicus and *A. fumigatus* var. arvii were placed here. Other strains from clinical specimens were divided into three clades (clades II, III and IV) and there was no strain from ones in clade V. The strains in clades II and III exhibited conidia with microtuberculate ornamentations, while those in clades IV and V had conidia with lobate-reticulate ornamentations. Clade III is clearly distinguished from the other clades and the strains included in this clade are considered to be a new species.

PS1-85-0397

Phylogenetic Studies Of The Section Rimosae (Agariacales, *Inocybe*)

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Inocybe (Agariacales) is a large genus with more than 300 species distributed all over the world. The subgenus *Inosperma* is divided into two sections, Rimosae and Cervicolores. Rimosae, characterized by rimose to rimulose pileus, is the larger of the two sections but recent studies indicate that it is polyphyletic. In this study a broad taxon sampling of the section is used to address the issue of polyphyly and the constitution of the different clades. A special emphasis is put on I. rimosa (Bull.: Fr.) Kumm., since different authors have different opinions of how to delimit the units of this species complex and the taxonomy of this and closely related species is far from clear. To address these questions a phylogeny of the section is inferred using data from the ITS1-5,8S-ITS2-LSU region of nuclear rDNA. The data is analyzed using both maximum pasimony and bayesian methods. It is confirmed that the section is polyphyletic and that the section is divided into two distinct clades. Both I. *obsoleta* Romagn and I. *perlata* (Cooke) Sacc. which has been synonymized with *I. rimosa* (Bull.: Fr.) Kumm. are identified as distinct species. I. *rimosa* (Bull.: Fr.) Kumm. is very variable in the sequences and it is likely that there are several still unidentified species sorted under this name. The taxonomy of the sections within Inosperma needs to be revised. Here a phylogeny of the subgenus is presented.

PS1-86-0399

Molecular-based detection and quantification of causal agents of Sigatoka disease complex of banana

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The Sigatoka disease complex, consisting of *Mycosphaerella fijiensis, M. musicola* and *M. eumusae*, is the most important disease of banana worldwide. The disease is mainly controlled via fungicide applications, which cause for great concern because of the environmental and ecological consequences, and the high percentage it represents in production costs (up to 45% in commercial plantations due to 40+ yearly applications). Disease diagnosis is based on the presence of host symptoms and fruiting structures of pathogen, which hamper preventive control strategies. In present study we developed rapid and robust molecular-based diagnostic tools for detection and quantification of *M. fijiensis, M. musicola* and *M. eumusae*. TaqMan real-time PCR assay was developed based on the ,-tubulin gene that could detect up to 1 pg/µl DNA for each *Mycosphaerella* species, and were validated using naturally infected banana leaves. Furthermore, conventional species-specific PCR primers were developed based on actin gene that could detect as little as 100, 1 and 10 pg/µl DNA from *M. fijiensis, M. musicola* and *M. eumusae*, respectively. TaqMan real-time PCR quantitative data now could be implemented in developing a chemical management system for the Sigatoka complex in banana.

PS1-88-0417

Some new species and new records of discomycetes from Taiwan

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Four new records including one new variety of *Lachnum* and one new species are described and illustrated from Taiwan. All the species reported were growing on a great diversity of substrates, such as: deciduous trees, herbaceous plants and bamboo. They were collected from the mountain areas with altitude 550-1500 m. The new species, *Hyalorbilia arcuata* occurs on the moist decayed bark of broad-leaved trees while the new variety, *Lachnum hyalopus* var. *miscanthi*, was on the dead culms of *Miscanthus floridulus*. The other two species recorded from Taiwan for the first time are *Lachnum lushanense* and *Stictis radiata. Lachnum lushanense* were from southern mountain area of Taiwan while *S. radiata* were from northern mountain area of Taiwan.

PS1-89-0431

Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders

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Molecular phylogenetic analyses for the gomphoid-phalloid fungi (Agaricomycetes, Basidiomycota) were conducted based on the 5-gene-dataset (nuc-LSU-rDNA, mt-SSU-rDNA, atp6, RPB2 and tef1) with extensive taxon sampling of 231 taxa. The results of both parsimony and Bayesian analyses strongly supported the monophyly of the gomphoid-phalloid clade, and four well-supported major subclades within the gomphoid-phalloid clade were recognized. Three of the four subclades (Geastrales, Hysterangiales and Phallales clades) were entirely represented by gastroid taxa, while only the Gomphales contained both gastroid and non-gastroid taxa. While the gastroid morphology, e.g., *Gautieria*, is derived from epigeous, non-gastroid taxa, e.g., *Ramaria*, in the Gomphales, the topology of the Phallales indicated that truffle-like form is an ancestral morphology of the stinkhorn fruiting bodies. Although basidiospore maturation occurs within the enclosed fruiting bodies of the stinkhorn, the elevation of the mature spore-producing tissue represents an independent origin of the stipe among the Basidiomycota. Comparisons are made between previous and new classification schemes, which are based on the results of phylogenetic analyses. Based on the results of these analyses, a new subclass Phallomycetidae, and two new orders, Hysterangiales and Geastrales, are proposed.

PS1-90-0432

Conservation of species names for economically significant mushrooms

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Principle III of the International Code of Botanical Nomenclature (ICBN) establishes the principle of priority of publication. Notwithstanding Prin. III, Art. 14.1 allows for the conservation of names of families, genera and species as nomina conservanda in order to avoid disadvantageous nomenclatural changes. However, very few fungal species epithets have been proposed for conservation as reflected in Appendix IIIB of ICBN, as most, 20 out of 28, listed in the St. Louis Code (2000) are lichens. The Vienna Code (2006) will include one mushroom name, Armillaria matsutake, conserved over Armillaria nauseosa, allowing for continued use of Tricholoma matsutake, for the Matsutake. This year proposals have been submitted or will be submitted to stabilize names for the commercial mushroom *(Agaricus bisporus), Poria cocos (Wolfiporia cocos), Agaricus lepideus (Neolentinus lepideus, Lentinus lepideus), Pleurotus japonicus (Lampteromyces japonicus, Omphalotus japonicus), Agaricus tigrinus (Lentinus tigrinus), Agaricus crinitis (? Lentinus crinitis)*, among others. Details are provided with rationales. Through traditional type studies several names have been competing for decades while others were only recently discovered to be superfluous. Now molecular based phylogenies are placing some species in large genera where the resultant binomials are pre-occupied leading to loss of both the traditional generic name and the long established species name simultaneously, e.g. *Polyporus gerdai*, replacing *Lentinus tigrinus*. Greater use of Art. 14.1 is recommended in cases of high profile species.

PS1-91-0442

Fungal species associated with three phytophagous insects of gorse

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Fusarium tumidum is a potential mycoherbicide for gorse control. Phytophagous insects naturally found on this weed, may serve as deliberate vectors of *F. tumidum* spores for gorse biocontrol. In this study, diversity of fungi on the surfaces of three phytophagous insects of gorse was studied by direct culturing and PCR-RFLP of ITS methods.

Fungi on cuticles of *Apion ulicis* (weevil), *Cydia ulicetana* (moth) and *Epiphyas postvittana* (moth) collected from three sites, were isolated through washing and plating on Potato Dextrose Agar. PCR products of amplified DNA obtained from the washing of the insects were cloned in pGEM-T easy (Promega) vector in Escherichia coli strain INV·F'. Plasmids were extracted from 141 clones (Wizard® Plus SV Minipreps *DNA purification kit*). PCR products from both cultured and cloned amplicons were digested with restriction enzymes: *Hin6I,* MboI, *BsuRI* and *HinfI* (Fermentas) and representative RFLP groups were sequenced and compared with the GenBank database using BLAST search programme.

Cladosporium, Penicillium, Phoma, Alternaria, Aspergillus, Aureobasidium pullulans, Beauveria, Epicoccum purpurascens, Pithomyces, Sclerotinia, Verticillium and yeast species were present on all three insect species. *Cladosporium* was the most prevalent genus. *Fusarium lateritium, F. tricinctum Gibberella pulicaris, Acremonium strictum* and *Ulocladium spp*. were found on the moths only. The yeast species included *Metschnikowia pulcherrima, Pseudozyma fusiformata, Rhodotorula mucilaginosa and Sporobolomyces ruberrimus*. The largest RFLP group among the cloned amplicons was Nectria mauritiicola which accounted for 92% of the cloned amplicons. Ten different strains of *N. mauritiicola* were identified by RFLP analysis. The most common strain on all the insect species was NHRC-FC042 (accession number AJ558114). The moths carried significantly more distinct fungal groups than the weevil, while the three collection sites had similar fungal diversity.

The results indicate that these insects harbour multiple microbial taxa on their cuticle. However, more genera of fungi were recovered using the direct culturing technique than from the cloned amplicons. Most fungi were ascomycetes with four basidiomycetes isolated. Only one zygomycete *(Mucor hiemalis)* was isolated from *Epiphyas postvittana.* The moths may have more potential to act as vectors of *F. tumidum* as they naturally carry *Fusarium spp*. Moreover, pheromone is available for attracting male moths to *F. tumidum* inocula. Further research into the antagonistic effects of the surface microflora on *F. tumidum* spores is ongoing to select the best vector for an auto-inoculation programme for gorse biocontrol.

PS1-92-0451

Another Teleomorph For *Nimbya* – The First From Amaranthaceae

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Undescribed species of *Nimbya* found causing leaf and stem lesions on alligator weed (*Alternanthera philoxeroides*) in Australia were tested for mating compatibility in the laboratory.
The type culture of *Alternaria alternantherae* (= *Nimbya alternantherae* ATCC 32833), seventeen Australian isolates from alligator weed along with one isolate from *A. denticulata* and two isolates from *Gomphrena celosioides* were paired on alligator weed stem agar.
Five combinations of five alligator weed isolates and the one *A. denticulata* isolate produced an ascomatal state. In addition, two of these alligator weed isolates produced infertile ascomata when paired with the *Gomphrena isolates*. There were no successful matings with the *N. alternantherae* type culture.
Sub-epidermal perithecia were formed on stem tissue at the junction of two compatible isolates after 40 days incubation. Mature ascospores were observed after a further period of one to two months.
The teleomorph was characterised by dark, globose, uniloculate and ostiolate perithecia 155 to 248µm diameter, at first sub-epidermal, later breaking through the epidermis often with a slight beak. Short setae resembling those of *Setosphaeria* were occasionally observed.
The short stalked bitunicate asci were cylindric-clavate, with an average measurement of 93.5 x 13.6µm. The phragmosporous and fusiform ascospores were hyaline, 3 (-5) septate, constricted at the septa without a gelatinous sheath and averaged 24 x 7µm.
Single ascospores plated to potato carrot agar produced typical *Nimbya* cultures and conidia.
Known teleomorphs of *Nimbya* are currently placed in the dictyosporous genera *Macrospora* and *Pleospora*. No teleomorphs are known for the six published species on *Amaranthaceae*. The new fungus falls within the Pleosporaceae but is clearly unable to be placed within either of the above genera.

PS1-93-0464

Synnematous fungi from Taiwan (1)

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The fungi in Taiwan were well listed in several books: e.g., Wang et al. (eds.), 1999, List of the fungi in Taiwan (ISBN 957-02-5216-2), Tzean et al. (eds.), 2005, Fungal flora of Taiwan, 1st ed. (ISBN 986-00-1786-7), etc. We recently investigated microfungal flora in Taiwan as a part of the project entitled "Cooperative research on fungal diversity in the Asian tropical monsoon areas". A part of the results is reported here, especially focusing on synnematous fungi.
Between March 2003 and November 2005, we conducted four field trips to collect fungi mainly on dead plant material from northern (Yangmingshan National Park, Taipei) to southern (Kenting National Park, Tainan) parts of Taiwan. Materials were collected and brought back to Japan with appropriate permits, and fungi were observed and isolated using conventional isolation and incubation techniques. Voucher specimens and cultures are presently in personal collections in Japan, but will be deposited soon in public herbaria and culture collections in Taiwan and Japan.
The synnematous fungi detected include: *Antromycopsis macrocarpa** (Ellis & Everh.) Stalpers, Seifert & Samson 1991; *Arthrobotryum stilboideum* Ces. 1854; *Bactrodesmium longisporum* M.B. Ellis 1976; *Endocalyx melanoxanthus* (Berk. & Broome) Petch 1908 *var. melanoxanthus; Endophragmia* atra (Berk. & Broome) M.B. Ellis 1959; *Kostermansinda* magna* (Boedijn) Rifai 1968; *Melanographium selenioides* (Sacc. & Paol.) M.B. Ellis 1963; *Phaeoisaria clavulata** (Grove) E.W. Mason & S. Hughes 1953; *Podosporium beccarianum* (Ces.) Seifert & G. Okada 1990; *Sarophorum palmicola** (Henn.) Seifert & Samson 1986 (1985); *Stilbella bambusae** (Pat. & Gaillard) Seifert 1985; *Stilbella clavulata** (Mont.) Seifert 1985; *Tretopileus sphaerophorus* (Berk. & M.A. Curtis) S. Hughes & Deighton 1960; *Tubercularia lateritia (*Berk.) Seifert 1985; Unidentified synnematous fungus 1 (with conidiogenesis similar to that of Torula species). *: Not listed in Wang et al. (1999).
Previous reports on synnematous and related hyphomycetes of Taiwan were published by H.S. Chang, J.L. Chen and co-workers. Although investigation is still incomplete, we found interesting synnematous fungi from plant materials collected in Taiwan. Some species are known to be strongly associated with particular host plants: e.g. Endocalyx *melanoxanthus* var. *melanoxanthus* (on palms), *Melanographium selenioides* (on the palm, *Arenga engleri*), *Podosporium beccarianum* & *Stilbella bambusae* (on bamboos), *Sarophorum palmicola* (persimmon seeds and fruits). The unidentified synnematous fungus with *Torula*-like conidiogenesis was collected on freshly cut stem of *Amischotolype chinensis*, probably showing its strong host specificity. Because Taiwan is rich in natural areas, it surely offers much potential for studies of fungal biodiversity. Cultures of the unique fungal species of Taiwan should be better represented in Taiwanese public resource centers for future studies, as should dried herbarium specimens.

PS1-94-0466

Macromycetes of Pinacate and Great Altar Desert biosphere reserve, Sonora, Mexico

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Mexico ranks among the first three places in the world as far as biological wealth is concerned, with over 12% of the world's biota. In general, its topography and geographical location on the boundaries of confluence of the neo-arctic and neo-tropical zones, explain the large variety of ecosystems and the consequent biological diversity. The Pinacate and Great Altar Desert biosphere reserve (PBR) is located 31°30'-32°30' N and 113°00'-114°30' W, with an area of 714,556.5 ha. Due to scarce records and in order to contribute to the knowledge regarding the diversity of macromycetes in this reserve, the present study registers 27 species, three of them being new to the Mexican mycobiota.

The survey was realized at PBR, conducting seasonal samplings from fall 2003 to summer 2004, on four types of vegetation: microphyllous desert scrub, sandy desert vegetation, mezquital, and sarcocaule scrub. The 10 sites studied were geo-referenced. Sampled sites were characterized by biotic and abiotic factors based upon topographic and subjet charts, and physical and chemical soil analyses. The edaphologic analysis was done on 12 to 15 samples (ca. 4 kg) randomly taken at each site at a depth of 0-30 cm. Threefold determinations were made, analyzing pH, electric conductivity, sodium absorption ratio, organic matter, nitrates, phosphates and texture.

Twenty seven taxa were determined: Order Phallales: Geastraceae (4); Order Agaricales: Agaricaceae (5), Phelloriniaceae (1), Lycoperdaceae (4), Schizophyllaceae (1) and Tulostomataceae (12). Five taxa were determined for Agaricaceae, with Montagnea arenaria and Podaxis pistillaris outstanding as they are present in all sites, and moreover throughout the year in several localities. On the contrary, other species of Agaricaceae had a restricted distribution: *Longula texensis, Chlorophyllum molybdites* and *Endoptychum arizonicum*. Regarding Phelloriniaceae, *Phellorinia herculeana* was the only species observed growing in the microphyllous desert scrub, solitary during winter. As far as Lycoperdaceae is concerned, the four species determined had a restricted distribution, growing especially during summer.

Montagnea arenaria and *Podaxis pistillaris* had the widest distribution and the highest number of collections; on the contrary, species of Geastraceae and Lycoperdaceae reported a rather restricted distribution. Tulostoma was the most represented genus with 12 taxa. *Geastrum berkeleyi, G. schmidelii* and *Tulostoma mohavei* are registered for the first time to the Mexican mycobiota.

PS1-95-0474

The anamorph of *Amphiporthe aculeans*, a pathogen of sumac and other Rhus species

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Amphiporthe aculeans (Schw.) Barr is a common fungus on dead branches of Rhus species (in particular on *R. typhina* in North America and on *R. javanica* in Japan). Its anamorph is usually reported as Stilbum rhois Berk. & Curtis (or S. rhoidis), but the teleomorph-anamorph connection has not previously been proven using single-ascospore isolations. *Stilbum Tode*, typified by *S. vulgare* Tode, is a heterobasidiomycetous genus (Oberwinkler and Bandoni 1982). Seifert (1985), in revising the species ascribed to *Stilbum* and its hyphomycetous counterpart *Stilbella* (anamorphic Hypocreales), excluded *S. rhois* from *Stilbella* because of its synnema anatomy, the brownish colour of its conidia and conidiophores, and its teleomorph (Diaporthales). We further discuss here the taxonomic disposition of the anamorph of *A. aculeans*.

The holotype (no. 1445 in Herb. Berk., K) and several supplementary collections of *Stilbum rhois* were examined, as well as teleomorphic specimens. Using freshly collected materials, the anamorph-teleomorph connection was confirmed by single-ascospore isolations using a Skerman's micromanipulator. Conventional methods were used for microscopy and cultural studies.

The anamorph-teleomorph connection between *A. aculeans* and S. *rhois* was confirmed using single ascospore and single conidium isolates from Canadian and Japanese materials. The anamorph produces synnematous conidiomata covered with minute crystals on the host, phialidic conidiogenous cells both on the host and in culture, and ellipsoidal conidia enveloped in a dense, dark slime mainly on the host. Cultures from North American specimens that are not rigorously single spored usually are contaminated or overgrown with a Phomopsis species, which has sometimes been reported to be an anamorph of A. aculeans.

The anamorph of *A. aculeans* cannot be included in any previously described hyphomycete genus. Several anamorph genera have species producing dark synnemata, phialidic conidiogenous cells, and ameroconidia: e.g., *Phialographium (= Pesotum, teleomorphs Ophiostoma, Ophiostomatales; Okada et al. 1998), Crinula (teleomorph Holwaya, Helotiales*), Stromatographium (teleomorph Fluviostroma, Trichosphaeriales), Acrostroma (teleomorph *Batistia, Sordariales*). The anamorph of A. aculeans differs from these genera in several respects, including details of synnema anatomy, the production of crystals on the synnema stipe, and the dark pigmentation of the conidia. Most known anamorphs of the Diaporthales are coelomycetes, and none of these genera have characters consistent with the anamorph of A. aculeans. Therefore, we intend to propose a new anamorph-genus for this anamorph. At present, we have no European strains of this fungus, a necessary prerequisite to a re-evaluation of the species concept and a determination of its phylogenetic relationships.

PS1-96-0488

Coniochaeta species from Iran

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Coniochaeta gamsii and *C. velutinosa* have been isolated from barley leaves, described and reported from Iran recently (Nova Hedwigia 82: 227–236). A new *Coniochaeta* species was recently isolated from pistachio (Pistacia vera) twigs from Tehran Province. The new taxon is closest to *C. gamsii* based on colony morphology, ascocarps, asci, ascospores and paraphyses. The major distinguishing feature is distinctive protruding ends of ascospores measuring up to 1.7–2.5 µm in length. They are also distinct by the size of ascocarps that is smaller in new *C*. sp.; absence of setae, peridium with a regular texture, shorter and wider asci and longer ascospores in *C. gamsii* are other distinguishing features. Crystals were not produced by *C. gamsii* but they were abundant in the new species obtained from pistachio twigs when grown on Leonian's agar. The anamorph state is Nodulisporium-like that is mainly characterized by proliferating conidiophores producing 2-3 conidiogenous loci and 2–3 blastoconidia (8–11 ? 2–3 mm) at the tips in sympodial succession with distinctive attenuated bases measuring 0.5–1.2 mm. In contrast blastoconidia in *C. gamsii* are mostly aggregated at the tips of conidiophores and have less distinctive attenuation at the bases.

PS1-97-0489

Molecular identification of fungi in rot types from logs in *Eucalyptus obliqua* forest in Tasmania.

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Biodiversity of decay fungi in Tasmania's eucalypt forests is of interest to conservation and land managers. Rot types have been characterised using simple criteria to assist rapid assessment of decay stages in logs in Tasmania's Eucalyptus obliqua forests. Fungi have been isolated from the different rot types and grouped based on morphology. In this study we aim to identify fungi from cultures and examine the feasibility of identifying fungi directly from rot samples using PCR and DNA sequencing. In the longer term, we plan to examine the correlation of fungal species with particular rot types.
DNA was extracted from mycelia and also directly from decayed wood. The ribosomal DNA internal transcribed spacers were amplified using primers ITS1-F and ITS4 and sequenced. Sequences were also obtained from fungal fruit-bodies. Public and private sequence databases were searched with the sequences derived from cultures and wood. Morphological groupings of cultures were largely supported by DNA sequence data. Database searches enabled identification usually to genus level, but occasionally a high percentage match indicative of con-specificity was obtained. Sequencing directly from decayed wood samples was successful in only a few samples, due to the presence of multiple fungi in the samples. Commonly isolated species include species of *Ganoderma, Phellinus* and *Postia*.
Detection of fungi by PCR and sequencing directly from decayed wood samples will avoid the laborious step of isolating and maintaining pure cultures. It is apparent, however, that a cloning step to separate the different fungal templates before sequencing is necessary in the majority of samples, and this is planned. The need for expansion of current databases to include sequences from a greater range of identified decay fungi, particularly of Australian taxa, is also highlighted. Broad identifications provided by lower DNA similarity enables the targeting of families and genera for more intensive sampling.

PS1-98-0490

Aschersonia badia from Thailand: one or more species?

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Petch (1921) monographed the genera *Hypocrella* and *Aschersonia* and recognised several species with brown-coloured stromata. Of these, *Aschersonia badia* was described from south east Asia and *Hypocrella palmicola* from Trinidad and Madagascar. Samples from numerous sites in Thailand matched with Petch's general description for *Aschersonia badia* although we recognised three major colour variants – yellow-brown, cream-brown and chocolate-brown. These three variants also produced Hypocrella states that produced whole ascospores as described for Hypocrella discoidea by Hywel-Jones & Evans (1993).
The complete ITS1&2 region (including the 5.8S gene) and partial b-tubulin sequences were used to construct phylogenies for Aschersonia species with whole-spored Hypocrella's. The ITS and b-tubulin phylogenies demonstrated clearly two sub-clades of the brown Aschersonia samples from Thailand. From these results we conclude that two distinct species are present in Thailand.
One of the clades produced cream-brown stromata and conidia 12-14 µm. A second clade had chocolate-brown stromata with conidia 5-6 µm matching a description by Petch (1921) for *Hypocrella palmicola*. Furthermore, Petch's description of the *Hypocrella* state indicated part-spores of 5-7 µm. Thai material of the small-spored *Aschersonia* produced and discharged whole ascospores. We conclude that a re-assessment is needed of Old World and New World species of *Aschersonia* and *Hypocrella* which produce brown stromata and *Hypocrella* states with whole ascospores.

PS1-99-0492

Phylogenetic analysis & identification of Antarctic microfungi by PCR of the ITS1, ITS2, mitochondrial small subunit rDNA & beta-tubulin gene

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Phylogenetic analysis was performed on various species of Antarctic microfungi utilizing the conserved regions of the highly evolving ITS1 and ITS2 as well as the Mitochondrial Small Subunit rDNA (mtSSU rDNA) and beta-tubulin gene. The study aims to characterize the microfungi based on the divergence of the proposed conserved genes as well as to detect polymorphism in the conserved genes. Established pure or single strain fungal cultures were isolated from various regions in the Antarctic. Soil samples were cultured and maintained on Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) according to the soil plate method of Warcup (1950). DNA extraction was performed using standard procedures followed by PCR amplification of the conserved genes. Primers used were as follows:- ITS1 region - ITS5-F & ITS2-R (White et al.,1990); ITS2 region – ITS86-F & ITS4 (Turenne et al.,1999 and Ferrer et al., 2001); mtSSU rDNA – MS1-F & MS2-R (White et al.,1990); beta-tubulin gene – T1-F & T22-R (White et al.,1990). PCR products were sequenced, aligned and used for phylogenetic tree construction. Polymorphism was imminent in all the conserved genes. The ITS1 and ITS2 showed gene sequences ranging from 250-350 bp in length showing divergence in both sequence and length of the gene. The polymorphic mtSSU rDNA was detected in Ascomycota 1, *Antarctomyces* sp., *Aureobasidium*-like sp., *Geomyces cretaceous*, *Mucor* sp., *Thelebolus sp., Trichosporiella cerebriformis* and Species 9 ranging from 600-700 bp in length. PCR amplification of the beta-tubulin genes showed 4 distinctive variations of the gene, namely 280 bp, 400 bp, 600 bp and 1500 bp thus prompting the speculation at least 4 different types of beta-tubulin genes occurring in the same genome. These were observed in *Ascomycota 1*, Antarctomyces sp., *Geomyces cretaceous*, *Thelebolus* sp. and *Trichosporiella cerebriformis* thus suggesting the occurence of random intron insertions or deletions in the gene.

PS1-101-0499

A five-gene phylogeny for *Hypocrella* and its anamorph *Aschersonia*

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The genus Hypocrella is the teleomorph of *Aschersonia* (Petch, 1921) About 400 isolates of *Hypocrella* and *Aschersonia* were collected from various sites in Thailand over a seventeen year period. There were eighteen taxa identified including: H. *discoidea*-A. *samoensis*; H. *raciborski*-A. *placenta*; H. *oxystoma*-A. *oxystoma*; H. *hypocreoidea*-A. *hypocreoidea. H. mollii*-A. *confluens*; A. *badia; H. reineckiana*-A. *marginata; A. cf samoensis; H. javanica*-A. *coffeae*; *H. schizostachyi* and *H. scutata*. The last two species are not known to produce an Aschersonia state in culture. However, they both produced a none-*Aschersonia* coelomycetous anamorph in culture.
A five gene phylogeny was constructed for representatives of these taxa (H. *scutata* was not available for study). The genus Hypocrella is split into two major clades: those that release whole ascospores and those that eject part-spores. The five-gene phylogeny clearly demonstrated a split between these two major groups. One group is centered on the type of the genus – H. *discoidea* – and also includes *A. badia* and *Aschersonia cf. samoensis*. The 'part-spore' group was further split into two sub-clades: flattened pale or whitish species (based on H. *raciborski, H. mollii* and *H. hypocreoidea*) and hard, hemispherical species (based on *H. javanica, H. reineckiana* and H. schizostachyi).
We conclude that Hypocrella forms a monophyletic clade which is sister to the plant-pathogenic Clavicipitaceae (including *Balansia, Claviceps* and *Epichloë*). The phylogeny does not however support the separation of *Hypocrella* based on whole ascospores versus part-spores. Furthermore, it does not support the separation of *Hypocrella* based on *Hypocrella schizostachyi* producing a non-coelomycetous anamorph in culture.

PS1-102-0505

Fungi isolated from an Attelabid beetle and its leaf roll

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Attelabid beetles are characterized by their elaborate oviposition behavior to construct leaf rolls (called 'cradles') in which the hatched larvae grow. The attelabids of the genus Euops have been considered to be strongly associated with fungi, because the female adults transport fungi in specialized organs termed mycangia, and fungi observed in their cradles are morphologically similar to the mycangial fungi. However, there are few reports about fungal isolation from both the mycangia and the cradles. In this study, species composition and frequency of occurrence of fungi associated with Euops splendidus VOSS were investigated.
Fungi were isolated from the mycangia of dispersing female adults, which were making their cradles in the fields, and the cradles at egg stage. These adults and cradles were collected in a stand of Reynoutria japonica SIEB. et ZUCC, which is one of the host plants of E. splendidus, in Toyota and Nagoya, Aichi Prefecture, central Japan. A total of 44 adult beetles and 47 cradles were used for isolations. Inocula were placed on potato-dextrose agar and malt agar and incubated at 25 C and 20 C in darkness, respectively.
Two *Penicilium* species, *Penicillium* sp. 1 and *Penicillium* sp. 2, dominated consistently in the isolates from the mycangia. It is noted that these two fungi never coexisted in a single adult. A number of fungal species were isolated from the cradles, and the species composition and frequency of occurrence of each fungi were varied among sites. However, *Penicillium* sp. 1 and *Penicillium* sp. 2 were also detected from the cradles, demonstrating that these two fungi were released from the mycangia and colonized in the cradles. As the result of fungal isolation from the mycangia, either *Penicillium* sp. 1 or *Penicillium* sp. 2 was found consistently in a single cradle. These results suggest that E. *splendidas* has a close association with the two *Penicilium* species. In addition, the species of the associated fungi may differ among individuals.

PS1-103-0510

Four species of Trichomycetes collected in Thailand

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A project study on fungal diversity in monsoon Asia has conducted in Thailand from 2003 to 2005. One of the purpose of that study was to record Trichomycetes (Zygomycota) in Thailand. There is little study on Trichomycetes in Thailand. Collection trips were held in 2003 and 2004 in northern part of Thailand. Trichomycetes is a group living in the gut tube of arthropods attaching its thalli to the lining. Insects were collected and were observed their guts to be found Trichomycetes after dissection. Four species of Trichomycetes were derived. *Harpella melusinae*, *Orphella haysii*, and both *Leidyomyces* sp. and *Enterobryus* sp. were derived from digestive tracts of black fly larvae (midgut), stonefly nymphs (hindgut) and adults of passalid beetle (hindgut), respectively. *Harpella melusinae* was the second record from Thailand. And the other two genera are new to Thailand. Larvae of black fly were collected from surface of submerged stones in streams in Mt. Doi Inthanon, and nymphs of stone flies were from between decayed leaves in a stream in Queen Sirikit Botanical Garden. *Leidyomyces* sp. was discovered from *Aceraius helferi* (Coleoptera: Passalidae) collected in Mt. Doi Sutep, Chiang Mai, A pair of the beetle was collected from a decayed log. Lining of the hindgut were observed after rinsing with distilled water. Several tufts of no-branching hyphae that were sharing a holdfast (anchor structure) were observed. This fungus was observed only at the former part of the hindgut. The tufts were consisted of over fifteen hyphae mass. This fungus was identified as a genus *Leidyomyces* by both its feature of holdfast morphology and living site of the gut. Diameter of the attaching part of holdfast was 11-25 um, length was 11-32 um. Hyphae were up to 750um. Thallus was septated and showed a series of cell. Each of them produced a cylindrical spore (primary infestation sporangiospore) inside; length x diameter= 26-41?14.5-18 (Ìm), ave.3 3.5?15.8 (Ìm), (N=16). The genus *Leidyomyces* is consisted of only one species *L. attenuatus*. The size of the spore of our specimens was larger comparing with that of *L. attenuatus*. The fourth species, Enterobryus sp., was also observed sharing the same hindgut with Leidyomyces. However, the attaching position to the gut of this *Enterobryus* sp. was at the latter part of the hindgut.

PS1-104-0537

Ceratocystis and *Ophiostoma* species associated with wounds on native South African trees

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Ceratocystis and *Ophiostoma* spp. are ascomycetes collectively referred to as Ophiostomatoid fungi. They are dispersed by insects and infect wounds on trees made or visited by these vectors. These fungi include many species of economic importance such as the Dutch elm disease fungi *O. ulmi* and *O. novo-ulmi* and *C. fimbriata*, which causes canker and die-back of many woody plants worldwide. There have been few reports of Ceratocystis and *Ophiostoma* spp. from South Africa, especially from native trees. In this study, a survey of *Ceratocystis* and *Ophiostoma* spp. associated with wounds on native tree genera in South Africa was conducted. Both morphological observations and DNA sequence comparisons using portions of the ITS, tubilin and elongation factor1-· gene regions were used to characterize the fungi. The pathogenicity of selected species was also assessed using artificial inoculation studies, under greenhouse conditions. *Ceratocystis* and *Ophiostoma* species were commonly isolated from wounds on most trees investigated. In many cases, they were associated with stain of the xylem tissue surrounding the wounds. *Pesotum quercus*, *P. fragrans, P. pluriannulatum, C. albifundus* as well as an undescribed *Ophiostoma* sp. and two undescribed *Ceratocystis* spp. were collected. In the pathogenicity tests one of the *Ceratocystis* sp. resulted in obvious lesions on Rapanea melanophloeos, while the other *Ceratocystis* sp. produced very small lesions on Acacia nigrescens and *Sclerocarya birrea* trees. This study provides concrete evidence that the diversity of the *Ceratocystis* and *Ophiostoma* spp. is incompletely understood in South Africa. The fact that potentially serious and previously unknown pathogens, have emerged from a relatively limited survey emphasizes the importance of continued surveys for these fungi in the country.

PS1-105-0545

Study on the double-stranded RNA elements in different isolates of *Rhizoctonia solani* AG-1 IA

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According to the result of our research that the genetic element with dsRNA may appear under a circum strand of high frequency. Besides, as what we find is that some fragments of dsRNA belong to *Rhizoctonia solani* will diminish the virulence, but another of it will enhance the virulence and the other seems to be no difference.
On the other hand, the size of the dsRNA are separated to be 2.0 kb, 2.5kb, 3.0kb, 14.0kb etc. and the point is that we find the dsRNA of 2.0kb are existed in Rs1E-2, Rs1F-1, Rs1H, Rs1M-3, Rs1N-2, Rh-Ta, PR-06, Rh-cabbage, Rh-Tsau(3) etc. Above of it, there are fifteen isolates, which is Rhizoctonia solani AG-1 IA, were collected from Taiwan, and after recognize of plant infected with 15 teat the result of that process is the same with the 15 tested isolates originally. Furthermore, the dsRNA of Rs1M-3 and PR-06 are found in the debris and microsomall fractions of cell.
The way by which we prove to confirm the dsRNA is to find that via serially transferred up to four times of subculture on PDA that there are Rs17 and Rs1M-3 which contain dsRNA stably. Finally, after anastomosis test show that 14.0Kb dsRNA of Rs17 and 2.0Kb dsRNA of Rs1M-3 could be the evident to prove that the 2.0kb dsRNA of Rs1M-3 could be transmitted by hyphal fusion. Although the amount of pigment and the number of sclerotia produced by six selected isolates with 2.0Kb dsRNA are minor than other two isolates without dsRNA, the growth rate of those isolate seems to be nothing sophisticated difference. The molecule weight of its coat protein is closed between 60 and 70kDa and the way is used by SDS-PAGE, and the dsRNA segments isolated from purified particles have the same weight with Rs1M-3.
Above all, the conclusion is that to have dsRNA of AG-1 will let it to be suffered of pigment and sclerotia. In depth, dsRNA are commonly associated with R. solani AG-1IA.

PS1-106-0547

Ophiostma spp. associated with wounds on native broad-leaved trees in Norway

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Ophiostoma spp. include some of the most important tree pathogens and many fungi that cause sap stain in lumber. Four anamorph genera including *Sporothrix, Leptographium, Pesotum*, and *Hyalorhinocladiella* are typically associated with *Ophiostoma* spp. These fungi infect wounds on trees and are often carried by insects that may also feed on them and on which they depend for their dispersal. Considerable information is available regarding Ophiostoma spp. on coniferous hosts in the Northern Hemisphere including Norway. However, very little research has been done regarding the occurrence of *Ophiostoma* spp. on native broad-leaved trees in Norway. In this study, a survey of wound-infecting *Ophiostoma* spp. on native broad-leaved trees was carried out in different geographical areas of Norway. Collecting sites stretched from Lyngdal in the south to Sørreisa in the north. Comparisons of DNA sequences from the ITS gene region were used to confirm the identity of the fungi collected. Many *Ophiostoma* spp. and especially their Pesotum anamorphs were common on wounds on the trees sampled. In most cases, they were associated with blue stain on trees such as *Betula pubescens*, *Populus tremula* and *Quercus robur*. Species including *P. quercus, P. catonianum* and *P. pluriannulatum* were consistently isolated. This is the first report of these fungi from these trees in Norway. These results suggest that the diversity of *Ophiostoma* spp. on broad-leaved trees is incompletely understood in the country.

PS1-107-0548

Two new ammonia fungi from Japan

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Ammonia fungi (Sagara, 1975) are known to form a fungal community that appears in disturbed conditions and also to appear restrictedly at sites of animal-related matter (carcass, dung) decomposed places. This group is suggested to be important in the ecosystem and is ubiquitously distributed in terrestrial places around the world. About 50 species have been recorded as ammonia fungi, but the species composition are different in each place. Our group investigated this fungal community for three reasons; to study species diversity (myco-flora, species composition) in different places, at different ecological sites of the world; the second reason is to study the biogeographical distribution of fungi because ammonia fungi are comparatively easy to detect from soil in artificial manner; the third reason is to study fungal species speciation through ammonia fungi, because this group occupies the special niche in the fungal community in ecosystem of the world and we can find different but related species composition set in different areas, suggesting that each community set is divaricated from each other. We have revealed hitherto that the biogeographical distribution is quite different between saprophytic and ectomycorrhizal fungi, i.e.; ectomycorrhizal ammonia fungi are distributed in close relationship with vegetation, such as Fagaceae or Pinaceae, but saprophytic fungi are distributed in a wider area and are less related with vegetation (Fukiharu and Horigome, 1996). We have also revealed that the ammonia fungus, *Coprinopsis phlyctidosora* is species complex, i.e.; this species has been believed to be a cosmopolitan species for a long time but our study of ammonia fungi revealed that this species separated into two groups, those distributed in the Southern and Northern Hemispheres, and the Southern Hemisphere group is a cryptic species (Suzuki et al, 2002). In this study, we found two new ammonia fungi, *Coprinopsis* sp. from Okinawa, southern part of Japan, and *Panaeolina* sp. from Hokkaido, northern part of Japan, and we show the details of these species in this presentation.

PS1-108-0550

Molecular phylogeny and morphology of aero-aquatic fungi, *Diplocladiella* and *Candelabrum*

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Aero-aquatic fungi are characterized by their growth of vegetative mycelia under water and formation of conidia with a special flotation forms only when the substrate, in which they grow, is exposed to a moist atmosphere (van Beverwijk, 1953; Fisher, 1977). Though the teleomorphs of some members of aero-aquatic fungi were revealed to be Pezizomycetidae ascomycetes, phylogenetic positions of this ecological group of fungi have not been well explored. In this study, we investigated the taxonomy and phylogeny of *Diplocladiella* and *Candelabrum*, which are frequently isolated from the submerged twigs or litters in?Japan, based on the sequences of 18S rDNA and D1/D2 region of 28S rDNA. In addition, the conidia produced?in culture or on natural substrates were observed with the light microscope and SEM for morphological studies.
The results suggest that the genus *Diplocladiella* is an anamorph with affinity to Dothideomycetidae and that two species are included in our isolates ; one is a new species, which is morphologically distinguishable in its smaller conidia from other known species, and the other is *D. alta*, which was isolated newly from Japan. Concerning *Candelabrum*, phylogenetic trees revealed that the genus is polyphyletic, having two different lineages in Ascomycota. One group including the type species, *C. spinulosum* clustered within the Leotiomycetidae (Helotiales) clade and the other group with *C. brocchiatum* nested in the Sordariomycetidae clade. A new genus should be established for the latter group on the basis of the differences from the *C. spinulosum* group in phylogeny and in the morphology of conidia, especially their shape and mode of branching. The *C. spinulosum* group (true *Candelabrum*) produces conidia with an H-shaped or clover-shaped basal plate from which branches come out, while those of the C. *brocchiatum* group lack the basal plate. The molecular data indicates two of our isolates belonging to the *C. brocchiatum* group are to be classified into a new species, which is also distinguishable in conidium morphology from *C. brocchiatum* and C. *microsporum*.

PS1-109-0554

Molecular analysis of bacterial communities in lichen thalli

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The diversity of lichenized ascomycetes is well known, but the occurrence of non-photosynthetic bacteria in long-living and stress-tolerant lichen thalli is still under-explored. In our preliminary work, we investigated the bacterial communities associated with 11 different lichen samples (belonging to 8 different species) from different habitats. The culturable aerobic-heterotrophic fraction of the bacterial communities was isolated from 9 lichen samples on protein-rich and sugar-rich/N-free media. Three genera of *Firmicutes*, 4 of Actinobacteria and 3 of Proteobacteria were identified, while 2 phylotypes, belonging to the phyla Actinobacteria and Proteobacteria, respectively, were not identified at genus level. Some bacterial taxa were retrieved frequently in different lichen species sampled in the same or different sites. *Paenibacillus* and *Burkholderia* strains seem to be common in lichens. In a cultivation-independent approach, total DNA was extracted from 11 lichen samples. Molecular fingerprints of the bacterial communities were obtained by PCR-amplification of the ITS region, and sequencing of selected bands indicated the presence of further bacteria. These initial results show that diverse bacteria are present in lichen thalli. This includes some bacteria already found in other fungal-bacterial associations as well as unknown and new lineages.

PS1-110-0555

Taxonomic revision of the genus *Sticta* (Lobariaceae, Lichenized Ascomycota) in East Asia

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The genus *Sticta* is one of the commonest foliose lichens, which is characterized by the tomentose lower cortex with cyphellae. The purpose of this study is to make a taxonomic revision to the genus *Sticta* in East Asia on the basis of morphological, anatomical, and chemical studies. We also carried out the phylogenetic analyses based on the sequences of the internal transcribed spacer (ITS) regions of the nuclear rDNA for clarified the phylogenetic relationships among the species of the genus *Sticta*, and the relationships between the genus *Sticta* and its allied genera *Dendriscocaulon, Lobaria, Pseudocyphellaria* belonging to the family Lobariaceae. As a result of this study, eleven species were proved to be distributed in East Asia. Characters of high taxonomic importance were recognized in the following morphological and anatomical elements: (1) the cortex tissues, (2) the margins of cyphellae, (3) the apothecia, (4) the thalloid exciples, (5) the ascospores, (6) the soredia, and (7) the isidia. Majority-rule consensus tree showed three distinct clades within the genus Sticta: *S. gracilis clade*, *S. wrightii* clade, and *S. nylanderiana* clade. Each of the clade had common morphological character states of the cortex tissues, the margins of cyphellae, the thalloid exciples, and the ascospores. The species included in *S. gracilis* clade had the following common morphological character states (1) the paraplectenchymatous upper cortex, (2) the cyphellae with prominent margins, (3) the sessile apothecia, (4) the absence of algal cells in thalloid exciples and subhypothecial medulla, and (5) the ellipsoid to fusiform ascospores. The species included in *S. wrightii* clade had the following common morphological character states (1) the paraplectenchymatous to scleroplectenchymatous upper cortex, (2) the cyphellae with non-prominent margins, (3) the stalked apothecia, (4) presence of algal cells in the thalloid exciples and subhypothecial medulla, and (5) the acicular to linear ascospores. The species included in *S. nylanderiana* clade had the following common morphological character states (1) the paraplectenchymatous to scleroplectenchymatous upper cortex, (2) the cyphellae with non-prominent margins, (3) the sessile apothecia, (4) the presence of algal cells in thalloid exciples and the absence of the algal cells in subhypothecial medulla, and (5) the acicular to linear ascospores. The four morphological characters: cortex tissues, the margins of cyphella, the thalloid exciples, and the ascospores were considered to be important characters which were reflected in the phylogeny.

PS1-112-0595

Genomic and EST sequencing of *Mycosphaerella* species will permit comparative analyses with related fungi and anamorphs

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Mycosphaerella and its associated anamorphs form one of the largest phylogenetically distinct groups of plant pathogenic fungi. A few species in this group also cause disease in humans and other vertebrates. Understanding the ecology and pathology of members of the genus *Mycosphaerella* will be greatly facilitated by a comparative genomics approach. To further this goal, two species of *Mycosphaerella, M. graminicola* and *M. fijiensis*, were selected by the International *Mycosphaerella* Genomics Consortium for complete genome sequencing. These species were chosen because they are two of the most economically significant pathogens of wheat and banana/plantain, respectively, and affect the global economy by causing huge losses annually to growers of these crops worldwide. A joint project between the USDA-ARS/Purdue University and Plant Research International B.V. was initiated to sequence both genomes, along with 40,000 ESTs from both *M. fijiensis* and the related maize pathogen Cercospora zeae-maydis. The work was conducted through the Community Sequencing Program sponsored by the U.S. DOE-Joint Genome Institute. The initial goals of the project are to: assemble 8¥ genomic shotgun sequences of *M. graminicola* strain IPO323 and *M. fijiensis* strain CIRAD 86; perform automated annotations of these genomic sequences as well as directed annotations using the 80,000 ESTs from *M. fijiensis* and C. zeae-maydis plus 37,000 ESTs from *M. graminicola* that will be made available by Syngenta; and make these sequences available publicly through a series of linked web sites for comparative analyses. Currently, the *8¥ M. graminicola* sequencing is complete and the raw data have been deposited in the trace archive at NCBI (SPECIES_CODE = 'MYCOSPHAERELLA GRAMINICOLA IPO323'). The 518,271 traces were assembled at JGI into 1008 contigs spanning 38.72 Mb, with an N50 contig number of 64 and an N50 contig size of 198 kb. These contigs were assembled into 129 scaffolds covering 41.86 Mb, so the genome size is slightly larger than estimated previously. The N50 scaffold number was 6 and the N50 scaffold size was 2.44 Mb, so at least half of the bases in the assembly are in a scaffold of that size or larger. A draft assembly of 43,962 bases appears to cover the complete mitochondrial genome. A community-wide effort for annotation culminating in an annotation jamboree will be open to all interested participants. This project will be coordinated with sequencing efforts being planned for other species of *Mycosphaerella* and its relatives to greatly increase the power of future comparative genomics analyses.

PS1-113-0604

Evolutionary patterns in *Microbotryum* - biological implications for basidiomycetous plant parasites

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The genus *Microbotryum* (Basidiomycota, Pucciniomycotina) comprises smut fungi that parasitise members of several eudicot host families. Sorus formation is often located in the inflorescence of the host, but also occurs in leafs and stems. The most prominent members in this group are the anther smuts of Caryophyllaceae, but parasitism also occurs on other families, e.g. Polygonaceae, Dipsacaceae, Asteraceae. Questions adressed in our study concerned species and genus delimitations, coevolution with hosts as well as the distribution of non-molecular characters in the group.
Based on molecular characters we inferred phylogenetic trees of the family of Microbotryaceae, the genus *Microbotryum* and the anther smuts. To conduct these studies the ITS and LSU regions of the rDNA were sequenced and analysed with several methods.
The Microbotryaceae were split in at least four distinct groups. *Caryophyllaceous anther* smuts thereby grouped into a monophyletic clade, and parasitism on Polygonaceae was shown to be the ancestral state of the genus *Microbotryum*. A second group of anther smuts was revealed, containing parasites on Dipsacaceae, Lamiaceae, and Lentibulariaceae. Combination of the molecular phylogenies with non-molecular data helped to understand the lineages of *Microbotryum* and led to the creation of some new taxa. We provide a conception of the species limits of anther smuts. To gain insight into the evolutionary processes that might contribute to the observed phylogenetic pattern we conducted more detailed analyses on closely related lineages in the caryophyllaceous anther smuts. Thus, *Microbotryum* may serve as a model for basidiomycetous plant parasites.

PS1-114-0615

Ceratocystiopsis and *Grosmannia* distinguished from *Ophiostoma* sensu stricto based on multigene phylogenies

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Many different classification schemes have been applied to species of *Ophiostoma* and these have typically utilised morphological characteristics of the teleomorph and anamorph states. Recent DNA sequence comparisons have shown that *Ophiostoma* sensu lato is strongly polyphyletic and comprised of different phylogenetic groups. In this study, DNA sequence data from combined partial nuclear LSU and ,-tubulin gene regions were used to consider the phylogenetic relationships of 50 *Ophiostoma* species, representing all the major morphological groups found in the genus. The data showed three well-supported, monophyletic lineages in *Ophiostoma*. Species with *Leptographium* anamorphs grouped together and the teleomorph-genus *Grosmannia* (type species G. penicillata), including 27 species and 24 new combinations has been re-instated to accommodate these species. Species that are cycloheximide-sensitive with short perithecial necks, falcate ascospores and Hyalorhinocladiella anamorphs formed another well-defined lineage. For this group, the teleomorph-genus *Ceratocystiopis* (type species *O. minuta*), including 11 species and three new combinations has been re-instated. A third group included species with either *Sporothrix* or *Pesotum* anamorphs. This group also included *O. piliferum*, the type species of *Ophiostoma*, and thus represents *Ophiostoma* sensu stricto. *Ophiostoma* has been redefined to reflect the changes resulting from new combinations in *Grosmannia* and *Ceratocystiopsis*. Our data have also revealed additional lineages in *Ophiostoma* linked to morphological characters. However, these species have been retained in *Ophiostoma* in anticipation of additional data for a larger number of species to confirm monophyly of the lineages.

PS1-115-0616

Identification of *Paecilomyces* spp. from wooden utility poles in South Africa

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The genus *Paecilomyces* section Paecilomyces includes species that are thermophilic or thermotolerant. These properties make it possible for some *Paecilomyces* spp. to survive in wood during the kiln-drying process. Some species of these fungi are also known to be tolerant to commonly used preservatives such as CCA, creosote and copper sulphate, applied to protect utility poles against fungal decay. Apart from degrading these compounds, some *Paecilomyces* spp. are also capable of producing soft rot cavities in wood. This type of decay can cause failure of the sapwood, thus dramatically decreasing the lifespan of utility poles. An extensive survey to better understand the failure of wooden utility poles in South Africa, yielded more isolates of *Paecilomyces* spp. than any other fungi collected. In this study, we identified the 172 Paecilomyces isolates collected during the survey. Based on morphology isolates could be assigned to three groups. Sequences of parts of the ITS and ,-tubulin gene regions of isolates selected from each of these groups were compared, revealing that at least five species were present. A PCR-RFLP method was then developed to rapidly distinguish between the 172 isolates. The PCR-RFLP results made it possible to assign all of the isolates to one of the five taxa revealed by DNA sequence comparisons. Further comparisons with published DNA sequences and species descriptions, confirmed that one of the species was the anamorph of *Byssochlamys laguncularia*e. Other species may represent new taxa and they are in the process of being fully identified. Laboratory tests have shown that these fungi only cause low levels of wood decay. However, they appear to play an important role in the degradation of preservatives applied to protect utility poles, and thus exposing the wood to other decay fungi.

PS1-116-0617

Ophiostoma spp. associated with *Scolytus ratzeburgii* infesting birch in Finland and Russia

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Several elm-infesting bark beetles belonging to the genus *Scolytus* (Scolytinae, *Coleoptera*) are vectors of *Ophiostoma* spp., most noteably the Dutch elms disease fungi, *Ophiostoma* ulmi and O. *novo-ulmi*. A less well known *Scolytus* species, *S. ratzeburgii*, attacks stressed or dying *Betula pendula*. Nothing is known regarding fungal associates of this beetle species. A preliminary survey was, therefore, conducted to determine whether *Ophiostoma* spp. are associated with *S. ratzeburgii*. Twelve galleries of *S. ratzeburgi*i were collected from birch logs in Finland, and incubated to allow fungi to sporulate. Spore masses from fruiting structures formed in the galleries and were transferred to malt extract agar containing cycloheximide. At the time of collection, living male and female beetles were taken from the galleries and squashed onto the same medium, selective for *Ophiostoma spp*. Living beetles obtained from birch in Russia, were treated in the same way. Resulting isolates were purified and grouped according to morphology. Partial ribosomal DNA sequences (ITS 1, 5.8S & ITS2) were determined for isolates representing each morphological group. Fungi resembling the *Pesotum* anamorphs of *Ophiostoma* were found in all the galleries and from all beetles. The cultures could be divided in three different morphological groups. ITS sequences confirmed that two isolates represented the anamorphs of *Ophiostoma quercus* and *O. catonianum*, respectively. However, both these species were isolated only once from each of two separate galleries. A third *Pesotum* species was isolated from all the galleries and all of the beetles considered in the study. Phylogenetic analysis showed that these isolates are related to, but clearly distinct from, hardwood species in the *Ophiostoma* piceae-complex, which includes the Dutch Elm disease fungi. This fungus appears to represent an undescribed species of *Ophiostoma.* The possible pathogenicity of the fungus, as well as, the nature of the apparent symbiosis between *S. ratzeburgii* and this *Ophiostoma* species, remains to be determined.

PS1-117-0624

NMR spectroscopy: a tool for rapid yeast characterisation and screening.

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Screening and identification of large numbers of yeast is essential for industrial, environmental and clinical applications. Increasing numbers of taxa and characters for their distinction are demanding on data management. Utilization of digitized data is of advantage for the characterization and identification of yeast.

NMR spectroscopy of whole cells has proven to be a rapid and robust technique to assess the phenotype of yeast [1]. These digital data allow the simultaneous determination of a large range of chemicals, the screening for particular metabolites and rapid assessment of the metabolom. The aim of this study was:

(1) to evaluate a hierarchical statistical classification strategy for a broad and extendable NMR-based identification and

(2) to evaluate NMR for screening of unknown, environmental yeast isolates.

A total of 1274 yeast isolates included the species *Candida albicans, C.dubliniensis, C.glabrata, C.parapsilosis, C.tropicalis, Clavispora lusitaniae, Cryptococcus neoformans, C.gattii, C.laurentius, C.humicolus, Issatchenkia orientalis, Pichia guillerimondii, Yarrowia lipolytica* and others.

NMR spectroscopy was performed on cell suspensions. A hierarchical identification system for clinical isolates was developed based on pair-wise classifiers [1, 2] that considered the taxonomic levels shown in Fig.1. This system was tested against pair-wise classifiers comparing all possible species combination seen in Fig.1 (requiring the development of an unfeasibly large number of 55 classifiers for the 11 taxa).

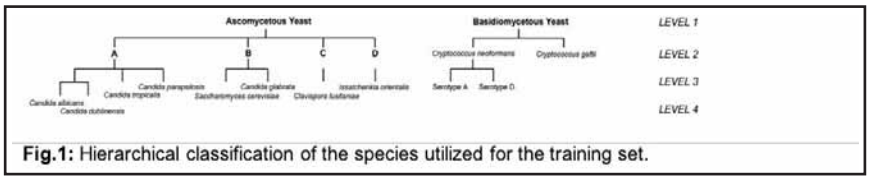

Fig.1: Hierarchical classification of the species utilized for the training set.

Fig.1: Hierarchical classification of the species utilized for the training set.

Similar accuracies were achieved when the conventional pair-wise classification (97% agreement with molecular identification) was compared with the hierarchical classification shown in Fig.1 (13 pair-wise classifiers, 95% agreement with molecular identification). The hierarchical system was also tested against species not included in the test set, but belonging to one of the "higher" taxonomic levels. In 90% of these cases, the respective isolates were assigned to the correct taxon, proving that this approach allows correct classification of species that were not part of the training process.

Further exploiting the potential of NMR, spectra of physiologically identical isolates of the genus Metschnikowia were compared by cluster analysis. Two well-separated clusters indicated the existence of distinct taxa, which were later confirmed by molecular tests, indicating the value of NMR spectroscopy for rapid screening in microbiology.

Statistical classification of NMR spectra is a suitable technique for rapid, robust and potentially automated characterisation and identification of yeasts that are otherwise difficult to distinguish.

[1] Himmelreich et al. Appl Environ Microbiol (2003) 69:4566-4574.

[2] Somorjai et al. in Artificial Intelligence Meth. & Tools for Syst. Biol. (2004) 5:67-85.

PS1-119-0642

Molecular characterization of *Fusarium commune* and F. *oxysporum* from a conifer nursery

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Root-rot disease caused by *Fusarium* spp. can cause severe loses in conifer nurseries. These fungi commonly occur in container and bareroot nurseries on healthy and diseased seedlings, conifer seeds, and in nursery soils. Over 100 isolates with F. *oxysporum*-like morphology were collected from a conifer nursery, Idaho, USA were selected. Two sets of isolates were used for molecular characterization. The first set comprised 32 isolates that were previously evaluated for pathogenicity (highly virulent vs. non-pathogenic) on Douglas-fir germinants in laboratory tests. The second set comprised isolates that were not tested for pathogenicity. Both sets of isolates were characterized by Amplified Fragment Length Polymorphism (AFLP) and DNA sequences (mitochondrial small subunit and nuclear translation elongation factor 1 alpha). Each isolate had a unique AFLP phenotype. A cluster analysis using AFLP data identified two groups of isolates. Group 1 isolates included all of the non-pathogenic isolates, whereas Group 2 isolates contained all of the highly virulent isolates. Phylogenetic analyses from DNA sequences showed very similar results with AFLP cladograms. Several isolates from the Group 2, including highly virulent isolates, shared identical sequence types with F. commune, a recently described species that is closely related to *F. oxysporum* and *Gibberella fujikuroi* species complexes. Phylogenetic evidence strongly suggests that these isolates are F. commune, but morphology of this group appears indistinguishable from *F. oxysporum*. Because morphological traits are often unreliable to identify Fusarium spp., we consider Group 2 isolates to be F. commune. This is the first report showing *F. commune* is associated with disease. For further validation, we are conducting pathogenicity tests for several Group 1 and 2 isolates that were previously uncharacterized for pathogenicity. Several AFLP genetic markers and DNA sequences offer potential as diagnostic markers to detect *F. commune* populations that are pathogenic within forest nurseries.

PS1-121-0660

Taxonomic Revision of *Cryphonectria*

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The taxonomy of *Cryphonectria* (Diaporthales) has been substantially revised during the course of the past few years. DNA sequence comparisons of different *Cryphonectria* species have revealed that *Cryphonectria* includes several genera that can be distinguished from each other based on robust and diverse morphological characteristics. Hence *Chrysoporthe* has been described for *Cryphonectria cubensis, Rostraureum for Cryphonectria longirostris*, Microthia for *Cryphonectria havanensis*, *Amphilogia* for *Cryphonectria gyrosa,* and *Holocryphia for Cryphonectria eucalypti*. The use of the name *Cryphonectria* is restricted to *Cryphonectria parasitica*, *Cryphonectria radicalis*, *Cryphonectria nitschkei* and *Cryphonectria macrospora*. Several newly discovered fungi have also been described in new genera. These include *Aurapex* and *Ursicollum*. *Cryphonectria* and allied genera are all united in the Cryphonectriaceae, a family of the Diaporthales recently described for the group. It is hoped that this substantially revised taxonomic scheme, based on extensive DNA sequence data sets and morphological comparisons, will aid mycologists and plant pathologists in the identification of the various genera and species in this group of fungi, which include some of the most important tree pathogens in the world.

PS1-122-0692

Characterization of *Septoria* using morphological, cultural and sequence data of the ITS region and partial calmodulin, actin and elongation factor-1 alpha genes

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Thousands of species have been described in the anamorph genus *Septoria* Sacc. Most are weak pathogens causing spots on leaves or stems. Taxa are mainly defined based on morphological characters of the conidiomata and conidia, and the identity of the host plant. Apart from some economically important species, very few *Septoria* sepecies have ever been studied in vitro, and isolates are difficult to keep viable. As a consequence, very little is known about the morphological variation in culture, and teleomorph connections. Sequence data are also scarcely available but will be of crucial importance for the purpose of corroborating currently used taxonomic concepts and the development of rapid and reliable identification tools. Furthermore, these data could be instrumental in research on behaviour of *Septoria* species in local flora's and assessment of risks imposed by these species on introduced crops. A phylogenetic study of *Septoria* species based on sequences of the 5.8 S rRNA gene and internal transcribed spacer (ITS) 1 and 2 showed that they group within the Mycosphaerella clade. Further work on a number of isolates identified to different species based on morphological characters and hosts, revealed (nearly) identical ITS sequences, indicating their very recent evolution. In order to test these species concepts and to find adequate molecular markers for their possible identification, we sequenced elongation factor 1-alpha (EF-1), calmodulin (Cal) and actin (Act) genes, using three groups of Septoria strains with 100% within-group ITS sequence homology, in total representing 40 morphological species.
Act sequences were sufficiently divergent to disciminate most of the species, with intraspecific polymorphism in some, viz., *S. chrysanthemella, S. galeopsidis* and S. *lamiicola*. Species could also be well resolved by EF-1 in two of the ITS groups (88, and 87 % minimal EF sequence homology for ITS group I and III, respectively). However, isolates of ITS group II identified as *S. taraxaci, S. calendulae, S. epilobii* and *S. stachydis*, could not be discriminated by EF-1 (100% homologous). Cal sequences also showed highest variation in isolates of ITS group III (around 75% min. homology), while those in ITS II showed least variation (95% min. homology). The variation in EF-1, Cal and Act provides additional support for the delimitation of most of the investigated *Septoria* species. However, the intraspecific variation observed indicates that more isolates need to be studied and additional loci investigated before more decisive conclusions can be drawn about the value of the present loci for delimiting natural entities in *Septoria*.

PS1-123-0694

TAXONOMY and phylogeny of *Entoloma* (Agaricales, Basidiomycetes) in Tasmania

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Intensive collecting of Entolomataceae (Agaricales) commenced in Tasmania in 1998 (G.G.), resulting in over 2000 collections, many of them representing undescribed species. Careful examination of the morphological characters and comparison with described species in the literature leads to the identification of many new species. The *Entoloma* flora of Tasmania has a characteristic composition with some rather enigmatic species that are difficult to classify using the current taxonomic framework. As such, Tasmania may well be considered a 'hotspot' for the genus. Character combinations occur which are unusual for this relatively well-known genus worldwide. Phylogenetic studies using molecular markers reveal that for some clades the distinctive morphology of the Tasmanian species adds considerably to our knowledge of the infrageneric classification of the genus and throws new light on the value of certain morphological characters and character states for classification purposes.

PS1-124-0698

A New Species of the Genus *Aprosporella* Parasitic on a Oriental Orchid, *Cymbidium kanran*

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Dark brown lesions appeared on foliar sheaths of an oriental orchid, *Cymbidium kanran,* and the diseased buds and young leaves were rapidly blighted. Fungal isolates from the lesions grew on potato dextrose agar (PDA) at 10-35 °C and optimum at 27-30 °C. The isolates produced blackish brown to black colonies consisted of dense mycelia on PDA, and formed scattered and solitary pycnidia on potato carrot agar (PCA). Pycnidia were subspherical to flask-shaped, black, uniloculed, 1-6 ostiolate, with papillate ostioles, with wall consisted of multi-layered cells, and 585-680 ? 520-560 µm. Conidia were holoblastic, solitary on the tips of cylindrical conidiogenous cells, which elongated from the most inner layer of pycnidial wall, obovoid to ellipsoid, hyaline, with many guttles at immature stages, becoming black, with papillate and pale brown bases, truncate at their bases, densely echinulate on their surfaces, and 46-62 ? 28-36 µm. Paraphyses were present among conidia. No teleomorph of the fungus was confirmed on the host or the artificial media, PDA and PCA. This fungus was judged as a new species of the genus *Aplosporella* on the basis of its morphology. The symptoms were reproduced by inoculation with the isolates. We propose that the disease is called as bud rot of *D. kanran*. In addition, the present fungus was found to attack *Cymbidium faberi* (cvs. Soshin group) too. Virulence of the fungus to other *Cymbidium* sp. and *Calanthe discolour* was not detected.

PS1-125-0699

A New Species of the Genus *Pseudocercospora* Isolated from *Dendrobium phalaenopsis*

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Spotting and blight of leaves on a orchidaceous plant, *Dendrobium phalaenopsis* were found in a greenhouse in Kagawa prefecture, Japan, in December 1997. Chlorosis to yellowing developing from lower leaves to upper ones occurred, and then light brown lesions with many small depressed hollows appeared on their leaves. The diseased leaves gradually turned grayish black, resulting in early desiccation and defoliation. A lot of small blackish granules appeared on reverse surface of the blighted leaves. When the defoliated leaves with those granules were kept in moist conditions, a dark mold grew up so densely from every granule. Reverse surface of their leaves looks very sooty. The granules were stromata with conidiophores and conidia of the pathogen. Conidiophores were fasciculate on stromata formed beneath stomata, cylindrical, light grayish brown, 6-10 septate, geniculate, occasionally branched, rounded and hyaline at the tips, with inconspicuous scars of conidial formation, 100-220 3.4-4.8 µm. Conidia were slender obclavate, pale olivaceous, straight or slightly curved, 1-5 septate, truncate and thin at the bases, pointed at the tips, smooth, 66-114 µm in length and 3.4-4.2 µm in width. This fungus was judged as a new species of the genus *Pseudocercospora* on the basis of the morphological characteristics, which was different from species of *Pseudocercospora* and *Cercospora* reported already as parasites on Dendrobium spp. Single spore isolates from conidia on the lesions grew slowly on potato dextrose agar at 10 to 30°C and optimum at 23 to 27°C. The symptoms were reproduced by inoculation with mycelial or conidial suspensions of the isolates to *D. pharaenopsis* leaves, and the isolates were re-isolated repeatedly from diseased plants inoculated. We, therefore, proposed to coin sooty leaf blight for the present new disease on *Dendrobium*.

P1-26- 0711

Molecular evolution and ecology of the basidiomycete genus *Calostoma* (Sclerodermatineae, Boletales).

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The Sclerodermatineae is a suborder of basidiomycetes within the Boletales that includes gasteroid genera with a wide array of fruiting morphologies. *Calostoma* is one of the more peculiar genera, forming fruiting bodies with gelatinized tissues and brightly colored peristome. The most recent study of the phylogeny of the Sclerodermatineae used 28S rDNA sequences and identified a relationship between morphologically diverse genera such as *Calostoma*, the earthstar like *Astraeus*, and the boletoid *Gyroporus*. However, some reports suggested that *Calostoma* is saprotrophic, which is inconsistent with its hypothesized relationship to the largely mycorrhizal Boletales. The goals of this study are: 1) Investigate the ecological role of *Calostom*a using a combination of isotopic and molecular tools. 2) Evaluate and establish the evolutionary relationships between *Calostoma* and its Sclerodermatineae relatives.
1) Molecular analysis of two *Calostoma* species (*C. sarasinii* from Malaysia and *C. cinnabarinum* from Eastern North America) are being performed using fungal and basidiomycete-specific primers. Isotopic profiles of d13C and d15N will be performed on both species and other mycorrhizal and wood/litter-decomposing fungi from their respective locales. These profiles will be used to infer the Calostoma species' mode of nutrient acquisition. The above methods have already determined *C. cinnabarinum* to be ectomycorrhizal. The mantle of *C. cinnabarinum* root tips form a gelatinous cuticle as well as gelatinous rhizomorphs. These characters have been found on ectomycorrhizae from soil cores collected beneath *C. sarasinii* from Malaysia. Isotopic and molecular analyses of *C. sarasinii* and *C. cinnabarinum* are currently in progress.
2) The evolution of *Calostoma* will be evaluated using a combination of nuclear ribosomal large subunit (LSU), RNA polymerase II subunit 1 (RPB1), and RNA polymerase II subunit 2 (RPB2). This study will also use a broader sampling of Sclerodermatineae taxa compared to previous analyses. Another goal of this study is to attempt to resolve the relationships among the Sclerodermatineae genera in order to generate a clearer picture of character evolution within the group. Preliminary analysis using 28S rDNA shows *Calostoma* is a monophyletic group within the Sclerodermatineae. This result is shared in separate RPB1 and RPB2 analyses with smaller data sets. None of these analyses however identify the sister group to *Calostoma*, nor clearly resolve the relationships between the genera. A broader taxon sampling in combination with ribosomal and protein coding genes, which is in progress in our laboratory, will help resolve these questions.

PS1-127-0723

A new species of *Rhizopus* from Iran

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A new species of *Rhizopus* is reported from Iran. An isolate was recovered from a fig fruit (*Ficus carica L.*) from Tehran, Iran. Major characteristics of this fungus, which distinguish this species from all members of the genus Rhizopus are: brownish black sporangia of various sizes (30–375 µm diam.), sporangiophores of two types (short 100–400 µm and long 750–2325 µm in length) arising directly from simple rhizoids or sometimes from weakly developed stolons (both types of sporangiophores are mostly recurved and the long ones are up to 66 µm wide), columella is variable in shape and size (from applanate, 22.5 v 25 µm, on short sporangiophores to strongly pyriform, 130 v 157 µm, on long sporangiophores), sporangiospores are variable in size (from 5 to 20 µm diam) and shape (fusiform, ellipsoidal to irregular), and finally the maximum growth temperature of this fungus that is 40°C. The most closely related taxon to this one is *R. stolonifer* (Ehrenb. : Fr.) Vuill. var. *lyococcos* (Ehrenb. : Fr.) Stalpers & Schipper (Studies in Mycology 25: 1–34, 1984). *R. stolonifer* var. *lyococcos* produces only one type of recurved sporangiophores (measuring up to 450 µm long), with an evident apophysis, smaller sporangia (80–240 µm), different columellae (cylindrical to obovoid) and its maximum growth temperature that is 36°C.

PS1-128-0728

Genetic diversity of *Rhizoctonia* species revealed by ITS sequencing

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Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a anamorphic plant pathogenic basidiomycetous fungus with a wide host range, including more than 500 genera of higher plants. Species cause disease in several important crops worldwide infecting the seeds, roots, leaves, stems and fruits. The genetic diversity of 274 isolates from *Rhizoctonia* species collected worldwide was characterized using ITS-5.8SrDNA-ITS2 sequence analysis to assess the degree of genetic variability within and among anastomosis groups (AGs). This included tester isolates of the respective AGs. Within the ITS tree, there was support for the separation of nine major groups. In general, the clustering of the *Rhizoctonia* isolates agreed with previously determined anastomosis groups. Nucleotide sequence similarity between isolates from the same host, but from different geographic origin was high.

PS1-129-0742

Molecular genetic classification of edible mushroom *Pleurotus eryngii* cultivated in Korea

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Verification of *Pleurotus eryngii* strains in Korean farm field has been accessed by using an internal transcribed spacer (ITS) sequence analysis and a random amplified polymorphic DNA (RAPD) fingerprinting. Sequence analysis of ITS1-5.8s rDNA-ITS2 regions of 24 strains of *Pleurotus* species, consisted of 22 strains of *P. eryngii* and control strains of each *P. ostreatus* and *P. ferulae,* demonstrated that the DNA regions shares mostly 99% sequence identity, indicating that the sequence-based analysis is not applicable for the verification of closely related mushroom strains. To verify the mushroom strains using RAPD method, we amplified DNA fragments from the chromosomes of 24 mushroom strains with 18 different random primers, yielding 538 distinct DNA fragments with the size ranging approx. from 200bp to 4000bp. Analysis of the DNA fragment pattern of mushroom strains showed that the 22 *P. eryngii* were clearly differentiated from their cousin *P. ostreatus* and *P. ferulae*, and could be categorized into 5 subgroups. Subsequent physiological studies on the development of fruiting bodies demonstrated the close correlation of the RAPD-based grouping with the phenotypical characteristics of mushroom fruiting bodies.

PS1-130-0779

Two novel ballistoconidia-producing yeasts species of the *Sporidiobolales* from aquatic environments in Patagonia, Argentina

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During a survey of carotenogenic yeasts carried out in north-western Patagonia (Argentina), several ballistoconidia-producing strains belonging to the order Sporidiobolales were isolated from aquatic environments. Five strains were found to represent two novel species, for which the names *Sporidiobolus longiusculus* and *Sporobolomyces patagonicus* are proposed, with CBS 9654T (=PYCC 5818T=CRUB 1044T) and CBS 9657T (=PYCC 5817T=CRUB 1038T) as the type strains, respectively. The elongated basidia, which are five to six times longer that those of the remaining species of the genus Sporidiobolus, are a particular micromorphological feature of *Sporidiobolus longiusculus*. On the basis of the sequences of the D1/D2 domains of the 26S rRNA gene, the species most closely related to Sporidiobolus *longiusculus* is *Sporobolomyces bannaensis*, whereas *Sporobolomyces marcillae* is the closest relative of *Sporobolomyces patagonicus*. Complete internal transcribed spacer sequence analysis confirmed the separate position of *Sporidiobolus longiusculus*, whereas for *Sporobolomyces patagonicus* no nucleotide differences were found with respect to *Sporidiobolus pararoseus* CBS 491T. Negative mating experiments between strains of *Sporobolomyces patagonicus* and strains of *Sporidiobolus pararoseus* together with the low DNA–DNA reassociation values for the type strains of the two species validated the proposal of *Sporobolomyces patagonicus* as a distinct species. Information on additional Patagonian ballistospores forming yeast isolates is also included in this report.

PS1-131-0793

Phylogenetic relationships among ophiostomatoid fungi associated with bark beetles colonizing white spruce in Eastern Canada.

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Ophiostomatoid fungi are the principal cause of sapstain of logs and lumber, which is a major concern for forestry in Canada. Many of these fungi are disseminated and introduced into the wood xylem by bark beetles (Coleoptera : Scolytidae). To our knowledge, no study has been conducted on the characterization of ophiostomatoid fungi associated with bark beetles in white spruce in Eastern Canada. The general objective of this study was thus to use molecular methods to characterize the principal ophiostomatoid species associated with bark beetles colonizing white spruce *(Picea glauca)* in Eastern Canada. Ophiostomatoid fungi isolated from bark beetles were first assigned to operational taxonomic units based on PCR-RFLP markers in the ribosomal DNA and beta-tubulin genes. Then, morphological characters were observed to confirm species identification. Finally, phylogenetic analysis based on portions of the rDNA large subunit and beta-tubulin gene sequences was carried out to establish relationships between taxa isolated in this study and known species. Analysis of the rDNA large subunit revealed that species found in this study belonged to two principal clades. Analysis of the beta-tubulin gene provided more details on the relationships among species. We obtained fourteen taxa in the genera *Ambrosiella, Hylorhinocladiella*, *Leptographium and Ophiostoma.* Within this group, seven taxa were closely related to known species but did not match any of them, and must be eventually characterized in detail.

PS1-132-0794

Molecular evolution of the hydrophobin gene family in the ectomycorrhizal fungus *Paxillus involutus*

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Duplications of genes or larger chromosome regions in combination with mutations that causes functional divergence of the duplicates is considered to be the most important mechanisms generating evolutionary novelties, including new gene functions and expression patterns. We have used DNA microarrays to screen for duplicated and rapidly evolving genes that could be associated with symbiotic adaptations in the ectmycorrhizal fungus *Paxillus involutus*. Strains of *P. involutus* with various abilities to form ECM were analyzed by comparative genomic hybridizations using a cDNA microarray containing 1076 putative unique genes. Approximately 17% of the printed genes were detected as rapidly and presumably non-neutrally evolving within *Paxillus*. Among them were several genes encoding hydrophobins. Hydrophobins are small, secreted proteins that are found as structural proteins located on the surface of filamentous fungi.

In this study we have examined the evolutionary mechanisms that could be responsible for generating sequence and expression divergence among members of the hydrophobin gene family in *P. involutus* in more detail. Seven hydrophobin genes (hydA to hydG) were characterized in *P. involutus.* A phylogenetic analysis showed that four of them have diverged rather recently, presumably after the divergence of the Paxillus clade from its ancestors within the basidiomycetes. To get further insights into the molecular evolution of these hydrophobins, orthologs of hydA to hydF were amplified from five strains of *P. involutus* and one strain of the closely related species P. filamentosus. Apart from the orthologs, several of the analyzed strains contained other hydrophobin genes, as well as pseudogenes with truncated sequences. The data suggest that the hydrophobin gene family in *P. involutus* evolves according to a birth-and-death model, in which new genes originated by gene duplication are maintained in the genome for a long time, whereas others are deleted or inactivated through deleterious mutations. Some of the new duplicates exhibited an initial phase of enhanced sequence evolution due to relaxed or positive selection. This was followed by an increase in the magnitude of functional constraints owing to purifying selection.

The expression patterns of hydA to hydG were analyzed in a wide range of tissues and growth conditions using microarray. The hydrophobin genes varied extensively in expression levels. However, this variation was not significantly related with their divergence in nucleotide sequences. In contrast, we observed that some of the hydrophobin genes had experienced a shift in their expression patterns following duplication. Such shifts indicate that the duplicated genes are preserved in the genomes by subfunctionalization and or/neofunctionalization.

PS1-133-0815

Characterization of root colonizing fungi in two species of *Shorea* seedlings using mycelial isolation and direct sequencing

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We examined root colonizing fungi of seedlings of two species tropical trees planted in soil collected from seven land use types (forest, rubber agroforest, rubber monoculture, oil palm plantation, cassava, annual crops and imperata grassland) in Jambi, Indonesia. Isolates of colonizing fungi were obtained from the roots of *Shorea lamellata* and *S. selanica* across different land use types. One hundred and ninety seven root colonising fungal isolates were isolated on Malt Extract Agar (MEA) and Corn Meal Agar (CMA). The land use type could be seen to affect the level of colonisation. Detection of taxa by direct internal transcribed spacer ribosomal DNA (ITS rDNA) sequencing of 170 root tips and 197 isolates detected a total of 55 taxa, including 7 known to be mycorrhizal. There was no overlap between two methods. Based on BLAST searching and alignments of these sequences, characteristic mycorrhizas from root tips were Thelephoraceae, "uncultured Ectomycorrhiza", *Pisolithus sp., Pisolithus tinctorius, Tomentella sp., Laccaria laccata,* and *Cortinarius callisteus*. Soil from Imperata grassland had moderate mycorrhizal inoculum (29.1 per cent colonisation) compared with soil from forest of Rantau Pandan dan Muara Kuamang-Kuning (38.4 and 34.0 per cent, respectively).

PS1-134-0829

Possible evolution of pedicillate teliospored rust fungi

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Rust Fungi are known to possess either sessile or pedicillate teliospores. Generally it is believed that pedicillate teliospored rusts have evolved from sessile teliospored rusts. During a study of the genus, *Melampsora* the author came across several teliospores being formed in older uredinia. The possible evolutionary significance of these teliospores is going to be presented.

Specimens of *Melampsora* on different host plants were obtained from various International Herbaria viz., B, BERN, C, DAOM, DAVFP, E, FH, G, HBG, HCIO, IMI, K, L, LE, LEV, LPS, PAD, PAV, PC, PDD, S, STE (U), UPS. Spore scrapings were mounted in Lactophenol or Lactophenol with Cottonblue. Both freehand and microtome sections were made. The processing and embedding of the microtome sections was done as per the procedure described by Johansen (1940). The teliospores of the genus Melampsora are single celled, sessile and produced in subepidermal or subcuticular non-erumpent single layered crusts. But during the course of the present investigation in some species they were found deep inside the cortex or deep inside the mesophyll tissue. In some species instead of single layered telial crust either two or three layered telial crusts were observed.

Interestingly in several specimens the teliospores were found coming in older uredinia. These teliospores have shown lot of variation in their shape. Based on their shape they were somewhat similar to the teliospores of *Puccinia, Uromyces, Diorchidium, Gymnoconia* etc.

It is a generally accepted theory that the pedicillate rusts have evolved from sessile teliospored rusts. The present study has shown that the teliospores that were produced in older uredinia resembled several pedicillate teliospored rusts. Since normally the teliospores of the genus, *Melampsora* are subepidermal/subcuticular and non-erumpent they never get an opportunity to take different shapes. But the teliospores once exposed are taking different shapes. So the author is of the opinion that in all probability the genus, *Melampsora* during the course of evolution might have given rise to pedicillate rusts.

PS1-135-0831

MOLECULAR studies support the interfertility data in solving taxonomic problems of Ganoderma boninense

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The systematics of *Ganoderma boninense* (Pat.), identified as the causal pathogen of basal stem rot of oil palms is rather inadequate, making identification of the species outside of its association with the oil palm *(E. guineensis)* host, very subjective. This has also led to some researchers stating that only 1 species is the infecting agent and others reporting that more than one species is responsible for the infection. Several DNA-based studies have been carried out in the past but no taxonomic conclusion could be drawn because of the complexities expressed from not being able to confirm the species as *G. boninense*. This is worse when the samples were obtained from other hosts or locations outside of Malaysia. This study established an interfertility database using selected monokaryons as testers based on the heterokaryotic, tetrapolar mating system of *G. boninense*. *Ganoderma* monokaryons that showed compatibility with the testers are confirmed as biological *G. boninense* while those that do not, as non-boninense *Ganoderma*. Then, molecular studies of RAPD and RAMS (random amplified microsatellites) were carried out on the samples, which consisted mainly of *Ganoderma* from and '*G. boninense*' from Japan, Taiwan, Sri Lanka and India for comaprison. Results showed that the clustering pattern of either molecular study was in total agreement with the interfertility data. This study shows that it is possible to use molecular data to determine species of *Ganoderma boninense*, using interfertility data as a reference.

PS1-136-0840

Smut fungi of Qilian mountains of China

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During the last three years, many specimens of smut fungi have been collected by the author and her colleagues from Qilian mountains and their adjacent areas in Gansu and Qinghai Provinces. Using light microscopy and scanning electron microscopy to study these specimens of smuts, 57 species in 10 genera of Ustilaginomycetes have been identified. Of these 8 smut species of the genera of *Anthracoidea, Urocystis* and *Ustilago* on plants of *Poaceae* and Cyperaceae on alpine regions are described as new to science. It contains a fairly respectable number of the genus of *Anthracoidea*. At the altitude of 3080 m from Sangke prairie of Gansu Province, owing to the geographical position as well as plentiful moisture and high altitude, this prairie is abundant in smut fungi. Poa plants were infected seriously by the common smut species *Ustilago striiformis* (Westend.) Niessl. Another species, *Urocystis hierochloae* (Murashk.) Ványky on *Hierochloa glabra Trin*., is very rich at the altitude of 2600 m. in the Mengda Heaven Pool of Xunhua county in Qinghai Province.

The smut flora is mainly composed of cosmopolitan species, north temperate and Eurasian temperate elements in Qilian mountains.

PS1-137-0847

Species Diversity of Phylloplane Yeasts: Do Nitrate-Negative *Cryptococcus* spp. Belong To Cr. *laurentii*

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A survey of yeasts from Mediterranean plants (mainly leaves) collected at the 'Arrábida Natural Park' (Portugal), yielded about 850 isolates, mostly of basidiomycetous affinity1. Amongst the latter, ca. 150 strains were initially assigned to *Cryptococcus laurentii* based on the results of key phenotypic tests: (i) production of white to cream-coloured colonies with butyrous to mucous texture; (ii) ability to produce starch-like compounds; (iiii) utilisation of D-glucuronic acid and inositol (C-source); and (iv) inability to assimilate nitrate (N-source). A representative subset of the isolates (62) was further characterised using a combination of conventional phenotypic tests with molecular methods (e.g. PCR fingerprinting and rDNA sequencing) to confirm their identity.
The fully characterised isolates were assigned to at least 17 distinct species, 13 of which seem to represent novel taxa (Fig. 1). Noteworthy, none of the isolates belongs to *Cr. laurentii*, which was previously considered a major component of the so-called phylloplane 'white yeasts'. The most abundant species on the phylloplane was *Cr. carnescens*, a former synonym of *Cr. laurentii* that was recently reinstated2. Our study confirms previous reports on the heterogeneity of "*Cr. laurentii*" and discloses a striking species richness among the phylloplane 'white yeasts'. The generally accepted ubiquity of *Cr. laurentii* on the phylloplane is therefore not confirmed by our results.
1Inácio et al. (2002) Microb Ecol 44: 344-353
2Takashima et al. (2003) IJSEM 53: 1187-1194
3Sugita et al. (2000) JCM 38: 1468-1471

PS1-138-0868

Some New Species of *Boletus* and *Cantharellus* from Korea

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Many higher fungi were collected from at Korea from 2002 to 2005. They were identified. As the result, three *Boletus* and one *Cantharellus* are new to the world. Among them, *Boletus tabicinus* D.H.Cho is pileus light yellow, stipe yellowish rough furrow-net, none exchanged color when burised. *B. alboporus* D.H.Cho is pileus darkish, rugolose, tubepores and stipe white. B. *chlorinus* D.H.Cho is pileus mixed green and yellowish, tubepores and stipe yellowish. *Cnatharellus minor f. pallid* D.H Cho is pallid color with white at edge.
Key words : New Species, *Boletus tabicinus, B. alboporus, B. chlorinus, Cnatharellus minor f. pallid*

PS1-139-0870

The Significance of the Anamorph in the Mega genus *Hymenoscyphus*

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The mega genus *Hymenoscyphus* is in the order Helotiales and known from a broad range of substrata and habitats in both terrestrial and aquatic environments. A group of aquatic hyphomycetes as anamorph stage in *Hymenoscyphus* (*Tricladium* spp. and *Varicosporium* spp.) is included for their relation and classification study. The result of molecular phylogenetic study based on ITS region of H. *varicosporoides* and their anamorph connections are proved to be polyphyletic. While rooted in the 18S rDNA in phylogram, the neighbourhood of their parsimonious clade is a sister taxon to the order Helotiales, and accommodates within the same order of Leotiomycetes Class. Hence, it is manifested that three chosen species embracing V. *giganteum* SS3012, V. *delicatum* SS3008, and T. *terestre* SS3011 are not monophyletic group. In addition, other anamorphic stages of H. *varicosporoides* SS76.01 and two strains of *Cudoniella indica* (SS708, SS3005) are clustered within the same fungal systematics. More related species will be added for further study of this mega genus.

PS1-140-0875

Using RPB1 and RPB2 sequences to improve the phylogenetic inference among *Candida* species

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Candida is an ubiquitous yeast that includes human pathogens, plant endophytes and environmental isolates. Previous phylogenies based on rDNA and actin sequences suggested that the genus is not monophyletic, and the relationships among related genera and species are not resolved. RNA polymerase II sequences have been useful to resolve taxonomic positions among a broad range of fungi. We investigate the evolution of *Candida* using sequences of the largest subunit (RPB1) and second largest subunit (RPB2) of RNA polymerase II gene in comparison with actin sequences with the same set of taxa using parsimony, and Bayesian analysis. The results from analyses of fifty taxa were congruent with actin phylogenies in showing that four phylogenetic groups are recognized but bootstrap support increased for some clades in RPB1 and RPB2. The phylogenetic positions of several taxa are also better-resolved using RPB1 and RPB2.

PS1-141-0876

Aquaphila has phylogenetic affinity in Tubeufiaceae inferred from rDNA sequences

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The teleomorph of *Aquaphila albicans* was discovered and illustrated from submerged wood collected in Thailand. It produces black, setose perithecia, bitunicate asci and hyaline, multiseptate ascospores. These features indicate its affinity to *Taphrophila* (Tubeufiaceae, Dothideomycetes). We therefore assessed the phylogenetic relationships of *Aquaphila and Taphrophila* among other fungi using ribosomal sequences from SSU, ITS, and partial LSU regions by parsimony, likelihood, and Bayesian analysis. An initial set of 40 taxa and related SSU sequences from GenBank, showed that they nested within the Tubeufiaceae with strong statistical support. A more detailed analysis of their placement with regard to *Helicoma, Helicomyces and Helicosporium* was inferred using ITS and partial LSU ribosomal sequences. Four isolates of *Aquaphila and Taphrophila clustered* as a strong monophyletic group and formed a sister relationship with *Helicoon gigantisporum and Helicoma chlamydosporum*. This is the first report of non-helicosporous fungi within the Tubeufiaceae, suggesting helicospores lost at least once within the family. Also perithecia bearing setae appeared to be a poor predictor of phylogenetic relationship in ascomycetes.

PS1-142-0877

Aspergillus ibericus: a new species of section *Nigri* characterised by MALDI-TOF MS

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Species of the *Aspergillus* section Nigri have been extensively used for various biotechnological purposes and are among the fungi best studied causing biodeterioration of commodities and food spoilage. Recently *Aspergillus ibericus* was described as a new species in the section. Taking into account the potentialities of Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry as a novel identification method based on a phenotype characterisation, this species was studied and compared with other black *Aspergillus*. MALDI-TOF mass spectrometry simplified the mass spectral analysis reducing the number of signals due to gentle ionization. Thus biomolecules to be analysed give either single and/or double charged ions. Therefore very complex samples like whole cells can be investigated. Employing unfractionated cell materials, organism-specific signal patterns ("fingerprints") in the mass range of 2.000 - 20.000 Da can be obtained. In filamentous fungi most signals correspond to membrane surface proteins. Their highly characteristic masses can be used for the identification and classification of organisms. Serra et al. (2006) showed that *A. ibericus* is closely related to *A. carbonarius* but is readily distinguished on the basis of smaller conidia size, absence of ochratoxin A production and DNA sequence analysis of two loci. In the mass spectra dendrogram obtained both species were successfully discriminated. Additionally, as it is described using tubulin gene sequence, *A. sclerotioniger* and *A. lacticoffeatus* showed relatedness with A. carbonarius and *A. niger*, respectively. The *A. niger* aggregate studied was composed by the type strains of *A. niger, A. phoenicis, A. tubingensis* and *A. vadensis* as well by strains of the molecular patterns N and T. All these species were aggregated in the same clade using MALDI-TOF analysis. Furthermore the uniseriate species *A. japonicus* and A. aculeatus were aggregated in a distinct clade from the bisseriate species, and the rare bisseriate species *A. ellipticus* with ellipsoidal conidia was classified separately from all other species in the section. In conclusion, results of MALDI-TOF analysis using mass range from 5000 – 20000 Da were similar to those of phylogenetic analysis giving a sound input for *A. ibericus* characterisation and showing the potentialities of the method for taxonomic purposes.
Serra, R., Cabañes, F.J., Perrone, G., Castella, G., Venâncio, A., Mule, G. & Kozakiewickz, Z. (2006) Mycologia 98: 295-306.
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PS1-143-0882

Taxonomy and Molecular Phylogeny of Orbiliaceae from China

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The family Orbiliaceae was established by Nannfeldt and is characterized by the small, waxy, light-colored and semi-translucent apothecia. As teleomorphs of the predatory fungi, comprehensive study on taxonomy of the group was neglected in the world. The nation-wide survey on the orbiliaceous fungi from 23 provinces and autonomous regions in China was conducted and more than 350 specimens were collected. Eight taxa of Hyalorbilia and 20 taxa of Orbilia have been identified. Among them, *Hyalorbilia brevistipitata, Orbilia bomiensis, Orbilia milinana and Orbilia querci were described as new species; H. andina, H. erythrostigma, H. fusispora, H. juliae, H. lunata, H. polypori, O. brasiliensis, O. cardui, O. crystallina, O. evonymi, O. fimicoloides, O. orientalis, O. rectispora, O. scolecospora, O. tenebricosa, O. tenuissima* were reported for the first time from China. Nearly 200 pure cultures were isolated from fresh specimens. Among them, 50 isolates with conidial production were assigned to *Arthrobotrys, Dactylella, Dactylellina, Dicranidion*, *Drechslerella and Trinacrium*, respectively. Fifteen teleomorph-anamorph connections have been confirmed. The connection between H. *brevistipitata* and *Dactylella brevistipitata* is the first evidence for *Hyalorbilia-Dactylella* connection, and *O. querci* and *Dactyelellina querci* is the first report on the connection between a knob-forming nematophagous hyphomycete with an *Orbilia teleomorph*. Phylogenetic relationship among species of the *Orbiliaceae* based on partial sequences of 28S rDNA and the internal transcribed spacer regions (ITS, including 5.8S rDNA) reveals that *Orbiliaceae* is a monophyletic family and supports the differentiation between Hyalorbilia and *Orbilia* based on the morphological characteristics such as the types of ectal excipulum, and cells asci arising from. Our phylogenetic tree also showed that species producing trapping devices to capture nematodes formed a monophyletic clade. The other clades seemed correlated to the shapes of ascospores.

PS1-144-0885

Ramaria in Australia

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Macrofungal research in Australia is unfortunately poorly represented, particularly in Queensland. The Queensland Herbarium is now making moves to address this imbalance in the study of groups of 'lower' organisms within this vast state. With the assistance of ABRS funding, Nigel Fechner and Dr. Tony Young are currently researching the genus Ramaria as it occurs Australia-wide. It is intended that taxonomic revision of existing species will be undertaken with all existing specimens from Australian herbaria. This will be supplemented with collection and identification of new material, description of new species, SEM analysis of spores, construction of interactive keys and distribution maps, and molecular and phylogenetic analyses.
Ramaria comprise a genus of erect, coralloid-branched basidiomycetes which occur on soil, humus or wood. They produce pale yellow to ochre brown spores which bear a variety of different ornamentation patterns including meandering ridges, warts, striations and echinulate or cog-like projections. Smooth spores can occur but this is very rare. A spore characteristic of this group is that the ornamentation is cyanophilic in cotton-blue stain. Species of *Ramaria* occur in a wide range of colours from white to dull browns and greys and also through pastel shades to very bright yellows, oranges, reds, pinks and purples. Some typically bear apices which are differently coloured from the branches. *Ramaria* species appear to be most abundant in Eucalypt forests/woodlands, but also occur in Pinus plantations, Kauri forests, heath communities and occasionally in rainforest.
There have been 35 taxa referred to Australia in local and world literature. Our research has demonstrated that in most cases the application of European nomenclature to Australian species has been erroneous. Currently, we have verified the existence of seven of those taxa:
Ramaria anziana R.H.Petersen
Ramaria australiana (Cleland) R.H.Petersen
Ramaria botrytoides
Ramaria capitata (Lloyd) Corner
Ramaria fennica (P.Karst.) Ricken var. fumigata (Peck) Schild
Ramaria gracilis (Pers. : Fr.) Quél.
Ramaria lorithamnus (Berk) R.H.Petersen
There is also currently 5-10 new species do describe. We estimate that there could be as many as 30 new taxa by the time the project finishes.

PS1-145-0895

The flora agaricina neerlandica project: the importance of morphological studies.

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The Flora agaricina neerlandica is a critical flora, covering the agarics and boleti occurring in the Netherlands and adjacent regions.
It is composed of contributions from various authors, edited by Noordeloos, Kuyper and Vellinga. It provides keys, extensive descriptions and illustrations of all taxa, and data on ecology and distribution. The flora is essentially based on own observations of the authors on fresh material and herbarium specimens collected by Dutch mycologists over more than 50 years, supplemented, if necessary, with herbarium specimens from abroad. The research for the Flora is carried out according to standard methods and it uses in principal a morphological species concept, though the results of molecular and other studies are incorporated if available. As such the flora is a standard work and can serve as a base for further research. The importance of thorough, standardized morphological studies is nowadays often underestimated by molecular mycologists.
The project has been accelerated by substantial financial support from the Kits van Waveren Foundation. Six volumes have been published. Volumes 7-11 will follow with an interval of 2-3 years.
Current projects which are carried out in connection to the Floraproject are:
Gymnopilus, Cystoderma, Lyophyllum, Tubaria, Squamanita - M. Nauta
Boletes - M.E. Noordeloos
Russulaceae - M.E. Noordeloos, A. Verbeken & J. Wisman
various genera of Cortinariaceae - E. Arnolds, N. Dam and Th. Kuyper

PS1-146-0897

New species and records of *Beltrania*-complex from brazilian semi-arid.

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The Brazilian Semi-arid region is located almost exclusively in the Northeastern part of the country.This large expanse of drylands stretches between 3-17o S and 35-45o W, covers almost 8% of the territory of Brazil and occupies about 900.000 Km2. Recents results of ongoing projects on fungi diversity in the Semi-arid region have shown that about 30% of collections represent new species for Brazil or new species to science. The results of collections of the Beltrania-complex are be presented. For a genus to belong to the *Beltrania*-complex, it must possess at least three of the following characters, (i) dark setae; (ii) setae or conidiophores with radially lobed bases; (iii) swollen separating cells; (iv) biconic conidia; (v) conidia with a hyaline equatorial band. There are currently six genus reconized in the Beltrania-complex, *Beltrania* Penz., *Beltraniella* Subraman., *Beltraniopsis* Bat. & J.L. Bezerra, *Pseudobeltrania* Henn. and Porobeltraniella Gusmão. Nowadays, the *Beltrania*-complex has about 39 accepted species. In the course of inventories of conidial fungi from semi-arid region in northeastern of Brazil, leaf litter was colleted in several localities and used as substrate. Leaf litter samples were placed in separate paper bags and taken to the laboratory. Samples were incubated in Petri dish moist chambers at 25 °C in polystyrene containers (170 L capacity) with 500 ml of sterile water plus 4 ml of glycerol and examined at regular intervals for the presence of microfungi. Mounts were prepared in polyvinyl alcohol resin (PVL) and measurements made at 1000 x magnification. All taxa were deposited in the Feira de Santana State University Herbarium (HUEFS). Eigth previous described species of Beltrania-complex were be found, *Beltrania africana S. Hughes, B. querna Hark*., B. *rhombica Penz*., *Beltraniella amoena* R.F. Castañeda, Canon & Guarro, B. japonica Matsuh., *B. portoricensis* (F.L. Stevens) Piroz., *Beltraniopsis esenbekiae* Bat. & J.L. Bezerra., *B. ramosa* R.F. Castañeda. Six new species are been proposed: *Beltrania bahiensis* sp. nov., *B. copaiferae* sp. nov., *B. flexuosa* sp. nov., *B. leandrae* sp. nov., *Pseudobeltrania* clusiae sp. nov. and *Beltraniella compacta* sp. nov.

PS1-147-0913

Redefinition of the genus *Cryptosporella* (Gnomoniaceae)

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A recent phylogeny of the family Gnomoniaceae placed the type species of the genus *Cryptosporella* Sacc. (C. hypodermia (Fr.) Sacc.) in a clade with the type species of the genus *Ophiovalsa* Petrak (*O. suffusa* (Fr.) Petrak). Species belonging to this clade have been considered to be pathogens, endophytes and saprobes on stems and branches of trees, mainly from the Betulaceae and Ulmaceae. Several nomenclatural issues are associated with determining the correct generic name for this clade. In addition, *Winterella* (O Kuntze) J. Reid & Booth has been considered a synonym of *Ophiovalsa*.

In this work we: 1) track the literature pertinent to the taxonomy of the species reported within these genera in order to assign a correct generic name; 2) compare the morphology and cultural characteristics of species falling within this clade; and 3) analyze DNA sequences of ribosomal and RPB2 gene regions to define this genus and the relationships of its species. A review of the literature demonstrates that the genus name deserving priority for species belonging in this clade is *Cryptosporella* Sacc. Our preliminary results indicate that shape and size of ascospores and cultural characteristics are useful for differentiating species within this genus. A redefinition of the genus based on molecular and morphological characters is presented and results in the need for at least five newly combined species epithets in *Cryptosporella*.

PS1-148-0926

Molecular and morphological discrimination of stipitate hydnoids in the genera *Hydnellum* and *Phellodon*

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Hydnellum and *Phellodon* species are found associated with a range of woody angiosperms and gymnosperms particularly within Fagaceae and Pinaceae, and are generally considered to be ectomycorrhizal. They appear to be declining in several European countries, including Britain, and the stipitate hydnoids are listed as priority species in the UK Biodiversity Action Plan. Understanding the ecology and determining the population trends and hence conservation status of these fungi is hampered by difficulties in discriminating some species: distinguishing between several *Hydnellum* and *Phellodon* species on morphological criteria is problematical, because of both the lack of consensus regarding the key discriminatory characters and difficulties with their interpretation and assessment. Neighbour-joining trees were constructed from DNA sequences of the internally transcribed spacer (ITS) regions and the 5.8S gene of the ribosomal gene cluster from 67 fruit body collections. This discriminated the known British species of *Phellodon* (*P. confluens, P. melaleucus, P. niger, P. tomentosus*), revealing more terminal clusters than currently recognised taxa, and prompted re-determination of one sample. The main focus of the study within Hydnellum was on the very similar species pair *H. concrescens* and *H. scrobiculatum*, though a few samples of *H. caeruleum*, *H. ferrugineum, H. peckii* and *H. spongiosipes* were also included. DNA sequencing of material identified on spore-based criteria as H. concrescens yielded two terminal clusters, but samples received as *H. scrobiculatum* were generally more variable. Of these, two were reassigned and the remaining group, with very similar spores (although shorter than in published descriptions of *H. scrobiculatum*), had highly variable sequence data. The results and conservation importance of these fungi highlight the need for a reassessment of *H. concrescens* and *H. scrobiculatum* collections from Britain and continental Europe using a combined molecular and morphological approach. Specific PCR (polymerase chain reaction) primers were constructed to discriminate fruit bodies of *P. niger* and *P. confluens* from each other and from other stipitate hydnoids.

PS1-149-0957

A preliminary analysis of *Lactarius* Subgenus *Piperites* from Northern Thailand based on morphology and nrITS sequence data.

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The genus *Lactarius* subgenus *Piperites* from Northern Thailand and is the first in a series resulting from a complete revision of the genus for the area. Five taxa are described as new for science: L*actarius formosus,* L. *austrotorminosus*, L. *austrozonarius*, L. *alboscrobiculatus* var. *alboscrobiculatus* and L. *alboscrobiculatus* var. *roseopurpureus*; three other new species are described and illustrated but not formally described. L. akahatsu and L. hatsudake, described by Hongo from Japan, are recorded for Thailand and L. *purpureus*, described by Heim from Thailand in 1966 is also listed in poster. The species concepts and the comparison with species of other continents are based on morphological and molecular data (ITS-region).

PS1-150-0962

The phylogeny and taxonomy of the thraustochytrid strains isolated from Malaysia (Labyrinthulomycetes, Stramenopiles).

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The class Labyrinthulomycetes in the Stramenopiles is characterized by the presence of the ectoplasmic net elements produced from the bothrosome and multi-layered cell wall. This class consists of a single order and two following families: 1) the Labyrinthulaceae consisting of a single genus *Labyrinthula*, whose cell moves inside the ectoplasmic net; 2) the cells of the Thraustochytriaceae with the rhizoid-like ectoplasmic network from a single bothrosome. Classification of six thraustochytrid genera is based on the cell morphology of various stages in the life cycle. However, recent molecular phylogenetic works clearly showed that these genera do not form monophyletic groups, so it is suggested that the taxonomical rearrangement might be necessary.
We isolated three thraustochytrid strains (RT0301, RT0304, RT0305) from the sediment samples, collected in Penang Island and Langkawi Island, Malaysia. We examined morphological features in the life cycle, the molecular phylogenetic analyses of 18S rRNA gene, and compared the chemotaxonomical features of poly-unsatulated fatty acids (PUFA) and carotenoid pigments.
On the zoospore formation of strain RT0301, the vegetative cell content move out of its cell wall as complete mass, and successively the naked protoplast divided into 16-32 zoospores. These features agree with those of Ulkenia sarkariana. The strain RT0304 was identified as Ulkenia radiata based on congruent morphological character in the amoeboid cell stage and the radical cell division in zoospore formation. The molecular phylogenetic tree of 18S rRNA gene shows that the members of genus Ulkenia appear in three independent groups. First group includes Ulkemia radiata (strain Raghukumar #16) and RT0304 (Ulkenia radiata). Second group includes RT0301 (Ulkenia sarkariana) and some original isolates. Third group includes Ulkenia visurgensis (ATCC20208, type strain), Ulkenia profunda (KMPB N3077a, type strain) and Japonochytrium sp. (ATCC28207). Each group is supported by 100% bootstrap value. Moreover, examined strains in each group share the common composition of carotenoids.
On the zoospore formation of strain RT0305, the vegetative cell divided into four to eight daughter cells, each of which changed the cell shape from sphere to dumbbell and subsequently progressed into the final division before zoospore formation. These dumbbell-shaped cells at zoospore formation have never been observed in the other thraustochytrids. The molecular phylogenetic analysis clearly shows that RT0305 form a newly recognized lineage with our original isolates from Hong Kong and Japan. All the members of this lineage share the above-mentioned unique cell division and the common compositions of PUFA and carotenoids. Therefore, we propose a new species of the genus Thraustochytrium for RT0305 and related strains.

PS1-151-0969

A new *Anhelia* (Myriangiales, Dothideomycetidae) species from the Brazilian cerrado.

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A new *Anhelia* species was found causing dark crusty lesions on leaves and stems of young Anadenanthera sp. (Fabaceae) plants. A close microscopic examination of the lesions showed that the fungus possessed deeply penetrating hypostromata with also superficial stromata containing multiple monoascous locules characteristic of an Anhelia species, which is now described as follows: Symptoms shown as stromatic crusts measuring up to 0.53 mm diam, crustose, brown to black, without a definite margin; Ascomata 80-230m high, 250-530m wide, eustromatic, pulvinate, multilocular, forming one layer of locules. Locules monoascal, 22-35 x 17-31 ?m, walls with textura angularis made of cells with 6-17m diam. Hypostromata 36-160 168-432m, epidermal, immersed. Asci 28-5 24-33 m, bitunicate, persistent, up to 8-spored, broadly clavate to globose, thick-walled particularly in the upper part, sessile or short pedicellate. Ascospores 13-19 ? 6-9 m, initially colourless, guttulate, becoming light brown, the end cells colourless, ellipsoidal to cylindrical, 1- to 5-transversal septa, and 1-4 longitudinal-septa, slightly constricted at the septa, however mostly phragmosporic. Two different species were recently described from Brazil: Anhelia tabebuiae on Tabebuia caraiba and A. verruco-scopiformans on Croton nigrans (Euphorbiaceae) [Inácio & Dianese, Mycol. Res. 102 (6): 695–708, 1997; Pereira & Barreto, Fungal Diversity 12: 155-9, 2003]. The specimen studied belongs in a new Anhelia species that will be published according with the International Code of Botanical Nomenclature.

PS1-152-0970

Novelties and conclusions from a monograph of the family Parmulariaceae.

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The Parmulariaceae is poorly known family in the Dothideomycetidae (Ascomycota), now including 34 genera and more than 100 species of mainly biotrophic fungi, with a pantropical distribution. Two new genera *Mintera* and *Viegasella* were added to the family. Dried specimens from herbaria were studied using optical microscopes and SEM, with all information stored in a suite of structured databases. Fresh material was collected from natural habitats, and attempts to grow these fungi on artificial media were unsuccessful. Some other genera were also studied, including *Kentingia, Parmulariella* and *Chaetaspis*, and new combinations in *Hysterostomella*, *Parmularia, Cycloschizon*, *Perischizon* and *Rhagadolobium* were made. New country records were recorded following field work in Panama and Brazil. A list of all members currently included in the Parmulariaceae will be presented, and some example genera will be discussed.

PS1-153-0973

A new trichomatous hyphomycete on *Emmotum nitens* (Icanaceae) from the Brazilian cerrado.

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The UB1432 exsiccate of *Emmotum nitens* (Icanaceae) deposited in Herbarium UB (Mycological Collection) showed leaf blades partially covered with a thin layer of light brown fungal growth. Under microscope was revealed a dematiaceous hyphomycete growing in association with the foliar trichomes, characterized as follows: colonies sparse, light brown to grayish brown; mycelium light brown thin, superficial, growing on the trichomes; hyphae 2 – 4 ?m wide, septate, light brown, prostrate; conidiophores up to 16m tall x 4m wide, septate, subhyaline, cylindrical, semi-macronematous or macronematous, erect or flexuous, non-scared; conidiogenous cells 3 - 5 (3) m long x 3 - 5 (4) m wide; conidia 19 - 67 (38) m long x 14 - 35 (17)m wide, dark brown, muriform, oblong, subovid to subglobose, rostrate, with rhexolytic secession. The fungus is similar to Pythomyces species as far as conidial secession and septation are concerned. It is also similar to *Trichomatomyces byrsonimae* Dornelo-Silva & Dianese, however the conidial shape is completely different although both are trichomatous fungi. Finally, the fungus studied is very similar in terms of conidial shape to *Monodictys* Hughes spp., however, the new fungus is a foliicolous epiphyte, or more specifically trichomatous, while *Monodictys* species are all saprophytic on wood and bark of trees, show micro to semi-macroconidiophores, and never forms rostrate conidia. Thus the new species may be admitted in *Monodictys widening* the concept of this genus or it can be allocated to a new generic taxon.
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PS1-47-0258

Molecular and morphological description of *Pestalotiopsis hainanensis* sp. nov., a new endophyte from tropical region in China

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Pestalotiopsis is an important group of endophytic fungi. Approximately 220 species of *Pestalotiopsis* were described (CABI Bioscience database, 2005), among them at least 46 *Pestalotiopsis* species have been reported as endophytes, some of which produce secondary metabolites with a great potential for anti-microbial and anti-tumor medicinal application. The traditional taxonomy of this genus was based mostly on morphology of conidia (Guba, 1961; Sutton, 1980; Nag Rag, 1993) and affinities of *Pestalotiopsis* species have been confused and equivocal. During a survey of the diversity of *Pestalotiopsis* species in the tropical region of China, a new endophytic fungus Pestalotiopsis *hainanensis* was isolated from the stem of *Podocarpus macrophyllus* at Xinglong Tropical Botanical Garden of Hainan Province which is morphologically distinguished from similar species *P. karstenii* in unbranched and short apical appendages, and from *P. heteroconis* in absence of basal appendages. Phylogenetic analysis based on ITS region (ITS1, 5.8S, ITS2) and beta-tubulin 2 gene (tub2) indicated that for 5.8S gene and ITS sequence *P. hainanensis* shared similarity of 97.4% with three *P. karstenii* strains and 96.1%-96.6% with three *P. heterocornis* strains; for tub2 gene sequence the new species shared similarities of 92.3%-93.8% with three *P. karstenii* strains and 94.1% with three P. *heterocornis* strains. Molecular results support that *P. hainanensis* is a new species which is distinguished from P. *karstenii, P. heterocornis* and other *Pestalotiopsis* species. It is suggested that when a new taxon of *Pestalotiopsis* is described, both of morphological characters and molecular phylogenetic information are necessary to prove the taxon unique from other known species.
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POSTER ABSTRACTS S9

1330-1500
POSTER SESSION 9 - MYCORRHIZAE

PS9-154-0016
Significance of arbuscular mycorrhiza for *P influx* of maize and groundnut in an Oxisol

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The contribution of indigenous arbuscular mycorrhiza (AM) on Phosphorus (P) uptake by maize and groundnut was examined in a low P field soil. The fungicide benomyl was applied to eradicate mycorrhizal infection The treatments consisted of three P levels viz. 0, 50 and 400 mg P kg-1 soil, with and without benomyl application. Maize and groundnut as test crop were sown two weeks after the application of benomyl and was harvested four times covering the whole growth period. At each harvest, the shoot yield, shoot P concentration, root length, soil solution P (CLi) and per cent root infection by AM was determined for benomyl treated and untreated soil at all P levels. Benomyl showed no effect on soil solution P concentration. When P was limiting, application of benomyl did reduce early maize and groundnut growth by 40-50% at P-0, and by 25-30% at P-50. At high P supply (P-400), benomyl had little or no effect on dry matter production. Thus, indicate that the effect of benomyl on plant growth was by its influence on P uptake from soil. Phosphorus supply affected percentage of root infected by AM which was 40% of the roots at P-0, and decreased to around 30% and 10% at P-50 and P-400 respectively in both the crops. In the early growing season, the P influx of maize was dependent on P in soil solution and the effect of AM was rather large. At high P supply, the contribution of AM to P influx showed a decrease. Without or low AM infection and at low P level, the P influx was 62% of that with AM. Groundnut, during early growth period showed a similar behaviour as maize at middle growth stage and without AM reduction of P influx, which was to an extent of 67%. In absolute terms AM is more important at maximum growth in the middle of the growth season for maize and only early growth season for groundnut. It is evident from the present investigation that AM may make an significant contribution by about 35 % to the P nutrition of maize and groundnut, but other factors, like P solubilization by root exudates, may be even more important.

PS9-155-0049
The *Peloton lysis* (Pattern) in some endangered and endemic Orchids of Eastern Ghat's, India

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Orchidaceae is one of the largest angiosperm families and the many species included within it occupy a variety of habitats globally. Although there is a huge diversity in morphological and reproductive characteristics, a unifying feature of the family is the production of minute seeds that must under natural conditions become associated very early in embryo development with fungi in order to germinate and establish the plant. Protocorm formation is development on fungal colonization. The emphasis in studies of orchid –fungal symbiotic associations has been biased in favour of the orchid, primarily because of the interest in propagation of commercially important and endangered species and because researchers studying pathogenic fungi do not usually study symbioses between orchids and fungi. It is clear from the evidence now available that the fungal species involved in this association are capable of degrading complex carbohydrates that exist in the soil and providing the heterotrophic protocorm stage with simple sugars required for growth and differentiation of a shoot and root system. In the cortical cells of protocorms and roots (of both land and epiphytic orchids) the fungal hyphae form coiled complexes called pelotons. One of the most striking events in the orchid mycorrhizal association is the lysis of the pelotons. The repeated formation of pelotons in the same host cell is a very interesting aspect of orchid mycorrhizal colonisation. A maximum of four generations of pelotons are digested in the same host cell during the life span of a root. Orchids viz. *Bulbophyllum kaitense, Gastrochilus acaulis, Hebenaria decipiens , Hebenaria rariflora, Malaxis rheedii* and *Vanda tesstellata* roots were collected during different seasons of the same year and subjected for the peloton studies. The present study points out that the mode of lysis of pelotons in orchid mycorrhiza follows a definite pattern and the pattern is specific to the orchid mycorrhizal partner. The different types of peloton lysis and the host mycorrhizae for each type of lysis are discussed in detail with reference to histochemical analysis. These patterns could also be used as one of the features to identify the fungal partner.

PS9-157-0166

Dynamics of arbuscular mycorrhizal fungal diversity and community in a hot and arid ecosystem during conversion from natural to arable and subsequently to restoration lands

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The spore density, species composition and diversity of arbuscular mycorrhizal fungi (AMF) in an arable land (AL), a restoring field (RF) and an indigenous plant community (IPC) which arrayed adjacently in a hot and arid ecosystem in southwest China were investigated. Spores in the rhizosphere soils of representative plants in the three habitats were extracted by wet-sieving and decanting methods. A total of 47 taxa of AMF including 31 taxa from the genus *Glomus*, 8 from *Acaulospora*, 6 from *Scutellospora*, 1 from *Entrophospora* and 1 from *Gigaspora* were isolated and identified morphologically. The spore densities were highest in IPC, slightly lower in RF and lowest in AL, which were opposite to diversity indices. No significant correlation between spore density and species richness was observed except in AL. The dominant species of AMF in different habitats were diverse in the three habitats, which indicated the possible adaptation of different AMF species to distinct habitats. *Glomus* and *Acaulospora* were dominant genera and the three species, *A. scrobiculata, G. claroideum* and *G. mosseae* were the dominant species in this hot and arid ecosystem. Cluster analysis based on the similarity in AMF community composition indicated that the distribution of AMF were not random over space and that AMF community composition associated with a given plant species was greatly habitat-dependent. According to the results of cluster analysis, we hypothesized that the effect of the habitat-dependence of AMF on their communities seemed to be higher than that of host preference in this hot and arid ecosystem. Not only in spore density and species numbers, but also in community composition, RF resembled more IPC than AL. This indicated that mixed (natural and artificial) restoration is an effective way for restoring AMF community composition from AL to IPC.
Keywords: Arbuscular mycorrhizal fungi; diversity; community; arid ecosystem; restoration

PS9-158-0170

Molecular and Morphological Diversity of Pezizalean Ectomycorrhiza

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Pezizales is the basal order of euascomycetes that includes mostly terrestrial or coprophilous saprobes and ectomycorrhizal (EcM) symbionts. Whereas EcM habit is well-known among hypogeous fruiting taxa, *Tuber, Genea, Terfezia*, etc., the trophic status of most epigeous fruiting taxa has remained unstudied. Recent molecular research has revealed a high diversity of pezizalean ectomycorrhiza (EcM), but most remain unidentified at the genus or species level. This study aims at identifying EcM-forming taxa within *Pezizales* and describing their mycorrhiza. EcM-forming *Pezizales* were revealed by morphotyping and sequencing of EcM root tips from forests in Estonia and Denmark. The taxa on EcM root tips were identified using phylogenetic analyses of LSU rDNA sequences derived from sporocarps of 301 pezizalean species, and comparisons with ITS rDNA sequences. Thirty-three species were suggested as EcM symbionts, representing all three major clades of Pezizales including the genera: *Genea, Geopora, Humaria, Tarzetta, Trichophaea* (pro parte), *Wilcoxina, Helvella, Hydnotrya, Tuber, Pachyphloeus, Peziza, Sarcosphaera* and two *Pezizaceae* anamorphs. EcM of most Pezizales spp. are easily distinguishable by anatomy, particularly thick cell walls and stout hyphae. EcM of the sister genera *Humaria* and *Genea* are nearly identical, sharing a red-brown mantle with large round cells and wide thick-walled hyphae. EcM of *Helvella* spp. and *Tuber* spp. share an epidermoid mantle structure, but all studied *Helvella* spp. lack cystidia. This study demonstrates that *Pezizales* spp. constitute a considerable proportion of the mycobionts in EcM fungal communities in mature boreal deciduous and coniferous forests in several soil types. EcM-forming lineages have likely evolved several times independently from saprotrophic ancestors. The results suggest that EcM lifestyle seems to be a precondition for the switch to hypogeous fruiting in both ascomycetes and basidiomycetes.

PS9-159-0171

Partial Mycoheterotrophy in Some Boreal Ericoid Subshrubs: Isotopic Evidence

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Some phototrophs obtain supplementary carbon via partial heterotrophy. In terrestrial ecosystems, this so-called "mixotrophic" strategy is only reported in xylem-tapping hemiparasites and some green orchids. The latter acquire carbon from their mycorrhizal fungi that in turn obtain it by forming ectomycorrhizae with overstorey trees. Pyroleae spp., common boreal ericaceous subshrubs, were hypothesized to be mixotrophic based on ecophysiological and evolutionary traits: low and unplastic photosynthesis, the presence of aphyllous forms, close evolutionary relationship to mycoheterotrophic Pterosporae and Monotropae, and root symbiosis with common ectomycorrhizal fungi. Trophic status and mycorrhizal associations of three pyroloid species, *Orthilia secunda, Pyrola chlorantha* and *Chimaphila umbellata* were determined in a sparse mature podzolic Pinus sylvestris dominated forest, NW Estonia. Using the autotrophic *Arctostaphylos uva-ursi* and the mycoheterotrophic *Monotropa hypopithys* as reference plants, concentrations of 13C and 15N stable isotopes revealed that *Orthilia secunda* and Pyrola chlorantha acquire respectively 50% and 38% of their carbon from fungi, whereas *Chimaphila umbellata* was autotrophic. High N content supported that *O. secunda and P. chlorantha* partly feed on fungal organic matter. Molecular identification of root fungi revealed 39 species, mostly endophytic or ectomycorrhizal, including frequent *Tricholoma* spp. These fungi potentially link *Pyroleae spp.* to surrounding trees that are likely their ultimate carbon source. Similarly to mixotrophic orchids, *Pyroleae spp.* harboured a moderately diverse community of ectomycorrhizal fungi, suggesting that lack of specificity may support mixotrophic lifestyle, whereas complete abandonment of photosynthesis likely requires elevated functional compatibility that may be achievable with only a limited number of fungal hosts. The high local abundance and year-round respiration of *Pyroleae spp.* may result in a considerable carbon cost to their autotrophic host plants.

PS9-160-0172

Ectomycorrhizal Fungi in Seychelles

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The granitic islands of Seychelles represent the remains of a microcontinent that was separated from the subcontinent of India c. 60 million years ago. Their position between Madagascar, Africa and Sri Lanka, and some remaining indigenous vegetation render Seychelles as a valuable area for biogeographic studies. Among the native plants, three extant genera, including *Intsia* (Caesalpiniaceae), *Pisonia* (Nyctaginaceae) and the endemic *Vateriopsis* (Dipterocarpaceae) support ectomycorrhizal (EcM) fungi. So far, A. Ashford and W. Allaway have recorded two unidentified species possibly belonging to Thelephoraceae on root systems of *Pisonia grandis*, a guano-inhabiting coastal shrub. The aim of this study is to document the EcM root symbionts of all ectomycorrhizal, keystone plant species in Seychelles using both morphological and molecular analyses of fruit bodies and root tips. The preliminary results suggest that species richness of ectomycorrhizal fungi is low (32 spp.) despite c. 150 collected root samples. The isolated populations of *Intsia bijuga* and *Vateriopsis seychellarum* hosted slightly overlapping communities of ectomycorrhizal fungi. Interestingly, the introduced eucalypts were also mostly associated with these fungi, whereas *Pisonia grandis* and the introduced *Pinus caribbea* displayed no such species overlap. The "inconspicuous-fruiting" members of Thelephoraceae built fruit-bodies up to 40 cm in diam. and substantially dominated both in "above-" and belowground surveys (14 spp on root tips), followed by members of the boletoid and hymenochaetoid clades. Of the latter, *Coltriciella* spp. are proven ectomycorrhizal for the first time. Confirming earlier findings on *Salix* spp., two species of *Sordariales* formed tiny black mycorrhizas on *Vateriopsis*. The low diversity of ectomycorrhizal fungi in Seychelles likely results from the long-term isolation of the microcontinent rather than the extreme rarity of the host species due deforestation. Phylogeographic analyses will reveal the biogeographic relations of Seychelles´ ectomycorhizal fungi when additional sequence data from other tropical regions accumulate.

PS9-161-0176

Mycelial response of ectomycorrhizal and saprotrophic fungi of coniferous forest soils to selected monoterpenes

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Monoterpenes are volatile secondary plant compounds and are found in high concentrations in coniferous trees. In coniferous forests, ectomycorrhizal (ECM) and saprotrophic (SP) basidiomycete fungi dominate the microbial community and are the main drivers of carbon and nutrient cycling processes. Monoterpenes are present in the litter layer where both ECM and SP fungi can be found and, although they have been shown to influence certain nutrient cycling processes and also have insecticidal, antibacterial and antifungal properties, little is known about their effects on the fungi that occur in forest soils and litters. If monoterpenes have differing effects on different fungal taxa or functional groups, this could in turn impact upon carbon and nutrient cycling in forest ecosystems.
In the work presented here, the monoterpenes present in green needles and fresh litter of *Pinus sylvestris, Picea abies* and *Picea sitchensis* were identified and quantified using gas chromatography with flame ionisation detection and gas chromatography-mass spectrometry. The rate of loss of monoterpenes from needle litter was also monitored over 6 months during a litter bag experiment.
The total monoterpene concentrations in freshly fallen litter of *P. abies and P. sylvestris* were 1179 ± 122 µg g-1 dry wt and 1635 ± 102 µg g-1 dry wt respectively. The total concentration in P. sitchensis litter was at least 15 times lower, with a total monoterpene concentration of only 80 µg g-1 dry wt ± 11. After 6 months, total concentrations had reduced to 1% and 5% of initial litter concentrations in *P. abies and P. sylvestris* respectively, and to 66% in *P. sitchensis.*
The most abundant monoterpenes in the conifer litters analysed were alpha-pinene, beta-pinene and 3-carene. These were consequently chosen for fungal exposure studies. A range of isolates of both ECM and SP litter-degrading fungi was grown in liquid culture and exposed to monoterpene vapours. All three of the chosen monoterpenes significantly inhibited growth in several of the species tested.
Further studies have been undertaken to test the effects of monoterpenes found in conifer litters on the infection rate of seedling roots by ECM.
The results of this work demonstrate that monoterpenes can occur in high concentrations in the litter layer of coniferous forests and that they have the potential to affect fungal community structure by differentially inhibiting different species. This could have important implications for the fundamental ecosystem processes of which fungi are the main drivers in coniferous forest systems.

PS9-163-0290

Interactions of soil fungal communities with ectomycorrhizal host plants in an alpine environment

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Fundamental knowledge about the biodiversity of soil fungal communities and their functional impact, especially for ectomycorrhizal (ECM) fungi in alpine habitats, is still scarce. However, this belowground biodiversity is essential for establishment and maintenance of ECM plant communities. The comparison of soil fungal communities (= the mycorrhizal potential of the soil) with the actual mycorrhizal status of ECM host plants will provide evidence of mechanisms affecting the mycorrhization processes.
The Rotmoosferner glacier forefront (46°50´N, 11°03´E) is a primary successional site located in the Austrian central Alps at an altitude ranging from 2280 to2450 m a.s.l. We selected an area of about 20000 m2 behind the moraine ridge of 1920 for our investigations. The herbaceous plant *Bistorta vivipara* (Polygonaceae), the grass Kobresia myosuroides (Cyperaceae) and the dwarf shrub *Salix herbacea* (Salicaceae) occur together in this area in a patchy distribution. Fife sampling plots (1 x 1 m) were selected for each plant, and fife root samples were taken from each plot. Soil samples were taken directly in hole of the root samples.
Soil fungal communities were investigated using direct DNA isolation, PCR of internal transcribed spacer rDNA genes and sequencing of cloned fragments. Fungal biomass was assessed by ergosterol contents. The mycobionts of each ectomycorrhizal morphotype were identified by molecular methods (rDNA ITS sequencing). Fungal fruit bodies collected and identified at the sampling site were used as reference material.
There is a high overlap in species richness- and diversity of mycobionts between the three host plants. Comparing root- and soil fungal communities indicates links between the fungal species composition on mycorrhized roots and the soil fungal community. This elucidates potential roles of plants in stimulating the establishment and development of ECM fungi, or vice versa.

PS9-164-0316

Ectomycorrhizal communities associated with a *Pinus radiata* plantation in New Zealand

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In Forestry, seedling quality is critical for a successful plantation establishment and is greatly dependent on mycorrhizal symbiosis. Poor mycorrhizal colonisation is detrimental as nutrient deficiency and reduced growth of stock causes poor plant establishment and subsequent growth. This study investigated ectomycorrhizal (ECM) communities in a *Pinus radiata* plantation in the North Island of New Zealand in five stands of different age, ranging from the nursery through to immediately pre-harvest, using molecular identification methods of both sporocarps and ECM root tips.
Field Sites: Five stands of different ages were used for sporocarp and ECM root tip surveys. Sporocarps were surveyed at fortnightly intervals between April and July 2005. Collected specimens were identified to genus level and tissue samples were taken for DNA analysis. Soil core surveys were conducted in July and December 2005. ECM root tips were extracted from the soil cores and grouped into morphotypes. DNA from sporocarps and ECM root tips was amplified using the ITS1-F/ITS4 primer set and PCR products were then subject to RFLP pattern analysis using the restriction enzymes *AluI, HinfI* and *MboI*. PCR products were also sequenced; sequence search was performed using the NCBI-GenBank service.
A total of 13 ECM species were collected as sporocarps across the survey. In contrast 15 ECM root tip types were morphologically identified, with molecular identification in progress. For both sporocarp and ECM root tips, species diversity increased from nursery to harvest, with species diversity being higher for ECM root tips compared with sporocarps at each age group. Sporocarps morphologically identified as *Hebeloma crustuliniforme*, *Tricholoma pessundatum* and *Laccaria laccata*, have been reported to be mycorrhizal associates of P. radiata in plantations in New Zealand in the past. Sequence analyses have identified species collected in this survey as Hebeloma oculatum, Tricholoma populinum or *T. ustale* and *Laccaria proxima*, respectively. The present sporocarp survey also identified Lactarius rufus, previously unreported from New Zealand. *Thelephora terrestris*, *Tomentella* sp. and *Tuber* sp., known symbionts of P. radiata in New Zealand, were collected during the soil core survey but not in the sporocarp survey. *Wilcoxina* sp., *Phialophora finlandia* and *Pseudotomentella mucidula* were collected in the ECM root tip survey and are known ECM fungi, but have not previously been reported in New Zealand.
Discussion
The findings of the sporocarp survey in 2005 generally agree with what has been reported on ECM associates with P. *radiata* in New Zealand; however, sequencing of sporocarp material resulted in clarification of several species present in New Zealand. The preliminary molecular results imply that the underground ECM community is more diverse than what indicated by the sporocarp survey, further sampling of sporocarps and ECM root tips is currently underway to confirm results.

PS9-165-0376

The functional diversity of *Caladenia tentaculata* (Orchidaceae) mycorrhizal fungi from six distinct populations within Victoria, Australia.

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As mycorrhizal infection is necessary for orchid seed germination, seedling growth and survival of adult plants, current conservation practices involve propagation of symbiotic seedlings for re-introduction. However, little is known of the functional diversity of these mycorrhizal fungi. *C. tentaculata* is a common species with a wide distribution reflecting the habitats of many endangered *Caladenia* species. Six study sites were selected, varying in climate, soil type and soil nutrient content. A fungal isolate from each site was used to germinate seed from its own and the five other sites: it was hypothesised that the isolate giving the highest seed germination would be the isolate collected from the same site as the seed. However, this was only found to be the case for half the sites. ITS sequencing showed that three fungi were identical and five were closely related (97-99%) to Sebacina vermifera. The other isolate showed 94% similarity to a fungus isolated from *C. formosa,* and interestingly was the only isolate to germinate seed from all six sites. Preference for simple and complex carbon and nitrogen sources was also investigated (dry weight on MMN liquid medium). Considerable functional variation was observed with all isolates studied, including the isolates identical to each other and closely related to *S. vermifera.* The implications of this functional diversity for the conservation of *Caladenia species* across different habitats are discussed.

PS9-166-0383

Low doses of fungicides influence competition and functioning of arbuscular mycorrhizal fungi

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Most plant species including important agricultural crops, form arbuscular mycorrhizas (AMF). The functioning of the symbiosis depends on the composition of the community of AMF colonizing the plant, but little is known about how mycorrhizal fungi co-exist in plant roots. Fungicides have been shown to affect AMF, by reducing colonization and hyphal P uptake, but may also influence the competition between the fungi and thereby the composition and function of a field population. The study showed that closely related AMF from the same field had different effects on the host plant performance. The relative colonization by the fungi was strongly influenced by the combination of fungi, and the relative abundance of the fungi influenced both plant biomass and P uptake by the plant. Low doses of the fungicides Carbendazim and Mancozeb influenced the relative abundance of the fungi and the plant performance. Though fungicides may not have measurable effects on AMF community composition in the field due to large spatial variation, fungicides may influence local competition between AMF and thus plant performance.

PS9-167-0393

Relation between alkaline phosphatase and polyphosphate in arbuscules of arbuscular mycorrhizal fungi

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Introduction Arbuscular mycorrhizal (AM) symbiosis is characterized by a specific organ, the arbuscule, which is formed in root cortex cells by the penetration of the finely branched hyphae of AM fungi. Host plants acquire phosphate (Pi) from AM fungi via Pi-transporter on the arbuscule-cortical cell interface. Alkaline phosphatase (ALP) has been thought to have an important role in the transfer of Pi from AM fungi to host plants, although recently there is a theory that acid phosphatase (ACP) is more important than ALP because most arbuscules show ACP activity. Although there have not been direct evidences, it is thought that polyphosphate (polyP) is hydrolyzed by ALP, ACP or other polyphosphatase in arbuscules. In this study, we analyzed the localization of ALP activity and polyP by the fluorescent dual-staining method to investigate the relation between ALP and polyP in arbuscules.
Methods Root pieces of *Lotus japonicus* colonized with *Glomus intraradices* were sectioned and labeled by DAPI, ELF97, or these dual staining. The labeled sections were observed using fluorescent microscope.
Results ALP activity was high in the fine branches of mature arbuscules and low in the center of them. Most of immature or collapsed arbuscules did not show ALP activity. PolyP was detected mainly in the center of mature arbuscules and in immature or collapsed arbuscules. PolyP was not detected in the area showing high ALP activity.
Discussion Our results showed that every arbuscules did not have high ALP activity. ALP activety was mainly detected in mature arbuscules. It is known that Pi-transporters of host plants are localized around mature arbuscules. From these things, ALP rather than ACP may have function in Pi-transfer from AM fungi to plants. Furthermore, from the result that polyP was hardly detected in the fine branches of mature arbuscules, polyP may be hydrolyzed in mature arbuscules. The positional relation between ALP activity and polyP suggests that ALP may be directly or indirectly involved in polyP metabolism in arbuscules.

PS9-168-0447

Molecular Diversity Of Ectomycorrhizal Fungi Associated With Southern Beech In New Zealand

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New Zealand only has a small number of native ectomycorrhizal trees: the southern beeches *(Nothofagus spp.)*, manuka (*Leptospermum scoparium*), and kanuka *(Kunzea ericoides*). However, extensive forests of these species contain very high cryptogamic biodiversity, and are thus highly significant components of the NZ flora. The diversity of ectomycorrhizal fungi in native New Zealand trees has to date been assessed largely by the collection of identification of epigeous and some hypogeous fruitbodies, or rarely by morphotyping of excised root tips. Thus current knowledge of species diversity is likely to be an underestimate of the true diversity, due to poor correlation between fungal reproductive structures and below-ground diversity. Using ITS-RFLP, cloning and DNA sequencing, we have assessed the diversity of fungi associated with excised root tips of silver beech (*Nothofagus menziesii*) and mountain beech (*N. solandri* var. *cliffortioides*) in selected NZ forests. The diversity of these sequences is compared with the taxonomic diversity of previously described species in each genus detected. Preliminary results indicate that the most common phylotypes found are sequences with high similarity to the genera *Russula* and *Cortinarius*, but also indicate the presence of sequences with high similarity to genera previously unrecorded in New Zealand. Ongoing work is focussed on using molecular community profiling techniques (denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment polymorphism analysis (T-RFLP)) to make broader assessments of fungal diversity in native plant communities.

PS9-169-0448

Fungal discrimination amongst three epiphytic Aeridinae orchids of south-eastern Australia

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Orchid seeds depend on colonization by an endophytic fungus for germination. Epiphytic orchids also need a substrate, for example a host tree, upon which to establish. Earlier investigations into three co-occurring, epiphytic, Aeridinae orchids of temperate Australia demonstrated distinct distribution patterns within the forest ecosystem. Two of the orchid species, *Sarcochilus olivaceous and Plectorrhiza* tridentata utilized only a subset of the potential host tree species available, whilst the third, *Sarcochilus hillii*, approximated a random distribution. The aim of this study was to identify the orchid-mycorrhizal fungi (OMF) of these orchid species. We hypothesise that orchid species in a common environment would utilise all OMF within that environment.
We sampled 60 fungi from the three orchid species on their two most prevalent tree hosts across two sites (including one tree species shared by all three orchid species). Identifying these OMF involved their isolation from orchid roots, functional verification through germination trials, and genetic sequencing. To determine the identity and relationships of the isolated fungi: the nuclear ribosomal internal transcribed spacer region (ITS) and the mitochondrial large subunit (ML) were used. Phylogenetic analysis of sequences was conducted using PAUP*.
Sequence data showed that all the OMF belong to the genus Ceratobasidium. Analysis of ML sequences revealed two distinct clades of OMF. ITS supported the ML data but indicated the presence of many genotypes within each ML clade. Cloning of PCR products revealed different copies of ITS within 40 of the individual fungal isolates. Fungi from both ML clades were found at each site and on the common tree species. Sarcochilus olivaceous and *P. tridentata*, were found with OMF from only one of the ML clades whilst *S. hillii*, was found with OMF from both ML clades.
In conclusion, while the fungi themselves appear to be widely distributed, these orchid species do not appear to associate with all OMF within their local environment, with the exception of *S. hillii*. *Sarcochilus hillii* utilises both the widest range of tree species and the greatest diversity of OMF; whist *S. olivaceous* and *P. tridentata* appear more restricted in both tree and fungal associates. We hypothesise that the distribution of the OMF plays a role in the distribution patterns of these three orchid species.

PS9-170-0450

Effect of Trichoderma bio-inoculants on ectomycorrhizal colonisation of Pinus radiata seedlings

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Trichoderma based bio-inoculants are applied in commercial *Pinus radiata* nurseries to provide protection once seedlings are planted into forestry plantations to soil pathogens, such as, *Armillaria*. Mechanisms employed by *Trichoderma* species, such as mycoparasitism, antibiosis, and their highly competitive saprophytic nature could have inhibitory, non-target effects on ectomycorrhizal (ECM) colonisation of *P. radiata* seedlings.
A trial was conducted to examine the effect of *Trichoderma* inoculation on the overall percentage ECM colonisation of P. radiata seedlings. Further, a dual agar plate bioassay experiment was conducted to assess individual interactions between five ECM species, known to be early colonisers of *P. radiata*, and six *Trichoderma* isolates.
A *Trichoderma* isolate specific effect on the overall percentage colonisation of ECM was observed. One isolate of *Trichoderma* increased percentage ECM colonisation while the other isolates, including a composite mix of all isolates, had no effect over the control. In the dual plate bioassays, the ECM species Suillus granulatus was not mycoparasitised or out-competed by any of the six *Trichoderma* isolates. While *Rhizopogon parksii* was out-competed by all Trichoderma isolates and, although not mycoparasitised, was extensively overgrown by *Trichoderma* hyphae.
While one *Trichoderma* isolate on its own increased ECM colonisation, this isolate in a composite mix had no effect on ECM colonisation. Changes in the diversity of ECM species colonising the roots could have occurred, as indicated by the dual plate bioassays where differential isolate specific interactions between the two classes of fungal symbionts occurred. This is currently being investigated in a pot trial assessing the effects of Trichoderma application on the colonisation of *P. radiata* by five ECM species *(Suillus granulatus, Suillus luteus, Rhizopogon parksii, R. luteolus*, and *Rhizopogon spp.*) which have been artificially inoculated into the potting mix.

PS9-171-0463

Mediterranean serpentine and non-serpentine ectomycorrhizal fungal communities

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Serpentine soils are extreme environments characterized by a unique combination of chemical and physical stressors including low levels of macro and micronutrients, drought, and high levels of heavy metals. This study focused on the ectomycorrhizal (ECM) fungal communities of a serpentine *Quercus rotundifolia* forest and a non-serpentine *Quercus pyrenaica* forest in northeastern Portugal. These forests are often found next to each other, and though very different in soil type and vegetation, host some common fungal species and have ECM fungi as a major component of their macrofungal communities (Branco, unpublished).

ECM community assessment was based on the collection and analysis of fungi colonizing root tips. Soil cores from 20 oak trees were collected in each forest. ECM roots of each core were sorted into morphotypes and one tip of each morphotype in each core was identified molecularly by terminal restriction fragment length polymorphism analysis (TRFLP) of the nuclear rDNA ITS region.

Forty-nine different ECM morphotypes were found in the *Q. pyrenaica* forest, almost twice as much as the serpentine *Q. rotundifolia* (26 morphotypes). In both forests the morphotype frequency showed a similar structure, with a few common and many rare morphotypes. A total of fifty-seven *Q. rotundifolia* and seventy-four *Q. pyrenaica* tips were analyzed molecularly, giving sixty-seven and seventy-nine TRFLP profiles respectively. The combination of morphological and molecular data indicated most types were rare, with only six types detected twice.

Based on morphological data alone the serpentine oak forest was less diverse, a finding consistent with the trend detected previously by fruitbody collection (Branco, unpublished). However, a combined morphological and molecular analysis indicated that both ECM communities were very species rich. This might result from a significant number of cryptic species not distinguishable by morphology, under-sampling, DNA amplification of contaminant fungi, and/or inherent problems with the TRFLP technique or molecular marker. Further sampling as well as the refinement of the techniques employed will continue to unravel the structure of ECM communities of these two interesting habitats.

PS9-172-0470

Mycorrhizal associations of *Borya mirabilis* a critically endangered Australian native.

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Borya mirabilis Churchill, otherwise known as the Grampians Pincushion Lily, is a Critically Endangered (ANZECC, 1999) plant of the Gariwerd/Grampians National Park. It is a resurrection plants. Previously thought to be extinct, this plant has been rediscovered in a small 20 m by 60 m area on top of a rocky peak. Currently there are only five separate clumps within this area and recovery plans focus on ex situ propagation and translocation. In many Australian native plants, successful growth requires effective mycorrhiza, a compatible association between plant and mycorrhizal fungi, to obtain adequate nutrition.

The mycorrhizal associations of *Borya mirabilis* were examined seasonally during 2004-5. Roots where collected, cleared and stained to observe mycorrhizal type and by inoculating ex situ plants with habitat soil to see growth effects.

Roots were mycorrhizal over all seasons and comprised mainly arbuscular mycorrhiza (AM) to about 50 % infection of the roots, with only about 5 % infection by ectomycorrhiza (EM). AM infection occurred in small nodules resembling truncated lateral roots. AM occurred in *Borya mirabilis* roots at all seasons, but infection occurred as Paris-type, with coils in winter and spores in summer.

Previous examination of roots of *Borya* sp. suggested that it has both EM and AM in the field (Conran and Temby, 2003), as found here, but did not comment on the nodular structures that contained the arbuscular mycorrhiza.

PS9-173-0479

The influence of urbanisation and management practices on ectomycorrhizal fungal associates of two native species of eucalypt.

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Eucalypt species growing in the city of Melbourne form ecomycorrhizae, however little is known about the species richness of the fungal symbionts. Recent evidence from Europe and North America suggests that urbanisation may have a negative impact on ECM fungal diversity. We sampled soil cores from beneath *Eucalyptus obliqua* and *E. camaldulensis* in urban, suburban and rural sites of equivalent habitat to assess fungal species richness and to identify trends associated with urbanisation. At each site trees were chosen with natural or mown understory. Mycorrhizal root tips were sorted from the cores and categorized based on morphology. Overall, 124 morphotypes were found, 91 associated with *E. obliqua* and 33 with *E. camaldulensis*. Species richness was significantly lower at the urban end of the gradient, accounting for only 12% of morphotypes. Mowing also affected fungi with the intact understory having greater richness at 5 of the 6 sites. The results indicate that some factors of urbanisation influence the community structure of ectomycorrhizal fungi, and that management practices can alter species richness.

PS9-174-0485

Ectomycorrhizal fungal communities in rehabilitated bauxite mine sites and adjacent forest.

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Current post-mining rehabilitation procedures at bauxite mines in the northern jarrah *(Eucalyptus marginata*) forest in the south-west of Western Australia aim to establish a stable forest ecosystem in which rehabilitated areas and undisturbed stands are integrated to the maximum extent possible. Research to validate and improve on current rehabilitation techniques and outcomes is ongoing and has a strong focus on biodiversity. This study compared the species richness and composition of ectomycorrhizal fungi in rehabilitated mine sites of three different ages with that of the surrounding forest.
Sampling plots of 50m by 50m were established in forest and rehabilitated mine sites. Fruit-bodies and root tips were collected for 3 and 2 years respectively. Fruit-bodies were identified morphologically and representative herbarium specimens retained. Root tips were identified by PCR-RFLP and DNA sequencing. Species composition was analysed by de-trended correspondence analysis.
A trend of increasing species richness with age of rehabilitated sites was observed. Species richness in 16 year old rehabilitated mine sites approached that of un-mined jarrah forest. Rehabilitated sites of similar age clustered together in the de-trended correspondence analysis and species composition became closer to the native jarrah forest as time since rehabilitation increased, though some species abundant in the native forest were absent from even the oldest (16 yo) rehabilitated sites. These included five *Russula* species. In un-mined forest, species composition of fungal communities in the wetter, western region was different from the drier, eastern region.
Current rehabilitation techniques, including topsoil management, appear to result in the re-establishment of a rich ectomycorrhizal fungal community in the regenerated forest. Further study is necessary to determine at what age, if any, the 'missing' species may re-establish. This study indicates the possibility that certain species have the potential to be used as reliable indicators of the progress of fungal community rehabilitation.

PS9-175-0495

Recognition of cryptic species and host specificities in the ectomycorrhizal genus *Strobilomyces* using molecular markers

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The systematics of higher fungi has been undeveloped due to the lacks of useful morphological characters for taxonomy. Such a problem is especially serious in ectomycorrhizal (EM) fungi, in which crossing experiments are usually difficult to perform. Host specificities in EM fungi were considered to be low. However, confusion of several cryptic species might cause the underestimation of host specificities. It is remarked that roles of EM fungi are important in ecosystems. Thus, it is necessary to improve the systematics of EM fungi and to demonstrate the true degrees of their host specificity. We tried to detect cryptic species in EM fungi using codominant DNA markers instead of performing crossing experiments. We elucidated that *Strobilomyces* confusus complex, which is characterized by spiny basidiospores, including two known species, *S. confusus* Sing. and *S.seminudus* Hongo, contained four distinct clades. Then, we performed PCR-RFLP analysis of nuclear single copy genes and evaluated amount of gene flows among the four clades at Mt. Yoshida, Kyoto city, where the four clades were shown to coexist. We detected high gene flows within the four clades, but not among them. These results strongly suggested that each clade of fungi might be independent biological species. We collected the EM of four clades of the complex, and identified the host plants using their nucleotide sequences of chloroplast DNA. We found that three clades constructed EM only with Fagaceae species while the other clade constructed with both Fagaceae and Pinaceae species. This result contradicted the conventional hypothesis that Strobilomyces fungi are generalists in host association, and instead suggested that most *Strobilomyces* fungi might possess more or less host specificities.

PS9-176-0533

Basidiomycetous fungi involved in orchid mycorrhiza

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Orchid is a group of terrestrial plants, which forms a unique type of mycorrhiza. In this mycorrhizal association, orchid is myco-heterotrophic, especially in seed germination and the following protocorm stage. This study aims to clarify both the species of mycorrhizal fungi and the roles of the fungi in some orchids.
Three kinds of orchid species were collected in Japan; *Epipogium roseum*, *Chamaegastrodia sikokiana* and *Cephalanthera falcata*. The first two are achlorophyllous species. Mycorrhizal fungi were isolated from the colonized roots to obtain pure cultures except in *C. falcata*. ITS region of rDNA was amplified by PCR, and then the PCR products were sequenced. Some phylogenetic analyses were carried out with some downloaded data from GenBank to reveal the identity of the mycorrhizal fungi. In E. roseum, mycorrhiza formation was attempted between orchid and the isolated fungus. On the other hand, in *C. sikokiana*, mycorrhiza formation was attempted between the host tree *(Abies firma)* and the fungus.
In *E. roseum,* the mycorrhizal fungi were found to belong to *Coprinus* or *Psathyrella* in Coprinaceae, which indicates that the mycorrhizal fungi work well to obatain carbon compounds from the surrounding fallen logs or leaves and to supply them to orchid. In this association, whole developmental process from seed to flowering was successfully completed under controlled condition. In *C. sikokiana,* the mycorrhizal fungi were found to belong to Ceratobasidiaceae, and could form ectomycorrhiza with seedlings of Abies firma. In *C. falcata,* the mycorrhizal fungi were some species of Thelephoraceae or Russulaceae, and those fungi were supposed to from ectomycorrhiza with *Quercus serrata*. In those latter two mycorrhizal associations, the fungal hyphae could work as a channel connecting between photosynthetic plant roots and the sink orchid roots.
These results suggested that diverse basidiomycetous fungi could be involved in orchid mycorrhiza.

PS9-177-0535

Role of mycorrhizae of grasses and ferns in controlling hill erosion in Mountainous areas of Pakistan.

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The present investigation concentrates on studying the role of mycorrhizal communities to cutting and grazing stress, since under field conditions these fungi are crucial for the transfer of minerals from the soil solution to tree roots (Harley & Smith, 1983). Effects of disturbances like excessive felling of trees and overgrazing have shown to reduce root growth and hence affect the picture of mycorrhizal communities. Indirect effects of cutting and grazing in reducing photosynthesis and hence carbon allocation to the root system may also inhibit mycorrhizal development. In nutrient poor environments mycorrhizae contribute not only to plant nutrition but also to process of soil stabilization by binding sand grains into water stable aggregates (Koske et al., 1975; Forster & Nicolson, 1981). They do so by binding the soil particles together. (Miller & Jastrow, 1992). Koske et al. (1975). Extensive hyphal networks of mycorrhizal fungi binding soil among plant roots. Lynch & Bragg (1985) suggested an indirect role of the fungi in soil binding. They emphasized that the hyphae may be serving as substrates for other polysaccharide-producing microorganisms. The bacterial polysaccharides cement the soil particle together, (Tisdall & Oads, 1979).
Four different stands in the Northern areas of Pakistan were sampled and analyzed using simple ecological methods. The stands sampled were situated in Ayubia and Nathia Gali, and Dunga gali. The stands included undisturbed natural vegetation stands and disturbed stands. The disturbed stands were characterized by excessive cutting and felling of trees and overgrazing leading to entirely changed picture of the plant communities and associated mycoflora. For mycological studies the root of plants of the above mentioned stands along with the rhizosphere soil were sampled and processed. It was recorded that the types of ectomycorrhizal fungi varied as the forest stands matured. The fungi almost tend to disappear when the forest trees are cut. The number of root tips of 25 ferns species with ectomycorrhizae decreased when the stands were disturbed. So was the case with r arbusculr mycorrhiza forming endogonaceous spores number in the rhizosphere soil of 150 various types of grasses found in the area. The weight of water stable aggregates also reduced in the soil of disturbed stands. These disastrous situations then end up with removal of rest of the forest vegetation and excessive erosion or removal of top fertile soil. This can be avoided with proper management of these fungi.

PS9-178-0538

Response of wheat (*Triticum aestivum* cv. Pak 81) grown in open top chamber system to various arbuscular mycorrhizal treatments.

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The experiments were conducted in open top chamber system installed at the University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. The wheat (*Triticum aestivum* Var. Blue silver) seeds were sown in earthen pots and were kept in filtered air (FA), unfiltered air (UFA) and ambient air (AA). The plants were grown in three different sets with three different inoculations for arbuscular mycorrhizal fungi. The ozone concentration was monitored daily during 1000hrs till 1600hrs. The data for light intensity and relative humidity was also regularly collected. The sets of plants growing in FA chambers (without ozone and dust particles) responded well as regards growth and yield are concerned. There were statistical differenced among sets with variable inocula for mycorrhizal fungi. The species of mycorrhizal fungi sensitive to tropospheric ozone failed to reproduce in ambient air and unfiltered air chambers (without dust particles).
Out of a total of 33 species, twenty-five species belonged to the genus *Glomus*, three each to *Acaulospora* and *Sclerocystis* and one each to *Gigaspora* and *Scutellospora*. The total number of species was variable during the growth phase. The total number of species reduced in soil of UFA chambers with the passage of time in all mycorrhizal treatments. The number of species reduced to almost half in UFA plants as compared to FA plants.
Maximum number of species (33) at each harvest was recorded in the case of T1 treatment in FA chambers, where the root pieces were used as inoculum. At first harvest the number of species for T2 and control sets of FA chambers was different i.e. 25 and 28 for the two respectively, which was same (24) at the time of third harvest.
Species of the Genus *Glomus* were highly abundant species at various harvests in all mycorrhizal and air treatments. Amongst most abundantly recovered *Glomus species were G. fasciculatum, G. mosseae, G. aggregatum, G. caledonicum, G. deserticola, G. geosporum, G. monosporum, G. multicaul, and G. reticulatum. The pattern of abundance kept on varying at various harvests for different air and mycorrhizal treatments. In the case of plants of UFA treatment, only two species of Glomus were abundant namely G. fasciculatum and G. geosporu*m. Species of Acaulospora and Gigaspora in particular and Scutellospora and Sclerocystis in general were sensitive to polluted air.

PS9-179-0565

Diversity And Germination Efficacy Of Mycorrhizal Fungi From Caladenia formosa G.W. Carr (Orchidaceae)

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Mycorrhizal fungi extracted from the orchid *Caladenia formosa* were compared across the developmental season using Random Fragment Length Polymorphism (RFLP) and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. The fungi isolated were closely related (94-100% homology), suggesting clones that persevered in the soil or tuber throughout the season. There was no relationship within the isolates between ITS homology and effectiveness in germination of seed. All fungi from *C. formosa* formed one clade, shared up to 83% homology with Sebacina vermifera and clustered within Sebacinaceae. The sebacinoids clustered away from other known orchid mycorrhizal fungi (*Tulasnella* spp., *Ceratobasidium* spp. and *Thanatephorus* spp.), with less than 69% homology within the nrDNA ITS region. Effective fungi with varying germination abilities isolated from *C. formosa* are thus likely to be in the Sebacinaceae, but sporulation is required for confirmation of their identity and speciation.

PS9-180-0566

Meliniomyces variabilis (Variable White Taxon), isolated from a fruitbody of *Hydnotria tulasnei*, forms ericoid mycorrhiza and changes morphology of *Betula, Picea* and Pinus roots

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The attempts to isolate ascomycetous *Hydnotria tulasnei* Berk. & Br. from fresh hypogeous fruitbodies into a pure culture yielded beside *H. tulasnei* also the mycelium of *Meliniomyces variabilis* Hambleton & Sigler (=strain MV2). M. variabilis belongs to the *Hymenoscyphus ericae* (Read) Korf & Kernan [=*Rhizoscyphus ericae* (Read) Zhuang & Korf] aggregate, which comprises fungi forming ericoid mycorrhiza (ErM) and/or ectomycorrhiza (EcM). MV2 was nearly identical to another *M. variabilis strain* (=MV1), isolated previously from a non-mycorrhizal root tip of Picea abies (L.) Karst.
Recent publications indicate that members of H. ericae aggregate isolated from roots of ErM hosts rarely form EcM and vice versa. Therefore, it seemed interesting to investigate mycorrhizal potential of MV1 originating from a non-mycorrhizal *Picea* root tip and MV2 from a hypogeous fruitbody of the EcM fungus. For the comparison, we also employed a strain of ErM fungus *R. ericae*, originally isolated from roots of *Calluna vulgaris* Hull.
All three fungal strains were aseptically inoculated to ericaceous Vaccinium corymbosum L. and Rhododendron ponticum *L. coniferous P. abies* and *Pinus sylvestris* L. and broad-leafed *Betula nana* L. seedlings.
All three fungal strains formed typical ErM in V. corymbosum and R. *ponticum* roots and also substantially changed the morphology of typically EcM species. At first glance, the root colonization of *P. abies, P. sylvestris* and *B. nana* inoculated with *R. ericae* resembled EcM. However, transversal sections of roots revealed absence of Hartig net and sometimes abundant intracellular colonization of turgescent root cells by fungal hyphae.
To our knowledge, this is the first report about the isolation of ErM fungus from a fresh, healthy fruitbody of an EcM species, which extends the known range of habitats of ErM fungi and indicates interesting interaction between ErM and EcM fungi. In our study, ErM fungi isolated from non-ErM root tip and EcM fruitbody formed ErM and non-specifically colonized roots of potentially EcM plants without forming EcM, but colonizing them intracellularly, changing their morphology in a similar manner as EcM fungi. Roots from field samples resembling EcM should be carefully screened for the presence of ErM fungi to reveal whether the association between ErM fungi and typically EcM hosts is under natural conditions regular or exceptional.

PS9-181-0567

The Mycorrhizal Associations Of *Borya Mirabilis* A Critically Endangered Australian Native Plant

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Borya mirabilis Churchill, otherwise known as the Grampians Pincushion Lily, is a Critically Endangered (ANZECC, 1999) plant of the Gariwerd/Grampians National Park. It is a resurrection plant. Previously thought to be extinct, this plant has been rediscovered in a small 20 m by 60 m area on top of a rocky peak. Currently there are only five separate clumps within this area and recovery plans focus on ex situ propagation and translocation. In many Australian native plants, successful growth requires effective mycorrhiza, a compatible association between plant and mycorrhizal fungi, to obtain adequate nutrition. Therefore the mycorrhizal associations of *Borya mirabilis* were investigated seasonally during 2004-5. Roots were collected cleared and stained to observe mycorrhizal type and by inoculating ex situ plants with habitat soil to see growth effects. Roots were mycorrhizal at all seasons and comprised mainly arbuscular mucorrhiza (AM) to about 50 % infection of the roots, with only about 5 % infection by ectomycorrhiza. AM infection occurred in small nodules resembling truncated lateral roots. AM occurred in B. *mirabilis* roots at all seasons, but infection occurred as Paris-type, with coils in winter and spores in summer. Previous examination of roots of Western Australian *Borya* spp. suggest that they have both ectomycorrhiza and AM in the field (Conran and Temby, 2003), as found here, but did not comment on the seasonal changes or nodular structures that contained the AM. Plants grown from cuttings in a glasshouse were inoculated with soil from around roots at the field site and effects on plant health and vigour were evaluated.

PS9-182-0577

Diversity of Mycorrhizal Fungi and Associated Plants Growing in Copper-rich Abandoned Mine Site

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Abundance of mycorrhizal fungi was investigated in this study to determine their diversity and association with plants that are potential in bio-rehabilitation of copper-rich ecosystem. A quadrat sampling technique was conducted to evaluate fungal population and plant composition within a portion of the 35,000-hectare abandoned mining site. Five transect measuring a kilometre each was established randomly in vegetation cover surrounding mine pits and dumpsites. Five sampling quadrats were alternately located along each transect at an equal interval. Trees, intermediate plants and undergrowth layer were identified from sampling plots measuring 10 x 10m, 3 x 3m, and 1 x 1m, respectively. Plant community type and diversity values were determined. Soil samples from each layer were collected to analyse copper content. Fine roots were cleaned and cleared with potassium hydroxide prior to staining in tryphan blue for assessment of mycorrhizal infection. A separate 10g soil sample from each plot was wet-sieved and decanted to isolate endomycorrhizal spores and compute for the density. Ectomycorrhizal fungi found under plantation within the mine site were likewise identified.

The area was generally covered by vegetation classified as a disturbed grass-shrubland-agroforest plant community growing in soil with copper content averaging 320 mg kg -1 dry soil. A total of 69 plant species, 66 genera and 35 families were identified with diversity index of 1.81 – 3.12. Among these, Acacaia mangium and four undergrowth plants (*Lycopodium, Saccharum, Nephrolepis, Stachytarpheta*) were heavily colonized by vesicular-arbuscular mycorrhizal fungi. All roots from *Stachytarpheta jamaicensis* were infected solely by *Glomus* species. Endomycorrhizal spores isolated from soil around *Pithecellobium dulce* harboured the highest density (2,575 spores/plant/30g soil), consisting of *Glomus, Acaulospora and Entrophospora*. *Muntingia calabura* was the only intermediate plant associated with *Glomus, Gigaspora, Scutellospora, Acaulospora and Entrophospora*, but with low spore population. Fruiting bodies of ectomycorrhizal fungi like *Pisolithus, Scleroderma, Thelephora* and *Bolettellus* were also found under plantation of *Acacia auriculiformis, A. mangium, Eucalyptus urophylla* and E. *camaldulensis.*

The diversity of mycorrhizal fungi and associated host plants were bio-indicators of their important ecological role in the rehabilitation of abandoned mining site. These fungi should be harnessed by testing their effectiveness and efficacy in the restoration of disturbed ecosystem like copper-rich areas.

PS9-183-0580

Differences In Groups Of Orchid Mycorrhizal Fungi Infecting Groups Of Caladenia Species In Australia

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Mycorrhizal fungi are vital for the survival and health of terrestrial orchids, in particular *Caladenia* in Victoria. Single-peloton isolates from common and threatened species were compared for effectiveness in germination, cross-infection and molecular phylogeny. Mycorrhizal isolates from common species (*C. tentaculata* and *C. phaeoclavia*) resulted in greater and more consistent germination rates than isolates from taxonomically distant rarer species (*C.fulva, C. hastata, C. robinsonii* and *C. venusta*). Cross-inoculation was largely unsuccessful and germination rates were inconsistent, especially in the rarer species. Both RAPD and ITS-PCR-RFLP suggested that most isolates contained more than one fungus. The culture R8 was isolated by dilution-plating of the monilioid cells from a previous isolate from *C. tentaculata*. Its ITS sequence was 99% similar to that of *Serendipita vermifera (Sebacina vermifera* Oberwinkler sensu Warcup and Talbot, 1967; Roberts, 1993), originally isolated from *C. dilatata* (from which *C. tentaculata* and *C. phaoclavia* were speciated). The R8 sequence was used to design specific primers, which produced amplicons with all mixed isolates but which could be sequenced. Two distinct groups of fungi were described by the sequence data. One group was isolated from *C. tentaculata* and *C. phaeoclavia* plants and was most closely related to *S. vermifera* (99.3-100% homology in the rDNA ITS region). The other group contained fungi isolated from *C. venusta, C. fulva, C. hastata* and *C. robinsonii* and remained unidentified, not having a close match in the databases. This latter group was still most closely related to *S. vermifera* (using the GenBank and EMBL fungal database), although sequences were only 29-35% homologous. These specific primers could be used to screen the external environment for the distribution of this fungus on the site and to screen potential translocation sites for the presence of a compatible orchid mycorrhizal fungus.

PS9-184-0590

Orchids and larger fungi: does a relationship exist above ground

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The Orchidaceae - due to their rarity and high endemizing capabilities - have been included in the lists of endangered/protected species at world and national level and protected under several international statements. An extensive and integrated project investigating the influences of genetic and ecological factors on reproduction and colonization success of Mediterranean orchids involving five Universities is in progress in Italy.
In particular, the interaction of *Limodorum abortivum* Battandier, a mixotrophic orchid, and its symbiontic associated fungi have been investigated. The analysis ranged from the real photosynthetic capacity in the various growing stages to roots fungal diversity by morphological and molecular characteristics. From the latter results, in addition to the well-known endophyte *Rhizoctonia*, another symbiont complex belonging to the *Russulaceae* was identified (Girlanda et al., Molecular Ecology 2006).
In order to see if this is reflected in the fungal community above ground, fruiting bodies of epigeous macromycetes were surveyed in semicircular sample plots of increasing distances from the selected orchid population.
Data from 2 years of observation, carried out in 7 areas in Tuscany (Italy), chosen in different plant communities and environments along an altitudinal transect from the mountains to sea level, are reported.
About 270 species were identified and nearly half of them were ectomycorrhizal growing principally at a distance of 10-20 m from the orchid. Dominant were Cortinariaceae followed by Russulaceae. Among these, *Russula delica* Fr. and R. chloroides Peck, also described in the root symbiotic complex, were only found near orchids confirming the tight trophic relationship. To note that various fruiting bodies of *R. delica* were counted nearby L. abortivum cultivated for an ex-situ conservation in the Botanical Garden of Siena, surely an artificial environment.
Thus, the results obtained open interesting research prospectives on the ecological and evolution aspects of the mycorrhizal association for this orchid and for the definition of efficient conservation measures in-situ and ex-situ in addition to projects for reintroduction.

PS9-185-0603

Inoculation with species of *Rhizopogon* and *Scleroderma* to improve the quality of forestal plants produced in nurseries

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Introduction:

Forest plantations in the North of Spain are generally submitted to rather mild climates with high precipitation rates. Nevertheless, they suffer marked periods of drought at irregular intervals, which affect negatively on growth and survival of seedlings. In this context, utilization of species resistant to low water availability seems to be an advisable practice.
Resistance to water stress in plants can be achieved by a series of morphological and physiological features and responses which can, to a great extent, be conditioned in the nursery by certain cultural practices (Villar-Salvador et al. 1999). Among these practices, controlled mycorrhization by inoculating nursery-growth seedlings with selected fungal strains, is an interesting alternative that can improve plant-water relations, therefore enhancing the plant drought tolerance (Davies et al. 1996).
Many native ectomycorrhizal fungi present in the north of Spain are able to form mycorrhizas under controlled conditions, and different fungal associations do not provide the same benefit to the host obtaining a pronounced variability in the response depending on the nature of the fungal-plant associations (Stenström & Ek 1990, Guehl et al. 1990). Among the tested fungal strains, some species of *Rhizopogon* and *Scleroderma* are early ectomycorrhizal root-colonizers of great interest as inoculants.
The purpose of this work was to study the response of *Pinus radiata, Pseudotsuga menziesii, Castanea sativa* and *Quercus robur* seedlings to the inoculation with different fungal species of the genus *Rhizopogon* and *Scleroderma,* making a special point of the tolerance to drought conditions.
For this study four species of *Rhizopogon* were used: *R. colossus* A.H.Sm. and R. subareolatus A.H.Sm. for the inoculation of Douglass-fir and *R. luteolus* Fr. and *R. roseolus* (Corda) Th. Fr. for *Pinus radiata*. In the case of *Castanea sativa* and *Quercus robur Scleroderma citrinum* Pers. and *Sclerodema verrucosum* (Bull.) Pers. were used. In all cases inoculation was performed using sporal inoculum. 8 months after inoculation the mycorrhizal infection was tested and mophological parameters were measured: stem height, stem diameter, root and shoot dry weights. Moreover, the plants were subjected to water stress, imposed to gradually attain a moderate and severe water deficit. The physiological status of the plants was evaluated on the basis of gas exchange and chlorophyll fluorescence parameters.
The effects of nursery ECM inoculation on seedling growth depended on the fungal species, but in general, plants showed a significantly higher shoot growth and all plant-fungus combinations showed higher root dry weights relative to non-inoculated plants. Likewise, inoculation of seedlings with *Rhizopogon* and *Scleroderma* species had marked positive effects on the tolerance to drought conditions. All tested species improved quality of host plants, but we propose *Rhizopogon* roseolus and *Scleroderma* citrinum as promising fungal species for inoculation programs in container nursery.

PS9-186-0614

Arbuscular mycorrhizal fungi of wheat in northeast of Iran

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The Golestan Province, northeast of Iran near the Caspian Sea, is an important wheat product area in Iran . Arbuscular mycorrhizal fungi(AMF) can increase water and nutrient (especially P) uptake , and therefore yield of this crop. For collection and identification these useful native fungi, during springs of 2004 and 2005 , wheat fields in this area surveyed and root cores sampled. Spores of AMF extracted by wet sieving and decanting method followed by sucrose centrifugation . Spores mounted in PVLG and crashed in PVLG+ Meltzer's reagent and studied under calibrated bright-field microscope. Eighteen AMF belong to 3 orders, 4 family and 4 genus, phylum *Glomermycota* identified as fallows: *Entrophospora infrequens* (Hall) Ames and Schneider, *Glomus caledonium* (Nicol.& Gerd.)Trappe & Gerd., *G. clarum* Nicol. & Schenck, *G. constrictum* Trappe, *G. deserticola* Trappe, Bloss & Menge, G. eburneum Kenn., Stutz & Morton, *G. etunicatum* Becker & Gerd., *G. geosporum* (Nicol. & Gerd.) Walker, *G. gibbosum* Blaszk., *G. globiferum* Koske & Walker, *G. intraradices* Schenck & Smith, *G. microcarpum* Tul. & C. Tul., *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. multifoum* Tadych & Blaszk., *G. rubiforme* (Gerd. & Trappe) Almeida & Schenck, *Glomus sinuosum* (Gerd. & Bakshi)Almeida & Schenck, Paraglomus occultum (Walker) Morton & Redecker and *Scutellospora dipurpurescens* Morton & Koske. All of these fungi are new for wheat mycorrhizal flora in this area. *G. eburneum , G. globiferum* and *Paraglomus occultum* probably are new for wheat mycorrhizal flora in Asia.

PS9-187-0621

Frantic diversity of resupinate thelephoroid fungi on ectomycorrhizal tree roots

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We will demonstrate the extremely high species richness of the resupinate thelephoroid fungi based on four independent studies: two from Danish beech forests, one in an Estonian wooded meadow and one from a Californian oak forest. In these studies ectomycorrhizal (EcM) tree roots were sampled by using slightly different sampling design and efforts. Sampled EcM were segregated into different morphotypes and further into anatomotypes based on the structure of mantle, cystidia, emanating hyphae and rhizomorphs. One or two root tips of each anatomotypes were subjected to DNA extraction and sequencing. The ribosomal DNA internal transcribed spacer (ITS) sequences of the EcM fungi were obtained for all analysed root tips. Root tip sequences of the resupinate thelephoroid fungi were separated from the rest and were aligned against an appropriate data matrix of fruitbody ITS sequences. Based on the sequences a *Pseudotomentella, a Thelephora/Tomentella* and a *Tomerntellopsis* data matrix were compiled. Software packages Modeltest, Paup, Mr. Modeltest and MrBayes were used for the phylogenetic placement of unknown EcM sequences with sequences of known species of resupinate thelephoroid fungi. In addition we selected 98.0% of ITS sequence identity as species criterion. We did not find any ITS sequences with pair-wise identities between 97.0 to 98.5% but some morphospecies displayed sequence similarities as low as 95%. However, in these cases the fruitbody sequences of the same morphospecies are falling into separate and well supported cryptic species which have identity >98.0%. We also created species accumulation curves for the EcM resupinate thelephoroid fungi to demonstrate species richness vs sampling effort at different sites and to estimate the number of unseen species.
In three of the studied sites the resupinate thelephoroid fungi was the most species rich EcM clade exceeding 60 species per site in Estonian wooded meadow. Despite the large number of fruitbody sequences generated during this and previous studies most EcM ITS sequences remained unidentified on species level. Because they rarely matched to any fruitbody sequences with >95% identity.
Our study show that temperate deciduous or mixed forests hosts unprecedented high species richness of resupinate thelephoroid fungi.

PS9-188-0782

Soil organic carbon decline and the importance of arbuscular mycorrhizal fungi

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Soil carbon is crucial to soil quality and losses of carbon through cropping contribute significantly to greenhouse gas emissions. Increased production of greenhouse gases are causing acute environmental changes including; temperature increases, rise in sea levels due to polar ice cap meltdown, and increases in climatic variability. The depletion of soil carbon stores, via erosion and oxidation resulting from agricultural management practices, accounts for the release of 1 to 2 billion tonnes of C from soil a year. There is an urgent need to address the continued soil carbon decline in cropping systems.
Arbuscular mycorrhizal (AM) fungal structures and AM associated glomalin (an undefined proteinaceous substance) are common in soil. AM may have the potential to increase soil carbon levels by: (i) diverting fixed carbon to a more stable carbon pool; (ii) increasing the structural stability of soil, which in turn slows the rate of degradation of soil carbon from other sources; and (iii) acting as a carbon sink that gives negative feedback to atmospheric CO2.
To investigate the effect that cropping may have on the AM fungal contribution to soil carbon, field surveys of both native and cropped soils were conducted and samples analysed for AM hyphal length, Bradford reactive soil protein (BRSP), organic matter, organic carbon, water stability of aggregates and basic soil chemistry (total N, nitrate, total P, pH, exchangeable cations K+ Ca2+ Mg2+ Na+, electrical conductivity and residual CO3).
To determine whether AM fungi have the potential to increase soil organic carbon levels a split-root, compartment pot experiment lasting 12 months and using six different soil types (3 with very low and 3 with higher initial carbon and protein levels with varying soil type and chemistry) was conducted. The four compartments (AM+,roots-; AM+,roots+; AM-,roots+; and AM-,roots-) allow the contribution of AM fungi to soil carbon to be distinguished from that of root turnover, root exudates and the rest of the microbial community.
These studies aim to determine the significance of AM fungi to soil carbon sequestration with the intention of ameliorating soil carbon decline in cropping systems through management practices that favour the persistence of AM fungi and the accumulation of associated recalcitrant carbon in soil.

PS9-189-0858

Finding a mycorrhizal fungus for reintroductions of the threatened terrestrial orchid *Diuris fragrantissima*

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Australian terrestrial orchids rely on associations with suitable mycorrhizal fungi for in situ seed germination and establishment, an important prerequisite for self sustaining populations. Finding an appropriate mycorrhizal fungus is therefore imperative to successful reintroductions. Reintroductions have been planned to conserve the terrestrial orchid *Diuris fragrantissima*, which is Critically Endangered in Victoria, Australia, having been reduced to less than 25 plants at a single site. This study investigated the presence of a suitable mycorrhizal partner for *Diuris fragrantissima* in situ, ex situ and from closely related species, for use in reintroductions. Six hundred seed baits were placed at three original sites of *D. fragrantissima* but did not recover a single germinant. Twenty-two fungi isolated from D. punctata, *D. dendrobioides* and *D. chryseopsis*, and ten fungi isolated from *D. fragrantissima* in ex situ collection were used in germination trials with seed of *D. fragrantissima.* Three isolates initiated germination, including fungi isolated from 'asymbiotic' ex situ *D. fragrantissima*. Germination rates were always below 30%. Fungal isolates were identified by direct sequencing of the nuclear internal transcribed spacer and large subunit regions of DNA. All isolates were closely related to Tulasnella calospora. Evolutionary relationships between fungi and their orchid hosts across Victoria are discussed.

PS9-190-0927

The composition and distribution of Arbuscular Mycorrhizal Fungi (AMF) under different ecological conditions.

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The presentation highlights distribution of AMF as affected by landscape, farming systems and plant species. It is a summary of studies undertaken in three countries in Africa. The selected studies selected are: (1) The effects of land use type on AMF, (2) The effect of farming systems (agroforestry) systems in Malawi on AMF, (3) AMF species associated with habitats of rare and endangered plant species in Kenya, (4), AMF in banana plantations in Kenya and Uganda and (5) Mycorrhizae dependency of banana cultivars. AMF species and abiotic and biotic conditions will be presented.
AMF species were assessed in seven land use types along a land use gradient from forest to farm lands in Kenya. AMF species displayed preference for land use type. In agroforestry systems in Malawi, AMF species diversity was dependant on the type of agroforesty system while studies on AMF in habitats of rare tree species showed AMF associations to have heritable traits and habitat and root characteristics to play a major role. Studies undertaken in banana plantations showed the genera *Glomus* and *Acaulospora* to dominate. The banana plantations in Uganda with predominantly conventional cooking and beer banana cultivars had higher species diversity compared to tissue culture banana plantations in Kenya. A green house study showed nine banana cultivars to depend and specific on AMF, with growth and nutrient content enhanced by three *Glomus* species while a *Gigaspora* species failing to colonize all banana cultivars. The studies display ecological trends and preference in the distribution and associations

of AMF species.

PS9-191-0936

Composition of arbuscular mycorrhizal fungal communities in saline soil in Wagga Wagga, New South Wales (NSW), Australia

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Dryland salinity is a devastating environmental problem that affects millions of hectares of land, reducing land productivity and biodiversity. The introduction of European farming systems involving large-scale removal of native vegetation is a major contributor to dryland salinity in Australia. Salinity is an extreme abiotic stress that adversely affects plant productivity, but less is known about how salinity directly affects the growth and the genetic and functional diversity of mycorrhizal fungi in Australia.

Mycorrhiza, or 'fungus-root' (Greek mikès and Latin rhiza), is the symbiotic association between plant roots and fungi that colonise the majority of land plants worldwide. The two most common types of mycorrhiza are ectomycorrhizal fungi and arbuscular mycorrhizal fungi, of which the latter will be the focus of this investigation. Mycorrhizas are known to enhance plant growth by increasing the uptake of nutrients through the development of a hyphal network in soil and subsequent nutrient translocation to the host plant. Some research indicates that the fungi may also improve salt tolerance and plant growth in highly saline soils.

The aim of this investigation is to improve the understanding of the genetic and functional diversity of mycorrhizas in saline soil in southern NSW. A field site consisting of pasture land with varying saline levels and an adjacent revegetated plot was selected for the study. A hierarchical sampling method is being employed for soil sampling and, where possible, plant root collection, to analyse spatial and temporal variation of mycorrhizal fungi. Denaturing gradient gel electrophoresis, of DNA extracted from isolated spores, roots and bulk soil, is being used to analyse the genetic diversity of mycorrhizal fungi.

Complementary studies of fungal morphology (both spores and intraradical structures) and population densities in soil are being performed in conjunction with the genetic analyses. Field-plant roots (weeds rather than pasture species due to drought conditions) were analysed for the presence of mycorrhizal fungi. Preliminary results revealed no colonization, with the exception of some ectomycorrhizal colonized woody plants in the revegetated plot. Plant pot cultures were subsequently prepared to trap the mycorrhizal fungi and induce colonisation and sporulation. Functional diversity (e.g. the role of mycorrhizas in plant accumulation of organic solutes) and infection efficiency of the isolated fungi will be investigated to determine the best performing indigenous species in saline soil. Results from this study may enable better sustainable resource management, successful rehabilitation of degraded ecosystems and may increase agricultural productivity in the future.

PS9-192-0963

SEM And PCR Study Of Mycorrhizal Fungi Isolated From The Australian Terrestrial Orchid: *Prasophyllum*

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Most members of the genus *Prasophyllum* (Leek Orchids) are endangered and restricted in distribution in Australia. *Prasophyllum* species are obligate mycotrophes and current conservation protocols for terrestrial orchids in Australia require propagation with symbiotic mycorrhizal fungi. Unfortunately there is a paucity of knowledge regarding the mycosymbiont in this genus, hampering conservation and re-introduction efforts. Before recovery plans can be implemented for this genus basic biological information is required about the nature of the mycorrhizal relationship. This study used two *Prasophyllum* species: *P.* sp. aff. *validum* and P. *diversiflorum*, both from south-west Victoria close to the Grampians National Park. Prasophyllum sp. aff. validum located at Deep Lead, in a low open grassy heathland and *Prasophyllum diversiflorum* (Gorae Leek Orchid) located on roadsides and private properties near Dunkeld and Hotspur on seasonally inundated mixed alluvial silty black basalt/loams in open grassy/swampy vegetation. Underground plant parts were collected for mycorrhizal isolation and Scanning Electron Microscopy (SEM) studies. Mycorrhizas were isolated from adult plants at four times during the year leafing (Autumn), budding (Winter), flowering (Spring) and dormancy (Summer). SEM was used to determine the location, type and amount of mycorrhizal colonisation. Mycorrhizal efficacy was tested with seed collected from plants in 2005. As most mycorrhizal fungi from Australian terrestrial orchids do not sporulate and therefore cannot be identified by normal taxonomic means, DNA from isolates were ITS-sequenced and closest GenBank matches determined. The information gained in this study will provide the basis for further re-introduction and conservation studies.

S6IS1 - 0179

Phylogeny and systematics of the yeast genus *Pichia* from multigene sequence analysis

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Pichia is the largest of the ascosporic yeast genera with over 100 presently accepted species. Single gene analyses have demonstrated *Pichia* to be polyphyletic with species distributed throughout the Saccharomycetales. However, single gene analyses are insufficient to provide strong phylogenetic placement, which is required if classification is to be based on natural relationships. In the present study, species of *Pichia* and related taxa were compared from multigene sequence analyses to develop a phylogeny-based system of classification.

Gene sequences compared were the entire large subunit rDNA, small subunit rDNA, translation elongation factor-1alpha, mitochondrial small subunit rDNA and cytochrome oxidase II. Both DNA strands of each gene were sequenced using ABI technology. Sequence data were analyzed phylogenetically using maximum parsimony and neighbor-joining.

Major clades in *Pichia* are represented by *P. membranifaciens*, which includes species assigned to *Issatchenkia*, *P. anomala, P. angusta* (*Hansenula polymorpha*), the latter clade representing the majority of methanol assimilating species, and smaller clades centered on *Hyphopichia burtonii*, *Kodamaea ohmeri, Komagataella pastoris* (also methanol assimilating), *Kregervanrija fluxuum*, *Kuraishia capsulata*, *Starmera amethionina*, *P. heimii*, *P. stipitis*, *P. guilliermondii*, *P. scolyti* and several other species. Proposals for reclassification of these clades as new genera and as new combinations in other phylogenetically circumscribed genera will be presented. In this latter context, *Pichia tannicola* and *P. ofunaensis* were shown to be members of the *Zygoascus* clade.

The *P. membranifaciens* clade, as well as species now assigned to *Issatchenkia*, represent *Pichia* sensu stricto. Phenotypically, these species are similar to those of the *P. anomala* clade. The combination of expanded gene sequence datasets and the discovery of additional new species has strengthened earlier proposals that *Hyphopichia*, *Kodamaea, Komagataella* and *Kuraishia* represent distinct genera. The earlier all inclusiveness of the genus *Pichia* resulted from the phenotypic similarity of most of the species, which has masked the great amount of phylogenetic diversity present. Not surprisingly, the phenotypes of newly circumscribed genera will often overlap, which will require reliance on molecular methods for accurate identification.

S6IS2 - 0066

Sex, endemism, and gene flow in natural yeast populations

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Haplontic *Metschnikowia* species associated with nitidulid beetles of ephemeral flowers form pairs of large, needle-shaped ascospores that arise during the first meiotic division and are the site of genetic recombination. Successful mating followed by ascospore formation can be used to circumscribe these species based on the biological species concept, although evidence that sexuality is in fact operative in nature has hitherto been lacking. Here we address this question in *Metschnikowia lochheadii*, which is represented by isolates collected in diverse localities in Guanacaste Province of Costa Rica and five Hawaiian islands.

Polymorphic DNA markers were screened by the arbitrary primer pair method of Burt et al. (1994 Mol Ecol 3,523), using three strains selected on the basis of variation in IGS/ETS rDNA sequences. From 66 pairs of 12 primers (GC-rich decamers), nine polymorphic loci were identified in *M. lochheadii*. Locus-specific primers were then used to genotype 32 isolates by single-strand conformation polymorphism electrophoresis (SSCP - Orita et al. 1989 Genomics 5,874) using the GenPhor system (Amersham/GE), and if necessary by sequencing.

We identified 14 distinct DNA haplotypes in 32 strains. In most cases, the DNA loci were independent from the mating type alleles, indicating that significant sexual recombination occurs in local Costa Rican populations of *M. lochheadii*. However, isolates recovered from five Hawaiian islands had the same mating type and DNA marker alleles, confirming the earlier hypothesis that they were introduced recently from the American continent as a single founder population. In the Mesoamerican isolates, many of the DNA markers exhibited strong linkage disequilibria amongst one another. The best explanation is that the species is subdivided into local demes. Consistent with this, many alleles behaved as apomorphies, being strictly divergent. However, the distribution of some alleles was better explained as the result of genetic recombination between strains. This was particularly evident in two isolates that shared alleles of two clearly distinct origins.

Microbial biogeography can be difficult to document because of an often intractable species concept and the scarcity of DNA variations that rise above background noise. The present study shows that DNA markers can be used to test biogeographic hypotheses in a yeast that truly fits the definition of a biological species. Enough genetic variation exists within *M. lochheadii* to start addressing the difficult but biogeographically pivotal questions of sampling density and isolate independence with some degree of satisfaction. The results are consistent with the view that *M. lochheadii* is a subdivided Mendelian population whose geographic distribution reflects an evolutionary history and not a random diffusion model. We look forward to extending this approach to *Metschnikowia hawaiiensis*, a sexual, single-island endemic, and to *Candida ipomoeae*, a more vagile, asexual species whose range extends beyond the Neotropics.

S6IS3 - 0771

Molecular taxonomy of the medically relevant yeasts *Malassezia* and *Trichosporon*

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Malassezia species are part of the human cutaneous microflora, and colonize lipid-rich areas, especially the head and neck. These microorganisms are associated with several skin diseases, such as seborrheic dermatitis, pityriasis versicolor, and atopic dermatitis. In 1996, *M. furfur* was divided into five species based on rDNA sequence analysis. Subsequently, our research group discovered three novel species from patients with skin disease. At present, eleven species are accepted in the genus *Malassezia*. Analysis of the ITS sequences of the rRNA gene is widely used for species identification, and more than 99% DNA sequence similarity is generally found within a yeast species. However, the ITS sequences of *Malassezia* show remarkable intraspecies diversity, and more than 10% differences are found within a species. This is an exception in the world of yeasts.

Trichosporon species are characterized morphologically by the production of arthroconidia, and are widely distributed in the environment. Some species in the genus are involved in infection and allergy. In the third edition (1984) of The Yeasts, the genus included only five species. In the fourth edition (1997), the number of accepted species increased to 19. This did not result from the discovery of novel species, but was because *Trichosporon cutaneum* sensu lato was divided into more than ten distinct species based on rDNA sequence analysis. Approximately twice as many species are listed in the forthcoming fifth edition because a number of novel species were subsequently isolated or were transferred from other genera (*e.g.*, *Apiotrichum*, *Cryptococcus*, and *Hyalodendron*). Many new candidate species remain, and more than ten species will be added to the genus *Trichosporon* within the next 1-2 years. Generally, the DNA sequences of D1/D2 26S rDNA and ITS region are widely used for the molecular taxonomy of yeast. The DNA sequences of the IGS region, which is located between 26S and 18S, enable discrimination between species that are difficult to distinguish using ITS or D1/D2 26S rDNA sequences analysis. The accumulation of DNA sequence data for the 18S, D1/D2 26S rDNA, ITS, and IGS regions in the genus *Trichosporon* make this genus useful for investigations of molecular taxonomy.

S6PS1 - 0407

Multi-locus sequence typing of the *Cryptococcus neoformans – Cryptococcus gattii* species complex

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Cryptococcus neoformans and *Cryptococcus gattii* are pathogenic basidiomycetous yeasts, once thought to belong to the same species. Recently, *C. neoformans* var. *gattii* (serotypes B and C) has been raised to species level because of differences in morphology, molecular characteristics and ecology.
We used multi-locus sequence typing (MLST) as a tool to study the species complex in more detail. 140 Strains of different origin (clinical, environmental, geographical) were included in our study.
Eight different regions were selected for our MLST approach: the mitochondrial regions ATP6 and mitochondrial large ribosomal subunit RNA (mtLrRNA); the ribosomal DNA regions intergenic spacer (IGS) and internal transcribed spacer (ITS); nuclear genes laccase (LAC), translation elongation factor 1 alpha (TEF-1alpha), the largest subunit of RNA polymerase II (RPB1) and the second largest subunit of RNA polymerase II (RPB2).
MLST showed that the *C. neoformans – C. gattii* species complex could be divided into six haploid and two hybrid groups. The two haploid subgroups within *C. neoformans* corresponded to the varieties *grubii* (serotype A) and *neoformans* (serotype D). Within *C. gattii* four haploid subgroups could be distinguished, but these have not been recognized as separate taxa. In addition to these haploid groups, two hybrid groups could be recognized. AD hybrids are hybrids between the two varieties of *C. neoformans* and BD hybrids are hybrids between *C. gattii* and *C. neoformans* var. *neoformans*.
The presence of hybrids complicates the classification of the *C. neoformans – C. gattii* species complex.

S6PS2 - 0506

Diversity of yeasts from gastopods in Gunung Halimun national Park, West Java, Indonesia

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Gunung Halimun National Park is the largest area of tropical rain forest remaining in Java. The forest of Gunung Halimun is an interesting place for exploration of fungi because it has a wide range of habitat types and a high diversity of plants and animals. The purpose of this study was to elucidate the yeast diversity from gastropods in Gunung Halimun National Park in West Java, Indonesia. Our study was based on altitudinal approach on forest vegetation, and samples were collected from the hill forest (500 m above sea level), submontane forest (1,000 m asl) and montane forest (1,500 m asl). We collected 85 samples of gastropods which belonged to *Bellamya javanica, Brotia spadicea, Pupina junghuhni, Melanoides* sp., *Gyraulus convexiusculus, Helicarion albellus, Liardetia convexoconica*, and *Helicarion radiatus*. Treatment of samples was done by removing the gastropod shells, and the remaining parts was mashed. The yeasts were isolated by direct and dilution methods. A total of 112 yeast isolates was obtained from samples. Molecular analysis based on the sequence of D1/D2 of LSU rDNA, ITS regions and conventional identification method were used to elucidate the diversity of yeasts from gastropods in Gunung Halimun National Park, West Java, Indonesia. Identification results from 25 selected yeast isolates showed that they consisted of the following species: *Candida apis* (1), *C. pseudointermedia* (1), *C. valdiviana* (1), *Cryptococcus aerius* (1), *Cryptococcus flavus* (1), *Cryptococcus laurentii* (1), *Cryptococcus podzolicus* (4), *Pseudozyma antarctica* (1), *Pseudozyma fusiformata* (1), *Rhodotorula glutinis* var. *dairenensis* (1), *Ustilago maydis* (1), and *Williopsis saturnus* var. *saturnus* (2). Nine isolates were identified at the genus level e.g. *Candida* sp. (1), *Cryptococcus* sp. (1), *Filobasidium* sp. (1), *Geotrichum* sp. (1), *Metschnikowia* sp. (1), *Tilletiopsis* sp. (1), *Ustilago* sp.1 (1), *Ustilago* sp.2 (1), and *Ustilago* sp.3 (1). They could not be assigned to any known species. Our study detects the presence of new taxa of yeasts which needs further investigation. This is the first report on the diversity of yeasts from Gastropods in Indonesia.

1530-1730

SYMPOSIUM 7 - *Transcriptome Analyses of Fungal Pathogens during Infection*

S7IS1

DNA microarray analysis of signal interference of the dimorphic transition of *Candida albicans*

Haibao Zhang

Abstract not available.

S7IS3 - 1013

Transcriptome study of *C.albicans* genese important for infection and virulence?

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Candida albicans is currently one of the most prevalent fungal pathogens in humans, causing life-threatening infections in immunocompromised patients. When *C. albicans* cells are exposed to human body fluid, particularly serum, many cellular functions and processes pertinent to infection of host tissues and survival of host defense are activated. One well recognized and extensively researched process is the yeast-hypha growth switch, which facilitates tissue penetration and escape of phygocytic destruction. We have used DNA micro-array to look for genes that control the growth transition, aiming at understanding the molecular mechanisms underlying polarized morphogenesis. On the one hand, we have found a key regulator of hyphal growth, which is a cyclin-related gene named HGC1. We have demonstrated that HGC1 is hypha-specific and essential for hyphal growth under both in vitro inducing conditions and in mice; we have also identified the signaling pathway and transcription regulators that control HGC1 expression. On the other hand, we have been greatly puzzled by the observation that among the numerous hypha-specific genes found so far, only HGC1 has a clear role in morphogenesis. Other genes are related with diverse functions such as cell adhesion, matrix penetration, protein degradation, and iron acquisition. This has led us to hypothesize that cellular events important for establishing polarized morphogenesis may not be controlled by hypha-specific genes. I will present evidence that some key cellular events essential for hyphal morphogenesis occurs immediately after hyphal induction and independent of hypha-specific gene expression

S7IS3 - 0862

Transcriptome dynamics in barley powdery mildew: insights into development and pathogenicity of an obligate biotroph

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Blumeria graminis f.sp. *hordei* is an obligate biotrophic pathogen that causes barley powdery mildew. We have analysed the dynamics of the *Blumeria* transcriptome as the fungus grows and infects its host. Probing cDNA microarrays with RNA extracted throughout its life cycle has revealed clusters of genes that are "co-expressed", i.e. they have common expression profiles during development. Some of these clusters include a high proportion of genes that are know to be associated or even necessary for pathogenicity in other fungi; this identifies novel potential candidate pathogenicity and virulence genes. Another finding has been that many of the genes that encode enzymes on specific metabolic pathways also appear to be co-expressed. The patterns of co-expression lead to inferences about the metabolic status of the fungus as it germinates and infects a host plant. Thus, for example, lipid and glycogen stores are actively broken down following germination to feed growth and host penetration via glycolysis. Once an infection is established, glucose taken up from the plant cells is fed to the epiphytic hyphae where new lipid and glycogen store are laid down in the newly formed conidia. The picture that emerges is that of a pathogen whose development and metabolism are tightly controlled in response to plant-derived cues. This leads us to propose that the obligate nature of *Blumeria*, and possibly that of other pathogenic and mutualistic fungi, is due to the loss of the ability to control their metabolism in the absence of a host, rather than loss of metabolic pathways *per se*.
We are currently attempting to exploit the phenomenon of co-expression to identify clusters of genes whose expression profiles are altered when *Blumeria* undergoes abortive development on non-host surfaces. We expect this eventually to lead to the identification of downstream targets of the signalling pathways that control pathogenic development and metabolism.

S7IS4 - 0763

Microarrays meet pathogenicity: gene regulation during the early infection phase of *Ustilago maydis*.

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In the phytopathogenic fungus *Ustilago maydis*, plant infection is initiated by fusion of two haploid, yeast-like sporidia. The resulting dikaryon grows as a filament on the plant surface, but cell proliferation is stalled until the fungus has successfully penetrated the plant epidermis. The key regulator for the switch from saprophytic to biotrophic growth is a complex of the two homeodomain proteins bE and bW encoded by the *b*-mating type locus.
To get insight into the processes at the early stages of plant infection, we performed a microarray analysis of *U. maydis* cells on the plant surface, comparing a pathogenic strain harbouring an active bE/bW heterodimer with a non-pathogenic wild type strain. The genes identified have putative functions in plant cell wall degradation, transport or transcriptional regulation. Interestingly, 41 % of the genes induced in the pathogenic strain encode *U. maydis* specific proteins, most of which are predicted to be secreted. We presume that these proteins have distinct functions during the infection process, such as surface attachment, host recognition, or the suppression of plant defence reactions.
Four of the genes induced on the plant surface encode putative transcription factors. Of particular interest is Biz1, a C_2H_2 zinc finger protein.When Biz1 is overexpressed, 130 *U. maydis* genes are induced. The deletion of *biz1* does not lead to any detectable phenotypic alterations during axenic growth. However, mutant cells show a severe reduction in appressoria formation and plant penetration. Mutant hyphae that invade the plant are arrested directly after plant penetration. Microarray analysis revealed that *biz1* is both required and sufficient for the induction of 14 genes during growth *on planta*. Systematic deletion analysis of these genes led to the identification of *pst1* as a novel pathogenicity factor. With the exception of an N-terminal secretion signal, Pst1 has no similarities to known proteins or protein motifs. The deletion of *pst1* leads to a phenotype similar to that of *?biz1* strains, implying that the observed pathogenicity defect is due to the absence of *pst1*. In wild type strains 81 of the 130 *biz1*-regulated genes are induced after penetration. Thus, it is conceivable that Biz1 is also required for the regulation of these genes at post penetration stages.

S7PS1 - 0523

Transient gene silencing in the oomycete, *Phytophthora infestans*, for determination of gene function

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Phytophthora infestans, the causal agent of potato and tomato late blight, produces several different cell types prior to penetration of the host plant and during the early stages of infection. Cell types including sporangia, zoospores, germinating cysts, and appressoria can be generated in the absence of the host plant and so form the basis for stage-specific gene discovery.
Gene discovery strategies such as amplified fragment length polymorphism based mRNA fingerprinting (cDNA-AFLP), suppression subtractive hybridisation (SSH), and EST sequencing were used to target transcripts specifically up-regulated during cyst germination and appressoria formation; structures formed just prior to infection of potato. These structures are likely to contain many transcripts involved in successful penetration of the host, and establishment of a compatible interaction. Expression of genes was quantified by microarray and real-time RT-PCR in cultured vegetative mycelium, sporangia, zoospores, germinating cysts, germinating cysts with appressoria and at several time points post-inoculation of susceptible potato cultivar Bintje. Based on the expression profile of the identified transcripts, they were prioritised for functional analysis by dsRNA induced transient gene silencing to determine their role in the pathogen lifecycle and interaction with the host plant, potato. Real-time RT-PCR has been used to confirm the level of gene silencing associated with the observed phenotypes. Proteins of interest will then be localised by translational fusion to fluorescent proteins, and observed under confocal microscopy.
Transcripts identified to date encode proteins potentially involved in adhesion, host cell wall degradation, signalling, virulence, amino acid and protein biosynthesis, stress response, and detoxification. Over 60 percent of gene transcripts do not exhibit homology with sequences held in databases. Real-time RT-PCR confirmed that transcripts identified through cDNA-AFLP and SSH were all up-regulated prior to host plant infection, with a smaller subset also up-regulated during host infection. Hybridisations to a 15 k unigene microarray have yielded over 200 genes that follow a similar expression profile. Transient gene silencing of selected genes is in progress and has generated phenotypes to date that include distorted or burst appressoria, loss of pathogenicity, and burst hyphal tips.
Our preliminary results show that both formation of functional preinfection structures and pathogenicity require the action of many genes.

S8IS1 - 0898
Diversity of microfungi of the brazilian semi-arid northeastern region
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The Brazilian Semi-arid region is located almost exclusively in the Northeastern part of the country.This large expanse of drylands stretches between 3-17o S and 35-45o W, covers almost 8% of the territory of Brazil and occupies about 900.000 Km2. Unlike many other Semi-arid region of the world, this area is densely populated, with over 20 million inhabitants, representing more than 10% of the total population of Brazil. The annual preciptation varies around 300-500mm, and it is usually restricted to just a few months (seven to nine). Several vegetation types were observed in the Brazilian Semi-arid , rock fields (Campo Rupestre), savana-like (Cerrado), as well as, semi-deciduous and deciduous forests. These last two forest types delimit the Semi-arid region and together they constitute the Caatinga Biome. This Biome is divided in eight proposed natural ecorregions. The predominant vegetation type comprises several forms of Caatinga and covers about 735.000 Km2. It is the most degraded vegetation type and less than 1% of it is protected in permanent reserves. Three strategies were used to collect information on the fungi in this region: 1. information from the list of municipalities in the States that composing the Semi-arid region published by the government (SUDENE); 2. information from the Herbaria and/or culture collections that have to fungi deposited from the Semi-arid region; 3. all bibliographies referring to the fungi of this region. However, only fungi collections published and deposited in a Herbaria/culture collection were included in the databases. A total of 466 species were be found among 186 genera of conidial fungi in the Brazilian Northeastern Semi-arid region. These genera were subdivided into 34 Coelomycetes (20%) and 152 Hyphomycetes (80%). For these genera, 91 have holoblastic (48,4%), 73 have enteroblastic (38,8%) and 3 have talic (1,6%) conidiogenesis; 21 were undetermined (11,8%). In some States there were no registers at all of microfungi, while other had dezens of species. This reflects the great need to build human resources in mycology taxonomy. Recents results of ongoing projects on fungi diversity in the Semi-arid region have shown that about 30% of collections represent new species for Brazil or new species to science.

S8IS2 - 0983
Rust fungi from Northwest Argentina
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Northwest Argentina is a subtropical area with high levels of climatic and biological biodiversity. A study of the rust fungi of Northwest Argentina was based on collections made in the region as well as herbarium specimens from the Arthur Herbarium, Botanical Research Institute of Texas (BRIT), Instituto Spegazzini (LPS), and the U.S. National Fungus Collection (BPI). Samples with signs and symptoms typical of rust fungi were collected in the provinces of Catamarca, Jujuy, Salta and Tucumán during the period 1993 to 2000. The material was dried, hosts were identified, and the rusts were examined using a stereomicroscope, compound microscope and scanning electron microscope. Macro and microphotographs were taken and at least 20 measurements of the different fungal structures were made. The material was deposited mainly in BPI and Instituto Miguel Lillo (LIL). One hundred eighty species of rust fungi belonging to 30 genera were identified from the 635 samples collected. Sixty of those species were new records for Argentina and 10 of these were new species. Two hundred fifty seven hosts species were identified belonging to 51 host families of wild and cultivated vascular plants. Eighty-four of these plants were new host records for Argentina, and from those 50 were new hosts for rust fungi (Hernández 2000, Hernández & Hennen 2002a). Many of the rusts caused conspicuous galls, witches' brooms and other abnormal growths (Hernández and Hennen 2003). Many species of *Ravenelia* were collected and an interactive key to those species was created for the species in Northwest Argentina as well as other parts of the world (Hernández & Hennen 2002b, Hernández et al, n.d.). In addition to having a diverse flora and climate, Northwest Argentina is an area rich in diversity of rust fungi as reflected in the results of this research.

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S8IS3 - 0992

Diversity of Discomycetes in Venezuela

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Data on the biodiversity of discomycetes in Venezuela were compiled to find out the number of species present in the country and their geographical distribution. Future studies will be discussed based on an analysis of these data, which revealed the gaps of our knowledge, both geographically and taxonomically speaking. The advance on discomycete studies for the last 40 years will be analysed, comparing Dennis´ classical work in 1970 with a recent publication (Iturriaga & Minter, 2006) shown in www.cybertruffle.org.uk/venefung. Ninety-eight species were treated by Dennis (1970) while Iturriaga & Minter (2006) have shown that 782 species (244 non-liquenized and 538 liquenized) are known today, an increase of 684 species studied on the last 36 years. Finally, an overview of the endemic discomycetes from Venezuela, will be presented emphasizing the main species.

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Iturriaga, T. & Minter, D.W. 2006. Hongos de Venezuela / Fungi of Venezuela. www.cybertruffle.org.uk/venefung [an interactive, bilingual website]

S8IS4 - 0757

Microfungi of the Brazilian Cerrado: example of Neotropical mycodiversity.

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The Brazilian savanna, designated as Cerrado, covers 2.5 million sq. Km, or over 20% of the national territory. Today it is a front where agriculture and pastures are quickly replacing the rich native vegetation. The local flora contains almost 7,000 known plant species distributed in 170 different families and 1144 genera. At the same time, the plant associated mycobiota shows a significant diversity of microfungi that can be verified in two ways: studying the micodiversity associated to a host family/genus or surveying the ability of member of a fungal taxon (genus, family, or order) or group (*e.g.* cercosporoid fungi) to connect with a variety of host species. An example will be discussed based on the study of the mycodiversity associated with a limited number of specimens belonging in four myrtaceous host genera. The *Myrtaceae* is the seventh largest family present in the entire Cerrado bioma with 212 known species, besides having significant economical potential. The study of exsiccates of fungi growing on leaves of *Blepharocalyx, Eugenia, Gomidesia*, and *Psidium* deposited in Herbarium UB (Mycological Collection) revealed 33 different microfungi, but only seven were known species, however these were all first records for the respective hosts. Six of them were ascomycetes (*Aphanostigme anonnae* on *Psidium pohlianum*, *Asterinella sublibera* on *Eugenia bimarginata*, *Didymella glaciales, Johansonia pandani, Schizothyrium coutarae*, and *Stomiopeltis andina* on leaves of *Eugenia dysenterica*), and one hyphomycete (*Janetia euporbhiae* on *Gomidesia brunea*). Eleven new species were detected: two hyphomycetes in genera *Trichosporodochium* and *Cercospora*; three coelomycetes belonging in *Chaetosticta*, *Phaeoseptoria* and *Stigmopeltis;* and six new ascomycetes in *Arnaudiella, Phaeosaccardinula, Staibia, Scolecopeltidium, Polycyclinopsis*, and *Tubeufia*. However, the most striking result was the presence on *Blepharocalyx salicifolius, Eugenia klotszchiana, Eugenia lutescens, Gomidesia brunea e Psidium pohlianum* of a total of fifteen new genera which included four hyphomycetes, four coelomycetes, and seven ascomycetes. The data suggests the need for intensive microfungal survey in the Cerrado region of Brazil where the fungal population is estimated in between 70,000 and 100,000 species, but just over 1,000 species are presently known.

1530-1730
SYMPOSIUM 9 - *The fungal septum and associated organelles*

S9IS1 - 0729

Woronin bodies: Crystalline peroxisomes close the door at the septal pore

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The Euascomycetes are a monophyletic group of filamentous fungi characterized by a septal pore associated organelle known as the Woronin body. Because of its restricted distribution and unique cellular function, understanding this organelles structure and function promises to deepen our understanding of fungal cell biology, physiology and evolution. The Woronin body functions to seal the septal pore in response to cellular damage and in this manner supports the syncytial hyphal organization of the Euascomycetes. It is known to be peroxisome derived and centered on a self-assembled structural protein called HEX-1. The HEX-1 crystal structure has been solved at a resolution of 1.8 Å and self-assembly-defective mutants have shown that the HEX-1 protein lattice is required for Woronin body function in vivo. Also, several lines of evidence support a model for the evolutionary origin of *hex-1* through gene duplication of an unrelated function, *eIF-5A*, in the ancestral Euascomycete.
Within the Euascomycetes distinct structural variation in Woronin bodies has been noted-with *Neurospora crassa* Woronin body having a hexagonal crystal structure and *Aspergillus oryzae* with a spherical Woronin body. While the hexagonal crystalline Woronin bodies were clearly evident by light microscopy the spherical Woronin bodies could be visualized by flourescent microscopy. Three-dimensional fluorescent microscopy in *A. oryzae* clearly revealed the sealing of the septum by the Woronin body. Moreover the existence of alternatively spliced forms of Hex-1 in some Euascomycetes also seems interesting.
Current research is focused on determining the cellular and genetic mechanisms controlling Woronin body genesis and function and some of this work will be presented.

S9IS2 - 0684

Structure and biochemical characterization of septal pore caps in basidiomycetes

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The septal pore cap (SPC) or parenthesome is a membranous structure located at the dolipore septum within the hyphae of certain groups of basidiomycetous fungi. Based on ultrastructural studies, several SPC types can be recognized, namely a tubulate, vesiculate, nonperforate and perforate type. The different morphologies of the SPC can be used as a taxonomic marker and follow to a certain extent the phylogeny based on molecular data. Although the ultrastructure is well described in many species, the composition of the SPC and its precise function in the fungal cell are still unknown.
In our study, we used mainly the plant pathogen *Rhizocotonia solani*, which has wide hyphae with well-described large SPCs of about 1600-2000 nm, and the model basidiomycete *Schizophyllum commune*, with smaller SPCs of about 600 nm. In the fungal tree of life, these two species occur rather distant from each other. However, both species have perforate septal pore caps that are continuous with the endoplasmic reticulum (ER). Ultrastructural studies on the SPC-dolipore structure showed the presence of an extensive fibrillar network connecting the SPC and the pore plug region in both *R. solani* and *S. commune*. Based on the connection with the ER and its localization at the dolipore, we suggest that SPCs are involved in the plugging process of the dolipore septum.
We used a combination of microscopic and biochemical methods to study the ultrastructure of SPCs and to enrich them. A preparation method was developed to enrich SPCs from cell extracts by the use of isopycnic centrifugation. Thereafter, fractions were studied with a transmission electron microscope and were subjected to protein analysis. An 18kD glycoprotein and a 14kD protein were isolated from the *R. solani* and *S. commune* SPC-enriched fractions, respectively. These proteins were further investigated and used for antibody production to perform immuno-labelling experiments. We will present the results of our research and give an overview of the current knowledge on septal pore caps in basidiomycetes.

S9IS3 - 0753

The MTOC associated protein ApsB interacts with the peroxisomal Woronin body protein HexA in *Aspergillus nidulans*

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Polarized growth is a key feature of both yeasts and filamentous fungi. Microtubules and microtubule-dependent motor proteins are key players, whose arrangement, interaction and contribution to polarized growth are in the focus of interest. Microtubules are produced from microtubule-organizing centres (MTOCs) and display dynamic instability. Using the kinesin motor *KipA* we have identified MTOCs at the nuclei, in the cytoplasm and at septa of *Aspergillus nidulans* and characterized the protein *ApsB* as a novel MTOC-associated protein (Konzack et al., 2005; Veith et al., 2005). The absence of *ApsB* affected the activity of the septal MTOC more than the nuclear-bound MTOC. To understand the role of *ApsB* near the septa, we performed a yeast two-hybrid screen with ApsB as bait and identified HexA, the constituent protein of the Woronin body. The interaction was confirmed *in vivo* using the bimolecular fluorescence system (split GFP). Both proteins, *HexA* and *ApsB*, localized to peroxisomes and were transported along microtubules. However, ApsB and HexA were not found in all peroxisomes but only in subfractions. Colocalization of the two proteins was also only found in certain peroxisomes. At the septa HexA localized to the Woronin body, whereas ApsB localized to distinct spots close to the Woronin body, suggesting exclusion of ApsB from the HexA-containing peroxisomes during maturation of the Woronin bodies.
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S9PS1 - 0324

SEPTAL seal: certified and secure host invasion

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The Woronin body is a peroxisome derived dense-core vesicle that is associated with septal pores in filamentous Euascomycetes, and is required to maintain hyphal integrity during cellular damage. The Hex1 protein serves as a major constituent of the Woronin body and is responsible for self-assembly of the dense-core of this organelle. Using a lesion in the *Magnaporthe grisea HEX1* locus, we define a dual and essential function for Woronin bodies during the pathogenic phase of the rice-blast fungus. Our data indicates that the Woronin body function of sealing septal pores is initially required for proper development of infection structures (appressoria) and subsequently necessary for survival of infectious fungal hyphae during host invasion and colonization. Fungal mycelia lacking Woronin Bodies were unable to survive nitrogen starvation stress, and to cope with nutritional stress encountered within the host. Thus, Woronin body- dependent septal and hyphal integrity provides the blast fungus with an important defense against the antagonistic and nutrient-limiting environment encountered within the host plant.
These results will be discussed in conjunction with our recent characterization of a peroxisome biogenesis mutant (*pex6*delete) and an acetyl-CoA transport mutant (*crat1*delete), which suggests that peroxisome function is necessary for cell wall synthesis and integrity during the appressorium-mediated host penetration.

S9PS2 - 0513

Polarized localization of chitin synthases in *Aspergillus nidulans*

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Chitin is a beta-1,4-linked homopolymer of *N*-acetylglucosamine and one of the major components of the fungal cell wall. Chitin is thought to be very important for the morphogenesis of filamentous fungi. We have isolated six chitin synthase-encoding genes, *chsA, chsB, chsC, chsD, csmA*, and *csmB*, whose gene products belong to classes II, III, I, IV, V, and VI, respectively, from the filamentous fungus *Aspergillus nidulans* and investigated their functions.
In the course of our study, we found that septa of *chsA chsC* double deletion mutants (delta AC mutants) were abnormally thick and had a large pore. Spatial distribution of some septa in the delta AC mutant was aberrant. *chsA* or *chsC* single deletion mutants did not exhibit those defects, suggesting that *chsA* or *chsC* gene product is essential for the formation of normal septa. To investigate the function of *chsA* and *chsC* in the septum formation, we constructed strains that produce HA-tagged ChsA (HA-ChsA) and FLAG-tagged ChsC (FLAG-ChsC) instead of ChsA and ChsC and determined their localization in hyphae. Both HA-ChsA and FLAG-ChsC localized at forming septa and their localizations were partly overlapped (1). FLAG-ChsC also localized at tips of hyphae.
We have also determined the localization of ChsB, CsmA, and CsmB in hyphae using the strains that express GFP-tagged ChsB (GFP-ChsB), HA-tagged CsmA (CsmA-HA), and FLAG-tagged CsmB (CsmB-FLAG), respectively. All these three chitin synthases localized at hyphal tips and forming septa. CsmA-HA and CsmB-FLAG localized near actin structures at septation sites (2, 3). We investigated the movement of GFP-ChsB during septum formation in living hyphae.
These results suggest that chitin synthesis during septum formation is a complex process and several chitin synthases function in it.
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1530-1730

SYMPOSIUM 10 - *Population Genetics of Fungi*

S10IS1 - 0366

GENETIC structure of fungal plant pathogens on wild and cultivated host populations at the host center of origin

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The genetic structures of populations of *Mycosphaerella graminicola* and *Rhynchosporium secalis* originating from the Fertile Crescent were determined using a combination of RFLPs, microsatellites, and DNA sequences in selected and neutral loci in the nuclear and mitochondrial genomes. The genetic structures of populations at the hosts' centers of origin were compared to the structures found in other populations originating from both natural and agricultural systems around the world. For both pathogens, there is evidence for restricted gene flow between host-specialized populations, consistent with the hypothesis that speciation was driven by host-specialization. In the case of the wheat pathogen *M. graminicola*, it appears that host and pathogen populations co-evolved in the Fertile Crescent, with host-specialization occurring during the same time frame as the domestication of wheat. In the case of the barley pathogen *R. secalis*, it appears that host-specialization occurred after barley was introduced into the pathogen's center of origin, i.e. host and pathogen populations did not co-evolve in the Fertile Crescent. Both pathogens show evidence for recent population expansions on the cultivated host, consistent with the hypothesis that the rapid expansion of agriculture enabled a significant expansion of the associated pathogen populations.

S10IS2 - 0332

The impact of the domestication and cultivation of maize on the origin and evolution of the corn smut fungus, *Ustilago maydis*

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Although the genetic consequences of domestication and cultivation on crop species are well described, similar historical contingencies have been largely ignored in studies of the evolution of crop pathogens. Hypotheses for the origin of agricultural crop pathogen species and their population structure have focused almost solely on the impact of current agricultural practices. We investigated the effects of the domestication and cultivation of maize on the origin and evolution of *Ustilago maydis*, a fungal pathogen of maize and the wild ancestors of maize, the teosintes. First, to determine whether agriculture played a role in the origin of *U. maydis*, we used phylogenetic methods to date the divergences of *U. maydis* from related smut taxa. The estimated dates for these divergences are millions of years prior to the domestication of maize 8-10,000 years ago, indicating *U. maydis* did not diverge from its ancestor as a result of agriculture. Second, in order to evaluate the effects of domestication and agriculture on the population structure on *U. maydis*, we developed simple sequence repeat markers and used these to evaluate genetic variation among collections from maize and the teosintes in Mexico, and from maize in South America and the United States. Population genetic analyses of these data resulted in the identification of five genetically distinct populations: one in the United States, two in Mexico, and two in South America. One of the populations in Mexico was comprised solely of collections from one teosinte population, while the other population in Mexico was comprised of collections from maize and the teosintes. Our results suggest that the Mexican maize- and teosinte-infecting population is a descendent of populations that were carried through the maize domestication. Patterns of genetic diversity, linkage disequilibrium and genetic bottlenecks within each of these populations suggest that, like maize, *U. maydis* originated in Mexico, was later founded in South America, and was most recently founded in the United States. Together, our findings indicate that *U. maydis* has coevolved with maize through the domestication and cultivation of maize.

S10IS3 - 0214

The impact of plant population structure on disease epidemiology and pathogen evolution in the *Linum marginale – Melampsora lini* interaction

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Over the past 20 years we have used the association between *L. marginale* and *M. lini* as a model for studying the ecological and evolutionary dynamics of a naturally occurring host-pathogen interaction. Here we provide an overview of a range of epidemiological, experimental and modeling studies that reveal some of the impacts of plant population structure on the pathogen. Much of this work has focused on a single clearly defined metapopulation system where surveys of multiple host and pathogen populations have shown significant spatial structure in the distribution of resistance, but not virulence, reflecting the pathogen's greater mobility. We discuss these patterns and show that host resistance variation is correlated with epidemic development. Results from a multi-year metapopulation experiment provide further evidence of how among-population processes such as pathogen colonization and extinction are strongly influenced by spatial structure. At this larger metapopulation scale, our analyses of natural populations have also demonstrated strong local adaptation by *Melampsora* to its host populations, an effect which is greatest at regional spatial scales, as predicted from the broader dispersal of *M. lini* relative to *L. marginale*. Perhaps the clearest demonstration of how host population structure can influence patterns of variation in the pathogen comes from work showing that pathogen populations attacking more susceptible host populations are less virulent but more aggressive than those occurring on more resistant populations. This trade-off has important implications for adaptation at the level of individual populations as well as for the structure and evolutionary trajectory of the pathogen metapopulation as a whole.

S10PS1 - 0156

Within and between bean field phenotypic variation of a fungal biotroph – estimation of populations

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There is a dearth of information on pathogen variation, either genotypic or phenotypic, within an individual field or defined ecological site. In this study, pathogenic variability of *Uromyces appendiculatus*, cause of bean rust, within individual fields was investigated. Six bean fields from tropical, subtropical and temperate regions within and outside of the two centers of common bean domestication, Andes and Middle America were sampled. Ten bean leaf samples with rust uredinia were collected in different sites of each field. The standard set of 12 bean differential lines/cultivars containing Andean and Middle American derived rust resistance genes was used to differentiate 65 bean rust pathotypes from 380 isolates. Pathotype diversity was detected among bean rust pathogen isolates collected in different sites of a field and among isolates from different geographic regions. The number of pathotypes within individual fields varied from 2 to 25 and the number of pathotypes at individual sites within a field varied from 1 to 8. Pathotype variation among bean rust isolates from different geographic regions was found, and pathotypes found in fields from tropical and subtropical regions were more virulent and diverse than those found in fields from temperate regions. The Middle American differentials were resistant to more of the 380 isolates compared to the Andean differentials. Each of the 12 bean differentials was able to differentiate pathotypes of *U. appendiculatus*, and therefore are useful for phenotypic evaluation of pathogen populations. The variance components between fields was greater than the variance within field based on mean disease score on the 12 differentials, but when the number of pathotypes was considered, the variance components within field were greater than the variances between fields. This is the first report that multiple site samples are needed to represent the fungal pathotype variation in a diseased field. Thus, more than one site in a field needs to be evaluated for virulence phenotype. In developing sampling plans, the cost of sampling one field is higher than the cost of one sample, therefore, we recommend selecting fewer fields and collecting more samples per field. In this way the cost of sampling can be minimized while obtaining a better estimate of pathogen variation.

S10PS2 - 0558

Speciation And Gene Flow In The *Botryosphaeria parva-B. ribis* Complex On Native And Introduced Hosts In South Africa

D Pavlic, B Slippers, T Coutinho, M J Wingfield
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Botryosphaeria parva and *B. ribis* are cryptic species with *Neofusicoccum* anamorphs. They occur widely on both native and introduced plants in various parts of the world. In South Africa, these fungi are known from introduced plants such as *Eucalyptus* and mango (*Mangifera indica*). Recently, they have also been identified as the most abundant *Botryosphaeria* spp. on native *Syzygium cordatum* in South Africa. Species boundaries within the *B.parva-B.ribis* complex are not always clearly defined. The aim of this study was, therefore, to study the species boundaries between *B. parva* and *B. ribis* obtained from different hosts and different geographical regions of South Africa. Isolates used in this study were obtained from native *S. cordatum* and introduced *Eucalyptus* and mango growing in different geographical regions of South Africa. Populations were characterised using seven simple sequence repeat (SSR) polymorphic loci combined with PCR-RFLP fingerprints and multiple gene genealogies. Preliminary results show that there are distinct groups within *B. parva-B. ribis* complex in South Africa. How the isolates representing these groups relate to taxonomic entities is not clear. Our results also indicate that gene flow is occurring between these fungi on native and introduced hosts in South Africa. Further analyses will aim to determine whether these groups represent different species or distinct populations within the same species.

1900-2000 - 0899

Honorary Lecture - MYCOLOGY AND MYCOLOGISTS

D L Hawksworth
MycoNova, Madrid, Spain

If fungi had been treated by Linnaeus in a *Species Fungorum* and not buried in *Species Plantarum* in 1753, mycology perhaps could have avoided relegation to recesses in botany and subsequently microbiological departments and courses. As fungi comprise perhaps 10-15 % of all species on Earth, and as global geochemical cycles and human well-being are dependent on them it deserved better. Yet we still know so little of the fungi with which we share the planet, and an analysis of the numbers described over the last 25 years have remained almost constant, though worryingly they are generated by a declining workforce.

Yet mycology has undergone a renaissance in the molecular era, adding to fundamental aspects of cell cycles and genetic processes relevant to all cellular organisms — something recognized in the award of a Nobel prize in Physiology and Medicine to Paul Nurse in 2001. There has also been an exponential growth in aspects of mycology concerned with emerging plant diseases and the conservation of biodiversity. Yet there is a major and growing mismatch between the people who really know and can recognize fungi and the demands of all who wish to work with them, something recognized since the 1940s but still not being addressed. This has repercussions for GenBank, on which so many now depend, but where perhaps 20-25 % of fungal entries are under erroneous scientific names.

Many of the pioneers in mycology were not professionally employed to work on fungi, but were driven by personal fascinations, and this remains a key aspect of mycology to this day. But the skilled enthusiasts depend on the back-up of professionals and, in the computer age, increasingly also on comprehensive and authoritative databases that can only be maintained in the long-term by concerted international action. Examples of major initiatives such as *Index Fungorum, MycoBank,* and *Fungal Planet* designed to organize and facilitate the exchange of information on the world's fungi, and the contribution of individuals to those, are highlighted.

Mycologists need to stand up and to be counted, to take action individually to address the current situation the subject faces, and not to expect "Somebody" to do something.

The series of IMC's was initiated in 1971 as a platform for the re-launch of mycology, as a key but independent component of biology. The content and range of participants at this Congress expresses this vision and enthusiasm so many have for fungi, and this is what we need to learn from and pursue in our diverse personal situations. We are the mycologists of 2006, and the future of the subject is our mandate.

Tuesday 22nd August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**Symposium 11** *Hall D* **Ascomycete Phylogenetics** Kevin Hyde (Hong Kong) Joseph Spatafora (USA)	**Symposium 12** *Halls A & B* **Proteome Analysis** Jim Kronstad (Canada) Scott E. Baker (USA)	**Symposium 13** *Hall C* **Mycorrhizal Ecology** Tom Bruns (USA) John Kilronomos (Canada)	**Symposium 14** *MR 1 & 2* **Propagation Strategies of Fungi** Akira Suzuki (Japan) Felix Baerlocher (Canada)	**Symposium 15** *MR 3 - 5* **Emerging and Reemerging Pathogenic Fungi** Hester Vismer (South Africa) Sue Coloe (Australia)
10:00	**Coffee Break** – *Hall 2*				
10:30	**Plenary 2:** John Taylor (USA) **Species Concepts in Fungi** *Halls A & B*				
11:30	**Lunch [pre purchase]** – *Hall 2*				
12:00	**Poster Session 2: From Genomics to Proteomics** **Poster Session 6: Food Mycology and Mycotoxins**				
13:30	**Symposium 16** *Halls A & B* **Fungal Phylogenomics** Teun Boekhout (Netherlands) Bernard Dujon (France)	**Symposium 17** *Hall C* **Signal Transduction during Pathogenesis** Jinh-Rong Xu (USA) Marty Dickman (USA)	**Symposium 18** *MR 3 - 5* **Veterinary Mycology** Kishio Hatai (Japan) Mark Krockenberger (Australia)	**Symposium 19** *Hall D* **Substrate Colonisation and Succession in Wood Inhabiting Fungi** Jan Stenlid (Sweden) Lynne Boddy (UK)	**Symposium 20** *MR 1 & 2* **Fungi of Monsoon Asia: - Linking Diversity and Ecosystem Function** Morten Christensen (Denmark) Seiji Tokumasu (Japan)
15:30	**Coffee Break** – *Hall 2*				
16.00	**Symposium 21** *Halls A & B* **DNA Barcoding for Fungi** Richard Summerbell (The Netherlands) Andre Levesque (Canada)	**Symposium 22** *MR 1 & 2* **Polyketides, Non-Ribosomal Peptides, and Terpenes as fungal signal molecules** Jens Frisvard (Denmark) Barry Scott (New Zealand)	**Symposium 23** *Hall C* **Adhesion of Fungi to Plant or Animal Hosts** Nick Talbot (UK) Ester Segal (Israel)	**Symposium 24** *MR 3 - 5* **Protein Secretion in Fungal Biotechnology** David Archer (UK) Merja Penttila (Finland)	**Symposium 25** *Hall D* **Evolution of Symbioses** Dominik Begerow (Germany) Martin Grube (Austria)
18:00	**Supper [pre purchase] / "Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
18:30	**Poster Session** *Hall 2*			**Roundtable 1** *Halls A & B* **Is it Time for a Mycological Code of Nomenclature?** Pedro Crous, David Minter, Amy Rossman, Franz Oberwinkler, John Taylor, Tsuyoshi Hosoya	
20:00	**MSJ Editorial Meeting** *MR8*				
20:30	**Evening Free**				

0800-1000 **Hall D**

Symposium 11: Ascomycete Phylogenetics

Chairs: Kevin Hyde (Hong Kong) / Joey Spatafora (USA)

Summary: This symposium summarizes major advancements in our understanding of the evolutionary relationships among Ascomycota. Talks presented represent current research on some of the major classes of the Ascomycota. For each class, speakers will present new results, with particular emphasis on multigene studies and their impact on our understanding of fungal biology and classification of the Ascomycota.

0800-0823 IS1 - 0440

Phylogenetics in Pezizales emphasizing Pyronemataceae

Karen Hansen (USA)

0823-0846 IS2 - 0540

Investigating Dothideomycete evolution using multi-gene sequence data

Conrad Schoch (USA)

0846-0909 IS3 - 0751

Phylogenetic relationships and evolution of lifestyles within the Eurotiomycetes (Fungi, Ascomycota)

Cécile Gueidan (USA**)**

0909-0932 PS1 - 0233

Molecular phylogenetic analyses reveal adaptive evolution occurred in the powdery mildew fungi (Ascomycota: Erysiphales)

Susumu Takamatsu (Japan)

0932-0955 PS2 - 0647

Multigene phylogenies in the systematics of Sordariomycetes and Loculoascomycetes

Rajesh Jeewon (Hong Kong)

0800-1000 **Halls A&B**

Symposium 12: Proteome Analysis

Chairs: Jim Kronstad (Canada) / Scott E Baker (USA)

Proteomics methods, using 2 dimensional gel electophoresis (2DGE) and/or liquid chromatography - mass spectrometry (LC-MS) allow researchers a snapshot of the proteins present at a given time in the life a fungus. As the number of sequenced fungal genomes increases, so too does the number of "hypothetical" or "conserved" proteins in gene databases. The global nature of the protein profile generated by proteomic research gives clues to the functions of "hypothetical" or "conserved" proteins and serves as a platform for the development of novel hypothesis driven research across all fields of mycology.

0800-0830 IS1 - 0988

Non-isotope-based quantitative proteomics in the absence of genomic sequence information

Scott E. Baker (USA)

0830-0900 – IS2 - 0851

The mixed xylem sap proteome of Fusarium oxysporum-infected tomato plants

Martijn Rep (The Netherlands)

0900-0930 – IS3 - 1003

Evolution Of The Ascomycotan Proteome

Barbara Robbertse (USA)

0930-0945 – PS1 - 0297

The proteome of Peronospora viciae: from spore to endophytic hyphae

Peter Spencer-Phillips (UK)

0945-1000 – PS2 - 0584

Protein profiling of the dimorphism in the fungal pathogen, Penicillium marneffei

Chet Cooper (USA)

0800-1000 **Hall C**

Symposium 13: Mycorrhizal Ecology

Chairs: Tom Bruns (USA) / John Kilronomos (Canada)

Mycorrhizal fungi form complex, species-rich communities. Their structure, function, successional patterns, and interactions with plant communities is slowly becoming known. In this symposium we highlight some of the recent work in this area and outline some of the remaining challenges.

0800-0830 – IS1 - 0221

Structuring of Mycorrhizal Fungal Communities

Roger Koide (USA)

0830-0900 – IS2 - 0256

Ectomycorrhizal symbioses and vegetation development in the primary successional volcanic desert on Mount Fuji

Kazuhida Nara (Japan)

0900-0930 IS3 - 0795

Dynamics of ectomycorrhizal fungal communities in a Scots pine chronosequence L

Luis Villarreal-Ruiz (Mexico)

0930-0945 PS1 - 0480

The ectomycorrhizae of Nothofagus cunninghamii and host shifting by the exotic fungus Amanita muscaria

Christopher W Dunk (Australia)

0945-1000 PS2 - 0624

Distribution And Function Of The Mycorrhizal Fungus Associated With Caladenia fulva

Ann Lawrie (Australia)

0800-1000 Meeting Room 1&2

Symposium 14: Propagation Strategies of Fungi

Chairs: Akira Suzuki (Japan) / Felix Baerlocher (Canada)

The success of any species depends on finding and exploiting resources. Fungi are heterotrophs lacking active mobility. They have evolved several distinct strategies to search for, acquire and defend resources, and to use them as a staging point for further dispersal. For the initial phase, fungi may rely on sexual or asexual propagules or on mycelial extension. These structures differ in longevity and the distances they can travel to colonize new resources. Relative investments in dispersal strategies /have been shaped by natural selection and will depend on the temporal and spatial distribution and the quality of the resource. The goal of the symposium is to bring together results from a variety of different systems and to search for common themes.

0800-0830 IS1 - 0334

Propagation strategy of ammonia fungi

Akira Suzuki (Japan)

0830-0900 IS2 - 0381

Reproduction and dispersal in aquatic hyphomycetes

Felix Baerlocher (Canada)

0900-0930 IS3 - 0619

Mycelia foraging strategies of saprotrophic cord-forming basidiomycetes

Lynne Boddy (UK)

0930-0945 PS1 - 0524

Propagation strategy patterns of Thai freshwater fung

Somsak Sivichai (Thailand)

0945-1000 PS2 - 0571

The effect of selection against sexual recombination on the diversity of A. areolatum mating-type genes

Margriet van der Nest (South Africa)

0800-1000 Meeting Room 3-5

Symposium 15: Emerging and Reemerging Pathogenic Fungi

Chairs: Hester Vismer (South Africa) / Sue Coloe

Humans are affected by an impressive diversity of fungal pathogens, some of which are either considered emerging or reemerging. Apart from infections caused by the anthrophilic dermatophytes, fungal diseases caused by pathogenic fungi, are not contagious and do not regularly cause epidemics. Emerging and reemerging fungal diseases are influenced by several factors and share some common features such as changes in host-pathogen interactions, e.g. the HIV/AIDS pandemic that may lead to dramatic increases in fungal disease incidences; pathogen evolution due to drug resistance; changes in agricultural practices; natural disasters (floods, earthquakes) that may enhance proliferation of the fungus (dependent on the source or natural habitat of the fungus) or increased risk of humans to come into contact with the fungus and contracting disease.

0800-0830 IS1 - 0994

Sporothrix schenckii infections in South Africa - A clinical, epidemiological, ecological and molecular taxonomic overview

Hester F. Vismer (South Africa)

0830-0900 IS2 - 0951

PENICILLIUM MARNEFFEI infection and current knowledge on its potent virulence genes

Nongnuch Vanittanakom (Thailand)

0850-0915 IS3 - 1004

An update on human pythiosis

Angkana Chapraisert (Thailand)

0915-0940 IS4 - 1011

Emerging Coccidioidomycosis and Cryptococcosis gattii in Brazil

Luciana Trilles (Brazil)

0940-1000 IS5-1019

Dermomatophyte demographics downunder: Melbourne Australia

Sue Coloe (Australia)

1030-1130 - 0819 **Halls A&B**

Plenary 2: Species Concepts in Fungi

Species of fungi: their recognition, maintenance and utility

John Taylor (USA)

1200-1330

Poster Session 2: From Genomics to Proteomics

Poster Session 6: Food mycology and mycotixins

1330-1530 **Halls A&B**

Symposium 16: Fungal Phylogenomics

Chairs: Teun Boekhout (The Netherlands) / Bernard Dujon (Paris)

The increasing number of fungal genomes being sequenced, yield unparralleded possibilities for a beter understanding of fungal diversity. This ranges from improved insights in how the fungal cell evolved and is functioning, the underlying processes of genome evolution, and, eventually, a more complete view of fungal evolution. In this symposium, results will be presented from comparative studies on genome evolution of yeast species, comparative genomics of filamentous ascomycetes (with emphasis on Aspergillus species) and finally how the genomics data can contribute to improve our knowledge of the Fungal Tree of Life.

1330-1400 IS1 - 0986

Comparative genomics of yeasts illustrates eucaryotic genome evolution

Bernard Dujon (Paris)

1400-1430 IS2 - 0589

Fungal Phylogenomics: from Kingdom to Species

Eiko Kuramae (The Netherlands)

1430-1500 IS3 - 0985

Genome structure, gene redundancy and gene expression of A. oryzae

Masayuki Machida (Japan)

1500-1530 PS1 - 0215

A Phylogenomic Analysis Of The Ascomycota

Barbara Robbertse (USA)

1330-1530 **Hall C**

Symposium 17: Signal Transduction during Pathogenesis

Chairs: Jin-Rong Xu (USA) and Marty Dickman (USA)

Fungal pathogens must be able to recognize a variety of extracellular signals to regulate different infection-related developmental and differentiation processes. Several signal transduction pathways have been implicated in fungal pathogenesis. Recent advances in several representative human and plant pathogenic fungi will be presented by the speakers. Topics that will be covered in this symposium include characterization of various signaling cascades, regulatory networks, and downstream targets.

1330-1400 IS1

A SAGE approach to investigate cAMP signaling in basidiomcyete pathogens

James Kronstad (Canada)

1400-1430 IS2

Signaling pathways and infection-related morphogenesis in Magnaporthe grisea

Jin-Rong Xu (USA)

1430-1500 IS3 - 0148

Dissecting the role of signal transduction in Stagonospora nodorum during infection on wheat"

Peter Solomon (Australia)

1500-1515 PS1 - 0812

Conidial germination in the dimorphic pathogen Penicillium marneffei

Kylie Boyce (Australia)

1515-1530 PS2 – 0014

Hard-surface dependent or thigmotropic cue regulates the G-protein cascade during blast-disease initiation

Naweed Naqvi (Singapore)

1330-1530 Meeting Room 3-5

Symposium 18: Veterinary Mycology

Chairs: Kishio Hatai (Japan) / Mark Krockenberger (Australia)

Fungi can be important in production, companion and wildlife animal species. This symposium features emerging fungal pathogens of animals, both 'new' pathogens and new manifestations of 'old' foes. Disease is a result of interactions between the host, pathogen and environment. This is especially relevant in potential pathogens that have a strong environmental component, such as fungi. A number of host species including aquaculture species will be considered and it will be seen that disease differences in different host species can lead to novel insights into the pathogen itself. Molecular techniques are utilized in detecting and characterizing various fungal diseases as well as identifying specific pathogen factors in the production of disease, but can also be used to give clues about the behaviour of these pathogens in their environmental niches.

1330-1400 IS1 – 0666

Emerging Fungal Infections in Animals (in Japan)

Ayako Sano (Japan)

1400-1430 IS2 – 0850

Koala Cracks Cryptococcus Case Wide Open

Nathan Saul (Australia)

1430-1500 IS3 – 0915

Comparative aspects of aspergillosis in dogs, cats and people

Carolyn O'Brien (Australia)

1500-1515 PS1 – 0556

Fungal infection in cultured marine fishes caused by Imperfecti fungi

Chutharat Munchan (Japan)

1515-1530 PS2 – 0425

Molecular detection of Aphanomyces astaci from Norwegian crayfish plague outbreaks in the time span from 1971 to 2005

Trude Vrålstad (Norway)

1330-1530 Hall D

Symposium 19: Substrate Colonisation and Succession in Wood Inhabiting Fungi

Chairs: Jan Stenlid (Sweden) / Lynne Boddy (UK)

The vast reservoir of carbon and mineral nutrients held in wood is recycled largely via the activities of Basidiomycota and xylariaceous Ascomycota. The communities that develop depend on physical and chemical attributes of the resource, microclimate and biotic interactions, and change with time. Fungal community composition therefore differs between tropical , temperate and boreal forests, between tree species, and depending on whether wood is decomposing whilst attached to the standing tree or fallen to the forest floor. This symposium reviews fungal community development in wood in a diverse array of habitats.

1330-1350 IS – 0715

Fungal dispersal and succession in boreal forests

Jan Stenlid (Sweden)

1350-1410 IS – 0618

Interspecific mycelial interactions: major drivers of colonization and succession of wood-inhabiting fungi"

Lynne Boddy (UK)

1410-1430 IS – 0633

Wood inhabiting fungi on decomposing logs in three Venezuelan forests

Teresa Iturriaga (Venezuela)

1430-1450 PS1 – 0034

Diversity and ecological patterns of wood decay fungi in a temperate, deciduous forest canopy

Martin Unterseher (Germany)

1450-1510 PS2 – 0159

Small-scale variation in chemical properties within logs of Japanese beech in relation to spatial distribution and decay ability of fungi

Yu Fukasawa (Japan)

1510-1530 PS3 – 0641

Community analysis of wood-inhabiting fungi using fruit bodies, culturing, and rDNA

Daniel Lindner Czederpiltz (USA)

1330-1530 Meeting Room 1&2

Symposium 20: Fungi of Monsoon Asia - Linking Diversity and Ecosystem Function

Convener: Mmakoto Kakishima (Japan)

Chairs: Morten Christensen (Denmark) / Seiji Tokumasu (Japan)

The monsoon Asian region lies to the north side of tropical rain forest area. In the last glacial period, there was a big land that is called Sundaland in this region. The temperate biological community in Asia had been able to endure this climate change by the existence of the land. Biodiversity in this region has risen further by repeating the contact between the biological communities in the tropics and the temperate zone. In addition, present climate distribution and many high mountains in the region are contributing to the maintenance of biodiversity. Information on the fungi diversity in this region is becoming abundant every year. However, there are too many kinds of fungal groups that should be examined inhabit in the region. Some topics concerning the diversity of the fungi and its ecosystem function in the Asian tropical monsoon region are presented in this symposium

1330-1400 IS1 – 0331

Why is the species diversity of fungi in tropical monsoon Asia high?

Seiji Tokumasu (Japan)

1400-1430 IS2 – 0127

Fungal decomposition of lignin in leaf litter: comparison between tropical and temperate forest soils"

Takashi Osono (Japan)

1430-1500 IS3 – 0160

Diversity of ecto-mycorrhiza fungi in Nepal - relation to forest types and management

Morten Christensen (Denmark)

1500-1515 PS1 – 0115

Effect of physico-chemical parameter on fungus in water bodies of Jabalpur(M.P)-India

Rashmi Chouksey (India)

1515-1530 PS2 – 0501

Tracking down beauvericin-production in the genus Isaria using molecular phylogenetics

Ja Jen Luangsa-ard (Thailand)

1600-1800 Halls A&B

Symposium 21: DNA Barcoding for Fungi

Chairs: Richard Summerbell (The Netherlands) / André Lévesque (Canada)

Phylogenetic techniques have allowed the study of fungal evolution, taxonomy and synecology to enter a golden age. One part of the fungal phylogenetics community, the „tree of life community, took on the resolution of the deep branches in fungal evolutionary history, giving us a vivid new perception of fungal relationships. This „vertical perspective required looking at several genes per fungus, and thus could encompass only moderate numbers of taxa. A complementary interest lay in obtaining a maximally broad-ranging molecular overview of fungal biodiversity, giving new insight into the full adaptive landscape explored by evolving fungal genomes and facilitating molecular ecological studies of large-scale biodiversity. This „horizontal perspective gave rise to the DNA barcoding community often strongly overlapping with the tree of life community with its ideal of sequencing one or two key genes in a stringently quality-controlled way, ultimately for all fungi. To deploy barcode, information to make fungal identification accessible to all scientists, barcoders have also stressed the use of oligonucleotide microcodes, in rapid identification systems. In this symposium, brief discussion of the basic principles and international collaborations involved in DNA barcoding will lead to examples of barcode database development in morphologically (near-) intractable fungal groups (Eurotiales, Hypocreales), accompanied by discussions of oligonucleotide selection for use in a wide range of successful rapid identification tools.

1600-1630 IS1 - 0606

The Canadian BarCode of Life Network and CoxI Barcoding of Penicillium

Keith Seifert (Canada)

1630-1700 IS2 - 0357

An oligonucleotide BarCode for species identification in Trichoderma and Hypocrea

Irina S. Druzhinina (AUSTRIA)

1700-1730 IS3 - 0836

DNA barcoding,' 'accelerated ecology' and the acremonioid fungi

Richard C. Summerbell (The Netherlands)

1730-1745 PS1 - 0591

UNITE - reliable identification of ectomycorrhizal fungi - DNA barcoding in action

Urmas Kõljalg (Estonia)

1745-1800 PS2 - 0765

Barcoding identification of Penicillium species occurring in cork bark of Quercus suber trees using calmodulin, B-tubulin and ITS and LSU rDNA sequences

Rita Serra (Portugal)

1600-1800 **Meeting Room 1&2**

Symposium 22: Polyketides, Non-Ribosomal Peptides, and Terpenes as Fungal Signal Molecules

Chairs: Jens C. Frisvad (Denmark) / Barry Scott (New Zealand)

Despite the fact that activities of certain fungal-derived metabolites are well documented with respect to their interactions with other organisms, the physiological significance of most of these small molecules to the producing fungi is largely unknown. Characterization of genes encoding enzymes for production of secondary metabolites helps us ascertain what their secondary metabolite products are doing in (and for) the fungal cell. It is becoming apparent that secondary metabolite biosynthetic enzymes are purveyors of small molecules for both basal metabolism and for specialized environmental niches. These molecules may be required as nutrient Œgatherers, and are often, but not always, associated with stress conditions [e.g., low or high iron, oxidative or nitrosative stress, on the plant host, when mating, when the fungal population is too high (or too low), etc]. This session includes talks on a variety of genes encoding enzymes for secondary metabolite (polyketides, non-ribosomal peptides, terpenes, etc) biosynthesis, their diversity, their evolutionary origins, their chemistry, and their biological function.

1600-1630 IS1 - 0672

Biological roles of fungal non-ribosomal peptide synthetases: elemental and diverse

Shinichi Oide (USA)

1630-1700 IS2 - 0849

Chemical diversity in Penicillium and Aspergillus: do all species produce terpene, non ribosomal peptide and polyketide secondary metabolites?

Jens Frisvad (Denmark)

1700-1730 IS3 - 0800

The genetic basis for indole-diterpene chemical diversity in filamentous fungi

Barry Scott (New Zealand)

1730-1745 PS1 - 0303

An endophyte nonribosomal peptide synthetase in siderophore biosynthesis is essential for mutualistic interactions with grasses

Linda Johnson (New Zealand)

1745-1800 PS2 - 0361

Polyketide and cytochalasin production during stromatal ontogeny of the Hypoxyloideae

Marc Stadler (Germany)

1600-1800 **Hall C**

Symposium 23: Adhesion of Fungi to Plant or Animal Hosts

Chairs: Nick Talbot (UK)

In order to cause diseases in animals and plants, fungi have evolved mechanisms by which they are able to adhere tightly to the surfaces of their hosts. Fungi deploy a variety of methods to facilitate surface attachment, including the use of amphiphilic proteins called hydrophobins, which are found at the host-fungal interface, and the secretion of specific adhesive molecules. Attachment to host surfaces triggers infection-related morphogenesis by many fungal species by stimulating signal transduction cascades associated with fungal development. This Symposium will present a variety of studies of fungal attachment mechanisms from human pathogenic, phytopathogenic and free-living fungal species and investigate how these attachment processes lead to specific developmental pathways and the formation of structures such as appressoria.

1600-1630 IS1 - 0787

Oral adhesion of Candida albicans - a cellular and molecular view

Richard Cannon (New Zealand)

1630-1700 IS2 - 0993

Sticking, Sensing, Starting Signal Relay and Stress in the Cereal pathogens Blumeria graminis and Magnaporthe grisea

Sarah Gurr (UK)

1700-1730 IS3 - 0867

Investigating the structure-function relationships of the EAS hydrophobin

Matthew Templeton (New Zealand)

1730-1800 PS1 - 0137

Invasive hyphal growth: an F-actin depleted zone is associated with invasive oomycete hyphae.

Ashley Garrill (New Zealand)

1600-1800 **Meeting Room 3-5**

Symposium 24: Protein Secretion in Fungal Biotechnology

Chairs: David Archer (UK) / Merja Penttilä (Finland)

The Symposium will address the importance of the process of protein secretion by fungi in relation to biotechnology. Many fungal species are exploited for their capacity to secrete a wide range of both native and heterologous enzymes but yields are often limited, especially with heterologous proteins, by the secretory system. The recent availability of genome sequences for some fungal species, together with gene arrays, has enabled genome-wide analysis of the secretory system in fungi as well as the response to secretion stress. This symposium will therefore focus on the genome-wide aspects of protein secretion and aim to provide new insights and avenues for improvement of protein secretion by fungi.

1600-1630 IS1 - 0675

Genome-wide analysis of secretion stress in Aspergillus niger

David Archer (UK)

1630-1700 IS2

Genome-wide analysis and physiology of protein production

Merja Penttila (Finland)

1700-1730 IS3 - 0834

Expression of a shark antibody using Trichoderma reesei as a heterologous host

Helena Nevalainen (Australia)

1730-1745 PS1 - 0352

Isolation and characterization of genes related to growth of Lentinula edodes on lignocellulose

Iris S. W. Kwok (China)

1745-1800 PS2 - 0125

Detection of extracellular proteases produced by ectomycorrhizal fungi

Cajsa Nygren (Sweden)

1600-1800 **Hall D**

Symposium 25: Evolution of Symbioses

Chairs: Dominik Bergerow (Germany) / Martin Grube (Austria)

Different kinds of symbioses act as driving evolutionary forces since early stages of life established on earth. Especially, fungal life experienced substantial organismal diversification through symbiotic interactions with other organisms. The symposium aims at discussing the evolution of different fungal symbioses and their reciprocal effect on the evolution of the partners involved. As mutualism and parasitism cannot be sharply distinguished in many cases, we want to include here all forms of fungal associations. We intend to cover the evolution of fungal parasites, mycorrhizal fungi, lichen symbionts and interactions of fungi with bacteria.

1600-1630 IS1 - 0441

Quantifying the species composition, density, spatial extent, and longevity of Rhizopogon spore banks in pine forests

Tom Bruns (USA)

1630-1700 IS2 - 0185

Coevolution of plants and pathogens in a metapopulation context

Jeremy Burdon (Australia)

1700-1730 IS3 - 0654

Endobacteria and arbuscular mycorrhizal fungi: symbiosis in evolution?

Paola Bonfante (Italy)

1730-1800 PS1 - 0632

Leaves and lichens are cradles of fungal diversification

Jolanta Miadlikowska

1830-2030

Poster Session

1830-2030 ***Halls A & B***

Roundtable 1: Is it Time for a Mycological Code of Nomenclature?

Pedro Crous (The Netherlands) / David Minder (UK) / Amy Rossman (USA) / Franz Oberwinkler (Germany) / John Taylor (USA) / Tsuyoshi Hosoya (Japan)

2000-2200 **MR 8**

MSJ Editorial Meeting

Prof Akira Nakagiri (Japan)

Free Evening

0800-1000
SYMPOSIUM 11- *Ascomycete Phylogenetics*

S11IS1 - 0440
Phylogenetics in Pezizales emphasizing Pyronemataceae
Karen Hansen, Brian A. Perry, Andrew W. Dranginis, Donald H. Pfister
Harvard University Herbaria, Cambridge, United States

The Pezizales, the only order in the Pezizomycetes, are characterized by the presence of an operculate ascus. Molecular phylogenetic studies indicate that the Pezizales (along with Orbiliaceae) are a sister group to all other Euascomycetes. Several groups within the Pezizales have been the subjects of intense phylogenetic studies, but the largest and most heterogeneous family, Pyronemataceae, is poorly understood and central to the understanding of the Pezizales as a whole. In the most recent Outline of Ascomycota the family includes 78 genera, encompassing c. 500 species (approx. half of the known species within the order). These fungi occur primarily in temperate and boreal regions, and are ecologically diverse. The majority of species have traditionally been considered saprobic. However, a few species are known to parasitize bryophytes, and a growing body of research is discovering that some of these species are mycorrhizal. For many species the biological activities remain unknown. The Pyronemataceae has been a "default" family for pezizalean taxa with uninucleate spores and iodine negative asci that lack distinguishing morphological characters by which they could be segregated into natural families. In a recent study, we conducted phylogenetic analyses of nuclear LSU rDNA sequences from a broad sampling of pyronemataceous taxa (including 143 species, representing 57 genera), to test the monophyly of Pyronemataceae, resolve boundaries and relationships of the closely related families, Ascodesmidaceae, Glaziellaceae, Sarcoscyphaceae and Sarcosomataceae, and establish intergeneric relationships. These results suggest that the Pyronemataceae is not monophyletic; Ascodesmidaceae is nested within it and several species of the Pyronemataceae fall outside the core group of the family. The analyses identified 11 clades within the family that are moderately to strongly supported, but did not resolve the relationships among these clades with confidence. Several genera were suggested to be non-monophyletic. To improve resolution and support within the Pyronemataceae, and fully resolve family boundaries, we have obtained partial sequences from three nuclear protein-coding genes, RPB1, RPB2 and EF-1, for a sub-set of the species sampled in the LSU analyses, including representatives from other families of the Pezizales. Preliminary analyses of the combined LSU, RPB1, RPB2 and EF-1, including 40 species, using parsimony and Bayesian approaches, show promise for increased resolution and confidence of many clades. Morphological characters, such as hair morphology, spore guttulation and presence or absence of carotenoids may prove to be phylogenetically informative. Trophic strategies such as mycorrhizal and bryophilic associations will be discussed within a phylogentic context.

S11IS2 - 0540
Investigating Dothideomycete evolution using multi-gene sequence data
CL Schoch 1, J Kohlmeyer 2, B Volkmann-Kohlmeyer 2, JW Spatafora 1
1 Dept Botany & Plant Pathology, Oregon State University, Corvalis, Oregon, United States, 2 Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina, United States

Members of the Dothideomycetes are often found as pathogens, endophytes or epiphytes of living plants and also as saprobes degrading cellulose and other complex carbohydrates in dead or partially digested plant matter in leaf litter or dung. Until recently very few large scale phylogenies of this class combining samples from multiple orders have been done. Incorporating data from the Assembling the Fungal Tree of Life Project (AFTOL) we present an expanded large scale phylogeny containing more than 100 taxa representing a large amount of the diversity in this class. We also incorporate a number of sequences obtained from strains isolated from marine environments This is done by analysing sequences obtained from nuclear ribosomal, as well as protein coding genes. Initial results strongly support separation of orders corresponding to presence or absence of pseudoparaphyses clustered with the main lineages of the Dothideales and Pleosporales. This will be discussed in context with other non-Dothideomycete fungal groups containing bitunicate asci and/or ascostromatal development.

S11IS3 - 0751

Phylogenetic relationships and evolution of lifestyles within the Eurotiomycetes (Fungi, Ascomycota).

C Gueidan 1, C Ruibal 2, GS de Hoog 2, F Lutzoni 1

1 Duke University, Durham NC, United States, 2 CBS, Utrecht, Netherlands

The class Eurotiomycetes is composed of two sister-taxa, the cleistothecial and prototunicate Eurotiomycetidae, including medically important genera such as *Aspergillus* and *Penicillium*, and the perithecial bitunicate Chaetothyriomycetidae, including some pathogenic species known as black yeasts as well as some lichenized species. Since morphological data and first molecular analyses did not seem to support the close relationship between these two subclasses, this sister relationship has been very controversial. However, recent molecular studies, including broad scale sampling and multigene analyses, supported the monophyly of the class Eurotiomycetes. Recent molecular studies also suggest that some taxa previously classified as incertae sedis, such as the order Coryneliales or the family Strigulaceae, belong to this class. A molecular phylogeny of the Eurotiomycetes will be presented here in order to summarize recent contributions in the phylogenetics of this class. Eurotiomycetes includes fungi with very diverse lifestyles. The Eurotiomycetidae occur as saprobes, plant parasites, mycorrhizae, or pathogenic anamorphs on animals. The Chaetothyriomycetidae includes two orders of mostly mutualistic lichenized taxa, Verrucariales and Pyrenulales, as well as one order that is strictly non-lichenized, the Chaetothyriales. The latter includes saprophytic fungi and anamorphic pathogens on animals. Recent studies also showed that some slow-growing melanized fungi inhabiting rocks in harsh environments, also called microcolonial fungi, belong to this order. The diversity of lifestyles within the Eurotiomycetes provides evidence that host-switches and changes in nutritional habits occurred frequently in this class. A molecular phylogeny of the Eurotiomycetes will be used to reconstruct ancestral character states of lifestyles in order to better understand the transitions between saprophytism, mutualism and parasitism in this group.

S11PS1 - 0233

Molecular phylogenetic analyses reveal adaptive evolution occurred in the powdery mildew fungi (Ascomycota: Erysiphales)

S Takamatsu1, Y Sato2

1Mie University, Tsu, Mie 514-8507, Japan, 2 Toyama Prefectural University, Kosugi, Toyama 939-0311, Japan

Powdery mildew fungi (Erysiphales), which consist of 15 genera and about 650 species, are obligate holobiotrophs of plants. The Erysiphales forms a distinct monophyletic clade within the Leotiomycetes. This indicates that the holobiotrophy, as well as morphological characters unique to this fungal group, was acquired only once in the ancestor of the Erysiphales. They infect up to ten thousand angiosperm species, and do not infect gymnosperms and ferns. Molecular clock analyses using ssu and lsu rDNA regions revealed that the origin of the Erysiphales can be traced back to the late Cretaceous, after the origin of the angiosperms. Aceraceae, Fagaceae, Ulmaceae, and Salicaceae may be the early host families of the Erysiphales. It is noteworthy that most species of these plant families consist of deciduous trees, which indicates that the early host plants of the Erysiphales were deciduous trees. These data suggest that Erysiphales expanded their host ranges onto herbs numerous times during the Tertiary. Hirata (1976) pointed out that the Erysiphales are divided into two distinct groups of genera based on their host ranges, i.e., tree-parasitic genera and herb-parasitic genera. All of the herb-parasitic genera have mycelioid appendages on ascomata, whereas deciduous tree-parasitic genera represent various types of appendages such as dichotomously branched, uncinate to circinate, clavate, or bristle-like. The ascomata are considered an organ that endures the winter season in the Erysiphales. Ascomata of deciduous trees are easily dislodged and blown off the leaf surface by wind or rain after maturing, where upon they adhere to the bark of twigs by the appendages, and function as primary infection sources for the next year. On the contrary, ascomata of herbaceous- and evergreen tree-parasitic genera remain on the leaf surface even after maturing. Thus, the overwintering behavior of ascomata differs markedly between herbaceous and evergreen tree-parasitic genera and deciduous tree-parasitic genera with the morphology of appendages considered to be a result of adaptation by ascomata for overwintering. Molecular analyses show that the genera having mycelioid appendages are polyphyletic. This may indicate that the mycelioid appndages are derived characters as a result of simplification of appendages that occurred multiple times as an adaptation to herb-parasitism.

S11PS2 - 0647

Multigene phylogenies in the systematics of Sordariomycetes and Loculoascomycetes

R. JEEWON, L. Cai, A.M.C. Tang, B.D. Shenoy, R. Kodsueb, S. Thongkantha, K. D. Hyde
1Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity,The University of Hong Kong, Hong Kong, China, 2Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Most contemporary fungal classification schemes are either based on morphology or phylogenies derived from single gene dataset (especially from rDNA) and from a limited number of taxa. We have reevaluated the systematics of major groups of fungi within the Sordariomycetes and Loculoascomycetes based on phylogenetic analyses of 18S rDNA, 28S rDNA, Beta Tubulin and RPB2 DNA sequences. Multigene phylogenies indicate that the Sordariomycetes is a well-established class with 3 subclasses (Hypocreomycetidae, Sordariomycetidae and Xylariomycetidae). The Boliniales appears to be related to the Chaetosphaeriales and Sordariales. Phylogenetic relationships within the Xylariomycetidae are still ambiguous due to lack of statistical support among the major nodes. Lulworthiales does not seem to belong to any of these subclasses. The family Sordariaceae is a well-defined monophyletic group when *Apodus* and *Diplogelasinospora* are excluded. The latter should possibly be transferred to Lasiosphaeriaceae. Similar results are reported for *Zopfiella* and there is substantial statistical support to justify *Schizothecium* as a distinct genus. 28S rDNA phylogenies reveal that Magnaporthaceae should possibly be accommodated in a new order. Among the Loculoascomycetes, the Pleosporaceae and the genus *Pleospora* appear to be polyphyletic in its current sense while *Pyrenophora* constitutes a monophyletic group within Pleosporaceae. The familial circumscription of Tubeufiaceae within Pleosporales is justified and it shares close phylogenetic affinities to the Venturiaceae. The phylogenetic affiliation of several anamorphic fungi was also investigated. *Sporidesmium* and *Sporidesmium*-like genera are polyphyletic and species were distributed in the Sordariomycetes and Loculoascomycetes whereas *Chalara* species were found to belong to the Helotiales (Leotiomycetes).

0800-1000

SYMPOSIUM 12 - *Proteome Analysis*

S12IS1 - 0988

Non-isotope-based quantitative proteomics in the absence of genomic sequence information

Ellen Panisko, Don Daly, Kevin Anderson and Scott Baker
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Mass spectrometry (MS) based proteome analysis generates an enormous amount of data in the form of a "snapshot" of peptides present in a sample and is therefore an attractive platform for discovery and assessment of protein-based signatures of environmental change. One major hurdle for the implementation of MS based proteomic analysis of environmental samples is a paucity of genomic sequence information for organisms that may be present in a given sample. Another complicating factor derives from the fact that most quantitative proteomics strategies rely on differential isotope labeling of samples. We are developing a proteomic fingerprinting method for complex environmental samples that does not require genomic sequence for the organisms present. Instead this method relies on a comprehensive generic peptide sequence database, rigorous experimental design and statistical analysis. We are validating the system using two strains of a single organism, *Trichoderma reesei.* Because the *T. reesei* genome has been sequenced we can run a non-genome sequence based analysis and compare/validate our findings with a genome sequence based analysis. Following validation on *T. reesei*, we will apply our method to complex, multi-organism environmental samples.

S12IS2 - 0851

The mixed xylem sap proteome of Fusarium *oxysporum-infected* tomato plants

M. Rep1, P.M. Houterman1, D. Speijer2, H.L. Dekker1, H.C. van der Does1, M. Meijer1, C.G. de Koster1, B.J.C. Cornelissen1
1Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands, 2Medical Biochemistry, Academic Medical Center, Amsterdam, Netherlands

Fusarium oxysporum f. sp. *lycopersici* (Fol) causes vascular wilt disease in tomato. The fungus invades the plant via the roots and subsequently colonizes the plant via the xylem vessels. Resistant plants 'trap' the pathogen after it has entered a xylem vessel, and at least one resistance gene is specifically expressed in xylem parenchyma cells. Proteins that play an important role in the interaction between plant and pathogen are therefore likely to be secreted into the xylem sap.
We have identified close to 30 proteins that accumulate in the xylem sap of tomato upon infection with Fol with two-dimensional gel electrophoresis, peptide mass fingerprinting (MALDI-MS) and mass spectrometric sequencing (LC-MS/MS). We had shown earlier that a number of pathogenesis related (PR) proteins from tomato and the virulence and avirulence factor Six1 ('Secreted in xylem 1') from Fol accumulate in xylem sap after infection (1,2). Here we report the identification of additional tomato and Fol proteins. From tomato we identified a polygalacturonase (endoPG), a number of peroxidases, a xylogucan-specific endoglucanase inhibitor protein (XEGIP) and a xyloglucan endotransglycosylase (XET). With degenerated PCR based on peptide sequence obtained with MS/MS and screening of a Fol BAC library, we have obtained coding sequences of five novel Fol proteins secreted into xylem sap during colonization: two enzymes and three small proteins (Six2, Six3, Six4) without similarity to known proteins. We now focus on the uncovery of the biological and biochemical functions of the Fol proteins.
References: 1. Rep et al. (2002) Plant Physiology 130: 904-917
2. Rep et al. (2004) Molecular Microbiology 53(5): 1373-1383

S12IS3 - 1003
Evolution of the ascomycotan proteome
Barbara Robbertse

The recent increase in fungal genome projects has made it possible to compare the proteomes of a diverse group of ascomycete fungi. We developed a pipeline of perl scripts that connects exiting programs such as BLAST, Tribe-MCL and CLUSTALW to perform a phylogenomic analysis and produce a reliable phylogenetic tree. The phylogenetic relationships represented in the tree and the protein clusters identified by Tribe-MCL was used as a guide to characterize each ascomycete proteome into phylogenetically informed clusters. This process was used to compare the profiles of multi-copy proteins and singletons in different ascomycete proteomes. Duplications before and after species divergence were identified in a high through put manner using the program Inparanoid comparing their different profiles. This allowed us to gain valuable insights into fungal proteome evolution.

S12PS1 - 0297
The proteome of *Peronospora viciae*: from spore to endophytic hyphae
R.C. Amey, R. Smith, T. Schleicher, M. Lewis, H. Macdonald, S. Neill, P.T.N. Spencer-Phillips
University of the West of England, Bristol, United Kingdom

Downy mildew, caused by the oomycete pathogen *Peronospora viciae*, is the most common foliar disease of pea (*Pisum sativum*), with up to 55% losses in yield observed where host resistance and chemical control are ineffective. We have used two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry to compare the protein profile of the pathogen, from freshly harvested conidia through to endophytic hyphae within pea leaves. Proteins were isolated from three pre-invasion developmental stages: 1) conidia from sporulating infections; 2) germinating conidia; 3) conidia with germ tubes and appressoria. A map of the proteins identified and a quantitative analysis of changes in their abundance through the pre-invasion stages of *P. viciae* development are presented. Typically 2% of the proteins could be identified by comparing MALDI-TOF peptide mass fingerprints to published databases, whilst 8% were identified using Q-TOF analysis of peptides to provide amino acid sequence data, although 90% remained unidentified. For 32 of the most abundant proteins from freshly harvested conidia, 9 gave no match to oomycete or plant proteins, 12 were of plant origin, whilst 11 matched oomycete proteins. The latter included those involved in: protein and amino acid synthesis (ketol acid reducto-isomerase, glutamine synthase, translation elongation factor); protein binding, transport and folding (heat shock proteins, molecular chaperones); glycolytic, metabolic and energy pathways (enolases, glyceraldehyde 3-phosphate dehydrogenase); cytoskeleton, shape, form and organisation (actin, beta-tubulin). Although infected leaves were examined at 4, 7 and 10 days post-inoculation, no proteins were detected from the endophytic hyphae. In contrast, many host proteins, such as the abscisic acid response proteins ABR17 and ABR18, the pathogen induced protein PI176, and cytosolic and chloroplastic glyceraldehyde 3-phosphate dehydrogenases, showed a differential increase in abundance as infection progressed.

S12PS2 - 0584
Protein Profiling of the Dimorphism in the Fungal Pathogen, *Penicillium marneffei*
C. R. Cooper, J. M. Chandler, E. R. Treece, T. D. Kim, G. R. Walker
Youngstown State University, Youngstown, Ohio, United States

Penicillium marneffei is a dimorphic fungus and a significant pathogen of immunosuppressed patients, especially in Southeast Asia. Like other *Penicillium* species grown at 25°C, *P. marneffei* exhibits the mold morphology typical of the genus. However, unlike other species, incubation of *P. marneffei* at 37°C causes the fungus to undergo phase transition to form single-celled yeasts. These yeasts mimic the in vivo form of *P. marneffei*, which thrives within the intracellular environment of macrophages. Hence, dimorphism in *P. marneffei* not only represents a unique model in which to study cellular development, but also understanding this process may provide insights into the underlying molecular mechanisms of pathogenesis.
Presumably, differential protein expression is a consequence of dimorphism in *P. marneffei*. Therefore, efforts in our laboratory have been directed towards establishing protein profiles of *P. marneffei* during phase transition. Using two-dimensional gel electrophoresis, the proteomes of the mold and the yeast phases of *P. marneffei* were resolved over a range of pI values. Several proteins unique to the yeast phase were isolated and analyzed using mass spectrometry. A subsequent Mascot search of known data bases demonstrated that a number of the isolated proteins possessed significant homologies to known fungal heat shock proteins as well as enzymes involved in glycolysis and the Krebs cycle. In addition, one particular protein exhibited very significant homology with known G-proteins having an intimate role in nuclear division among a variety of fungi. We have tentatively designated this protein as PmRanA due to its high level of peptide identity to the RanA protein, a GTPase, from *Aspergillus nidulans*. The apparent up-regulation of PmRanA during the growth of *P. marneffei* at 37°C appears to correlate with previous observations that nuclear and cellular division become coupled during mold-to-yeast conversion in this fungus. Current efforts are being directed towards developing a broader protein profile of dimorphism in *P. marneffei* and the potential role of phase-specific proteins in both morphogenesis and pathogenesis of this important fungal agent of disease.
(These investigations are supported by the National Science Foundation, Award No. DBI - 0330883)

0800-1000
SYMPOSIUM 13 - *Mycorrhizal Ecology*

S13IS1 - 0221
Structuring of Mycorrhizal Fungal Communities
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We have investigated factors that influence mycorrhizal fungal communities. In an ectomycorrhizal fungal community we monitored species frequencies over time, at different soil depths, and in different forest floor substrates. In arbuscular mycorrhizal fungal communities we monitored species frequencies in different fields consisting of two distinct soil types. In the ectomycorrhizal fungal community, soil depth, forest floor substrate and time significantly influenced species frequencies. Competition among species may have also occurred. In the arbuscular mycorrhizal fungal communities, soil type and competition significantly influenced species frequencies. We also present some evidence for the role of dispersal in structuring the arbuscular mycorrhizal fungal communities. Our results illustrate the roles of space, time and competition in structuring mycorrhizal fungal communities.

S13IS2 - 0256
Ectomycorrhizal symbioses and vegetation development in the primary successional volcanic desert on Mount Fuji.
K Nara
The University of Tokyo, NishiTokyo, Tokyo, Japan

Ectomycorrhizal (ECM) fungi are symbiotic soil fungi that colonize the roots of many tree species. Since ECM fungi provide their host plants with a significant amount of soil nutrients, these fungi are thought to be important for ecological processes. However, accurate quantification of their effects on hosts under field conditions is difficult because of the unavailability of non-mycorrhizal controls in most natural settings. In some primary successional settings, however, such non-mycorrhizal controls are available. Studies in these settings may provide valuable information about the ecological aspects of ECM symbioses and their contribution to vegetation development.
In the early successional volcanic desert on Mt Fuji, Japan, ECM fungal communities were studied in four host species using a molecular approach to below-ground ECM communities. Populations of three ECM fungal species were also analyzed using microsatellite markers. Spore germination was compared between major ECM fungal species. I also analyzed natural establishment patterns and the performance of transplanted seedlings with special reference to the ECM plants that established early.
Most ECM fungi initially appeared with *Salix reinii*, the first ECM host plant in this desert. With growth of this dwarf willow, ECM fungi showed a clear successional pattern. Sporocarp populations of two early-stage ECM fungi, *Laccaria amethystina* and *L. laccata*, were only composed of small genets (<1 m in largest distance), whereas many large genets (> 10 m) were included in the population of *Scleroderma bovista,* which usually followed the early-stage fungi in the successional sere. Spore germination rates were significantly high in early-stage fungi, compared to late-stage fungi. These results indicate that reproduction by spores is important for the early-stage ECM fungi. However, spores do little to help most current-year seedlings because of the late start of fruiting season and the absence of dormant spores. ECM colonization on current-year seedlings of major host plants, including *S. reinii* and late-colonizing timber species, was observed only near the early established *S. reinii*, where vegetative ECM mycelia were available. Naturally established individuals of timber species and *Pyrola incarnata*, a herbaceous Ericaceae species, were always accompanied by early-established *S. reinii* and shared common ECM fungi. These results indicate that early established *S. reinii* facilitate subsequent seedling establishment of many host plants and drive vegetation development by providing compatible and accessible ECM fungi.

S13IS3 - 0795

Dynamics of extomycorrhizal fungal communities in a Scots pine chronosequence L

L Villarreal-Ruiz 1, I Jalexander 2, I C Anderson 3
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3. Macaulay Institute, Aberdeen, Scotland, UK

Scots pine (*Pinus sylvestris* L.) is the second most important tree in boreal forests and the widest distributed pine in the world. However, the successional trends of their distinctive ectomycorrhizal (EcM) fungal biota have not been extensively studied with well-replicated experimental designs. The aim of this research was to study: (1) the biodiversity and ecology of EcM fungi, analyzing their dynamics in species richness, composition and relative abundance with stand age. (2) The possible effects of organic and mineral soil depth. (3) If these changes reflect changes in functional attributes of EcM communities as represented in the proportion of different exploration types.
EcM communities were studied in a native Scots pine forest in Glen Tanar Nature Reserve, northeast Scotland, combining field and laboratory approaches by: (1) using classical and molecular techniques in taxa detection and profiling. (2) Monitoring EcM communities above- and below-ground across a chronosequence with three replicated plots (50 m x 50 m) per stand age (0-10, 10-20, 50, 160 and 260-yr-old).
The overall species richness was 136 EcM taxa, representing 68% of species previously known to associate with *Pinus* in Britain. There was a rich epigeous and hypogeous EcM sporome community with 70 identified taxa and 30 identified to genus, of which *Cortinarius* was the dominant. Mean species richness increases rapidly from 0-10 to 50-yr-old stands, but levels off thereafter. Belowground surveys revealed an EcM community of 46 morphological groups recovered from >43,116 pine roots, of which 95.8% were found in the organic horizon. *Cortinarius* spp., *Russula sardonia*, *Tomentellopsis submollis*, *Cadophora* cf. *finlandica*, *Russula paludosa, Suillus variegatus* and *Pseudotomentella tristis* were the dominant species. Ordination showed that differences in EcM community structure and composition are related to stand age and to soil depth. A shift from EcM communities with long distance-medium fringe exploration types produced by members of Boletaceae and Cortinariaceae in 0-10 to 50-yr-old stands to contact exploration type formed by Russullaceae in the 160 to 260-yr-old stands was detected.
Because a pattern of change in compositional community structure of EcM fungi across the chronosequence was detected, is realistic to conclude that a trend in secondary succession of EcM fungi occurs in the studied pine forest.

S13PS1 - 0480

The ectomycorrhizae of *Nothofagus cunninghamii* and host shifting by the exotic fungus *Amanita muscaria*

CW Dunk 1, T Lebel 2, PJ Keane1
1La Trobe University, Bundoora, Victoria, Australia, 2 National Herbarium, Royal Botanic Gardens Melbourne, South Yarra, Victoria, Australia

The importance of fungi to ecosystem function is becoming increasingly apparent, yet Australian fungi remain a poorly studied group, with ectomycorrhizal research primarily focused on the timber industry. *Nothofagus cunninghamii*, an obligate ectomycorrhizal host, is a significant relic of Australia's Gondwanan floral heritage that has probably co-evolved with a unique fungal biota. The purpose of this study was to examine the ectomycorrhizal relationships of *N. cunninghamii* in order to gain an understanding of fungal community structure and diversity, and to investigate the apparent host switching of the introduced fungus *Amanita muscaria*.
Sporocarps of macrofungi found growing in close proximity to *N. cunninghamii* in two geographically distant regions of the plant's Victorian range were collected over a three year period. Mycorrhizal roots from soil cores taken beneath sporocarps were characterised by morphotyping, and their relative frequency in the cores recorded. RFLP analysis and direct sequencing of ribosomal nDNA were used to identify the fungal partners of the associations and confirm *N. cunninghamii* as the host.
The two locations exhibited a high degree of species richness both above and below-ground. Of the sporocarps, less than 10% of species occurred in both regions. This regional delineation was generally continued at the genus level, with the notable exception of *Cortinarius* which was dominant at all sites, accounting for over 1/3 of all ectomycorrhizal sporocarps collected. Ectomycorrhizal community structure showed a level of morphotype richness higher than reported for similar northern hemisphere studies (13-20 morphotypes per tree), with richness in disturbed areas significantly reduced (5-14 per tree).
An ectomycorrhizal morphotype found consistently beneath *A. muscaria* sporocarps was conclusively identified as an *A. muscaria-N. cunninghamii* association, using direct sequencing of both partners. This represents the first confirmation of an exotic ectomycorrhizal fungus successfully shifting hosts to become established in native vegetation. Moreover, morphotype richness of native fungi in the presence of *A muscaria* was significantly lower than that found at un-invaded sites. The implications of this invasion for fungal biodiversity, loss of species (many undescribed), management of remnant forests and timber plantations, and the potential long-term effects on the health of the *Nothofagus* forest community will be discussed.

S13PS3 - 0564

Distribution And Function Of The Mycorrhizal Fungus Associated With *Caladenia fulva* G.W. Carr

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Caladenia fulva is an endangered Australian orchid that grows in only a few conservation reserves in Victoria, Australia. Like other *Caladenia* spp., it is recorded as being reliant on infection by the basidiomycetous fungus Sebacina vermifera Oberwinkler (Warcup 1981). The recovery strategy for C. fulva calls for ex situ cultivation and translocation back to the site, but a major obstacle in this strategy is that most orchids in pot culture decline and die within a few years. *S. vermifera* has also been recorded as ectomycorrhizal in *Melaleuca uncinata* R. Br. ex Aiton f. (Warcup 1988). It is therefore possible that orchids, higher plants and their mutual fungi form a tripartite relationship, with the higher plants acting as 'nurse' plants for the orchids via their common mycorrhizal fungus. The prevalence of *S. vermifera* in green orchids and surrounding higher plant roots was investigated by PCR using *S. vermifera*-specific primers and by isolation and sequencing of fungi from orchids. Amplicons were found in tissue and fungal isolates of the orchid genera *Caladenia*, *Glossodia*, *Petalochilus* and *Cyanicula* and in roots of three shrubs (genera *Micromyrtis*, *Astroloma* and *Acacia*). *A. fulva* seeds inoculated with orchid fungal isolates showed that isolates from the genera *Caladenia* and *Glossodia* were effective in germinating seed of *C. fulva*, while those from the genus *Pterostylis* were not. Seeds of *C. fulva* and *Eucalyptus leucoxylon* (the dominant tree at the field site) were germinated and grown together with the fungi isolated from *C. fulva* in tissue culture pts of oatmeal or tap water agar for 3 weeks. The presence of orchids reduced the weight of eucalypts in tap water agar at p=0.086. Growing the plants together for a longer period might have yielded more significant results. Treatment with glyphosate killed eucalypts but not orchids in oat medium, suggesting the orchids relied less on photosynthetic activity than did the eucalypt. These results support the hypothesis of a tripartite mycorrhizal relationship and suggest that culture of *C. fulva* may be more successful in future if higher plants sharing their fungi are grown in the same pots to provide adequate organic nutrition from photosynthesis by the higher plant(s).

0800-1400

SYMPOSIUM 14 - *Propagation Strategies of Fungi*

S14IS1 - 0334

Propagation strategy of ammonia fungi

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Ammonia fungi are latent fungi that grow vigorously after an application of large amounts of ammonia or ammonia related substances in the field. An ammonia fungus would have a physiological advantage for colonizing disturbed habitats that are suddenly enriched with ammonium nitrogen under weakly alkaline to neutral conditions. Under these conditions, most saprotrophic and ectomycorrhizal ammonia fungi exhibit highly synchronized germination. Saprotrophic ammonia fungi grow vigorously at weakly alkaline or neutral conditions, while most mycorrhizal ammonia fungi grow better at acid conditions. Most ammonia fungi quickly colonize substrates without forming mycelial cords and establish their territories with low competition from other microbes that are under stress due to high nitrogen treatments. Pronounced intermingling among saprotrophic ammonia fungi enhances their ability to quickly establish themselves in competition against other fungal groups. In some saprotrophic ammonia fungi, stimulation of fruiting has been reported, but in most ammonia fungi this was not observed. A saprotrophic ammonia fungus, *Tephrocybe tesquorum*, starts to fruit during the linear growth phase and forms basidiospores on vegetative hyphae without forming basidioma. This rapid initiation of reproduction by ammonia fungi on natural and nutrient agar media suggests that they have adapted strategies suited for colonization a ruderal habitat. Further investigation on frequencies of colonization and longevities of mycelia and spores in the field are required to further elucidate the propagation strategy of ammonia fungi.

S14IS2 - 0381

Reproduction And Dispersal In Aquatic Hyphomycetes

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Aquatic hyphomycetes are a phylogenetically heterogeneous group of fungi that occur most commonly on deciduous leaves falling into streams. In temperate streams, leaf fall is strongly seasonal, which creates boom-bust cycles in the supply of substrates for aquatic hyphomycetes. Following fungal colonization, leaves are rapidly consumed by detritivores. This further shortens the period of abundant substrate supply. These conditions must have favoured life history traits emphasizing rapid colonization over defense of acquired substrates, and there appears to be extensive intermingling of hyphae belonging to different species. Within a stream, dispersal and colonization depend primarily on multiradiate (tetraradiate) or sigmoid conidia. Not surprisingly, ? 50% of fungal production on leaves is converted to conidia. However, the relatively delicate and hyaline conidia seem ill-suited for surviving periods of substrate scarcity (summer) or for long-distance dispersal. It has long been speculated that meiospores (primarily ascospores) may be involved in the latter. This may explain the world-wide distribution of several species of aquatic hyphomycetes, and contribute to the rapid recovery of fungal stream communities after initiation of leaf-fall. Recently developed molecular methods allow more accurate observation and quantification of the various reproductive and vegetative fungal states and their involvement in short and long range dispersal.

S14IS3 - 0619

Mycelia foraging strategies of saprotrophic cord-forming basidiomycetes

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In natural terrestrial environments nutrients are often patchily and sparsely distributed or not readily available, because they are locked in recalcitrant material (e.g. lignin), and microclimate is constantly changing both temporally and spatially. While the majority of fungi propagate as spores some can grow out of a resource as mycelium in search of new resources. The mycelium of these fungi typically aggregates to form linear organs, termed cords or rhizomorphs. In forest ecosystems they ramify at the soil/litter interface, interconnecting disparate litter components to form extensive (many metres or even hectares), long-lived (many years) systems. These mycelial systems form effective dispersal mechanisms in space and time.

Fungi that form mycelial cords operate two dispersal (resource capture) strategies, and these are not mutually exclusive: (1) a 'sit and wait' strategy, whereby a large mycelial network waits for resources to land on it and then actively colonizes those resources, often with responses occurring elsewhere in the system; (2) to grow and search actively for new resources, with an array of responses to finding them. With both approaches, mycelia are at risk or have nutritional opportunities from encounter with other organisms, both micro- and macro-, and they have evolved dramatic responses to such encounters.

The mycelial networks that develop are remodelled continuously in response to abiotic and biotic cues, including local nutrient supply, microclimate, interaction with other fungi, grazing by invertebrates or other destructive disturbance events. Remodelling occurs through a complex combination of hyphal growth, branching, fusion and regression in different parts of the mycelial system. Not only does morphology change but so also does physiology, especially the complex set of processes associated with uptake, storage and redistribution of nutrients throughout the network. These morphological and physiological changes are highly coordinated ensuring that environmental changes that occur on a local scale are responded to not only locally but also over a larger scale. Different fungi respond differently to abiotic and biotic variables leading to different long-term behaviour/foraging strategies. Thus for example, *Hypholoma fasciculare* often produces a dense space-filling mycelium in soil responding dramatically to encountering new woody resources, irrespective of the size of the latter, and might be considered to operate short-range foraging strategies. On the other hand, *Phanerochaete velutina* produces much more open systems, responds dramatically to large but not small new resources, and could be considered to be a long-range forager. This paper reviews foraging strategies, mycelial modelling and nutrient translocation during mycelial spread through soil.

S14PS1 - 0524

Propagation strategies patterns of Thai freshwater fungi

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Over 600 species of freshwater fungi were recorded in the last 7 years. The majority of the fungi collected were mitosporic fungi, with few ascomycetes and rarely any basidiomycetes. Most of these fungal spores adapted to aquatic environment with tetraradiate, branched or sigmoid conidia in aquatic hyphomycetes, or with appendages, sticky mucilaginous sheaths, polar pads and filament in ascomycetes. However, many of these have shown to produce another types of spores for propagation with secondary spores especially in the mitosporic fungi. Some of these examples for both anamorph and teleomorph spores and another morphological spores adaptation will be presented and discussed, especially in their attachment and entrapment for propagation on substrata in their freshwater habitats.

S14PS2 - 0571

The effect of selection against sexual recombination on the diversity of *A. areolatum* mating-type genes

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The homobasidiomycete fungus, *Amylostereum areolatum*, is characterised by an unusually low overall genetic diversity. This is surprising, since the fungus has a heterothallic tetrapolar mating system, with the capacity to generate numerous new genotypes per generation. However, the symbiont of *A. areolatum*, the *Sirex* wood wasp (*Sirex noctilio*), can efficiently spread asexual spores of the fungus, making basidiocarp formation energetically expensive and redundant. Such a selection against sexual recombination potentially impacts on the population diversity of the fungus, as well as on the diversity of the genes that control sexual compatibility. To study the effect of such selective pressures on the diversity of *A. areolatum* mating-type genes, we first characterized a pheromone receptor (PR) of the B mating type locus and then determined the distribution of PR alleles in isolates obtained from Northern and Southern Hemisphere regions. For this purpose, we used degenerate PCR primers and PCR-based genome walking to obtain the PR gene in a dikaryotic isolate of *A. areolatum*. Based on the resulting two allele sequences (Rab1.1 and Rab1.2) of the parental dikaryon that co-segregated with mating type, we designed PR-specific primers. After amplifying the PR region from various dikaryotic isolates with diverse origins, the PCR products were cloned and at least five clones per dikaryon were sequenced. Among the resulting sequences, we detected one additional PR allele, Rab1.3. All three alleles were present in different combinations in the Northern Hemisphere and only Rab1.2 and Rab1.3 in the Southern Hemisphere. These findings suggest that the *A. areolatum* mating system differs from those of other homobasidiomycetes with respect to the number of alleles per PR gene. Results of our study show that a reduction in sexual recombination is apparently linked to a reduction in mating-type allele diversity, which would potentially decrease the chances of mating-type compatibility and ultimately the overall population diversity of this fungus.

0800-1000
SYMPOSIUM 15 - ***Emerging and Reemerging Pathogenic Fungi***

S15IS1 - 0994
Sporothrix schenckii infections in South Africa – A clinical, epidemiological, ecological and molecular taxonomic overview

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Sporotrichosis, caused by the dimorphic fungus *Sporothrix schenckii*, is a common subcutaneous fungal infection in South Africa. Sporotrichosis was first diagnosed in the Guateng gold mines in 1914. Currently it is reported throughout South Africa with a higher incidence in the Northern Province, Gauteng, Mpumalanga and Kwazulu Natal. The disease occurs at all ages with males predominating. Exposure to sources of the fungus, either from recreational or occupational activities, is the main determining factor in acquiring the disease. Lymphocutaneous and localized forms of the disease are most often recorded, while extracutaneous and systemic disease is rarely encountered. Morphological differences have been recorded in strains of *S. schenckii* isolated from underground timbers, compared to strains isolated from the general population. The latter could be converted on moist wood to produce triangular and pigmented conidia, similar to those seen in the gold mine isolates. Larger conidium volumes constituted an increased cytoplasmic content and cell wall thickening in the older conidia, as confirmed by fluorescent nuclear DNA staining and electron microscopy. The two types of pathogenic strains could therefore be interconverted by altering nutritional conditions. Molecular research indicate that (i) Mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) in clinical strains of *S. schenckii* classified the species into 23 mtDNA types, and clustered them into two major groups by phylogeny, i.e. Groups A and B. Group A isolates are predominant in SA. Environmental isolates, morphologically identified as *S. schenckii*, are rarely identified as such by RFLP, while (ii) DNA sequence comparisons between human pathogenic and environmental strains, with similar morphology to *S. schenckii,* represent different species. (iii) 18s rDNA sequences reveal significant differences between *S. schenckii* and *Ophiostoma stenoceras*. The latter species was previously considered as the possible teleomorph of *S. schenckii*.

S15IS2 - 0951
Penicillium marneffei infection and current knowledge on its potent virulence genes

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Penicillium marneffei infection is an important disease among human immunodeficiency virus – infected patients in Southeast Asian countries. Several cases have been reported among HIV-infected persons from other countries after they visited the disease – endemic regions. Common manifestations of disseminated *Penicillium marneffei* infection in AIDS patients are fever, weight loss, lymphadenopathy, hepatosplenomegaly, respiratory signs, and skin lesions. Patients who do not receive the appropriate treatment have a poor prognosis. The severity of the disease depends on the immunological status of the infected individual. Rapid diagnostic tools have been developed to help the physician to make more rapid diagnoses and initiate an treatment early. The occurrence of natural reservoirs and the molecular epidemiology of *Penicillium marneffei* have been studied, however the natural history and mode of transmission of the organism remain unclear. Soil exposure, especially during rainy season, has been suggested to be a critical risk factor.
Penicillium marneffei is one of the facultative intracellular pathogens. The pathogen can usually survive and replicate as fission yeast inside the phagosome of macrophages, and is also found extracellularly in blood smears or host tissue. Surviving within the alveolar macrophage is a primary key to the success of *Penicillium marneffei* invasion. However, the mechanism of fungal survival under oxidative stress in this environment has not been elucidated. We have isolated and characterized some potent virulence genes from the yeast phase of *Penicillium marneffei.* They are catalase-peroxidase (*cpeA)* and copper, zinc superoxide dismutase (*PmSOD1*) genes. Catalase-peroxidase has been implicated as a virulence factor in some pathogenic fungi and mycobacteria. Copper-zinc superoxide dismutase (Cu, Zn SOD) has proved to be involved in the virulence of some pathogenic fungi. The *cpeA* and *PmSOD1* genes displayed a high level of expression, specifically being induced when the temperature was shifted to 37oC, the condition whereby the pathogenic yeast phase of *Penicillium marneffei* is formed. The high expression of the *cpeA* and *PmSOD1* mRNA transcripts at 37oC may contribute to the survival of this dimorphic fungus in host cells. The role of these genes in the resistance of macrophage antimicrobial activity and the pathogenesis of *Penicillium marneffei* requires further investigations.

S15IS3 - 1004

An update on human pythoisis

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Pythiosis is a disease in mammals, caused by *Pythium insidiosum*, a pseudofungus in the class Oomycetes. The disease has been more frequently reported in tropical and subtropical areas as infection in animals such as horses, dogs, cattle, cats and sheep. Human pythiosis was firstly reported from Thailand by Thianprasit in 1986. Majority of the cases were seen in Thailand and a few cases have been reported from USA, Australia and Malaysia.

The clinical features of human pythiosis in Thailand can be divided into 3 types; 1) subcutaneous or cutaneous infection; 2) systemic or vasculitis and 3) ophthalmic or keratitic infection. Almost all of pythiosis patients had remarkably thalassemia as the main underlying disease especially in the first two types. Many factors in thalassemia patients predisposed them to infection such as splenectomy, iron overload, severity of anemia and phagocyte dysfunction. Besides these, skin trauma and exposure to zoospores contaminated environment such as swampy area and animal farm have also been identified as risk factors of pythiosis.

Krajaejun (2006) has recently gathered all reported and unreported cases of pythiosis in Thailand and analysed these data. There were 102 pythiosis cases reported in 9 tertiary-care hospitals from all parts of the country. The percentage ratio of systemic form: ocular form: cutaneous form was 61: 33: 6. The proportion of case distribution in the central, the northeast, the northern, the southern and the eastern parts were 46%, 27%, 16%, 8%, and 3% respectively. Eighty-one percent of cases had haematological underlying. All patients with no underlying disease were ocular cases. Thalassemia, agricultural-related careers, ages of 20-60 years, and male gender are predisposing factors. Even under intensive cares, at least 71% of ocular patients lost eyes, while at least 74% and 31% of systemic patients were limb amputated and died, respectively.

Laboratory diagnosis of pythiosis can be done by microbiological, serological and molecular methods. Microscopic examination of clinical specimens such as pus, thrombus or tissue usually reveals large branching nonseptate with thin wall hyphae. Isolation of *P. insidiosum* is the gold standard of pythiosis diagnosis. Immunodiffusion test, ELISA and Western blot are favourite serological tests nowadays. Nested polymerase chain reactions based on ITS1 and ribosomal RNA operon were also developed for rapid detection and identification of *P. insidiosum*.

Almost treatment of pythiosis with recently available antifungal drugs reveals unsatisfactory outcome. Immunotherapy with *P. insidiosum* immunogens revealed 50% success rate in severe pythiosis cases.

1030-1130
PLENARY 2 - 0819

Species of fungi: their recognition, maintenance and utility

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All fungal species are recognized by phenotype (morphological species recognition) or, less commonly, by reproductive isolation (biological species recognition). Over the past decade, genetic isolation, as determined by concordance of gene genealogies (phylogenetic species recognition), has become a popular method of recognizing fungal species. Many phylogenetic species recognition studies have shown that morphological species recognition is overly broad, relative to phylogenetic species recognition. More recent work has shown that phylogenetic and biological species recognition find nearly the same species, and that genetic isolation precedes reproductive isolation.

Studies of biological species recognition in *Neurospora* have shown that barriers to hybrid sexual reproduction have developed following mating, which is unlike the situation in many animals and plants. Inability to find hybrid *Neurospora* individuals, combined with stronger barriers to hybrid mating between different sympatric, as compared to allopatric, species suggests that barriers to hybrid matings have been reinforced in sympatry.

Divergence dates for the two or more phylogenetic species typically discovered in a single morphological species can be an order or two of magnitude more recent than divergence dates between morphological species, leading to the conclusion that species divergence is far more common than species persistence. Lack of species persistence may be explained by extinction or by to merger of recently diverged, sister species. Allopatry and the aforementioned reinforced barriers to hybrid mating success among sympatric partners provide means of preventing fusion of recently diverged species, but can be defeated if recently diverged species come into sympatry before significant barriers to reproduction have evolved.

Failure to find natural hybrids of *Neurospora* species is at odds with reports for agriculturally or medically important fungi, perhaps because humans can bring together recently diverged, allopatric species that still are capable of producing hybrid progeny, and because human activity provides disturbed environments in which hybrid progeny are protected from competition with their parents.

Groups of fungi for which species have been recognized by phylogenetic means constitute powerful systems for investigating many aspects of evolution, including the evolution of different regions of the genome, measuring natural variation in transcription, detecting the effects of selection, and searching for evidence for adaptation. A major challenge will be to determine adaptive traits for fungi. Evolutionary studies of fungi are greatly aided by their haploid nature and virtual immortality, but their small size can make it difficult to fully understand their natural ecology.

POSTER ABSTRACTS S2

1200-1330
POSTER SESSION 2 – FROM GENOMICS TO PROTEOMICS

PS2-193-0073
A glance at *Blastocladiella emersonii* biology through a comparative EST analysis
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Some ancestral characteristics of fungi and animals, or fungi and plants could be more conserved in early-diverging fungi than in members of late-diverging fungal species. In this sense, we followed two approaches to identify genes in the chytridiomycete *B. emersonii* previously classified as proper of animals or plants (1) and conserved in fungi, but largely divergent (2). For pursuing goal (1), we carried out a comparative analysis of *B. emersonii* putative unique sequences (4,873 uniseqs) against protein and unigene sequences from 22 animal or plant species deposited in Genbank, using BLASTX and tBLASTX tools and an Evalue ≤ 10-5 as the cut-off. Uniseqs were grouped according to the presence or absence of homologues in the examined species and more than 170 sequences not previously identified in fungi were revealed during the analysis. This group included cDNAs encoding enzymes from coenzyme B12-dependent propionyl-CoA pathway, a metabolic route completely absent in fungi, whose expression was validated by Northern blots. To reach goal (2), we selected translated *B. emersonii* full-length sequences and their orthologues in the ascomycete Neurospora crassa and the basidiomycete Ustilago maydis. We compared the scores obtained against the same animal and plant sequences, using BLASTP and tBLASTN tools and an Evalue ≤ 10-5 as the cut-off, and analyzed orthologous pairs with highest score differences. Through these fungal sequence comparisons, we found 4 sequences from *B. emersonii* not found or highly divergent from those reported in other fungal species. Both methods allowed us to reach our goals, and in addition we could associate sets of genes with cellular structures and biological processes occurring in these organisms.

PS2-194-0205
Secondary structures of ITS2 nuclear ribosomal DNA of some species of *Cercophora* and *Podospora* (Lasiosphaeriaceae)
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Internal transcribed spacer 1 and 2 (ITS1 and ITS2) of the nuclear ribosomal DNA (nr DNA) have become important genetic markers for inferring phylogeny of fungi. Because they have stem and loop structures, identification of homologous regions for sequence alignment is critical in phylogenetic analysis. To provide a universal model for sequence alignment, we analyzed the secondary structure of the ITS2 (internal transcribed spacer 2) of 8 species of *Cercophora* and 23 species of *Podosproa* by the rule of minimum free-energy. The predicted model has four arms the third of which is the longest. The first and second arms are conserved and the third arm is instable among the species. Our model will be useful for phylogeny reconstruction in the two genera and possibly also in closely related taxa.

PS2-195-0246
Identification of *Sclerotinia sclerotiorum* genes expressed in early stages of *Brassica napus* infection
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The ascomycete *Sclerotinia sclerotiorum* infects over 400 species of dicot plants, causing significant damage to crops including canola, sunflower and beans. We aimed to identify and characterize genes involved in *S. sclerotiorum* infection of *Brassica napus* (canola).
Expressed Sequence Tags (ESTs) were examined from libraries prepared from three tissues: complex appressorium (infection cushions), mycelia grown on agar, and lesions formed on leaves of B. napus. Quantitative real-time PCR was used to analyse tissue-specificity and timing of transcription of particular genes.
Among the 650 ESTs sequenced, there was low redundancy within and between the three developmental stages. A high proportion of genes (68%) had not been previously reported for *S. sclerotiorum* in public gene or EST databases. Quantitative PCR analyses of particular genes revealed differing transcriptional patterns during vegetative growth, leaf infection and stem infection. Genes investigated in detail included those with best matches to MAS3 (appressoria-associated protein from *Magnaporthe grisea*), cellobiohydrolase I, oxaloacetate acetylhydrolase, metallothionein, pisatin demethylase, and an unknown gene with orthologs in fungal pathogens but not in saprophytic fungi.
The types of novel genes identified in the infection cushion library highlights the functional specificity of these structures, and similarities to appressoria in other fungal pathogens. Microarrays for *S. sclerotiorum* will facilitate genome-wide transcriptional studies of the infection process, about which little is known at a molecular level.

PS2-196-0326

Microarrays & gene silencing for functional genomics of *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum is a devastating, necrotrophic ascomycete that causes stem rot of many plants, including major crop species. We are utilising the genome database for *S. sclerotiorum* (Broad Institute) to identify genes likely to be involved in infection and survival of this fungus. Microarray analysis of gene expression is being utilised to identify specific gene targets for further functional genomics investigations.
Combimatrix oligonucleotide microarrays, with 12,000 probes, are being designed to analyse *S. sclerotiorum* gene expression during early stages of infection of *Brassica napus (canola)*. Fungal gene expression in vitro and at various early infection stages will be compared. Quantitative RT-PCR will be used to verify expression for genes of interest. Our research focus is on secreted proteins (effector candidates) and enzymes involved in biosynthetic pathways that are up-regulated specifically during host penetration/infection. Selected genes will then be targeted for genetic analysis using gene silencing and gene disruption.
Gene silencing has been demonstrated to be an efficient tool for gene analysis in a related fungus, Botrytis cinerea, however it has not been reported for *S. sclerotiorum*. Both fungi often have multiple nuclei and multiple copies of genes which can make gene disruption (by homologous integration) difficult. Gene silencing (RNA interference) overcomes this problem by targeting transcripts rather than the genomic copy of the gene itself. A biosynthetic gene for the pigment, melanin, is being targeted to test the efficiency of this system. *S. sclerotiorum* produces a vegetative survival structure (a sclerotium) that is protected against degradation in the soil by a tough, pigmented (melanised) coat. The sclerotia are an important source of inoculum and they survive in soil for many years.
We have produced a silencing construct, with a hairpin targeted to the putative *S. sclerotiorum* THN (tetrahydroxynaphthalene) gene in a Gateway vector. Various *S. sclerotiorum* genes have been identified based on sequence similarity to known pathogenicity genes from other fungi. These will also be targeted for gene silencing. Combimatrix microarray probes have been designed based on the genome sequence (Broad Institute). Genes putatively encoding secreted proteins have been identified (Broad Institute) using the software programmes SignalP and Target P.
Our initial aim is to establish whether gene silencing has utility for high-throughput genetic analysis of *S. sclerotiorum* genes. Novel genes that are up-regulated during infection of host tissues will then be targeted for further functional analyses.

PS2-197-0351

Transcriptome analysis and expressed sequence tags of differentially expressed genes in sporulating Shiitake mushroom *Lentinula edodes*

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Shiitake mushroom is a well-known edible mushroom. To improve its cultivation, we need to better understand its biology. To this end, we first studied its gene expression profiles in various stages of its life cycle. For comprehensive transcriptome analysis, serial analysis of gene expression (SAGE), based on a high-throughput sequencing of 10 – 15 tags derived from mRNAs, is commonly used. However, only a small number of genes were annotated in *L. edodes* and thus tag identification has been difficult. To solve this problem, we produced a reasonably large cDNA database with high through-put sequencing with the Genome Sequencer 20 (Roche). In this study, two LongSAGE libraries were produced from two different developmental stages of *Lentinula edodes* L54, i.e. fruiting bodies before and during sporulation were used as starting materials. Thousands of LongSAGE tags from clone sequences were extracted and identified using our primordium EST collection of *L. edodes* L54. About 4000 and 7000 tags were obtained from fruiting bodies before and during sporulation. Since the ESTs were generated from primordium, few tags could be matched to this database. We then generated a large number of cDNA contigs using the Genome Sequencer 20 that can produce sequences totaling 25 million bases in one 4-hour run using microfrabricated high-density picolitre reactors. cDNA pool from L54 fruit bodies before and during sporulation were generated and sequenced. About 7800 contigs were produced and 109 contigs were over 500bp. Most abundance tags were matched with the contigs. When comparing the expression profiles in different stages, many novel tags were found in sporulating fruiting bodies such as heat shock protein 12, NADH dehydrogenase, proteophosphoglycan, alpha-galactosidase, Glutamine amidotransferase and some hypothetical proteins. The LongSAGE results were validated by Real-time PCR of selected genes. By using LongSAGE and the Genome Sequencer 20, transcript profiles of spore formation process could be revealed. We thus have a better understanding about *L. edodes* biology in particular and in mushroom biology in general.

PS2-198-0364

Transcriptomic analysis of biotic actions of the ectomycorrhizal fungus *Paxillus involutus*

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Paxillus involutus, an ectomycorrhizal fungus, is known to be able to degrade and sequester elements from complex organic substrates. Preliminary work has shown that living mycelium of other fungi may be degraded and used as a source of nutrients (Lindahl et al., 1999 New Phytol. 144, 183-193). In soil microcosms, antagonistic reactions have been observed to take place between *P. involutus* and wood degrading *Hypholoma sp*..In a microcosm study, the extra-radical mycelium of *P. involutus,* growing out from *Pinus sylvestris* host seedlings, was confronted with *H. fasciculare* mycelium, growing out from a wood block. Soil containing interacting mycelium was sampled and mRNA extracted and purified. In order to analyse gene expression, sample cDNA was hybridized with micro arrays containing 3 500 ESTs, half of the estimated genes in *Paxillus involutus* genome (Johansson et al. 2004 MPMI 17-2 pp202-215). The EST's were gathered from 10 different cDNA libraries obtained from *P. involutus* mycelium under different environmental conditions and contain both mycelium from mycorrhizal root tips as well as extra radical mycelium. The aim of this study is to identify genes involved in antagonistic interactions including stress and defence related genes expressed by *P. involutus* in response to a confrontation with *H. fasciculare*. Furthermore, enzymes such as proteases and chitinases, involved in nutrient acquisition from interacting mycelium is expected to be highlighted. We hope to obtain new insights on the molecular mechanisms of biotic interactions as well as nutrient acquisition from organic substrates.

PS2-199-0402

Differential display, cDNA microarray, expressed sequence tags and serial analysis of gene expression (SAGE) reveal gene expression profiles of shiitake mushroom *Lentinula Edodes* development

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Mycelial growth and fruiting body development are important topics in mushroom biology and have been analyzed at the molecular level recently. We aim to characterize gene expression profiles during development of Shiitake mushroom Lentinula edodes. First, we used Differential Display method RNA fingerprinting with arbitrarily primed polymerase chain reaction (RAP) to isolate genes differentially expressed in fruiting. RAP was powerful in isolating gene fragments but down-stream works to isolate full-length cDNA are tedious. Over 100 genes were isolated and sequenced. Fifteen were studied further. Second, cDNA clones were randomly sequenced to generate about 1000 Expressed Sequence Tags (ESTs). Differential expression of the ESTs were analyzed by dot-blot hybridization and cDNA microarray analysis using total cDNA from mycelium and primordium as probes. Third, we used Serial Analysis of Gene Expression (SAGE) to determine the proportion of each mRNA among total transcripts in various growth stages,. About 30,000 transcripts were counted from seven growth stages. Expression profiles of the tags were clustered. To annotate these SAGE tags, we obtained sequences of over 13000 cDNAs from mycelium and fruiting bodies. Over 1400 tags could match to our cDNA sequence collections. The kind of genes differentially expressed at the initiation of fruiting body showed the following: (1) initiation—stress response and specific signal transduction, (2) reconstruction of proteome—protein degradation, modification and biosynthesis, and (3) switching of biochemical pathways and structural components like hydrophobins. The gene expression profiles during further development of the fruiting body and sporulation showed the occurrence of: (1) stress response—heat shock protein 12, (2) biosynthesis of DNA and chromosome—histones, (3) proteome reshuffling—proteases, cyclophilin, (4) production of specific enzymes and proteins—B2 aldehyde forming enzyme, Phosphatidylethanolamine binding protein, alpha-galactosidase, Proteophosphoglycan, and Glutamine amidotransferase. Gene expression profiles revealed by different approaches were compared and were generally consistent.

PS2-200-0420

The *Leptosphaeria maculans* genome initiative

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Genoscope (National Sequencing Centre), the French sequencing agency has been involved in large-scale sequencing of the genome of the Dothideomycete *Leptosphaeria maculans* since 2000. The first two collaborative projects between INRA and Genoscope consisted in precise sequencing, assembly and finishing of a 1.1 Mb genomic region in isolate v23.1.3, along with less precise sequencing of the corresponding region in two other isolates. These data enabled us to characterize the first known retrotransposons and avirulence genes in *L. maculans*. They also suggested a particular organisation for the genome of *L. maculans*, encompassing alternating regions of isochores, i.e., of long A+T-rich regions reminiscent of higher Eukaryote heterochromatin, and of G+C-equilibrated, gene-rich regions. With this preliminary snapshot of the genome as a basis, complete shot-gun genome sequencing strategy has been initiated in December 2005. Its goal is to release an annotated assembly with 10-x genome sequence coverage (600 000 reads) for *L. maculans* isolate v23.1.3. Additional sequencing of BAC-ends for physical mapping and ESTs from mycelia grown under three different conditions will also be performed (total of 50 000 reads). The current status of the sequencing project and first data from it will be presented.

PS2-201- 0438

Characterization of *Leptosphaeria maculans* gene that enables this fungus to Infect *Arabidopsis thaliana* ecotype Col-0

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Blackleg disease caused by the dothideomycete *Leptosphaeria maculans* is the most damaging disease of canola *(Brassica napus)*. The fungus can infect some *Arabidopsis thaliana* mutants (lms1, pad3-1, pmr4-1) but not the wild type accessions (Col-0 and Ler). *L. maculans* invades canola and *A. thaliana* mutants via the stomatal aperture, grows between the mesophyll cells and produces asexual spores on the leaf surface. We are studying molecular aspects of interaction between *A. thaliana* by screening for and characterizing T-DNA insertional mutants of *L. maculans* that can infect accession Col-0.

We have screened 140 *L. maculans* insertional mutants for successful penetration of *A. thaliana* leaves. Pycnidiospores were placed onto intact leaves of Col-0 in the presence of 1% glucose and tissue stained and examined by light microscopy.

One mutant (A22) penetrated the host via the stomatal aperture at a frequency eight times higher than that of wild type and caused more hypersensitive cell death than the wild type isolate. Further testing of the A22 mutant on another *A. thaliana* wild type accession (Ler) showed that the mutant penetrated stomata three times more often than the wild type. In contrast, both mutant and wild type isolates showed same pathogenicity on *B. napus*. In addition, mutant A22 was able to grow further into the mesophyll layer of *A. thaliana* penetration mutant pen1-1 (defect in syntaxin) than in pen 2-1 (defect in glycosyl hydrolase) or in Ler. The A22 mutant has a single T-DNA insertion in the open reading frame of a single copy gene, a22. The predicted protein encoded by a22 has sequence similarity to hypothetical proteins of other plant pathogenic fungi. Experiments are underway to determine the pattern of gene expression and in vitro morphological development of the mutant isolate.

This study is providing insights of how *L. maculans* responds to cues from its host, *B. napus* and from non-host plants.

PS2-202-0471

Distribution of optional mitochondrial introns encoding putative homing endonuclease genes in the *Fusarium oxysporum* complex

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Mitochondrial DNA Restriction Fragment Length Polymorphism's have often been used for population studies in the *Fusarium oxysporum* complex. These studies have demonstrated the *F. oxysporum* mitochondrial genome to vary in size from about 45 kb to 55kb. However, the nature of these length polymorphism's is not known. To determine whether optional introns are responsible for this variation, the distribution of introns in five genes was determined for 16 randomly selected Australian isolates of *F. oxysporum*. The genes selected were for apocytochrome b (cob), cytochrome oxidase c subunit 1 (cox1), cytochrome oxidase c subunit 2 (cox2), nicotinamide adenine dinucleotide ubiquinone oxidoreductase subunit 1 (nad1) and nad5. These genes were chosen based on the high frequency of introns reported in other ascomycetes. Long PCR was used to test for the presence of introns. Primers were designed using *F. oxysporum* mitochondrial genome sequences available on GenBank.

Group I introns were found in two of these genes. In all cases these introns encoded putative ORF's for homing endonuclease genes (HEG's). A HEG containing two LAGLIDADG motifs was found in the nad5 gene of all isolates. The distribution of HEG's in the cob gene was found to be variable. Nine isolates possessed a HEG with a GIY-YIG motif. Two of these isolates contained a second cob HEG. This second HEG had a LAGLIDADG motif.

HEG's were sequenced. They showed high homology to HEG's that have been found inserted in the same position in the mitochondrial genomes of other ascomycetes. Phylogenetic analysis of the isolates based on partial translation elongation factor gene sequences revealed a relationship between phylogeny and HEG distribution in the cob gene. The absence, or presence of one or two HEG's in the cob gene, correlated with previously reported *F. oxysporum* clades 2, 3 and 1 respectively.

The two optional cob introns were about 1.5 kb in size. Thus, the presence or absence of introns in the five genes examined accounts for only a proportion of the length variations reported in the *F. oxysporum* mitochondrial genome. The more interesting finding is that the distribution of these introns correlates with phylogenetic lineages in the F. oxysporum complex. These results provide further support for splitting *F. oxysporum* into a small number of species.

PS2-203-0518

Cloning and Characterization of Secondary Metabolite Biosynthetic Genes from Lichens

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It is well known that lichens are able to produce unusual secondary compounds, called lichen substances. These substances are thought to function to protect against excessive light, to keep moisture, and to inhibit growth of other organisms as antibiotic and/or allelochemical. Most of them are derived from the acetate-malonate biosynthetic pathway, also known as the polyketide biosynthetic pathway, and several metabolites are derived from the shikimate and mevalonate biosynthetic pathways. In the polyketide biosynthetic pathway polyketide synthase (PKS) may play major role, however, PKS genes from lichenized fungi are reported to be partially cloned for phylogenetic analysis. Little is known of biosynthesis-related genes in the other pathways. To investigate the molecular mechanism of the biosynthesis of lichen substances, the responses of mycobiont culture under different nutritional conditions or stress were analyzed firstly. The culture analyses revealed that the metabolism of Vulpicida juniperinus was effected by addition of ribitol, which is the sugar alcohol produced by green algae partner in thallus. Secondly, PKS and related genes were amplified by PCR with degenerate primers and by thermal asymmetric interlaced PCR (TAIL-PCR), resulting in cloning of DNA fragments for gene encoding ketoacyl synthase (KS) domain of PKS. The relationship between metabolites and gene-expression under modified conditions will be also discussed.

PS2-204-0529

SSH-based analysis of the genes expressed differentially in the primordia and basidioma of a medicinal mushroom *Hericium erinaceum*

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Currently, information regarding the molecular events occurring in the development of a mature basidiome from primordial tissue in H. erinaceum is limited. Furthermore, the differential expression of genes involved in the process is poorly understood. This study was performed to identify the genes that specifically expressed at two different stages of mushroom development in *H. erinaceum*. Developmentally expressed genes in primordial and basidiome cells of the cultivated mushroom were analysed by PCR-based suppression subtractive hybridization (SSH) technique and sequencing. *H. erinaceum* KUMC1008 used in this study was obtained from Odae mountain in Korea. . *H. erinaceum* was cultivated in tissue culture bottle(6?11cm) containing 150g of the oak sawdust and rice bran in a ratio of 4:1(v/v). Total RNA was extracted using the TRI Reagent and SSH was performed between RNA extracted from primordia(tester) and/or basidiome(driver).The SSH subtractive cDNA fragments were cloned into a cloning vector using the TOPO™ TA cloning kit and sequenced. Translated SSH clones were compared to protein sequence databases at the National Center for Biotechnology Information using the BLAST algorithm. In this study, we obtained 282 cDNA clones (137 and 145 from primordial and basidioma libraries, respectively). The size of the insert ranged from 90 to 1300 bp with an average of 300 bp for primordial and 370 for basidioma library. DNA sequences of the SSH cDNAs differentially expressed in primordia and basidioma of *H. erinaceum* was successfully analysed. 45% of the analyzed genes had nucleotide sequence homology with those of known fungal genes. More numbers of genes involved in metabolism and protein synthesis were expressed in basidioma than in primordia. More than 50% of the expressed genes are hypothetical protein-coding genes.

PS2-205-0534

Genome environment is instrumental in evolution towards virulence at the AvrLm1 avirulence locus of *Leptosphaeria maculans*

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The Dothideomycete *Leptosphaeria maculans* is the most damaging disease of oilseed rape (*Brassica napus*) worldwide. Genetic studies demonstrated the occurrence of gene-for-gene interactions in the *L. maculans / B. napus* system. In this model, avirulence (Avr) proteins produced by the pathogen directly or indirectly activate plant defense responses, such as the hypersensitive response, upon recognition mediated by matching plant resistance (R) proteins. To date nine avirulence genes (AvrLm1-9 genes) have been identified in the pathogen and the corresponding nine resistance genes (Rlm1-9 genes) were identified in the host plant. In France, disease control relies mainly on the use of disease-resistant cultivars. The Rlm genes effectively control the disease as long as the corresponding avirulent allele (AvrLm) dominates in the pathogen population. However, *L. maculans* has the ability to very rapidly adapt to the selection pressure exerted by a novel resistance gene as exemplified by the 3-year evolution towards virulence at the AvrLm1 locus in French field conditions. Here, we investigated the molecular mechanisms responsible for the gain of this new virulence in French field isolates. The AvrLm1 avirulence gene was recently cloned and shown to be a solo gene within a 269 kb non-coding, heterochromatin-like region consisting of mosaics of degenerated repeats. We fully or partly sequenced the AvrLm1 genomic region in one avirulent and two virulent isolates. The gain of virulence was linked in both cases with a 260 kb deletion of a chromosomal segment spanning AvrLm1 and deletion breakpoints were identical or similar for both the virulent isolates. Among 191 field isolates analyzed, a similar large deletion leading to chromosome length polymorphism was evidenced by multilocus haplotype analysis in 90% of the virulent isolates. Furthermore, deletion breakpoints were strongly conserved in all these virulent isolates leading to the hypothesis that a strong constraint in the genome environment is instrumental in generating a unique event of chromosomal rearrangement leading to virulence towards Rlm1 plants. Surprisingly, even though a large chromosomal fragment was lost to gain virulence, no effect on fungal and pathogenic fitness could be evidenced, either in vitro or under natural field infection conditions.

PS2-206-0827

Investigating gene structure and transcription in the intracellular plant pathogen, *Plasmodiophora brassicae*

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The plasmodiophorids are a group of intracellular parasites that were historically classed as fungi, but are now included within the eukaryotic supergroup, the Rhizaria. Although containing several important plant pathogens, there is almost no information on the genomic makeup of the plasmodiophorids. Indeed, little is known about the genomes of any protists from the Rhizaria. Plasmodiophora brassicae, the causal organism of club root disease, is a plasmodiophorid pathogen that infects plants in the Brassicaceae family, stimulating the formation of large hypertrophic root galls. Club root disease is a problem for growers worldwide. To locate P. brassicae genes transcribed in planta, we performed suppression subtractive hybridisation between RNA from *P. brassicae-infected* and uninfected *Arabidopsis* tissue. We also used an oligo-capping procedure to screen full-length cDNA clones from the infected tissue. In total around 80 new *P. brassicae* gene sequences were identified, most of which were extended to full-length at the 5' end using RACE amplification. Many of the unisequences were predicted to contain signal peptides for endoplasmic reticulum translocation, and hence may play roles in manipulation of the host by P. *brassicae*. Results of cDNA cloning and possible roles of genes in host-pathogen interaction will be presented. Using PCR walking techniques, genomic DNA sequences flanking 18 of the *P. brassicae* genes was retrieved. The presence of 9 new genes was predicted in these sequences by BLASTX and was then confirmed by RACE amplification. Evidence suggesting that the *P. brassicae* genome is compact but relatively rich in introns will also be presented.

PS2-207-0887

Phylogeny of gene clusters responsible for the biosynthesis of epipolythiodioxopiperazine (ETP) toxins

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Epipolythiodioxopiperazines (ETPs) are toxic secondary metabolites made only by filamentous fungi. The best-known ETP is gliotoxin, which appears to be a virulence factor associated with invasive aspergillosis of immunocompromised humans. Another well characterised ETP, sirodesmin, is a virulence factor of *Leptosphaeria maculans*, which causes blackleg stem canker of canola. Genes responsible for the biosynthesis of secondary metabolites are typically clustered in filamentous fungi. Recently the gene clusters involved in the biosynthesis of sirodesmin by *L. maculans* and of gliotoxin by *Aspergillus fumigatus* have been identified by bioinformatics analyses of genomic sequences containing open reading frames with predicted roles in ETP biosynthesis. Targeted mutation of a gene in each cluster resulted in mutants unable to produce the respective toxins.
Gliotoxin is also synthesised by other ascomycetes such as *Penicillium citreonigrum* and *Trichoderma virens*. The presence of clusters producing the same molecule in these distantly related fungi is intriguing. We are interested in the evolutionary origins of these ETP biosynthetic gene clusters. We have isolated putative gene clusters for biosynthesis of gliotoxin in *P. citreonigrum* and in *T. virens*. We have also examined the genome sequences of other filamentous fungi and identified putative ETP biosynthetic gene clusters in nine fungi. We are comparing the phylogenetic relationships between coding sequences of eight homologs from ETP-like clusters in these fungi, to predicted organismal phylogenies based on the 18S rDNA, Hsp70 and beta-tubulin genes.
Our preliminary results suggest that the evolutionary history of these eight genes within the ETP-like biosynthetic clusters is different to that predicted by the phylogeny of the fungal species. This would suggest that at least some gene clusters were not inherited by vertical transmission, but by horizontal transfer.

PS2-208-0900

Transformation of *Sclerotinia sclerotiorum* with the green fluorescent protein gene

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Sclerotinia sclerotiorum is an important pathogen of a wide variety of crops. To obtain a genetic marker to study the interaction of the pathogen with its hosts, isolates ND 21 and ND 30 were transformed using constructs pCT74 and gGFP both containing genes for the green fluorescent protein (gfp) and hygromycin B phosphotransferase. Plasmid DNA was produced and purified using standard methods. Protoplasts were generated and a protoplast-PEG (polyethylene glycol) transformation method was employed. Twenty hygromycin resistant putative transformants appeared on the surface of the selection medium in 7 to 12 days using ND 21 with pCT74. With ND 30, only four to five transformants were obtained with each construct. The sgfp gene was detected in seven stable transformants using sgfp specific primers. Southern analysis on transformants detected a single copy of the sgfp gene in the genome. The fluorescence of mycelial plugs was quantified using a Synergy HT multi-detection microplate reader with an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. Fluorescence values of Z4-1 and Y4-1 transformants from ND 30 and 3B-17 and 4A-14 from ND 21 were significantly higher compared to the wild types. In addition, protein extracts from mycelium of the transformants had higher fluorescence values compared to the wild types. Pathogenicity of four ND 30 and three ND 21 transformants on canola, dry bean, soybean, and sunflower were determined by measuring leaf lesion diameter. In dry bean and canola, ND 30 wild type had significantly higher lesion diameter compared to the transformants. However, on sunflower and soybean one ND 30 transformant (Y4-1) was as pathogenic as the wild type. Lesion formation by ND 21 wild type and the transformants was slower than ND 30. All ND 30 transformants were pathogenic on the four hosts, but pathogenicity of ND 21 transformants varied depending on the host. Infected tissues were sectioned and examined on a Leitz Wetzlar epifluorescence microscope, or a Nikon E600 CARV Confocal cell imaging system, both equipped with filters for gfp excitation and emission. Hyphae of transformants fluoresced in host tissue and could be distinguished from the plant cells. These stable gfp transformants are useful tools for studying the biology of this important pathogen, especially when examining the interaction with plants or other microorganisms.

PS2-209-0933

Functional analysis of hybrid histidine kinase genes of *Cryptococcus neoformans*

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Hybrid histidine kinases (HHKs) are known to regulate drug resistance, osomo- tolerance, pathogenicity and hyphal growth in fungal species. *Cryptococcus neoformans*, a human pathogen, possesses seven HHK genes in its genome. One of them, CnNIK1, has been functionally analyzed and its involvement in phenylpyroll resistance, pathogenicity and HOG1 MAP kinase regulation. Here we analyzed functions of the rest HHK genes, CnHHK2, CnHHK3, CnHHK4, CnHHK5, CnHHK6 and CnHHK7 by gene knockouts. *C. neoformans* B-4500 (mating type alpha, serotype D) derivative, an uracil requiring strain was used for genetic manipulation. CnHHK2, CnHHK5, CnHHK6 and CnHHK7 genes were successfully knocked out by gene replacement with a functional URA5 gene, and knockout strains were designated as TLHM2, TLHM3, TLHM1 and TLHM4, respectively. From TLHM3, TLHM1 and TLHM4, we could obtain strains with opposite mating type by sexual crosses with B-3502 (mating type a, serotype D), but not from TLHM2. Resulting progenies with each knockout alleles were designated as RLHM3a, RLHM1a and RLHM4a, respectively. Sexual mating assay revealed that crosses between TLHM3 x RLHM3a, TLHM1 x RLHM1a and TLHM4 x RLHM4a could all complete sexual development as that between wild type strains, suggesting that CnHHK5, CnHHK6 or CnHHK7 are not required for *C. neoformans* to develop hyphae, clamp connections, basidia and basidiospores.

PS2-210-0934

Cloning and characterisation of a transcription factor GC-ZXT in *Glomerella cingulata*

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Glomerella cingulata causes bitter rot in apples and fruit rot in other subtropical fruits. In response to environmental cues like contact with the host, *G. cingulata* forms a special structure called an appressorium, which accumulates glycerol and thereby generates a sufficiently high turgor pressure to push an infection hypha into the host tissue. Little is known of the transcriptional regulation for appressorium formation and function in *G. cingulata* or other appressorium-forming fungi.
A transcription factor from *G. cingulata* (named GC-ZXT) was cloned using degenerate PCR and subgenomic library screening. Targeted gene deletion was successful. Deletion mutants were isolated and characterised by Southern blot analysis. These mutants display many phenotypic changes. Complementation mutants were constructed to confirm the function of this gene. A full-length copy of this gene together with a second selection marker was reintroduced into the deletion mutant and the wild type phenotype was restored.
Deletion mutants form appressoria at the normal rate and with unaltered morphology. In comparison with the wild type, these appressoria did not generate high turgor pressure. This resulted in a defect in penetration of onion epidermal cells. Nor were they able to invade unwounded apples. Therefore, the *G. cingulata* GC-ZXT gene is required for appressorium function. In addition, deletion mutants displayed stunted aerial hyphae, "wettable" mycelium, reduced conidia production, and a defect in perithecium formation. These results suggested that the *G. cingulata* GC-ZXT has multiple roles in fungal development.

PS2-211-0944

Carbon source depending interaction of cAMP signalling pathway and light in *Hypocrea atroviridis*

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Light as a central abiotic factor is primarily known to influence general morphogenesis and both sexual and asexual sporulation in fungi, but it has also been reported to influence several metabolic processes. We used phenotype microarrays as a method to study the effect of light on the utilization of 95 different carbon sources by *Hypocrea atroviridis* (anamorph *Trichoderma atroviride*) and compared the results to those obtained with two mutant strains in which one of the two genes encoding the blue light receptor genes blr1 and blr2 had been deleted, respectively. Light enhanced growth on some carbohydrates (mono-, di- and polysaccharides), sugar acids, sugar amines and polyols to different degrees (1.5 – 2-fold), but not that on amino acids, aliphatic acids. This increase in the growth rate was not observed in the blr1 and blr2 delta strains. Light also leads to conidiation of *H. atroviridis* on some carbon sources, most of them were the same as those whose assimilation was stimulated by light. We hypothesized that the specificity of light on selected carbon sources involves signalling via cyclic AMP and performed experiments in which dibutyryl-cAMP and the phosphodiesterase inhibitor IBMX were added to the wild-type and the blr1 and blr2 mutants and incubated in light and in darkness on the phenotype microarrays. These results will be discussed with respect to a possible cross-talk between light and cyclic AMP in light-stimulation of growth and sporulation in *Trichoderma*.

PS2-212-0981

Evolutionary pressures on two pheromone receptor genes in heterothallic *Neurospora* species

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Pheromones play important roles in female and male behaviour in ascomycetes by mediating signal transduction to induce expression of mating genes. In *Neurospora* the pheromone receptor genes pre1 and pre2 are believed to function in mate recognition.
Pheromone receptor genes and other genes involved in reproduction appear to evolve faster than other genes in the genomes, as seen in a wide range of fungal taxa including *Neurospora*. This phenomenon may be important for the establishment of barriers to fertilization between outcrossing taxa, leading to speciation. However, the question remains whether the rapid evolution of reproductive proteins is a result of stochastic or deterministic processes. The aim of this study is to investigate whether the divergence in the pheromone receptor genes pre1 and pre2 is promoted by positive Darwinian selection.
For these purposes we amplified the entire coding region of pre1 and pre2, from 30 individuals belonging to the nine heterothallic species of *Neurospora*. We used likelihood-based methods to compare models of different types of selective pressure among codons to analyze the mode of evolution of the two genes. We found that pre1 appears to evolve under positive selection, while pre2 does not. The different selection pressures might indicate that the two pheromone receptor genes are involved in different functions apart from mate recognition.

PS2-213-0982

Proteome analysis of waito-c rice seedlings treated with culture fluid of gibberellin producing fungus, *Fusarium proliferatum* KGL0401

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Gibberellins are a large family of isoprenoid plant hormones, and the term GA was first used in 1935 to describe the substance produced by the fungus *Gibberella fujikuroi* that caused overgrowth symptoms in rice, referred to as bakanae disease. GAs control many aspects of plant development, including seed germination, shoot elongation, flower formation and development, fruit setting, seed development, sex determination, and chlorophyll content. Isolation of a new gibberellin producing fungus *Fusarium proliferatum* KGL0401, treatment of cultural fluid of isolated strain on the seedling of waito-c rice, and some protein spots upregulated by the culture fluid were presented.
The isolated *F.proliferatum* KGL0401 was cultured in Czapek's liquid medium, at 30C, 120rpm for seven days. The cultire fluid was then concentrated 30-folds and applied to 2-leaf waito-c rice seedlings that had been grown for 7 days at 30C. To determine the proteins related to the effect of the *F. proliferatum* KGL0401 culture fluid on the waito-c rice, the proteins were analysed by 2-D electrophoresis. The rice seedlings were grown for 7 days, then directly homogenated using a motor driven homogenizer in a sample lysis solution. The proteins were extracted for one hour at room temperature with voltexing. After centrifugation at 15,000 x g for one hour at 15C, the insoluble material was discarded and the soluble fraction was used for two-dimensional gel electrophoresis. A large number of protein spots were separated on the 2-D PAGE gel, and most of the proteins identified from the control treated with the medium and those from the sample treated with the culture fluid of F. proliferatum KGL0401 were separated with a similar electrophoretic mobility in the 2-D PAGE. However, 180 protein spots were overexpressed in the sample trated with the culture fluid. Gas chromatography and mass spectrometry(GC-MS) were used for identification of GAs produced by *Fusarium proliferatum* KGL0401. And 2-D elecrophoresis, MALDI-TOF analysis and database search were carried out to identify the upregulated proteins.
51 spots out of 180 upregulated proteins were characterized. Those were glutathione S-transferase, a hypothetical protein, heat shock protein, tubilin alpha-1 chain, ribulose-1,5 bisphosphate carboxylase/oxygenase(RuBisCo), RuBisCo activase, and unknown proteins.
Discussion: RuBisCo activase has been shown to be phosphorylated with Ca2+, Mg2+, and ATP from rice leaves grown in the presence of GAs, thereby suggesting a role in GA signaling. The tublin alpha-1 chain and actin have also been identified as GA3-upregulated proteins. Microtubules are involved in many cellular processes, such as cell devision, cell transport, and cell elongation in plants, and tubulins are the major protein in microtubules. Furthurmore, proteins of unknown function were also include in the list of GA-regulated proteins.
Little is still known about the proteins regulated by Gas in rice. The role of Gas in the germination of Arabodopsis has showed that alpha-tubulin, a cytoskeleton component, is a prime target of GAs in this system. Therefore the information revealed by the treatment of waito-c rice with the *F. proliferatum* KGL0401 culture fluid will be helpful in predicting the function of many other proteins in response to environmental challenges and for further studies related with GAs.

PS2-215-0990

A proteomic approach into biological control of sugar canegrubs

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Metarhizium anisopliae is a fungus that grows naturally in soils throughout the world and causes disease in various insects including the greyback canegrub *(Dermolepida albohirtum)*, a sugarcane pest in Australia. Even though there have been a number of gene-based approaches into identifying determinants for biological control and developing improved strains, our research bestows a comparative proteomics approach into identifying key proteins produced by the fungus during infection of greyback canegrubs. Simultaneously a proteomic map for the greyback canegrub larvae have been produced which is responsible for considerable crop loss.
Proteins related to pathogenicity from both liquid culture and solid culture approach was identified by mass spectrometry. Concept of solid culture approach is thought to give a more realistic view of infection process compared to liquid culture. Identification of novel target proteins and differential displays of proteomic maps of healthy/infected whole grub as well as healthy/infected cuticle were produced and evaluated via Progenesis image analysis software. Proteomic map of healthy fungus *(Metarhizium anisopliae)* (MY) was also generated successfully. A total of 156 protein spots on the healthy whole grub (HWG), 88 unique spots on infected whole grub (IWG), 17 spots from healthy cuticle (HC) and 14 spots from the fungus (MY) were analysed by mass spectrometry. Of these, 61 protein spots from HWG, 40 protein spots from IWG, 15 protein spots from HC and 9 protein spots from MY were confirmed by using cross species identification by mass spectrometry. Among the identified proteins were different isoforms of actin and tropomyosin, ATP binding protein, arginine kinase, formate dehydrogenalase, enolase, tara like protein isoform and heat shock proteins. Further identification for most of the protein spots has been hindered due to the limited number of suitable/accessible databases. *Metarhizium anisopliae* has been successfully transformed to benomyl resistance using pBENA3, a plasmid containing the benA3 allele from *Aspergillus nidulans* using particle bombardment as a preparation for introducing genes encoding the identified pathogenesis factors from both solid and liquid culture approaches into *Metarhizium*.

PS2-216-0991

Characterisation of the *Trichoderma reesei* proteasome

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The filamentous fungus *Trichoderma reesei* is one of the most efficient eukaryotic cell factories available. Considering its extraordinary secretion capacity, between 40 – 100 grams L-1 of protein, this species can be characterised as "a professional" protein secretor. Protein quality control is a crucial cellular function Ubiquitin-proteasome pathway plays a key role in the post –translational regulation in eukaryotic cells. Proteins that are not folded correctly or not fully assembled are recognized in the early secretory pathway featuring a large (approximately 2.5 MDa) multicatalytic protease, the proteasome.
The aims of this project are (i) to isolate the *T. reesei* proteasome and separate the subunits using 2-dimensional gel electrophoresis to produce a reference proteomic map for the fungal proteasome and (ii) to link the proteasome to ERADication of misfolded proteins from the secretory pathway using mutant forms of an endogenous efficiently secreted cellulase enzyme as experimental molecules.
An isolation method for the 20S proteasome of *T. reesei* and a 2D master map have been established. From this reference map, 13 of the 14 20S proteasome subunits and many related proteins that were co-purified with the 20S proteasome have been identified. We have also prepared a series of mutant forms of the main cellobiohydrolase enzyme CBHI to trace their secretion and presume degradation in the proteasome. The results will provide novel information of the involvement of the ubiquitin-proteasome pathway in the clearing of misfolded proteins in the highly secreting fungal cell.

PS6-217-0089

Control of spoilage and ochratoxin a (ota) production in moist grain for animal feed using the biocontrol agent *Pichia anomala*

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Introduction: The major hurdle in production of commercial biocontrol agents (BCAs) has been the lack of production of appropriate formulations. Of particular importance is the conservation of viability and ecological competence after application. *Pichia anomala* has been found to reduce mould growth and ochratoxin A (OTA) production. With this in mind studies were conducted to develop formulations of *P. anomala* which would have these attributes.
Methods: Studies were carried out on the fermentation of the yeast cells under different conditions in a molasses-based medium and the effect on endogenous reserved, quality and viability assessed. Studies were carried out with fluidised bed-drying to determine the temperature (30-70oC) which was optimum for conservation of the yeast cells as a dry powder. Several additives for conservation of viability were examined.
Biocontrol efficacy was determined with the best formulations in laboratory scale storage and in pilot scale silo studies over a complete season.
Results: About 50oC was found to be optimum temperature for fluidised-bed frying of yeast cells and for conservation of viability. Additives such as cotton seed flour+skimmed milk was best for conservation of yeast cell quality. The biocontrol efficacy of formulated *P. anomala* cells was found to inhibit mould growth and OTA production depending on storage water availability of wheat grain. Furthermore, modified yeast cells with increased levels of trehalose and arabitol gave similar efficacy as fresh cells. A subsequent pilot scale study, using malfunctioning airtight silos containing moist grain, showed that addition of fresh cells or formulated *P. anomala* cells both effectively controlled P. roqueforti.
Discussion: This study has demonstrated that using ecophysiological approaches to the production of formulations of biocontrol agents can improve the performance of formulations which can result in biocontrol efficacy as good as fresh cells. This can have a significant impact on potential commercialisation of such fungi.

PS6-218-0099

Manipulation of the toxigenicity of *Aspergillus flavus* soil populations to control aflatoxin contamination in peanuts

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Aflatoxin contamination of crops, which results from invasion and growth of the fungi *Aspergillus flavus* and *A. parasiticus*, is a major food and feed safety issue throughout the world. Among the crops affected is peanut (*Arachis hypogaea*), and aflatoxin contamination of peanuts not only threatens human and animal health, but it also causes great economic loss to peanut industries in most peanut-producing countries. Because preharvest aflatoxin contamination is associated with hot, dry weather conditions during the crop maturation period, the only method to control or prevent contamination has been irrigation, which is not an option for the majority of peanut growers. However, research in recent years has shown that biological control of aflatoxin contamination of peanuts can be achieved by applying a competitive, non-aflatoxigenic strain of *A. flavus* to soil during the middle of the growing season. A formulation for establishing that strain in the field was developed, approved as a biopesticide by the U. S. Environmental Protection Agency, and used commercially for the first time in 2004. Studies were conducted to determine the efficacy of the biopesticide for control of aflatoxin in peanuts grown in the southeastern U. S. during the 2004 crop year. The biopesticide was applied mid-season to approximately 2000 ha of peanuts at a rate of 22.5 kg/ha. Dilution plating of soil samples taken from representative fields of treated and untreated peanuts showed that the incidence of toxigenic strains of *A. flavus* was greatly reduced as a result of treatment. The mean incidence of toxigenic strains in untreated fields was 71.1% with a mean total *A. flavus* population density of 842 CFU/g. However, treatment with the biopesticide resulted in a mean incidence of 4.0% toxigenic strains with a mean total A. flavus population density of 7242 CFU/g. Samples of farmers' stock peanuts from treated (n = 404) and untreated (n = 178) fields were collected at seven different locations and analyzed for aflatoxin. Results showed that mean aflatoxin in treated peanuts (11.7 ng/g) was reduced by 85.2% compared with untreated peanuts (78.9 ng/g). The percentage of farmers' stock loads containing > 100 ng/g was also reduced by 86.5%. Highly significant ($P < 0.01$) reductions in aflatoxin were seen at all locations where appreciable levels of aflatoxin (> 20 ng/g) occurred. Treated and untreated peanuts were stored separately within the same warehouse at two locations and subsequently shelled to produce edible grade peanuts. Mean aflatoxin in commercial shelled lots at one location was reduced from 36.2 ng/g in untreated peanuts to 0.9 ng/g in treated peanuts. At the other location mean aflatoxin was reduced from 7.2 ng/g in untreated peanuts to 2.2 ng/g in treated peanuts. It is of significant economic importance that no shelled edible lots of treated peanuts were rejected for containing above the regulatory limit of 15 ng/g at either location. This is in contrast to rejection of 48.4% of untreated shelled lots at the first location and 15.8% at the second.

PS6-219-0131

Reduction of aflatoxins in wounded peanuts: role of competition by native *Aspergillus* species and applied nontoxigenic biocontrol strains

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Soil is a source of primary inoculum of *Aspergillus flavus* and *A. parasiticus*, fungi that belong to section Flavi and produce highly carcinogenic aflatoxins in peanuts. Aflatoxigenic fungi invade developing peanut seeds during conditions of late-season drought stress and elevated soil temperatures, and the highest concentrations of aflatoxins are found in damaged seeds. Biological control of aflatoxin contamination in peanuts has been achieved by applying competitive, nontoxigenic strains of *A. flavus* and/or *A. parasiticus* strains to soil. In this study, a laboratory procedure was developed in which viable fungus-free peanut seeds were wounded and inoculated with field soil containing natural populations of fungi, then incubated under optimal conditions (0.92 seed water activity; 30 °C) for invasion by section Flavi species. The procedure provided a model system for studying fungal competition, specifically the effect of soil density of (1) native *Aspergillus* species and (2) applied biocontrol fungi on the colonization of peanut seeds by aflatoxigenic *Aspergillus* species and associated aflatoxin formation. In an examination of 20 soils containing natural fungal populations, wounded peanut seeds were preferentially colonized by section Flavi species despite low soil densities of the section (2-1700 CFU/g) relative to the total numbers of filamentous fungi (≤ 1%). Colonization of seeds by individual section Flavi species (*A. flavus, A. parasiticus, A. caelatus, A. tamarii* and *A. alliaceus*) decreased as soil densities of other, potentially competing species within the section increased; a significant interactive effect ($P < 0.0001$) among soil densities in the colonization of seeds was detected. Soil densities of section Flavi species and A. niger showed a similar interactive effect ($P < 0.0001$). In a second series of experiments, conidia of two identifiable nontoxigenic biocontrol strains, *A. parasiticus* NRRL 21369 (orange-brown conidial mutant) and *A. flavus* NRRL 21882 niaD (nitrate-nonutilizing mutant), were separately added at increasing amounts to soil in which wild-type *Aspergillus* populations had been fully characterized according to composition, density, and toxigenicity. *Aspergillus* species colonizing peanut seeds were similarly characterized, and seeds were individually analyzed for aflatoxins. Aflatoxigenicity was high (> 98%) among wild-type strains of *A. flavus* and *A. parasiticus* from soils and on colonized seeds. Increasing soil densities of biocontrol strains resulted in decreasing incidences of aflatoxigenic *A. flavus*/*A. parasiticus* on seeds ($R2 = 0.91$ and 0.67 for NRRL 21369 and 21882, respectively; $P \leq 0.01$) and in decreasing seed concentrations of aflatoxin B1 ($R2 = 0.80$ and 0.68; $P \leq 0.01$). Increasing incidences of biocontrol strains on seeds similarly lowered incidences of wild-type *A. flavus*/*A. parasiticus* ($R2 = 0.65$ and 0.79; $P < 0.05$) and aflatoxin B1 ($R2 = 0.69$ and 0.90; $P \leq 0.01$). Predictive mathematical relationships indicated that a 1:1 ratio of NRRL 21882 niaD to wild-type A. flavus/A. parasiticus would reduce aflatoxin B1 in seeds by 50% whereas only a 1.6:10 ratio is required with NRRL 21369 for the same effect. Reduction of aflatoxin contamination in peanuts by nontoxigenic strains is strain dependent, and the effectiveness of strains depends upon the composition and toxigenicity of the native Aspergillus populations and on the densities of those populations relative to densities of applied nontoxigenic strains.

PS6-220-0355

Characterisation of the Fungal and Bacterial Diversity in Pig Feed Fermented with Whey, Wet Wheat Distillers´grain or Water at Different Temperatures

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Pig feed can be produced by fermenting mixtures of grain with liquid waste products from food industry or water. Pigs experience such feed tastier and show a healthier gastrointestinal tract, with reduced risk for infection with *E. coli* or *Salmonella*. The use of by-products in pig production improves the circulation of nutrients in agricultural systems and reduces the environmental load. The fermentation is a spontaneous process and not much is known about the microorganisms involved. We investigated the microbial population diversity in different fermented pig feeds.

Three different feeds were fermented at different temperatures and the growth of yeasts, moulds, lactic acid bacteria (LAB), total aerobic bacteria and Enterobacteriaceae was monitored by quantification on selective media. The diversity of yeasts and LAB was investigated using PCR-fingerprinting. The different fingerprint types were identified by rDNA sequencing. Moulds were identified by morphologic characterisation.

Microbial diversity was influenced by the substrate, but not by temperature. The time needed to obtain a stable microbial population was however, prolonged with lowered temperature. Dominating yeasts belonged to the genera *Pichia* and *Kluyveromyces*, bacteria mainly to *Lactobacillus* and *Pediococcus*. The diversity, but not the number of moulds decreased during fermentation, with *Eurotium amstelodami* and *Penicillium roqueforti* being the dominating organisms at the end of fermentation.

Although regarded as positive for animal health and nutrition, a general concern about the fermentation process is its uncontrolled nature. In this study we have identified yeasts and LAB in the fermentation. This provides the basis to produce starter cultures for running the fermentation in a controlled way. Identifying a microorganism that can control the growth of *E. amstelodami* and *P. roqueforti* will be advantageous for future fermentations.

PS6-221-0372

Diversity of moulds species in Norwegian drinking water

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Fungi are receiving growing attention as agents of human allergies and infections. Studies of potable water in hospitals and private dwellings indicate the public drinking water system as transmission route for allergenic, toxigenic and opportunistic fungi. In Norway, it is largely unknown which fungi are resident and capable to survive and contaminate the drinking water. Therefore data on the frequency of individual species throughout the water system is needed.
In order to determine the occurrence and significance of filamentous fungi in public drinking water systems in Norway, water sampling from 14 water supplies, both with surface and ground water source was performed. Frequencies (cfu/100 ml) of the most common fungal species and the species diversity in samples from raw water, treated water, and from home and hospital installations were determined on the basis of incubation of membrane-filtered samples on DG18 media. The moulds were phenotypically identified to species level. In addition, a few non-identifiable isolates that were molecularly identified by ITS sequencing.
Moulds were recovered from 70% of the surface samples, while 42% of the ground water samples were positive. The risk to recover moulds from surface water is three times higher compared to ground water, and it is more likely to detect moulds in cold water than in showers and hot taps. A total of 94 different mould species, belonging to 30 genera were identified. The mycobiota was dominated by species of *Penicillium, Trichoderma* and *Aspergillus*. Some of the species identified occurred throughout the entire drinking water system.
Several of the species identified are known to be able to cause allergic reactions or disease in humans; others are common contaminators in food and beverage industry, and some may have sensoric or technical concern in water distribution systems. Our results indicate that the mycobiota should be considered in terms of assessing microbiological safety and quality in drinking water.

PS6-222-0406

Mycological examination and biofilm formation in drinking water

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The occurrence of fungi in drinking water has been recognised for decades and growth has been described as "oligotrophic". The number of studies of the fungi in water is slowly increasing from an unsatisfactorily low level. Some of the problems associated with fungal growth may be (a) unsightly appearance, (b) blocked pipes in distribution systems, (c) odours, (d) pigments, (e) source of potentially-pathogenic and allergy-causing fungi, and (f) mycotoxin production. The possibility of drinking water being a target of criminal acts cannot be ignored in this security conscious age. Biofilm formation by bacteria in water systems is undesirable as the problems of contamination are compounded by the multi-microbial systems. It is only now becoming apparent that bacteria interact with other organisms especially in relation to biofilm formation, where the formation of lactone secondary metabolites have consequences on the ability of bacteria to form biofilms. Variation in colony counts were observed with session in Portuguese tap water and there was an inverse relationship with bacterial/yeast counts. *Penicillium* strains were isolated frequently including *P. brevicompactum* and *P. expansum*. *P. expansum* is associated with the mycotoxin patulin which can affect bacterial biofilms. *P. brevicompactum* is associated with mycophenolic acid production - another lactone compound. However, no potentially pathogenic Aspergillus fumigatus strains were isolated at the mesophilic temperatures employed. Fungal involvement in biofilms has not been demonstrated unambiguously. The detection of fungi by conventional methods is complex, indirect and time consuming. To overcome these problems a combination of two fluorescent techniques for direct detection was tested in the present work: (a) Fluorescence In Situ Hybridization (FISH) employing the universal rRNA probe EUK516, labelled with the red Cy3, followed by (b) staining with Calcofluor white M2R fluorescent dye which stains fungal cell walls blue. FISH demonstrated eukaryotic microorganisms after 5 hours while the Calcofluor method revealed chitinous or cellulosic filamentous structures in under one hour. In conclusion, FISH and Calcofluor staining provide rapid and direct information on the involvement of fungi in biofilms which form in water. Fungi have been isolated which may be intimately involved in the ecology of biofilm formation.
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PS6-223-0607

The Influence of Fungal Metabolites on the Flavour of Coffee Beverages

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To investigate the influence of fungi on the flavour of coffee beverages, samples of raw coffee beans of varying quality were collected from different region of Brazil. Samples were surface disinfected with 0.4% chlorine solution for 1 min, then 50 beans from each sample were plated directly (10 particles per plate) onto Dichloran 18% Glycerol agar, and incubated at 25ºC for 7 days. After incubation, plates were examined and all fungal species were first isolated onto malt extract agar plates. After growth, other culture media were used for identification. To study drinking quality, raw coffee beans from each sample were roasted and ground. The samples were evaluated in two different degustation test: infusion and espresso. The sensorial analyses were carried out, evaluating the quality of the beverage in respect to: body, aroma, acidity, bitterness, astringency and sweetness. Besides that, presence of positive flavours and aromas such as: bread toast, caramel, chocolate, floral; and/or negative character such as: immature, fermented, stinker, woody, rancidity, mouldy, rioy and smoke were also evaluated. The most common fungi isolated from raw coffee beans, which still presented a good clean beverage were: *Cladosporium* spp, *Fusarium* spp., *Penicillium brevicompactum* and *P. palitans*. On the other hand the most common fungi isolated from raw coffee beans which presented fermented, stinker and rioy taste were *Aspergillus niger, A. ochraceus, Fusarium* spp, *Penicillium brevicompactum* and others. More studies are being conducted comparing the fungal infection to chemical metabolites by Electrospray mass spectrometry (ESI-MS) and the sensorial evaluation of coffee beverage. ESI-MS is a fast and sensitive method that can be used for on-line monitoring of the beverage without any need for sample preparation or component extraction.

PS6-224-0610

Microbiological and nutritional properties of *Rhizopus oligosporus* fermented barley tempeh

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The zygomycete *Rhizopus oligosporus* has been used for centuries in Indonesia to produce soybean tempeh. During fermentation with *R. oligosporus* the soybeans are bound by the white mycelium to a cake, and enzymes released by the fungusmakes the protein-rich product more digestible. We have developed a process where intact cereal grain kernels can be fermented with *R. oligosporus* to barley tempeh (patent pending). The resulting product has a firm texture, a neutral taste and can used in many food dishes. During the original soybean process a multitude of microorganisms develops together with R. oligosporus, whereas our grain fermentation process originally was developed as an axenic system.

We have recently developed barley tempeh co-cultivation systems by introducing different lactic acid bacteria and yeast species. Among the lactic acid bacteria *Lactobacillus plantarum* grew to high numbers without affecting fungal growth. To evaluate the co-cultivation of *R. oligosporus* with the food-grade yeasts *Saccharomyces cerevisiae*, *S. boulardii, Kluyveromyces lactis* and the biocontrol yeast *Pichia anomala* it was necessary to develop a novel method to quantify fungal biomass. Determination of fungal colony forming units are suitable for the unicellular yeasts, but not to follow the mycelium development of *R. oligosporus*. Ergosterol is commonly used to quantify fungal biomass, but can not differentiate between different fungi inin mixed fermentations. Instead we developed a quantitative real-time PCR method where results were highly correlated with the ergosterol contents of R. oligosporus and with CFU numbers of *Saccharomyces cerevisiae*. Yeasts inoculated at 104 CFU g-1 grew to about 107 CFU g-1 within the 20 h fermentation, without reducing *R. oligosporus* growth.

The *R. oligosporus* fermentation to barley tempeh increases provitamin D (ergosterol) 100-fold and reduces the mineral binding phytate with 30-95%

(depending on process). The fungal fermentation of the cereals also gives products with high amounts of dietary fibers and a low glycaemic index, i e with slow carbo-hydrates. Additional data on nutrional properties will also be included in this presentation.

PS6-225-0612

Mycobiota of Cocoa Beans and Toxigenic Potencial of *Aspergillus* species

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To investigate the mycobiota and toxigenic species in cocoa beans, samples at different stages of fermentation, drying and storage were collected in Bahia, Brazil. Samples were surface disinfected with 0.4% chlorine solution for 1 min, then 33 beans from each sample were plated directly onto Dichloran 18% Glycerol agar, and incubated at 25ºC for 7 days. After incubation, plates were examined and all fungal species were first isolated onto Czapek Yeast extract agar (CYA) plates. After growth, other culture media were used for identification. The ability to produce aflatoxin and ochratoxin was tested using the agar plug technique and thin layer chromatography (TLC). The most common fungi isolated were: *Aspergillus* section Flavi, *Aspergillus* section Nigri, *Eurotium chevalieri, Penicillium roqueforti, Rhizopus* sp, *Mucor* sp, yeasts and dematiaceous fungi. All isolates of *Aspergillus parasiticus* and 30% of *A. flavus* tested produced aflatoxins. From *Aspergillus* section Nigri, 18% of *A. niger* and 100% of *A. carbonarius* tested produced ochratoxin A. *Aspergillus ochraceus* was isolated from only one sample and it was ochratoxin producer. The results of this work showed that toxigenic fungi can be found in cocoa beans and may be of concern for cocoa product consumers.

PS6-226-0623

Potential Inhibition Effect Of Essential Oils On *Aspergillus* Sección Flavi Growth In Maize Grain.

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Storage fungi, like *Aspergillus* section Flavi, are commonly controlled by synthetic compounds. Nowadays interest in the use of natural antimicrobials is growing, especially for herbs, plants, and spices. Plant extracts may provide an alternative method to protect food and feed from fungal contamination. This search was made with the objective to evaluate the effect of different concentrations of *Pimpinella anisum* L. (anise), *Pëumus boldus* Mol (boldo), *Eugenia caryphyllata* clove, Thymus vulgaris (Thyme) and *Lippia turbinate* var. integrifolia (griseb) (pennyroyal) essential oils on growth rate parameters of *Aspergillus* section Flavi. These parameters were measured in vitro in irradiated maize grains at three water activities: 0.982, 0.955 and 0.900 and 28°c. Irradiated maize grains (10 KGy) were dispensed as a monolayer in sterile Petri dishes. Water activity of sterile maize was adjusted with sterile distilled water before inoculation at 0.982, 0.955 and 0.900 aw. Appropriate amount of each essential oils were added to reach a final concentration of (500, 1000, 2000 and 3000 ppm). The plates were inoculated centrally with a 5 mm diameter mycelial disk of four *Aspergillus* section Flavi taken from the margin of a 7 day-old culture of each isolate grown on malt extract agar. Cultures were incubated at 25°C for 11 days in a chamber with a 98% 95% and 90% controlled relative humidity with K2SO4, to avoid changes in the equilibrium relative humidity; the saline solution was daily replaced with fresh solution. Fungal growth was examined daily; the diameter of the fungal growing colonies was measured in two directions at right angles to each other every day until the colony reached the edge of the plate. The growth rate (cm.day-1) was calculated by linear regression of the linear phase for growth and the line intercept the x-axis was used to calculate the lag phase in relation to strain, essential oils and water activity. Mycelial growth rate of *Aspergillus* section Flavi strains were affected by addition of essential oils. At three water activities assayed, the level of inhibition was proportional to the concentration used. Boldus and thyme essential oils produced total Inhibition of *Aspergillus* section Flavi growth at all aw and concetration assayed. Clove, anise and peniroyal was insufficient to affect growth of aflatoxigenic strains at lowest essential oils concentration assayed, significant inhibition growth occurred at 2000 ppm and 3000ppm and were able to completelly inhibit growth rate when aw was 0.982. Generally, the effect produced by essential oils on fungal growth was more effective at water activities 0.955 and 0.900 since total inhibition in this condition was observed at smaller concentration. Lag phase increased following the same general pattern of growth rates, concentration lower than 1000 ppm of essential oils did not show a significantly increase compared to lag phase of control.

Based in the results obtained, the use of some essential oils as an auxiliary treatments could be potential to control *Aspergillus* mycelial growth.

PS6-227-0625

Effect of Osmotic Potential on Growth and Endogenous Polyols and Sugars Accumulation by Toxigenic Strains of *Aspergillus* section Flavi from Peanut

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Populations of *Aspergillus* section Flavi have been isolated from different agroecosystems. Fungal growth and survival are markedly affected by water availability of natural subtrates. These xerofilic/xerotolerant fungi to reduce the internal water potential, are able to synthesize and accumulate compatible solutes (polihidroxyalcohols) which enable their enzyme systems to continue functioning under extreme conditions of enviromental stress (Magan, 2001). The objective of this work was to evaluate the effect of osmotic stress and temperature on growth and sugar alcohol (glycerol, erythritol, arabithol and mannitol) and sugar (glucose and trehalose) accumulation in aflatoxigenic strains of *Aspergillus* section Flavi isolated from peanut. *Aspergillus* section Flavi strains were cultured on water stressed meal peanut media with 15% agar by the addition of glycerol and NaCl to 0.982, 0.955 and 0.937 water activity. The plates were incubated at 18°C and 30°C. Growth rates were determined by linear regression. For polyols and sugar analysis the processed samples were analysed by HPLC. Our results indicate that the growth rate was faster at 30°C under both NaCl and glycerol. A. flavus did not grow in modified medium with NaCl at 0.932 at 18°C. There were not differences in the impact of NaCl and glycerol on accumulation of polyols and sugars. There was an increase in total polyols at 0.937 aw with both solutes. Thereby, the tolerance of *Aspergillus* section Flavi to modifications of water potential could increase the ability for spoilage and mycotoxin production in substrates with water stress. This knowledge is very important for understanding fungi physiological process of peanut aflatoxigenic species and in the development of prevention strategies in peanut.

PS6-228-0627

Effect of Natural Maize Phytochemicals, Nutrients And Water Activity on Sclerotium Formation of *Aspergillus flavus* and *A. parastiticus*

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It was important to know the impact of nutrient requirements, water stress and maize phytochemicals on sclerotia formation in spoilage fungi, for the management of prevention strategies in storage. This study was carried out to evaluate the impact of maize phenolic acids, nutrients and water stress of different culture media, on sclerotium formation, size, number and volume, on *A. flavus* (RCM89, RCM142) and *A. parasiticus* (RCM38, RCM108) strains, isolated from stored maize. These strains grew in three culture media: Czapek Dox agar (CD, specific medium for sclerotium formation), and two maize-based media, maize meal extract agar (MMEA) and MMEA with sucrose and NaNO3 (MMEA S/N), both compounds in the same concentration like in CD. Water activity of three media were modified with glycerol to 0.971, 0.955 and 0.937. Ferulic acid at concentrations of 1, 10, 20 and 25 mM and cinnamic acid at concentrations of 1, 5, 10 and 20 mM were used in these studies. Experiments were carried out at 30ºC. Sclerotia were collected after 15 days. All strains developed sclerotia on the three normal unstressed media (0.999 aw). RCM89 strain produced small sclerotia (<400 µm). It was the unique S strain. None strain produced sclerotia under the driest conditions (0.955 and 0.937 aw) with and without phenolic acids. These strains produced higher number of sclerotia on CD agar than in MMEA at 0.999 aw and without phenolic acids. These isolates were more sensitive at higher natural phytochemicals concentrations tested in all media. It was observed a reduction of sclerotium production (size, number and volume) when increase cinnamic acid concentration. This effect was higher in MMEA than in other two media. Ferulic acid presented a similar effect than cinnamic acid on sclerotium formation. Maize natural phytochemicals used may be an alternative strategy to try to control *Aspergillus flavus* and *A. parasiticus* species. The data showed that ferulic acid and cinnamic acid at higher concentrations could be considered as effective fungitoxicants for these species.

PS6-229-0629

Impact of Competitive Mycoflora on *Aspergillus flavus* and *A. parasiticus* Populations in Stored Peanut Pods

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The aflatoxigenic fungi, *A. flavus* and A. *parasiticus* infect a wide variety of crops, all of which produce oil-rich seed. A histological study of the host-pathogen interaction between stored peanut (*Arachis hypogaea L.)* and *A. parasiticus* demonstrated that the fungus colonized all tissues of the peanut pod and appeared to grain ingress through the corky layer of the pericarp. GUS activity was found in hyphae infecting the pericarp, embryo and cotyledons, indicating expression of aflatoxin biosynthetic genes in these tissues (Haixin et al, 2000). This fact appears to turn to peanut pods more susceptible to be infected by other fungal pathogens. Thus, the objective of the present work was to evaluate the behavior of *Aspergillus* section Flavi population relative to other natural competing mycoflora in peanut pods over a 5-month storage period. Two experimental polyethylene big bags were used to store 200 kg of peanut pods from July to December 2004. One of these was previously homogeneously inoculated with a spore suspension (104 spores ml-1) of a mixture of A. flavus CHG46 and A. parasiticus CHG24. Ten samples (250 g) were randomly collected at 30 days intervals using a sampling device. Temperature and humidity were monitored using a distance reading thermometer and hygrometer. To determine fungal colonization on peanut pods, 50 g of milled pods were shaken with 450 ml of peptone / water and spread on dichloran / rose Bengal / chloramphenicol (DRBC) and dichloran / glycerol 18% (DG18) media. The results were expressed as colony-forming units (CFUs) per gram of peanut pods. The fungal genera were identified according to Pitt and Hocking (1997) and Samson et al (2002). The results on mycobiota composition present in nature peanut pods corroborate that the predominant genera of potentially toxigenic fungi, during the five storage months, were *Penicillium* spp., *Aspergillus* section Flavi, *Aspergillus* section Nigri and *Fusarium* spp. In minor grade the genera isolated were *Eurotium* spp., *Trichoderma* spp., *Mucor* spp., *Absidia* spp., *Monascus* spp. and *Alternaria* spp. Whereas, during the 1th storage month, mycological analyses of inoculated peanut pods showed predominance of *Penicillium* spp. (4.5x104 CFU g-1) following by *Aspergillus* section Flavi (1.8x104 CFU g-1), Fusarium spp. (2.2x103 CFU g-1) and *Aspergillus* section Nigri (4x102 CFU g-1), a high prevalence of *Aspergillus* section Flavi (7.7x104 CFU g-1) following by Penicllium spp and Aspergillus section Nigri with count of 6.8x103 and 3x103 CFU g-1, respectively and absence of *Fusarium* spp was observed at the end of the storage period. Between the 1th and 5th month of storage, fungal count in both containers significantly increased (1 log unit) according enhanced the temperature (7.1 to 19.8ºC), while the humidity to be maintained relatively constant about of 8.2%. Significantly differences of 0.5 and 1 log units was observed when compared total fungal count between natural and inoculated peanut pods (Figure 1). This study showed the prevalence of *Aspergillus* section Flavi from 2th to 6th sampling in inoculated peanut pods demonstrating the high competition ability of this populations between *Aspergillus* section Nigri, *Penicillium* spp., *Fusarium* spp. and other fungal species for niche occupation and other interspecific interaction that could exert selection pressure on the mycota influencing the dominance of species.

PS6-230-0630

Fungal Population Succession in Stored Peanut Seeds

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Carcinogenic aflatoxin produced by *A. flavus* and *A. parasiticus* are common contaminants of peanuts. Soil serves as a reservoir for primary inoculum of *A. flavus* and *A. parasiticus* (Horn and Dorner, 1998) and peanut pods have direct contact with soil populations of aflatoxigenic fungi. Postharvest contamination may also occur when stored products are not maintained at a safe moisture and temperature level. Other fungi, dominated by the genera *Penicillium* and *Fusarium* colonized stored peanut seeds primarily at water activities (aW) and temperatures suboptimal for section Flavi species and A. niger. Thus the objective of the present study was to compare the native fungal population succession in silo control and silo treated with *A. flavus* / *A. parasiticus* mixture during 5 months of storage in peanut seeds. The environmental factors including aW, temperature and pH were monitored. *A. flavus* / *A. parasiticus* mixture (104 spores ml-1) was applied on 200 kg of stored peanut. A control peanut without inoculate was made. Representative samples (10 samples per month) were taken with a device once a month during five months. Temperatures changes were recorded once a week. Water activities (aW) and pH values of the grains were determined. Quantitative enumeration of fungal propagules was done on the solid media (dichloran-Rose Bengal-chloramphenicol and dichloran-18% glycerol) using the surface spread method by blending 50 g portion of each sample with 450 ml of peptone water solution. The results were expressed as CFU per gram of peanut. Taxonomic identification of the different genera was made according to Pitt and Hocking (1997) and Samson et al. (2002). When compared total fungal count between nature and inoculated seeds (Table 1), significantly difference was not observed during all storage period. However, fungal count increased 1 log unit from 1th to 6th sampling in both peanut containers. An increase of temperature from 7 to 20°C was registered along the experience, while aW and pH of the grains were relatively constant about of 0.73 and 6.7, respectively. Predominant genera in nature peanut seeds at 1th sampling were *Penicillium* spp. (81.7%) and *Aspergillus* section Flavi (18.3%) among other genus isolated in minor percentage. At the end of the storage period a high prevalence of *Penicillium* spp. (70.8%) following by *Aspergillus* section Flavi (35%), *Aspergillus* section Nigri (20.5%) and *Fusarium* spp. (4.2%) was observed. Mycological analyses in inoculated seeds showed similar results during the six sampling, with 47.6% of *Penicillium* spp., 38% of *Aspergillus* section Flavi and 4.8% of *Aspergillus* section Nigri. *Aspergillus* section Flavi population was isolated during all storage period in both natural and inoculated peanut seeds in the order of 1.5x103 CFU g-1, showing a high adaptability to environmental conditions. The inoculation not showed impact on mycological population succession. The results showed that peanut pods protect the entry of additional inoculum to the seeds and environmental conditions allowed to maintain the stability of inoculum level in the seeds.

Table 1. Comparison of means temporal changes in total fungal count (log CFU) between natural peanut grains and inoculated peanut grains over a 5-months storage period.

Treatments	**Log CFU / g**					
	1th sampling	2th sampling	3th sampling	4th sampling	5th sampling	6th sampling
Natural peanut	3.6x103 a	6.6x103 a	2.7x103 ab	1.9x104 a	9.4x103 ab	1.7x104 a
Inoculated peanut	1.9x103 ab	8.0x103 ab	2.7x103 b	1.1x103 b	2.1x103 ab	2.1x104 a

Data with the same letter for each count are not significantly different according to Tukey test (P<0.05).

PS6-231-0656

Inhibitory Effects Of Geranium Pelargonium Oil On Aspergillus Flavus Growth Rates

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Mycotoxin Are Metabolites Formed By Molds Growing In Food Stuffs , Fodder And Organic Waste Materials . All Molds Produce Specific Mycotoxins And Species Can Be Characterized By Their Mycotoxin Spectra As Well As Natural Habitation.
To Identify Preventive Maintenance Procedures Wich Limit Fungal Colonization Growth And Amplification / Toxicogenesis Of Natural Oils This Study Was Conducted . Hens Escential Oil Of Geranium Pelargonium Was Tested For Inhibitory Activity Against Aspergillus Flavus Isolated From Corn Bulks .
The Disc Diffusion Method Was Used To Evaluate The Zone Of Fungal Growth Inhibition At Various Concentrations Of The Oil So That The Minimal Inhibitory Corcentration (MIC) And Minimal Fungicidal Concentration (MFC) To Be Determined . It Was Found That Geranium (Pelargonium) Oil Has Static Effect At 1:4 Dilution Ratio As Well As Fungicidal Property At 1:2 Ratio So On .
The Extent Of Inhibition Of Fungal Growth Was Dependent On Used Concentration Of The Essential Oil , So , Substitution Of Currently Used Antifungal Chemicals By Natural Compounds Is Recommended For Animal And Human Food Or Crope Conservations .

PS6-232-0683

The efficacy of a cathodic treatment in controlling inherent mycoflora levels in soybean seeds.

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Many seed treatments have not been effective in eliminating seed-associated fungal pathogens, whilst simultaneously maintaining seed quality. While some of these treatments reduce the degree of fungal infection to levels low enough to prevent economically important losses, others cause seed injury or death although eliminating inherent fungi. This study sought to assess the efficacy of a cathodic treatment in eliminating inherent mycoflora from soybean seeds while maintaining high levels of seed vigour and viability (as measured by the germination index).
Soybean seeds from a stock batch were placed in specially constructed, sealed chambers that were capable of generating an evenly distributed electrical charge of –300 V. Control seeds were placed in identical chambers, but were not subjected to the cathodic charge. These stock seeds were considered to represent the natural state of seeds either in the field or in storage. A second batch of seeds – previously subjected to microwave-irradiation, which eliminated seed-associated fungi – was placed in identical chambers as described above. A third batch of microwave-irradiated seeds was experimentally inoculated with spores of *Aspergillus flavus*, a known soybean seed pathogen, and placed in identical chambers as described above. Seeds in all chambers were sampled at regular intervals for 12 weeks, and assessed for tissue water content, germination index, seed-associated mycoflora levels, and seed tissue ultrastructure.
Analyses indicated that the cathodic treatment had a significant effect on both seed embryonic axis and cotyledon water contents. The treatment also improved seed germination indices in addition to reducing seed mycofloral levels. Following the treatment, seed tissue ultrastructure was well preserved with few, if any, cellular abnormalities. Preservation of seed cellular structures was enhanced by a pre-treatment of microwave irradiation which also enhanced the storage capability of normally short-lived soybean seeds.
The application of a cathodic charge to soybean seeds drastically reduced the incidence of seed-associated fungi, many of which are known to be pathogenic. The treatment also enhanced seed storability, vigour and germination index. By achieving this, the treatment surpasses many others in eliminating seed-associated fungi while preserving seed quality.

PS6-233-0730

Occurrence of aflatoxin M1 in raw milk at cattle farms by ELISA in Babol, Iran.

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In the study, raw cow milk was collected from milk-tanks as samples from 30 Babol's cattle farms in winter 2006(a city of Iran). On the whole 90 raw milk-samples were tested for aflatoxin M1 (AFM1) contamination by competitive Enzyme Linked Immunosorbent Assay (Tecna, AFLA M1 - code MA418). In 90 of the 54 samples(60%) the presence of AFM1 was detected in concentration ranging between 50 to 297.1 ng/l and 36 samples(40%) contained AFM1 at levels of 4.3-50ng/l. therefore 60% of samples was above of European community regulations (50 ng/l). The AFM1 contamination levels (>50 ng/l) in January, February and March was 40%, 60% and 80% respectively. The highest contamination level was observed in March. The lowest contamination level was observed in January. Statistical evaluations show that there is not a significant relationship between AFM1 contamination and different months of winter. To decrease AFM1 in milk to the lowest point food stuff ration should be checked regularly, and food stuff should be kept away from fungi contamination.
Keywords: Aflatoxin M1, ELISA, raw milk, cow.

PS6-234-0731

Survey of the occurrence of Aflatoxin M1 in pasteurized milk consumption primary schools in Babol, Iran.

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In the study, during the winter of 2006, Saleh pasteurized milk package were collected from primary schools in Babol (a city of Iran). Totally, 72 samples were tested for AFM1 by competitive Enzyme Linked Immunosorbent Assay (Tecna, AFLA M1- code MA418). AFM1 were detected in 100% of all samples. 100 % of samples were above of European community regulations (50 ng/l). AFM1 contamination levels were range 193.6-253.5 ng/l (mean level: 230.82).Therefore more than 4.6 fold levels European community. There is not a significant relationship between AFM1 contamination level and different months of winter applying statistical test. The results showed that need to introduce safety limits for AFM1 levels in child milk under Food Legislative liable of Iran. Aflatoxin M1 contamination is a serious problem for public health, and it is potentially hazardous for human health.
Keywords: Aflatoxin M1, ELISA, pasteurized milk.

PS6-235-0805

Elimination of inherent seed-associated mycoflora of soybeans: efficacy of microwave irradiation versus cathodic protection.

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Soybean seeds are generally susceptible to invasion by a range of fungi during development and in storage. Most of these fungi cause significant losses within storage facilities and upon subsequent planting, resulting in low agricultural yields and contaminated products. Whilst various treatments limit these losses, microwave-irradiation and cathodic protection treatments have proven more beneficial. Both techniques are capable of significantly reducing the fungal loads of contaminated seeds, while maintaining a high degree of seed viability.
This study sought to compare the efficacy of both treatments, by assessing the effects on seed water content, seed vigour and viability, seed fungal loads and seed tissue ultrastructure.
Soybean seeds were treated with microwave radiation for 30 s and stored for 12 weeks at 22 ± 2 0C. Seeds from the same batch were treated with a cathodic charge of –300 V while being stored as previously described. Seeds were monitored for changes in water content, germination index, fungal loads, and tissue ultrastructure.
Microwave-irradiated seeds showed reduced water contents and germination indices over the storage period, whereas seeds subjected to the cathodic charge showed a general increase. Both treatments reduced the levels of inherent fungal contamination, indicating the capability of both treatments in completely eliminating most, if not all, seed-associated fungi.
Overall preservation of tissue ultrastructure was observed in seed tissues subjected to both treatments. Untreated material was characterised by cells with convoluted walls, abnormal nuclear morphology, lack of homogeneity of cellular, nuclear and nucleolar matrices, and a general lack of organelle definition. Microwave-irradiated material showed slight lobing of the nuclear profile – with this organelle appearing otherwise structurally unaffected. Middle lamellae of cell walls appeared distorted and wavy. Organelles were evenly distributed throughout the matrix, and a few mitochondria appeared to have patchy matrices with a few discernible cristae. In some cells, the plasmalemma appeared to have separated from the wall. Material subjected to the cathodic charge appeared to be free of any ultrastructurally visible abnormalities. Walls maintained their shape and integrity. There was evidence of considerable mitochondrial development, plastid starch accumulation, formation of small vacuoles, and endomembrane activity.
Damage to organelles observed in untreated material appeared consistent with free-radical induced damage. While microwave-irradiaton reduces fungal-associated damage and promotes short-term activation of repair mechanisms, cathodic treatments supply electrons that inactivate free radicals, reducing fungal contaminants and the peroxidation of macromolecules, ultimately enhancing seed storability to a greater degree than microwave radiation.

PS6-236-0807

Physiological relationship between food preservatives, environmental factors, ochratoxin and otapks gene expression by *Penicillium verrucosum*

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There has been significant interest in trying to understand the relationship between environmental factors, concentration of preservatives and expression of genes involved in mycotoxin production. Very little information is available on linking physiological stress factors and expression of genes responsible for mycotoxin production. There is pressure from consumers to reduce the use and concentrations of aliphatic acid-based preservatives. However, sub-optimal concentrations of fungistats in food can lead to a stimulation of growth and perhaps toxin contamination of foods. This study has investigated for the first time the relationship between sub-optimal concentrations of calcium propionate and potassium sorbate under different water availability conditions on the growth, ochratoxin and expression of otapks genes in Penicillium verrucosum.
A Yeast sucrose based medium was modified with either glycerol or NaCl to 0.98, 0.95 and 0.93 water activity. These treatments were further modified with either 150 or 300 ppm of Calcium propionate or Potassium sorbate. Cellophane overlays were used to enable removal of mycelial biomass for otapks expression analyses. The treatments were centrally inoculated with spores and measurements of growth carried out for up to 15 days. After 8-9 days mycelial biomass was frozen at -80oC for RNA extraction at a later date. Initial studies showed that 8-9 days was optimum for toxin gene experession. The biomass was also extracted for ochratoxin and analysed using HPLC.
There was a slight inhibition of growth as water activity and preservative concentration was increased, regardless of solute. There was a slight stimulation of OTA production at 150 ppm preservatives at 0.95 and 0.93 aw. The otapks copy number followed the same pattern showing that at the 150 ppm treatments there was a significant stimulation of activity which paralleled the OTA data.
This study has demonstrated that physiological stress factors influence toxin gene expression and is paralleled by the phenotypic changes observed in growth and mycotoxin production.

PS6-237-0852

Potential of food safety prediction by detection of volatile biomarkers by electronic nose technologies

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Volatile metabolites play an important role in food safety because they allow us to determine food spoilage and quality. Sensory panel evaluation and GC-MS analysis have been applied for detection of food spoilage and quality. Both methods suffer from being expensive and time consuming. Headspace analysis by electronic nose measurement has a potential in indirect determination of spoilage of food, more specifically production of mycotoxins by food spoilage fungi. This talk will illustrate how the electronic nose (e-nose) works and the potential of such e-nose technologies compared to traditional methods such as GC-MS analysis and LC-MS analysis.
A total of 20 isolates of the cheese associated species Geotrichum candidum, *Penicillium camemberti, P. nordicum* and *Penicillium roqueforti* and its closely related species *Penicillium paneum, Penicillium carneum* as well as the non cheese associated *Penicillium expansum* were investigated. The isolates were inoculated, in four replicates, on yeast extract sucrose agar in 20 ml headspace flasks and electronic nose analysis was performed daily for a seven day period. The results obtained from the e-nose analysis were correlated with analysis of mycotoxin content which was done by LC-MS analysis.
After Principal Component Analysis (PCA) was performed on the e-nose readings it was evident that the trend went from no separation of the species, neither from each another nor from blank samples on day one, to almost complete separation of all species from one another and from blank samples at day six. SIMCA analysis on fifth day measurements gave rise to an average of 68% correct classification.
Analysis for mycotoxin production performed by LC-MS showed that 14 mycotoxins were detected from the seven species. No mycotoxins were detected in samples from *G. candidum* and *P. camemberti*.
Differentiation of fungi to species level has been achieved for four of seven species by use of e-nose technology, thus permitting prediction of mycotoxin production by differentiating between mycotoxin producing species and non-toxenogenic species. Our findings support the hypothesis that volatile organic compounds can be used as biomarkers in differentiation of fungi to species level.

PS6-238-0892

Identification of black aspergilli species responsible for ochratoxin A contamination of food using qualitative volatile fingerprints

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Black aspergilli, including *Aspergillus carbonarius* and some *Aspergillus niger* aggregate species, are the main source of OTA in food commodities such as wine, grapes, and dried vine fruits. Traditional mycological methods are time consuming and require taxonomical and chromatography expertise. Molecular diagnostic methods for early detection, including PCR techniques, have been developed. Toxigenic and non-toxigenic strains of related species may use different biosynthetic pathways resulting in different volatile production patterns. The aim of this study was to evaluate an electronic nose-based system using metal oxide sensor arrays for the detection/differentiation of black aspergilli species responsible of OTA food contamination, based on the volatile fingerprints.
We have grown related a range of ochratoxigenic species on four different media using a spore lawn technique and analysed the volatile fingerprints after 48-120 hrs. Subsequent studies were carried out on maize grain to examine the potential for early differentiation of contamination with different ochratoxigenic species.
nitial in vitro studies showed that it was possible to use discriminant function analyses to differentiate between groups of specific species. The type of medium influenced the level of discrimination achieved based on the volatile fingerprints. The discrimination achieved between related mycotoxigenic *Aspergillus* section nigri species/strains was similar to that obtained using molecular techniques. Studies of ochratoxigenic species differentiation when inoculated on maize grain suggests potential for early differentiation between contamination with different ochratoxigenic species.
This study suggests that there is potential in trying to link qualitative volatile fingerprints with contamination by strains/species of mycotoxigenic fungi. Appropriate libraries of information need to be obtained before unknowns can be validated.

PS6-239-0953

Real-time PCR assay to quantify *Aspergillus westerdijkiae* in coffee beans

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Aspergillus westerdijkiae is a new species of fungi that was recently dismembered from *Aspergillus ochraceus* taxa (Frisvad et al, Studies in Mycology, 50: 2004). Most of the isolates of *A. westerdijkiae* are able to produce large amounts of a mycotoxin named ochratoxin A (OTA). OTA has been found in food and beverages, such as coffee. Evidently, actions to prevent OTA production in coffee beans would decrease the human exposure to this toxin. *A. westerdijkiae* is very similar to *A. ochraceus,* and several isolates previously identified as *A. ochraceus* is now identified as *A. westerdijkiae*. By using b-tubulin sequences, we analyzed several isolates obtained from Brazilian coffee bean samples, previously identified as *A. ochraceus*, to compare with those of *A. westerdijkiae*. In fact, most (84%) were recognized as *A. westerdijkiae*. Since this species consistently produces large amounts of OTA, we developed a specific primer-pair for detecting and quantifying it in coffee beans. The primers BT2-AW F (5'TGATACCTTGGCGCTTGTGACG) and BT2-AW R (5'CGGAAGCCTAAAAAATGAAGAG) provided an amplicon of 347 bp in all *A. westerdijkiae* isolates, and no cross-reaction was observed using DNA from A. ochraceus. The specificity of this primer-pair was also evaluated by Real-Time PCR. A Platinum SYBR Green qPCR SuperMix UDG kit (Invitrogen) was used in a 25-µl reaction. The PCR program consisted of a denaturation step (95°C for 5 min), followed by 40 cycles of 30s at 95°C, 30s at 62°C, and 30s at 72°C. A Real-Time PCR standard-curve was obtained by decreasing the initial amount of total DNA of *A. westerdijkiae* (r2=0.982; slope= –0.2951). Coffee beans were inoculated with *A. westerdijkiae* and DNA extraction and cfu assay were performed at each 48 hours, until 192 h. High correlation between the cfu data, and the fungal DNA content in coffee beans was detected. The method-sensitivity was assessed by using serial dilutions of the DNA extracted from coffee beans inoculated with *A. westerdijkiae*. A high correlation coefficient (r2 = 0.997) among all dilutions was observed, and Real Time PCR did demonstrate that it is possible to detect as little as 10 cfu into coffee beans.

PS6-240-0964

Invasion Pathway of Peanut Flower by Green Fluorescence Protein Aspergillus flavus

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Colonization of peanut seed by *Aspergillus flavus* and subsequent aflatoxin contamination is a serious worldwide problem. The development of *A. flavus* strain that produce green fluorescence protein (GFP) offers the opportunity to track pathways of infection which have not been clearly identified. Three peanut genotypes (511CC, 419CC and Tainan 9) were grown in a hydroponic system to determine flower and aerial peg infection by *A. flavus*. Peanut flowers were marked with colored thread and inoculated with 0.5 ml of GFP A. flavus spore suspension. By 24 and 48 hr after inoculation, inoculated flowers were separated into stigma, style, hypanthium and ovary for observation of fungal invasion and colonization. At 10 days after inoculation, aerial pegs were valuated for the incidence of fungal colonization. Observation with an UV-illuminated microscope showed conidia of GFP *A. flavus* germinated within 24 hr and extensively colonized stigma and style, especially near pollen grains. By 48 hr after inoculation, conidiophores and conidia had formed over the peanut flowers and the fungal hyphae grew down the style, eventually reaching the top of the ovary. However, visible fungal colonization in embryos was sparse. The highest incidence of fungal colonization was found in Tainan 9. This experiment compelling provides evidence seed infection by *A. flavus* may occur systemically directly through floral infection. Initial infections may take place from, i) infected pollen, ii) penetration through the stigma follow the part of pollen, and iii) penetration directly through the style and ovary wall.

1330-1530

SYMPOSIUM 16 - *Fungal Phylogenomics*

S16IS1 - 0986

Comparative genomics of yeasts illustrates eukaryotic genome evolution.

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With the rapidly expanding number of genomes sequenced, the features of molecular evolution can now be contemplated with levels of comprehensiveness and precision unknown before. Comparisons between distinct organisms from a same phylum help us disentangle the distorted and superimposed traces left in genomes by the successive, numerous evolutionary events. But the pictures obtained are clearer when some of the compared organisms are also amenable to experimentation. This is the case for the Hemiascomycetous yeasts. With their compact genomes, these yeasts cover a very broad evolutionary range, made of billions of successive generations, which remained unsuspected from their similar life styles and morphologies. Several yeast species are commonly used for genetic experiments while others are of biotechnological importance or of medical concern as infectious agents. Altogether, almost three dozens of Hemiascomycetous yeasts have now been sequenced, either totally or partially, the largest number from a single evolutionary phylum of Eukaryotes.
Comparisons of chromosome maps and genome redundancies reveal that yeasts have evolved through a remarkable interplay between distinct molecular mechanisms including tandem gene repeat formation, massive genome duplication, segmental duplications and extensive gene loss. Experiments with *Saccharomyces cerevisiae* reveal how the chromosomal dynamics characteristic of evolution can lead to the formation of novel genes, whereas observations from the pathogenic yeast *Candida glabrata* simultaneously reveal the loss of function and the formation of specific genes. Transposon-mediated single gene duplication, mitochondrial DNA transfer and horizontal gene transfer have also played a specific, though quantitively limited, role in evolution. With the help of yeasts, the consequences of these various mechanisms, which have their equivalents in other organisms, are now becoming more clearly understood.
With the help of developing technologies, comparative yeast genomics will rapidly enter the field of population genomics. Several dozens of *Saccharomyces* isolates are being sequenced and compared. At the same time, a greater emphasis on the genes encoding RNA molecules is needed. Preliminary data suggests how they differ from the protein-coding genes and allow interesting speculations that are amenable to experimental testing.

S16IS2 - 0589

Fungal Phylogenomics: from kingdom to species

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Our understanding of the fungal Tree of Life (TOL) is fragmentary. On the one hand, until recently molecular phylogeneticists built trees based on ribosomal RNAs and selected protein sequences that, however, usually suffered from lack of statistical support. To overcome this problem, we took the advantage of the available complete genome data and the assignment of their proteins to KOGs (euKaryote Orthologous Groups) to explore part of the fungal TOL. The concatenation of large data set of genes increased the phylogenetic signal and statistical support. On the other hand, the tree-like nature of the evolution of the organisms has been questioned and a 'web of life' has instead been proposed as a more adequate model. Phylogenetic analyses based on concatenation of the total core of 161 shared KOGs among 21 fungal, 3 animal and one plant genomes, and those based on the core of proteins with a similar evolutionary signal showed trees with excellent nodal support for each branch, although the tree topologies obtained from the individual protein groups were different. We interpreted our results as evidence for the presence of reticulate evolution also in eukaryotes. As an example, the phylogenetic position of the fission yeast *Schizosaccharomyces pombe* in the fungal TOL is still controversial. Three alternative phylogenetic positions have been proposed in the literature. We compared 91 proteins containing a single orthologue that are shared by 19 eukaryote genomes. The major part of these 91 orthologues supported a phylogenetic position of *S. pombe* as a basal lineage among the Ascomycetes, thus supporting one of the propositions. Interestingly, part of the orthologous proteins supported a fourth, not yet described alternative, in which *S. pombe* is basal to both Basidiomycetes and Ascomycetes. All phylogenetic trees were well supported. We believe that both reflect correctly the phylogenetic history of the species concerned. This apparent paradox may point to a heterogeneous nuclear genome of the fungi. Importantly, this needs to be taken in consideration for a correct understanding of the Tree of Life.
Finally, data on comparative genomic hybridization to elucidate the species complex, namely *Cryptococcus neoformans*, will be presented.

S16IS3 - 0985

Genome structure, gene redundancy and gene expression of *A. oryzae*

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Aspergillus oryzae has been extensively used in the Japanese traditional fermentation industries for alcoholic beverages (Sake and Shochu), soybean paste (Miso) and soy sauce. *A. oryzae* is also used in enzyme production in modern biotechnologySee 1 as an example). The whole genome sequencing of *A. oryzae* has been completed by the end of 20052). Approximately 12,000 genes encoding proteins with more than 100 amino acid residues were predicted in its 37.6 Mb genome. Of the three aspergilli, *A. oryzae*, *A. fumigatus* and *A. nidulans*, *A. oryzae* possesses the largest number of genes: comparison of genes in each of the COG functional categories revealed that *A. oryzae* contains a larger number of genes for metabolism than the other aspergilli and *N. crassa*. Synteny analysis showed that *A. oryzae* had non-syntenic blocks (*A. oryzae*-specific blocks) distribute throughout the genome in a mosaic manner. Mapping of ESTs to each chromosome revealed that the expression of genes in the *A. oryzae* specific-regions was lower than that in the regions common to the three aspergilli. Based on the genome sequence, the DNA microarray consisting of 11,000 oligonucleotides were prepared. The DNA microarray analysis confirmed the lower expression of the genes on the *A. oryzae*-specific blocks. As a part of evaluation of the DNA microarray, heat-shock response of *A. oryzae* was analyzed and compared with the results obtained from *A. fumigatus* and *S. cerevisiae*. Although the expression of most of the genes annotated as heat-shock protein genes from all the three species responded, significant number of species-specific genes responded to heat-shock existed. Temperature is one of the most important factors for fermentation in terms of high efficiency of the process, high quality of the products and so on. Comparative analysis of heat-shock response between *A. oryzae* and *A. fumigatus* may be useful to understand both pathogenicity and fermentation by these organisms.

1. Christensen,T. et al. Bio/Technology 6, 1419-1422 (1988).
2. Machida,M. *et al. Nature* 438, 1157-1161 (2005).

S16PS1 - 0215

A Phylogenomic Analysis Of The Ascomycota

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At the current rate of sequencing, the number of available fungal genomes will double in the next year or two. Using phylogenomics for phylogenetic reconstruction and predicting function of uncharacterized genes, thus require a fast and robust pipeline for analyses. We describe how existing bioinformatic programs were connected using perl scripts to: 1) identify ortholgous sequences from predicted protein sets, 2) produce a reliable phylogenetic analysis which resolved higher level taxonomic groups and 3) identify orthologous regions in an unannotated genome for use in a phylogenetic analysis. An all-vs-all BLASTP search was parsed and analyzed by the program Tribe-MCL which uses a Markov Clustering algorithm. After performing this analysis at different stringency levels and filtering for best hits within clusters, we identified orthologous protein clusters that were distributed in 17 proteomes. Each of the orthologs was aligned with ClustalW using the amino acid sequences reported in the BLASTP search and concatenated into a single alignment file that contained 201,419 amino acid characters after removing all gap-containing columns. The resulting 814 orthologous protein sets of 15 ascomycete fungi were analyzed in a series of phylogenetic analyses (ML, NJ distance and parsimony) with two Basidiomycota outgroups. All analyses resolved the two derived subphyla Pezizomycotina and Saccharomycotina, and *Schizosaccharomyces pombe* as a basal lineage of the Ascomycota. Importantly, these analyses resolved the Leotiomycetes as the sister group to the Sordariomycetes, a region of the Ascomycota phylogeny that has remained problematic in studies of more limited character sampling. Orthologous protein sequences were also identified in a high through put way from an unannotated Ascomycota genome eg. *Coccidiodes immitis.* A phylogenetic analysis of 600 clusters, including *C. immitis,* resulted in an identical topology to the previous 814 ortholog analysis and correctly placed *C. immitis* in the Eurotiomycetes, thus demonstrating the ability of this approach to incorporate unannotated genomic data into common phylogenetic analyses.

1330-1530

SYMPOSIUM 17 - *Signal Transduction during Pathogenesis*

S17IS1

A SAGE approach to investigate cAMP signalling in basidiomycete pathogens

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No abstract available

S17IS2

Signaling pathways and infection-related morphogenesis in Magnaporthe grisea

Jin-Rong Xu
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No abstract available

S17IS3 - 0148

Dissecting the role of signal transduction in *Stagonospora nodorum* during infection on wheat

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Stagonospora nodorum is a fungal pathogen of wheat that has the potential to severely affect the economic viability of Australia's wheat crop. Although it has been recognised as a serious wheat disease for many years, the molecular details of how the fungus and its host interact are poorly understood. A reverse genetics approach has been adopted to find genes involved in pathogenicity focusing predominantly on signalling and primary metabolism pathways. To uncover the role of signal transduction in pathogenicity, genes from the cAMP (*Gna1*), MAP kinase (*Mak2*) and calcium (*CpkA*, *CpkB* and *CpkC*) signalling pathways were characterised by gene disruption. Fungal strains developed with mutations in either *Gna1* or *Mak2* were severely reduced in pathogenicity and both were unable to sporulate. No effect on pathogenicity was apparent for the strains mutated for *CpkA*, *CpkB* or *CpkC* although the *CpkA* strain was unable to sporulate *in planta*. The impairment of each of the signalling pathways leads to several significant phenotypes *in vitro* including melanin synthesis deficiencies, sensitivity to osmotic stress, reduced protease production/secretion and sporulation. These results suggest that these key signalling genes control processes downstream which are required for pathogenicity. To determine what these processes are, a functional approach including proteomics and metabolomics has been adopted. A detailed analysis of these key signalling mutants and a synopsis of the functional approach to identify target genes will be presented.

S17PS1 - 0812

Conidial germination in the dimorphic pathogen *Penicillium marneffei*

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Penicillium marneffei is an opportunistic human pathogen with a thermally regulated dimorphic switch. At 25∞C, *P. marneffei* asexual spores (conidia) germinate to give rise to multinucleate, septate, branched hyphae. In contrast, at 37°C conidia germinate to produce arthroconidiating hyphae, in which nuclear and cellular division are coupled and cellular fragmentation occurs via double septation events and separation along the septal plane to generate uninucleate yeast cells which can consequently divide by fission. A second developmental pathway, conidiation, also occurs at 25°C and results in the formation of a complex multi-cellular structure termed a conidiophore and culminates in the production of uninucleate conidia capable of reinitiating the life cycle. The germination of conidia therefore gives rise to two different developmental pathways and cell types at 25°C and 37°C. To examine the regulation of conidial germination in *P. marneffei* we have investigated the role of the Ras GTPase RasA, the Rho GTPase CflA, the G-alpha subunit GasC and the newly isolated PAK kinase PakA during conidial germination at both 25°C and 37°C. Expression of dominant activated and dominant negative alleles of the Rho GTPase CflA results in accelerated or delayed germination at both 25°C and 37°C, respectively. In contrast, expression of dominant negative and dominant activated alleles of *rasA* results in a reduction in conidial germination at 25°C but not at 37°C. Interestingly, deletion of *pakA* results in a severe reduction in conidial germination at 37°C but not at 25°C. Therefore, although germinating conidia at 25°C and 37°C appear morphologically similar, this study shows that germination is differentially regulated at 25°C and 37°C. The generation of double mutants was used to analyse the epistatic relationships between these genes. The dominant activated *cflA* allele expressed in a D*pakA* strain exhibited reduced germination at 25°C and a severe reduction in germination at 37°C suggesting that at 25°C the accelerated germination observed in dominant activated *cflA* strains requires PakA. This result also suggests that at 37°C PakA acts downstream of CflA during germination.

S17PS2 - 0014

Hard-surface dependent or thigmotropic cue regulates the G-protein cascade during blast-disease initiation

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The germinating conidia of rice-blast fungus *Magnaporthe grisea* must differentiate into an infection structure called the appressorium in order to penetrate its host. Apart from hydrophobicity, the other host-surface characteristics that are responsible for the appressorium initiation and maturation are poorly understood. In a forward genetics approach, we identified Magnaporthe mutants that are defective in early signaling events in infection related development. This lead us to the identification of TMT1390 mutant (disruption in the *Regulator of G-protein Signaling / RGS1* locus) which was capable of forming appressoria on non-inductive surfaces both hydrophobic and hydrophilic but couldn't form appressoria on soft surfaces. Further characterisation, molecular identification and analysis of cells lacking *RGS1* function helped us identify and define a Thigmotropic response as being essential for initiation of pathogenesis. Involvement of a G-protein signaling network was identified as a downstream effector module of such early surface-hardness dependent signaling. Chemical genetic studies and global transcriptome analyses related to surface hardness indicated that such thigmo-morphogenesis is initiated within two hours after conidia germination and requires calcium and gravity as intricate signals. Biophysical analyses helped us in estimating the critical hardness necessary for efficient initiation of infection in Magnaporthe. Our preliminary results suggest a possible role for stretch-activated and mechano-sensitive ion channels during hardness sensing and host infection. These will be discussed along with a role for extra-cellular matrix proteins in blast disease initiation.

1330-1530
SYMPOSIUM 18 - ***Veterinary Mycology***

S18IS1 - 0666
Emerging Fungal Infections in Animals in Japan.
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Dermatophytosis and *Malassezia pachydermatis* infections are well known as superficial animal mycoses. Aspergillosis, candidiasis, cryptococcosis and zygomycosis are also well recognized as common systemic mycoses in animals. Recently, candidiasis caused by antifungal-resistant *Candida* spp. in small animals has become a serious problem.Besides the above mycoses, rare species of pathogenic fungi have also been isolated from animals and their environs:*Hortaea werneckii* was isolated from skin lesions of a guinea pig.
Chaetomium globosum was repeatedly isolated from a ringworm-like facial lesion of a 4-month-old male dog. Its colony on Sabouraud dextrose agar (SDA) plates produced no characteristic structures and looked like those of *Microsporum canis* in texture and color, while that on potato dextrose agar (PDA) plates produced black globose perithecia with coiled hairs.
Lecythophora hoffmannii caused fatal osteomyelitis in a 2-year-old spayed female dog. The colony on SDA plates was orange and looked like an environmental contaminant, suggesting that some isolates of *L. hoffmannii* from clinical materials might be thrown away unrecognized as a pathogen.
The thermo-tolerant fungal species *Ochroconis gallopava* is an important causative agent in both humans and animals. There are 3 human cases and 4 environmental isolates of *O. gallopava* in Japan. No animal cases have been documented up to now in Japan; however, such infections may break out in the near future.
Autochthonous highly pathogenic mycosis or so-called histoplasmosis caused by *Histoplasma capsulatum* in humans, dogs, horses and sea otters in Japan is impossible to ignore, although human cases have consisted of both imported and domestic ones. All 7 canine histoplasmosis were nationals and involved skin and occasionally internal organs, and might have been caused by fungal clones closely related to the *H. capsulatum* var. *farciminosum* genotype.
The recent development of people's close contact with household animals (pets) suggests the risk of fungal zoonosis in addition to diseases caused by microbial pathogens from animals.

S18IS2 - 0850
KOALA cracks *Cryptococcus* story wide open The importance of molecular typing in understanding cryptococcosis in Australia: the WA connection
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Cryptococcus gattii is a basidiomycetous yeast and one of the causative agents of cryptococcosis, a serious and potentially fatal disease of humans and animals. *C. gattii* is of importance in Australia as it causes significant morbidity and mortality, particularly in indigenous and rural populations, as well as in domestic and native animals, such as the koala. *C. gattii* is unusual in that it causes disease in a wide range of hosts, including amoebae, nematodes, horses, ruminants, carnivores and humans. Unlike the more common closely related species *C. neoformans*, it is rarely associated with immunocompromised hosts. Little is known about the epidemiology of *C. gattii* in Australia, or the strain diversity in relation to molecular type which may have an effect on disease manifestations, frequency of disease and prognosis. Preliminary evidence suggests that in Australia most *C. gattii* isolates belong to one of two major genotypes VGI and VGII. Genotype VGI is found throughout Australia and has been shown to have a strong association with *Eucalyptus* trees. Genotype VGII appears to be more restricted and is found predominately in Western Australia and the Northern Territory although VGII infections in animals have been recorded in Sydney and Queensland. We treated a koala with invasive cryptococcal rhinosinusitis that had recently been transported from Western Australia. The infecting strain of *C. gattii* had a VGII genotype. Preliminary data also suggests that the VGII genotype differs from the more common VGI in both its epidemiology and clinical presentation. We therefore hypothesised that the infecting strain was acquired in WA, and that colonisation progressed to subclinical and subsequently clinical disease, presumably as a result of the stressors associated with transport to the east coast and establishment in a new environment. Accordingly, we conducted a field trip to south west WA to obtain both environmental and clinical samples (blood, and nasal and paw swabs) to clarify the epidemiological associations of this case. Culture based techniques revealed an exceptional level of *C. gattii* colonising some environmental substrates. Ongoing studies are being conducted to assess the genetic diversity of the WA isolates using molecular techniques to analyse population phylogeny. There are two extremely valuable components of this work. Firstly, detailed investigation of this individual case illustrates the importance of the timing of the acquisition of infection in relation to the development of clinical disease and the importance of naturally-occurring disease in koalas as a model of the pathogenesis of disease in people. Secondly, investigation of cryptococcosis caused by the genotype VGII in endemic and outbreak situations in Australia will lead to a greater understanding of the environmental associations and epidemiology of this apparently more virulent genotype.

S18IS3 - 0915

Comparative aspects of aspergillosis in dogs, cats and people.

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As in people, *Aspergillus* species generally cause either respiratory tract or disseminated infections in dogs and cats.
In dogs, the most commonly recognised form of the disease is mycotic rhinitis generally caused by *Aspergillus fumigatus*, although infections with other species have been reported. The disease primarily affects young to middle aged, apparently immunocompetent, mesaticephalic and dolicocephalic (long-nosed) dogs. The infection is generally non-invasive, mainly causing destruction of nasal turbinates. Signs include chronic nasal discharge, facial pain and ulceration of external nares. Typically, the disease in dogs most closely resembles chronic erosive non-invasive fungal sinusitis in people. Treatment with systemic antifungal medications tends to produce disappointing results whereas the administration of topical enilconazole or clotrimazole is generally more effective.
In feline fungal rhinitis, proliferative disease, with penetration of adjacent bone and soft tissues is more of a feature. Nasal aspergillosis is rare in cats, thus epidemiological data are lacking, however cats with brachycephalic (short-nosed) facial conformation, such as Persians and Himalayans, seem predisposed. Most individuals are apparently immunocompetent. Cats usually require systemic antifungal agents to effect a cure, although cases without apparent invasion may respond to topical clotrimazole.
Allergic fungal rhinosinusitis and bronchitis are not recognised entities in companion animals.
Disseminated aspergillosis in dogs and cats, as in people, is recognised much more frequently in immunocompromised individuals. Most cases of canine aspergillosis occur in German shepherd dogs (GSD). Infections tend to be caused by *Aspergillus terreus*, although other species are occasionally involved. The underlying immunological defect in GSD is yet to be determined, however abnormal mucosal immunity, permitting penetration of fungal spores is suspected. Most cats with disseminated aspergillosis have concurrent immunosuppressive diseases.

S18PS1 - 0556

Fungal infection in cultured marine fishes caused by Imperfecti fungi

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In 2004 and 2005, fungal infections were found in Japanese flounder, *Paralichthys olivaceous* and striped jack, *Pseudocaranx dentex* in Japan. The fungal infections have annually occurred in both fishes between April and July. These fishes grossly showed swelling of abdomen and whitish nodules in internal organs such as kidney, spleen and liver. Numerous brownish hyphae were particularly observed in the kidney under a microscope. The fungi were purely isolated from the kidney of both fishes using glucose-yeast extract-peptone-seawater (PYGS) agar. The identification was mainly attempted from morphological characteristics of the fungi by using light microscope and scanning electron microscope. As a result, the fungi from Japanese flounder and striped jack were identified as the genera *Exophiala* sp. and *Ochroconis* sp., respectively. The optimum temperature for growth of *Exophiala* sp. NJM 0562 and *Ochroconis* sp. NJM 0472 was at 25 oC. The range of temperature and NaCl tolerance for the growth of both strains were 10-30oC and 1-9%, respectively. Moreover, artificial infection using two strains against original each fish was also conducted. Fungal hyphae and granulomas were histopathologically observed in the spleen, liver and kidney of striped jack and Japanese flounder intraperitoneally injected with conidia of *Ochroconis* sp. NJM 0472.

S18PS2 - 0425

Molecular detection of *Aphanomyces astaci* from Norwegian crayfish plague outbreaks in the time span from 1971 to 2005

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Aphanomyces astaci (Saprolegniaceae, Oomycetes) is the agent of crayfish plague, a disease lethal to freshwater crayfish of non-North American origin and in Norway classified as a group-A disease. European populations of the noble crayfish (*Astacus astacus*) have been drastically reduced due to this disease. According to the World Organization for Animal Health (OIE), the crayfish plague diagnosis requires isolation of *A. astaci* in pure culture followed by confirmation of morphology and virulence. The method is time consuming and will detect *A. astaci* under optimal conditions only. Crayfish plague has since 1971 repeatedly wiped out noble crayfish populations in Norwegian watercourses even though alien carrier crayfish species are missing in Norway. However, the attempts to isolate *A. astaci* in pure culture from Norwegian outbreaks in the period from 1971 to 2004 were unsuccessful. Diagnoses in these cases relied on the disease history and on observations of non-septate oomycete hyphae infiltrating the soft crayfish cuticle. Outbreaks of crayfish plague require rapid actions in order to prevent further spread of the disease. Consequently, faster and more reliable methods for confirmative detection of *A. astaci* are urgently needed. In the present studies we tested two methods for molecular detection of *A. astaci* directly from crayfish tissues: 1) Conventional PCR of the nuclear ribosomal Internal Transcribed Spacers (ITS1-2) with *A. astaci*-specific primers followed by DNA-sequencing to confirm 100% homology to reference strains of *A. astaci*; 2) Target taxon-specific real-time PCR (TaqMan MGB probe) detecting a 59 basepair ITS1-fragment unique to *A. astaci*. DNA was extracted directly from crayfish tissue (cuticle, eyes and muscle). The materials examined included 20 separate submissions of moribund or dead noble crayfish from Norwegian lakes and watercourses sent to the National Veterinary Institute for examination in 2005. Materials of ethanol fixed crayfish from previous Norwegian outbreaks dating back to 1971 were also examined. Intracuticular invasions of non-septate oomycete hyphae were observed in 45% of the 2005-submissions, but *A. astaci* was successfully isolated in pure culture from only one submission. In contrast, the molecular methods convincingly detected *A. astaci* from 65% of the submissions, and trace amounts of *A. astaci* were detected by real-time PCR in some additional cases. Furthermore, molecular analyses of material from earlier years verified the Norwegian crayfish plague diagnoses in the time span 1971-2004. Sufficiently validated, the power of molecular methods is demonstrated by their more reliable and faster detection of *A. astaci* than the conventional OIE method.

1330-1530

SYMPOSIUM 19 - *Substrate Colonisation and Succession in Wood Inhabiting Fungi*

S19IS1 - 0715

Fungal dispersal and succession in boreal forests.

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The abundance of many species of wood-decaying fungi has decreased dramatically in Swedish boreal forests over the last century. We have conducted studies to link spore deposition to known occurrences of wood-decaying fungi. Spore deposition was monitored in regions representing different impact on the ecosystems, using field sampling methods based on recording the dikaryotisation of monokaryotic mycelia on nutrient agar and wood discs (spore traps). The studied species, all mainly confined to Norway spruce in the boreal forest zone, were *Fomitopsis pinicola, Fomitopsis rosea*, *Gloeoporus taxicola*, *Phlebia centrifuga* and *Trichaptum laricinum*. Spore deposition for *F. pinicola*, *F. rosea* and *G. taxicola* was higher in plots (radius 2-km) with a high proportion of old Norway spruce forest (>80 years) than in plots with a lower proportion of old forest. There was a significant decline in spore deposition towards the south for *F. rosea* and *P. centrifuga*. No deposition was found south of the distribution borders. We also studied genetic variation in *P. centrifuga*. Consistent with the spore deposition studies, Fst values indicate a moderate genetic differentiation within northern Europe, and a reduction in genetic variation towards the edge of the species distribution. Limited spore dispersal implies a late colonisation outside the core area of distribution of a species. Early successional occurrence in a site would then be biased towards species with rapid and abundant dispersal. Species that arrive late would then have to fight their way into communities. Mycelial combat experiments were conducted and did reflect the position in succession. Generally, late stage decayers had a high ability to replace species known to occur in early stages of decay in the forest.

S19IS2 - 0618

Interspecific mycelial interactions: major drivers of colonization and succession of wood-inhabiting fungi

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Fungal communities development in wood follows a diverse array of pathways rather than occurring in simple stages in an exact ordered pattern. There are four driving forces for change: stress aggravation (worsening of abiotic environmental conditions), stress alleviation (improvement in abiotic conditions), disturbance and combat, the latter frequently being the main one. When decay fungi arrive at woody resources, either as spores or as mycelia, it is usually already colonized by other fungi. Thus they usually need to employ aggressive, combative mechanisms to effect entry. Once within they must defend against newly arriving fungi or attack further to gain more territory. Having control of space/territory effectively means having control of the carbon and mineral resources therein.

Recognition as non-self results in antagonistic responses observable as dramatic morphological, physiological, metabolic and enzymatic changes in both mycelia. Production of secondary metabolites is one of the mechanisms involved in 'attack and defence', and is often visually manifested as bright pigments in mycelium and culture substratum. Changes can also be seen distant from the site of interaction, indicating that the mycelium is responding elsewhere with changes in gene expression. The overall outcomes of combative interactions can be: deadlock, where neither fungus gains any territory; replacement, where one fungus replaces the other and gains its territory; partial replacement, where some but not all of the opponent's territory is gained; and mutual replacement, where one fungus gains some of the territory held by the other fungus and *vice versa*. Outcomes vary depending on the abiotic environment and the activities of other organisms. For example, grazing by invertebrates can completely shift the balance from one fungus replacing another to itself being replaced.

Within fungal communities in a particular woody resource there are clear hierarchies of combative ability. These hierarchies are broadly, though certainly not completely, correlated with position of fungi in the succession. Primary colonizers tend either to be already present in functional wood, as latent propagules, or to be prolific spore producers that arrive rapidly, germinate and grow rapidly but with limited combative ability. Fungi that arrive later are better combatants, and those that dominate even later are often better still at combat. However, why some extremely combative species tend only to be found at very late stages of decay is unclear - evidently combative ability is not the whole story. Some specific early decay agents appear to have a large influence on which late stage decay fungi establish. Wood previously occupied by some species seems actually to be stimulatory to the growth of other species, e.g. that of *Fomes fomentarius* stimulated growth of *Coprinus micaceus, Lycoperdon pyriforme, Mollisia cinerea* and *Nemania serpens.*

S19IS3 - 0633

Wood inhabiting fungi on decomposing logs in three venezuelan forests

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Species composition and diversity of wood inhabiting fungi growing on fallen, decomposing logs were studied in three different types of forests located in Venezuela: a tropical rainforest at Henri Pittier National Park, Aragua State; an Amazonian forest located at Cataniapo, Amazonas State; and a tropical sub-mesotermic forest at Gran Sabana, Canaima National Park, Bolívar State. For each site, a certain number of logs with a minimum of 40 cm diam x 5 m length were chosen randomly, selecting the same number of logs belonging to each of the three decay stages defined as: 1) wood hard through the whole log, cortex present; 2) cortex partially present, wood partly decayed; and 3) no cortex present, most of the wood decayed. Several collecting trips were made to each site in order to cover seasonal changes. It was not possible to determine the tree species of the logs. Fungi present were collected at least once, and a diagram of each log was made every time, showing position of each of the fungal species present. Fungal specimens were identified and deposited at VEN and O. Correlations between species richness, log decay stage, and seasonality was made for each study site. Comparisons of the three forests showed that the highest fungal species diversity occurred for the three sites in the beginning of the rainy season, being highest in the Amazonian forest (76 species on 50 logs, found on 3 collecting trips during 1 year), followed by the tropical rainforest (60 species on 51 logs, found on 5 collecting trips during 1 year). The lowest number of species was found in the tropical sub-mesotermic forest (81 species on 120 logs, found in 4 collecting trips during 1 year). For the three sites, most of the species belonged to the orders Poriales and Stereales of the Phylum Basidiomycota, and produced white rot. New species of corticoid and poroid fungi were found in Amazonas and Bolívar state's forests, showing that the mycobiota of these ecosystems is still poorly known, and merits more studies.

S19PS1 - 0034

Diversity and ecological patterns of wood decay fungi in a temperate, deciduous forest canopy

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Studies on fungal richness and ecology have been largely disregarded since the first intensive efforts to investigate organismal diversity in forest canopies. We used the Leipzig Canopy Crane research facility to sample wood-decaying fungi in a mixed deciduous forest canopy 10-30 m in height. The structural complexity of the canopy was analysed using different methods, including meteorological measurements. With respect to temperature and relative humidity, marked differences existed between forest floor and upper canopy layers that persisted on smaller scales. Of the 118 taxa found in 128 sample units, pyrenomycetes and corticioid fungi outnumbered other macrofungal groups. Fungal communities showed distinct variations both in species richness and composition with respect to substrate (tree species), height in the canopy, stage of decay, and branch diameter. Pyrenomycetes and their anamorphs dominated the mycobiota on thin, exposed twigs at great heights, indicating their ability to overcome extended periods of drought and high levels of solar irradiance. Other taxa of *Tremellales* (*Exidia* spp.), *Orbiliales* (*Hyalorbilia inflatula*, *Orbilia* spp.) or *Agaricales* (*Episphaeria fraxinicola*, *Cyphellopsis anomala*, *Lachnella* spp.) also exhibited features that enabled them to develop in lesser protected habitats within tree crowns.

S19PS2 - 0159

Small-scale variation in chemical property within logs of Japanese beech in relation to spatial distribution and decay ability of fungi

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Introduction: Fungi play a central role in wood decomposition in forest ecosystems. Wood decay fungi utilize wood chemical components in various proportions and change chemical property of wood. In forests, tessellated distribution of colonies of wood decay fungi exist even within a single log, forming decay columns that elongate along a longitudinal axis and are delimited by zone lines. It is necessary to reveal the spatial distribution of fungal decay columns and chemical property of wood of the columns in decomposing logs, in order to understand the importance of fungal community structure in wood decomposition in forest ecosystem.
Methods: The study site is a cool temperate deciduous forest dominated by *Fagus crenata* in Japan. A naturally fallen large bough (decay class 3) of *Fagus crenata* was used as the material. Five logs (30 cm long, diameter 10.7-20.5 cm) were cut out randomly from the bough. Each log was cut into ten successive disks (3 cm thickness), and one cut surface in each disk was observed for distribution of fungal colonies distinguished by zone lines. Fungi were isolated from each colony in each disk, and volume of each fungal colony was calculated. Contents of Lignin, carbohydrates and nitrogen of each fungal colony were analyzed. Relative content of total carbohydrate in lignocellulose matrix in wood was calculated as lignocellulose index (LCI). Wood decay ability of fungi isolated from disks was studied with pure culture decomposition test under laboratory conditions.
Results: Eight basidiomycetes, one mitosporic fungus, and one sterile fungus were isolated from decay columns. *Omphalotus guepiniformis* and *Trichoderma* spp. were isolated from all five logs. *O. guepiniformis* and *Antrodiella albocinnamomea* occupied the largest volume in the logs. LCI and nitrogen content were differed among decay columns colonized by different fungal species in each log. In the pure culture decomposition test, *O. guepiniformis*, *Stecchrinum rhois* and *A. albocinnamomea* decomposed lignin and carbohydrates simultaneously. *Psathyrella candolleana* decomposed carbohydrates selectively. In *O. guepiniformis*, LCI of decay column was correlated to that of wood blocks decayed under pure culture condition by the fungi isolated from the decay columns.
Discussion: The results of this study suggest that the small-scale variation in chemical properties within fallen logs of Japanese beech reflects the distribution and the decay ability of colonizing fungi. Revealing dynamics of fungal decay columns and its chemical properties along decomposition process of logs is necessary for understanding the process of fungal decomposition of wood.

S19PS3 - 0641

Community analysis of wood-inhabiting fungi using fruiting bodies, culturing, and rDNA

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Three sampling techniques were used to investigate the wood-inhabiting fungal community in *Picea* logs in Sweden and the Kenai Peninsula of Alaska. Collection and identification of fruiting bodies, culturing of wood samples on artificial media, and analysis of rDNA sequences were used to determine if sampling technique affected the observed species richness and species composition of wood-inhabiting fungi. This investigation is part of a larger project to document fungal succession and to determine whether the composition of the fungal community affects the rate of decay in *Picea* logs. Fruiting bodies were collected from logs and samples were drilled from the interior of each log for culturing and extraction of DNA. Genomic DNA was isolated from wood samples and the ITS region of rDNA was amplified using fungal specific primers. ITS amplification products were then cloned and sequenced for identification. Comparison of the three sampling methods indicated that each yielded a somewhat different set of species, and that unique species were observed with each technique. In general, cloning and sequencing yielded the highest number of taxa per log, followed by culturing and identification of fruiting bodies. No single technique yielded all of the species observed in a log, and each technique had advantages and disadvantages in terms of cost, time required to process samples, and the ability to assign ecological significance to the results. Although cloning and sequencing yielded the largest number of taxa, identification and delimitation of species using ITS sequences can be problematic. In addition, stringent negative controls are necessary to eliminate the possibility of sequencing contaminating DNA. DNA analyses have the potential to identify all of the species present in samples, but there are still many practical concerns that have yet to be addressed, including biases introduced through DNA extraction, PCR amplification, and cloning.

1330-1530

SYMPOSIUM 20 - *Fungi of Monsoon Asia - Linking Diversity and Ecosystem Function*

S20IS1 - 0331

Why is the species diversity of fungi in tropical monsoon Asia high?

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The history of the earth shows that relationships among organisms in a certain area had gradually become complex as time passes, and developed into a web of life adapted to the environment conditions of the place. As a result, the biodiversity in the area had continued to rise gradually in the period when the climate on the earth is steady. To the contrary, it had decreased in the climate change period. The climate change that started about two million years ago has decreased the biodiversity in the temperate zone. Pleistocene glacial cycle has forced temperate organisms to shuttle between their original habitats and lower latitudes. Many plant species became extinct, and it brought about species co-extinction. Many organisms with slow migration speed such as trees had been exterminated before reaching their safe refuges. Other species had been brought in danger of extinction by differences of important conditions in relation to sexual reproduction such as photoperiodicity between the habitat and the refuges.
In the tropics, the influences of Pleistocene glacial cycle have been comparatively slight because the tropical rainforest climate has continued without disappearing in the low latitude area. Most tropical organisms have survived a dangerous situation without migrating. Consequently, we can see the high biodiversity and a developed web of life in the rainforests. Therefore, the tropical rain forest regions have the highest in species diversity of fungi.
The monsoon Asian region has extended to the north side of tropical forests. The big land that is called the Sundaland appeared in this region at the last glacial period. The biological community that had developed in the temperate zone in Asia has been able to endure this climate change by the existence of the land bridge. Such a lucky path did not emerge in Europe and America. Movement towards the south was prevented by the Mediterranean Sea, the desert or the mountain range that runs east and west. The contact of different biological communities that have happened repeatedly might be the most important factor to raise the biodiversity in this region. Moreover, present distribution of the climate and the existence of high mountains in Malay and Indochina Peninsula are important elements to contribute to high biodiversity in this area. The latter becomes a refuge for many temperate plants. For the above mentioned reasons, the biodiversity including fungi has become very high in monsoon Asia area.

S20IS2 - 0127

Fungal decomposition of lignin in leaf litter: comparison between tropical and temperate forest soils

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Surface litter accumulation in undisturbed, non-water-saturated soils is generally lower in tropical forests than in temperate forests despite the fact that tropical forests have twice the litterfall amount. The lower accumulation in tropical soils has been attributed to faster rates of litter decomposition, which is generally 1.5 to 2 times faster than in temperate soils. Explanations for this difference in decomposition rates have included higher temperatures that mediate higher biological activities, lower litter quality, and differences in decomposer communities in the tropics such as the presence of termites. Recent studies have shown that lignin, a major structural component that is resistant to microbial decomposition, is actively removed from litter during decomposition on tropical soils, whereas lignin is decomposed slowly on temperate soils. Therefore, studies of fungi associated with lignin decomposition will provide further insight into the mechanism underlying the difference in decomposition processes between tropical and temperate forests. In this study, I focused on the occurrence of bleached portions on litter surfaces, which is associated with fungal decomposition of lignin. Fallen leaves with bleached portions were collected from four tropical forests located in northern Thailand and southern Japan and from four temperate forests located in central Japan and western Canada. Unidentified basidiomycetes and white sterile mycelium were responsible for bleaching in the tropical soils, and *Marasmius* sp., *Xylaria* sp., and *Coccomyces* sp. were responsible for bleaching in the temperate soils. Leaf mass per area (LMA) and lignin concentration was consistently lower in bleached portions than in surrounding, non-bleached portions of the same leaf, indicating the selective removal of lignin led to the enhanced mass loss in bleached portions in both tropical and temperate forests. The number of microfungal species isolated from bleached portions was generally lower than that from non-bleached portions. Changes in microfungal communities were studied for bleached and non-bleached portions on decomposing evergreen tree leaves in one tropical and one temperate forest. Decomposition of bleached portions was faster in the tropical forest than in the temperate forest. Fungal communities changed more rapidly in leaves on the tropical soil than in leaves on the temperate soil.

S20IS3 -0160

Diversity of ecto-mycorrhiza fungi in Nepal - relation to forest types and management.

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The mycota of Himalaya is poorly described and information on the diversity of ecto-mycorrhizal species in the region is very limited. Three years of intensive studies of the fruitbodies in different areas of Nepal has documented a very high diversity of mycorrhizal fungi. Systematic information collected from permanent plots in *Shorea*, *Castanopsis*, *Pinus*, and *Tsuga* forest is analysed and the species have been enumerated. Boletales (*Tylopilus, Suillus, Boletus, Austroboletus, Areoboletus, Pulveroboletus, Strobilomyces*), Russulales (*Russula, Lactarius*) and Amanitales (*Amanita*) are dominating in all forest types, and members of members of Cortinariales (*Cortinarius, Inocybe, Heboloma*) are numerous under *Pinus* and *Tsuga* but less common in the broadleaved forest. *Thelephora, Tricholoma, Laccaria, Scleroderma, Cantharellus, Craterellus* also occur in the studied forests.
An analysis of the influence of different forest management types on the diversity of ecto-mycorrhizal fungi show that traditional management types such as coppice often house a very high diversity compared to monoculture high forest.
The diversity found in the forest in Nepal is compared with studies from Japan, Europe and North America and affinity and similarities are discussed on species and genera level.

S20PS1 - 0115

Effect of physico-chemical parameter on fungus in water bodies of Jabalpur(M.P.)-India

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The present work deals with the study of ecology of water mycoflora of water bodies of Jabalpur city during the year Jan.2005 – May 2005.
Total eleven sites water bodies viz. Goratal, Hanumantal, Babatola , Sangram Sagar, Supatal, Devtal, Kohaltal, Goluva tank, Tilwaraghat (River – Narmada), Gwarighat (River – Naramada), Jilaharighat (River – Naramada) at Jabalpur were surveyed for water borne mycoflora.
Various observations delineate the behavior of water mycoflora in natural habitat, their interaction with chemical factors of their environment. The study in the laboratory conditions throws light on their cultural characters & growth response for various physical & chemical conditions.
The ecological survey of the water bodies resulted into the isolation of nine microflora these are *Aspergillus, Alternaria, Cladosporium, Cheatomium, Curvularia, Fusarium, Helminthosporium, Penicillium, Mucor* . Maximum number of fungi was colonized on decaying litter. True aquatic forms could not be isolated.
During our study I have noticed that number of microorganisms are more in Hanumantal, & Babatola water bodies which may be due to its chemical nature, to high degree of pollution or presence of rice debris. It was observed that quantitatively microorganisms are more during Jan-Feb. & their number reduces during April-May. Stagnant water favor large amount of organisms due to the optimum ecological factors (T.D.S, D.O, B.O.D, C.O.D).
During the course of present investigation it was also observed that factors viz. seasonal changes, concentration of 'H' ion, temperature, availability of plant litter (amount & diversity), quality of water (aeration, pollution status etc.) were directly responsible for the concentration & composition of water-borne microorganisms, of sampling site. A single factor did not play any significant role, in controlling frequency & abundance of aquatic fungi therefore it is difficult to pinpoint a single factor.

S20PS2 - 0501

Tracking down beauvericin-production in the genus Isaria using molecular phylogenetics

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Beauvericin is a bioactive cyclohexadepsipeptide mycotoxin originally described from *Beauveria bassiana* and is also produced by several *Fusarium* species. Members of the genus *Isaria* are also able to produce this metabolite. Thirty seven isolates of *Isaria* and its *Cordyceps* teleomorph including nine *Beauveria* species were selected, sequenced and tested for beauvericin production. From these isolates trees using ITS rDNA and b-tubulin sequences was constructed. A tightly knit group comprising *Isaria tenuipes* and its known teleomorph *Cordyceps takaomontana, I. japonica, I. fumosorosea,* and *Cordyceps* cf. *ninchukispora* showed positive beauvericin production which correlated well with both ITS rDNA and b-tubulin phylogeny. This demonstrates that within the genus *Isaria* the ability to produce beauvericin has either been lost by certain species, retained by some or re-acquired by others.

1600-1800

SYMPOSIUM 21 - *DNA Barcoding for Fungi*

S21IS1 - 0606

The Canadian barcode of life network and CoxI barcoding of *Penicillium*

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DNA barcoding employs a standard gene region to identify species across life. A 648 bp region of the mitochondrial gene cytochrome c oxidase 1 (COI) has been used with great success in animals. Within the last year, a well-funded network of Canadian researchers has been established, including several mycologists who will test this concept for fungi for the first time. We will present an overview of this network and the results of a pilot study applying COI barcoding to the ascomycetous mould genus *Penicillium*.

Primers for a 545 bp fragment of the COI of *Penicillium* and *Aspergillus* spp. were designed using mitochondrial genome sequences in GenBank. Sequences were generated for 358 strains from 58 species of *Penicillium* subg. *Penicillium*, and several outgroup species. Sequence divergence was compared with internal transcribed spacer (ITS) and partial beta-tubulin (*BenA*) sequences for the same taxa.

All COI amplicons were the same length so alignment was straightforward. Thirty-eight of 58 species in subgenus *Penicillium* formed cohesive assemblages with distinct barcodes from all other species. Most cases of sequence sharing involved known species complexes (4 complexes, a total of 17 species) where other genes tend also to have similar sequences. The mean infraspecies divergence of COI sequences was 0.1%, less than in ITS and *BenA,* whereas the mean interspecies divergence for COI was 5.7%, comparable to the ITS, but less than the 15% interspecies divergence seen in *BenA* sequences.

Penicillium subg. *Penicillium* is a harsh test for DNA barcoding in fungi. This is one of the few fungal groups where phenotypically-based species concepts are more finely dissected than those based on molecular data. COI data generated for subg. *Penicillium* resulted in species-diagnostic sequences (invariant among strains, or with some terminal branching within species) for 2/3 of the species. There were four complexes of species where all strains of each species either had identical sequences, or only minor variations that were not species specific. The observed infraspecific and interspecific variation was comparable to that for ITS. Despite frequent reports of introns in fungal mitochondrial genomes, we found only a few introns, allaying a main fear of the applicability of COI barcoding to fungi. The ease of amplification of COI contrasts to the more problematic *BenA*. Moreover, both ITS and *BenA* are laborious to align, so the ease of COI alignment is a major advantage. The value of employing a gene in fungi that is standardized for barcoding of other kingdoms of life cannot be overemphasized.

S21IS2 - 0357

An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*

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One of the biggest obstructions to studies on *Hypocrea/Trichoderma* has been the incorrect and confused application of species names to isolates used in industry, biocontrol of plant pathogens and ecological surveys, thereby making the comparison of results questionable. Here we provide a convenient, on-line method for the quick molecular identification of *Hypocrea/Trichoderma* at the genus, clade and species levels based on an oligonucleotide barcode: a diagnostic combination of several oligonucleotides (hallmarks) specifically allocated within the internal transcribed spacer 1 and 2 (ITS1 and 2) sequences of rRNA repeat. The first barcode version was developed on the basis of 979 sequences of 88 genetically characterized species which displayed in total 135 ITS1 and 2 haplotypes. Oligonucleotide sequences which are constant in all known ITS1 and 2 of *Hypocrea/Trichoderma* but different in closely related fungal genera, were used to define genus specific hallmarks. Verification of the DNA-barcode was done by a blind test on 53 unknown isolates of *Trichoderma*, collected in Central and South America. The obtained results were in a total agreement with phylogenetic identification based on *tef1* (large intron), NCBI BLAST of vouchered records and *postum* morphological analysis. The library of species-, clade- and genus-specific hallmarks is stored in the MySQL database and integrated in the *Trich*OKey v. 1.0 - barcode sequence identification program with the web interface located on www.isth.info. The original *Trich*OKey v. 1.0 identified 76 single species and 6 species pairs. The current version of the program has barcodes for 15 newly recognized species of *Hypocrea/Trichoderma.* We conclude that oligonucleotide barcode is a powerful tool for the routine identification of *Hypocrea/Trichoderma* species and should be useful as a complement to traditional methods.

S21IS3 - 0836

DNA barcoding, 'accelerated ecology'and the acremonioid fungi

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The fungi that grow and reproduce within relatively dense, semipermeable matrix materials – for example, soil or internal plant or animal tissues – are often remarkably difficult to identify. Adaptation to these circumstances causes many fungi to produce highly simplified reproductive structures and to reproduce mostly or entirely asexually. The genus *Acremonium* is a case in point. Species are simply structured, with long thin phialides not much different from hyphal side-branches, and small one-celled conidia. Some of these species are common in soil while some are common plant endophytes and/or invaders of the dense keratin of human nails. In sequence-based taxonomic studies, ITS and protein coding sequences of *Acremonium* species are mostly too divergent to align validly except within small subgroups, and LSU and SSU offer minimal clade resolution. A DNA barcoding approach, however, offers a practical way of dealing with these organisms while very challenging phylogenetic analyses are being worked up. All *Acremonium* species known in culture and many related and similar fungi were sequenced for the full length of the ribosomal ITS/5.8S locus. A particular priority was given to ex-type and other known or potentially nomenclaturally significant isolates (e.g., potential epitype and neotype isolates). The primers most commonly used were ITS-1F and ITS4; various others were used in cases where problematic primer affinity was suspected. A supplementary locus such as beta-tubulin, actin or elongation factor · was sequenced in groups considered to be possible species complexes based on ITS variability; priority in such cases was given to the locus giving the most reliable yield of high-quality sequences in the group in question. EF- · in particular was very problematic in some groups because of loop formation in the amplicons.
Distinct *Acremonium* species were clearly delimited by ITS sequences in most cases, and sequence-based identification was generally found to be easy, rapid, and unambiguous. Existing species names, however, often referred to several different species, most of which need new names. In some species complexes such as *A. sclerotigenum*, distinct lineages were found that did not correspond with morphological distinctions.
Barcoding will permit meaningful ecological work to be done with most of these species for the first time. Array technologies and other rapid barcode-based methods hold the promise of a great acceleration in the acquisition of reliable, identification-based ecological data on *Acremonium* species as well as other simplified fungi from matrical substrata such as soil.

S21PS1 - 0591

UNITE – reliable identification of ectomycorrhizal fungi - DNA barcoding in action

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UNITE is a DNA barcoding database of ectomycorrhizal (ECM) fungi.
Identification of ECM fungi is often achieved through comparisons of ITS sequences derived from environmental samples with accessioned sequences deposited in public databases. It has been shown in many ECM studies that ITS sequences can successfully discriminate between species, detect species previously not known to form ECM, and even discover potential cryptic species. A major problem encountered is that annotation of the sequences in public databases is not always complete or trustworthy. In order to overcome this deficiency we have developed UNITE, an open access database comprising fully annotated fungal ITS sequences from well-defined herbarium specimens including herbarium reference identification data, collector/source, ecological data, etc. The UNITE database comprise sequences from all major ECM fungal clades as well as multiple accessions of many individual species. UNITE can be searched either by taxon name or via a sequence similarity searches using *blastn*. The database also incorporates a phylogenetic species recognition tool - *galaxie*. *Galaxie* uses prealigned sequence files to place query sequences with known taxa. UNITE is a relational database built on a *MySQL/Apache-Linux* platform that communicates with the web interface through *PHP/Perl*. UNITE represents, in its conception and open-ended development, a good working example of DNA barcoding in action. At present (March 1 2006), the UNITE database includes 1441 ITS sequences of 753 species from 96 genera of basidio- and ascomycete fungi. The UNITE database is accessible through: http://unite.zbi.ee

S21PS2 - 0765

Barcoding identification of *Penicillium* species occurring in cork bark of *Quercus suber* trees using calmodulin, B-tubulin and ITS and lsu rDNA sequences

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Penicillium is one of the most frequently isolated fungal genera in cork mycobiota surveys. Species of the genus are relevant for mycotoxin and off-odor production, two important concerns for cork quality and safety, especially in the case of cork stoppers for wine bottles. Portugal is the main world producer of cork and the industry needs to assess the technological processes of cork manufacturing and implement HACCP programs. Part of the assessment is determining the fungal species in cork at harvest time.
The bark of *Quercus suber* trees was harvested normally, then peeled into two layers, one the outer bark and the other an internal layer. Cork from each layer was fine-ground and dilution plated on DRBC agar. Cultural and morphological traits were observed for cultures grown on standard media (MEA, CYA). Some *Penicillium* isolates were common and readily identified but others were impossible to identify using only morphological and cultural traits. Therefore DNA sequencing of these *Penicillium* isolates was performed. Calmodulin, B-tubulin and the ITS and lsu rDNA loci were amplified using published primers and protocols. Sequencing was performed using ABI kits and an ABI 3730 sequencer. Because a large and well documented database of *Penicillium* sp. sequences from these loci is available, we supplemented our phenotypic study with corroboration by DNA sequence identification.
Portions of the genus *Penicillium* have been examined using the geneological concordance phylogenetic species concept. For species studied in that way, identifications using DNA sequences were considered valid if all three loci were in agreement about the disposition of the isolate. Other areas of the genus are not as thoroughly studied, but single locus (ITS and lsu rDNA) sequences are publicly available for mosts species of *Penicillium*. Identifications of isolates where only single locus genetic data were available were made on the basis of the phenotypic data and the single locus sequences. Both approaches have been very useful in identification of isolates. The multi-locus approach provides the highest confidence for the identifications. We present the species identified and report on the occurrence of rare and undiscribed taxa isolated from cork bark.
Rita Serra was supported by grant SFRH/BPD/20827/2004 by Fundação para a Ciência e Tecnologia in Portugal.

1600-1800
SYMPOSIUM 22 - ***Polyketides, Non-Ribosomal Peptides, and Terpenes as Fungal Signal Molecules***

S22IS1 - 0672
Biological roles of fungal non-ribosomal peptide synthetases: elemental and diverse
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The genome of the filamentous ascomycete, *Cochliobolus heterostrophus* (*Ch*) carries 12 non-ribosomal peptide synthetase (NRPS)-encoding genes (*NPS*), of which four are conserved and eight are discontinuously distributed in filamentous fungi. Here, we report that alterations in morphology, asexual and sexual development, virulence, and in sensitivity to various types of stress are associated with absence of the four conserved *NPSs*. Deletion of *ChNPS6* causes reduced virulence to the host, maize, hypersensitivity to hydrogen peroxide, to the superoxide generator, KO2, to high pH, and to iron depletion. Deletion of *ChNPS2* causes defects in sexual development; when homozygous in a cross, no asci and ascospores are formed, although pseudothecia are present. Deletion of *ChNPS4* leads to loss of hydrophobicity. In nutrient-rich conditions, strains deleted for *ChNPS10* show abnormal aerial hyphal development and reduced asexual sporulation. Expression of *ChNPS2, 4, 6* and *10* is detected easily under most *in vitro* conditions and *in planta*. In contrast, expression of the eight *ChNPSs* that are discontinuously distributed is barely detectable, if at all, under all conditions tested. Most phenotypes associated with the *Chnps*-deletion strains are found also for corresponding *Fusarium graminearum* (*Fg*) and *Alternaria brassicicola* (*Ab*) deletion strains. HPLC analyses of *Chnps2* and *Chnps6*-deleted strains reveal the corresponding NRPSs are responsible for intra- and extra-cellular siderophore biosynthesis, respectively. Further characterization of *nps2*, *nps6,* and *nps2*;*nps6*-deletion strains of *Ch*, *Fg,* and *Ab* shows that siderophores play a central role in metabolism of the essential nutrient, iron. Overall, the *NPS2, NPS4, NPS6,* and *NPS10* data demonstrate that certain NRPSs play fundamental roles in development, accounting, perhaps, for their conservation. Those NRPSs unique to each genus, species, or strain likely play specialized roles, important for niche adaptation. Given that the majority of fungal NRPSs belong to the latter group, the diversity of roles NRPSs play in filamentous ascomycetes is potentially vast.

S22IS2 - 0849
Chemical diversity in *Penicillium* and *Aspergillus*: do all species produce terpene, non ribosomal peptide and polyketide secondary metabolites?
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Filamentous fungi are known to produce many different secondary metabolites, biosynthesized as polyketides (PKs), non ribosomal peptides (NRPs), terpenes (TPs), shikimic acid derivatives or even mixtures of these. We wanted to examine whether the ability to produce PKs, NRPs and TPs in the two common genera *Penicillium* and *Aspergillus* were a feature of all species, whether they were consistently produced within those species and whether there was a tendency of some or all of the species to produce several PKs, NRPs or TPs. All species in *Penicillium* (ca. 500 species) and *Aspergillus* (ca. 300 species) were inoculated on Czapek yeast autolysate (CYA) and Yeast extract sucrose (YES) agars for one week at 25 C in the dark and analyzed by high performance liquid chromatography, and the different compounds identified via their polarity, UV and mass spectra and by comparison to standards. With few exceptions, all species of *Penicillium* and *Aspergillus* produced a large number of secondary metabolite families and all species appeared to produce both PKs, NRPs and TPs (or mixtures of those). Some of these secondary metabolite families appear to be related to ecology rather than to phylogeny, as the same metabolites were found both in closely related species and in distantly related species. Some secondary metabolites were only found in cold-adapted species. For example the non ribosomal peptide families cycloaspeptides and psychrophilins were only found in Penicillia from arctic and alpine areas of the world. In conclusion the chemical diversity of *Penicillium* and *Aspergillus* species is extraordinarily high, but so is the chemical diversity in species in many other genera of filamentous fungi. There is still possibilities to find new drug leads among the filamentous fungi and new screening methods and production media may help in discovering those.
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S22IS3 - 0800

The genetic basis for indole-diterpene chemical diversity in filamentous fungi

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Indole diterpenes are a large, structurally diverse group of natural products synthesized by filamentous fungi. These metabolites all have a common cyclic diterpene skeleton derived from geranygeranyl diphosphate (GGPP) and an indole moiety derived from indole-3-glycerol phosphate. Using *Penicillium paxilli* as a model experimental system we have shown by deletion analysis that a cluster of seven genes is required for the biosynthesis of paxilline. Just four of these *pax* genes are required for the synthesis of the first stable indole-diterpene intermediate in this pathway, paspaline. A pathway for the biosynthesis of paxilline is proposed on the basis of deletion analysis, and genetic and chemical complementation. Homologues of the *pax* genes have been identified in *Aspergillus flavus* and in *Epichloë festucae*, organisms that synthesize aflatrems and lolitrems, respectively. The genes for aflatrem biosynthesis in *A. flavus* appear to be organized in at least two clusters, with cluster one containing core biosynthetic genes, and cluster two containing genes for later steps. Using a combination of PCR, SSH and chromosome walking a cluster of 10 genes has been identified for lolitrem (*ltm*) biosynthesis in *E. festucae*. These genes are organized in three mini-clusters that are separated by large blocks of retrotransposon DNA sequence. Deletion and complementation analysis has confirmed that six of these genes are required for lolitrem biosynthesis. Based on our knowledge of how paxilline is synthesized in *P. paxilli* a scheme will be presented to account for the indole-diterpene chemical diversity found in the *E. festucae*-perennial ryegrass symbiotum and the bioprotective role of these metabolites discussed.

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S22PS1 - 0303

An endophyte nonribosomal peptide synthetase in siderophore biosynthesis is essential for mutualistic interactions with grasses

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Siderophores are low molecular weight, iron-chelating agents that solubilize iron (III) and control intracellular iron levels. We have identified a novel siderophore from symbiotic fungal endophytes (genera *Epichloë* and *Neotyphodium*) of cool-season grasses. In nature, these fungi are never free-living, but form mutualistic associations where they are confined to the intercellular spaces (apoplast) of leaf sheaths and blades. We undertook a degenerate PCR approach to identify NRPS genes from endophytes with an aim to functionally characterise their role in symbiosis. One of these from *E. festucaë* has high amino acid identity to other NRPSs involved in siderophore biosynthesis. Functional analysis of this gene (termed *sidEf)* by targeted gene replacement eliminated biosynthesis of an extracellular siderophore that was induced under iron-depleted conditions. Structural characterisation by LC-MS-MS showed clear similarities to hydroxamate-type siderophores. To our knowledge this is the first siderophore detected from grass endophytes and from our studies appears to be the only one synthesised. Surprisingly, plants inoculated with strains carrying a targeted deletion in *sidEf* have a dramatic phenotype. Siderophore loss consistently caused de-regulation of fungal hyphal growth, plant stunting and sometimes even tiller death. Transmission electron microscopy revealed an altered hyphal ultrastructure, including uncharacteristic vacuolation. Additionally, in these plants, we also found significantly higher levels of the endophyte alkaloids ergovaline and lolitrem. These alkaloids are only produced *in planta*, but the mechanism for regulation is unknown. From our results, we hypothesise that competition for iron is a critical factor in keeping the grass-endophyte interaction mutualistic and that siderophore loss upsets the carefully controlled process of iron homeostasis in the whole symbiotum. We are now investigating the molecular mechanisms controlling global regulation of iron in the symbiotum, and how this influences endophyte secondary metabolism by using Affymetrix GeneChips. In addition to the transcriptomic analysis of plants infected with the siderophore knock-out, a detailed metabolomics analysis is also underway.

S22PS2 - 0361

Polyketide and cytochalasin production during stromatal ontogeny of the *Hypoxyloideae*

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More than 50 polyketide pigments and other secondary metabolites were isolated in the past years from stromata of the *Xylariaceae*. Among those are various novel types of azaphilones, which are concentrated in granules beneath the surface of ascigenous stromata. In addition, some pentaketides that are presumably derived from the 1-8-dihydroxynaphthalene melanin biosynthesis, proved to be preferentially located at the stromatal surface. These pigments were studied for antimicrobial and nematicidal activities, and most of them showed broad-spectrum biological effects. Along with the rather high yields (up to 5% of the dry stromatal biomass) this suggests a protective role of these metabolites against feeding enemies.
However, we recently even found first evidence that secondary metabolite production is correlated with certain developmental stages of these ascomycetes. In the common European species, *Hypoxylon howeanum* and *H. fragiforme*, a drastic shift in secondary metabolism was noted upon stromatal development by HPLC-UV and HPLC-MS analyses of samples taken during the vegetation period: Production of large amounts of cytochalasins (i.e. notorious mycotoxins that are not polyketides but derived from different biogenetic pathways) was found to be associated with the anamorphic stage, while mature, ascogenous stromata contained mainly the aforementioned azaphilones [1]. Interestingly, yet a different secondary metabolism was observed in laboratory cultures of these fungi, which are known to produce small polyketides (dihydroisocoumarins) that appear to be absent altogether in the fruitbodies.
As inferred from a HPLC profiling study that hitherto included about 3000 specimens and 200 taxa [2], this phenomenon is not restricted to *Hypoxylon* but rather appears to be widespread in the *Hypoxyloideae*. Different kinds of metabolites are involved according to the taxonomy of the producer organisms. Those taxa that are regarded as more advanced as inferred from morphological and molecular data have apparently adapted more sophisticated strategies, involving synthesis of more complicated chemical matters.
Since up to 150 different compounds were encountered in stromata and cultures of a single species by the above HPLC-based methodology, an intensified "mycochemical" evaluation, even of common and well-known taxa appears promising in a search for novel drugs and pesticides.
[1] M. Stadler et al. (2006) Mycol. Res.: in press.
[2] M. Stadler & V. Hellwig (2005) Recent Res. Devel. Phytochemistry 9: 42-93.

1600-1800

SYMPOSIUM 23 - *Adhesion of Fungi to Plant or Animal Hosts*

S23IS1 - 0787

Oral adhesion of *Candida albicans* – a cellular and molecular view

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The fungus *Candida albicans* is an opportunistic human pathogen. It causes a variety of infections in immunocompromised individuals - a common presentation being oral candidosis. In order to cause an infection *C. albicans* must first adhere to a host surface. Understanding *C. albicans* adhesion mechanisms may lead to novel treatments that prevent colonisation and hence preclude candidosis. Here we demonstrate how both a cellular and molecular approach can help elucidate oral adhesion and the role of adhesion in virulence. The adhesion of radiolabelled *C. albicans* cells to cultured epithelial cells, denture acrylic and to voice prosthesis silicone was measured. The effect of adding human saliva or anti-*C. albicans* antibodies on adhesion was determined. Salivary proteins that attached to *C. albicans* cells, or to adhesion substrates, were analysed by electrophoresis, immunodetection, and N-terminal sequencing. In a parallel approach, amplified fragment length poymorphism (AFLP) analysis was used to identify DNA sequences associated with a clade of *C. albicans* strains (GPG strains) that causes disease 10-100 times more frequently than other groups of strains.
Specific anti-*C. albicans* IgA antibodies inhibited *C. albicans* adherence to epithelial cell monolayers. Human saliva however, particularly from individuals with low titres of specific anti-*C. albicans* secretory IgA (sIgA), promoted adhesion in a dose-dependent fashion. Biotin labelled saliva proteins, and immunodetection, indicated that free secretory component (SC) from sIgA bound to *C. albicans* cells and promoted adherence. In contrast, other salivary proteins were selectively adsorbed to denture acrylic and to silicone rubber and *C. albicans* was shown to bind to at least one, which was identified as parotid salivary protein (PSP).
The AFLP screen identified mutations specific to GPG strains in two genes likely to be involved in adhesion, *ALS7* and *PNG2*. *ALS7* belongs to a gene family involved in adhesion and *PNG2* is predicted to be an adhesin. Many alleles were found for both genes, due to variations in tandem amino acid repeats. Certain allele combinations, however, were associated with GPG strains, even though the repeat regions rapidly generate new alleles. Thus the association of specific allele combinations with the more pathogenic GPG strains must be due to selective pressure, indicating a role for these genes in pathogenicity.
Conclusions: Salivary SC and PSP promote *C. albicans* adhesion to epithelial cells and denture acrylic, respectively, but exogenously added anti-*C. albicans* IgA can reduce adhesion. A genetic approach independently identified putative adhesins, and particular alleles of these, as virulence factors.

S23IS2 - 0993

Sticking, sensing, starting signal relay and stress in the cereal pathogens *Blumeria graminis* and *Magnaporthe grisea*

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The rice blast fungus *Magnaporthe grisea* (*Mg*) and the barley powdery mildew fungus *Blumeria graminis* f sp *hordei (Bgh)* cause significant crop losses worldwide. Upon germination of the asexual conidium each pathogen forms a specialised infection structure, the appressorium, and subsequently breaches the host cuticle and cell wall to establish infection. Much of our work has focussed on studies looking at sensing, perception and signal transduction in *Bgh* (Hall et al. 1999; Pryce-Jones et al. 1999; Hall and Gurr 2000; Zhang and Gurr 2001, Zhang et al 2001, Wheeler et al 2003, Zhang et al 2004, Zhang et al 2005). However, the truly obligate biotrophic nature of *B. graminis* precludes easy gene functional analysis. Therefore, we chose more recently to use the genetically tractable and culturable rice blast fungus as a surrogate to study early signal exchanges between plant and pathogen.
Firstly, we shall allude to some recent work which shows that deletion of a secreted catalase affects virulence in *Mg* by compromising fungal wall integrity. Secondly, we were curious as to why the *Mg* genome (http://www.broad.mit.edu /annotation/fungi /magnaporthe) carries so many putative cutinase genes when the fungus is well-known for generating a huge appressorial turgor pressure to mechanically penetrate its host (Dixon et al. 1999)? We sought to unravel the role that cutinases play in adhesion, in host signal perception and in the triggering of fungal signal transduction. As a prerequisite to gene functional analysis, we used qreal-time-RT-PCR to finely profile the differential expression of cutinase genes during *Mg* germling differentiation. Such studies have provided us with a ranked order of cutinase genes to knock-out and we shall present the data associated with one such mutant.

S23IS3 - 0867

Investigating the structure-function relationships of the EAS hydrophobin

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Class I hydrophobins are a remarkable family of proteins, unique to filamentous fungi, that form a polymeric, water repellent monolayer on the surface of structures such as spores and fruiting bodies. This polymer film is comprised of cylindrical rodlets with dimensions of approximately 10 ¥ 100–250 nm. Hydrophobin monolayers are amphipathic and particularly robust, and reverse the wettability of the surface on which they are formed. By changing the surface properties of the fungal cell wall, making it extremely hydrophobic, hydrophobins function in the formation of aerial hyphae, fruiting bodies and spore dispersal. Hydrophobins have been implicated in the adhesion of hyphae and specialised penetration structures such as appressoria to the plant and insect cuticle. There are significant similarities between hydrophobin polymers and amyloid fibrils. For example, hydrophobins give the characteristic green/gold birefringence with Congo Red observed with amyloid fibres. However, structural information on these proteins and the rodlets they form has been elusive. We have recently solved the structure of the hydrophobin EAS from *Neurospora crassa* using high-field nuclear magnetic resonance. EAS forms a beta-barrel structure punctuated by several disordered regions and displays a complete segregation of charged and hydrophobic residues on its surface. This is consistent with its ability to form an amphipathic polymer. Using this structure and available biophysical data, we have been able to propose a model for the polymeric rodlet structure adopted by these proteins. X-ray fibre diffraction data from EAS rodlets are consistent with our model. Our data provide the first molecular insight into the nature of hydrophobin rodlet films. This will be discussed with regard to the function of rodlets.

S23PS1 - 0137

Invasive hyphal growth: an F-actin depleted zone is associated with invasive oomycete hyphae

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Tip growth, the mechanism by which oomycetes and fungi extend is characterised by localised extension at the hyphal apex. Models of tip growth have typically stated that it is driven by turgor pressure, the force of which when exerted on the cell wall physically breaks bonds between wall polymers. This would also, given substrate attachment, provide a protrusive force, which along with sufficient enzymatic substrate breakdown would enable hyphae to force their way through substrata (i.e. grow invasively). These models become problematic for the oomycetes, as these are unable to regulate and thereby increase turgor. How then might these organisms grow invasively?
An oomycete, in the absence of an ability to increase turgor could grow invasively because of an increase in tip yielding. Such an increase could arise though cell wall softening and through cytoskeletal rearrangement. In the oomycetes there is evidence suggesting that microfilaments reinforce the hyphal tip, thus a reduction of F-actin at the tips could, along with cell wall softening, increase tip yielding and play a role in invasive growth.
In view of the above a comparison has been made of F-actin staining patterns, levels of turgor and tip strength in invasive and non-invasive hyphae of two species of oomycete. Hyphae were grown either in or on agar and were chemically fixed using a combination of formaldehyde and methylglyoxal. After staining using Alexa Phalloidin they were observed using confocal microscopy. Turgor and tip strength were measured directly using a pressure probe.
In *Achlya bisexualis* an F-actin depleted zone was present in the tips of 70% of invasive but only 9% of non-invasive hyphae. In *Phytophthora cinnamomi* these figures are 74% and 20% respectively. Thus the F-actin depleted zone appears to be associated with invasive growth. Measurements of growth rate and TEM images suggest that this F-actin depleted zone is unlikely to represent areas of vesicle accumulation. Measurements of turgor indicate no significant increase under invasive conditions (0.65 MPa (invasive) and 0.63 MPa (non-invasive)). Similarly no difference was evident in burst pressures (1.04 MPa (invasive) and 1.06 MPa (non-invasive)), although surrounding agarose may lead to overestimates of invasive tip strength.
An F-actin depleted zone has the potential to increase protrusive force in the absence of turgor increases. Previous studies have concentrated on the role of changes to the cell wall in regulating tip yielding. Our findings are consistent with an additional level of control in the hyphal interior.

1600-1800

SYMPOSIUM 24 - *Protein Secretion in Fungal Biotechnology*

S24IS1 - 0675

Genome-wide analysis of secretion stress in *Aspergillus niger*.

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The genome sequences of several *Aspergillus* spp. are either already available or on their way. The secretion of proteins is a core component of the lifestyles of *Aspergillus* spp. and their capacity for efficient secretion has underpinned their commercial exploitation. The genome sequences will provide a resource for the discovery of new enzymes and might provide insight into the role of secreted enzymes in the pathogenicity of *A. fumigatus*. Genome analysis and the use of transcriptomic techniques is beginning to provide insight into the mechanisms of protein secretion and the cellular stress responses, including the unfolded protein response (UPR), that are induced when *Aspergillus* spp. are used as cell factories for high-level enzyme production. This presentation will focus on what has already been learnt from the *Aspergillus* genomes about the molecular basis of protein secretion and associated stresses, taking *A. niger* as the main focus. Repression under endoplasmic reticulum (ER) stress (RESS) will be discussed in addition to the UPR, as will transcriptomic studies in *A. niger* using Affymetrix GeneChips. Stress was induced either by chemicals (e.g. DTT or tunicamycin) added exogenously to cell cultures or by the secreted production of a heterologous protein. But, not all heterologous proteins elicit stress to the same extent and it is not known why some proteins are secreted well whereas others are poorly-secreted and induce the UPR. The predicted proteins encoded by most of the stress-induced genes function as part of the secretory system including chaperones, foldases, glycosylation enzymes, vesicle transport proteins, and ER-associated degradation proteins. Several genes were down-regulated under stress conditions and these included some genes that encode secreted enzymes. We use these data to both provide insight into the molecular basis of protein secretion and secretion-related stress in an effective protein-secreting fungus, and to identify target genes for manipulation in strain improvement strategies.
We acknowledge funding from DSM Food Specialities and the Biotechnology and Biological Sciences Research Council.

S24IS2

Genome-wide analysis and physiology of protein production

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Finland

No abstract available.

S24IS3 - 0834

Expression of a shark antibody using *Trichoderma reesei* as a heterologous host

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The increasing demand on the manufacture of therapeutic proteins has lead to the use of microbial expression systems for efficient production of the desired protein. The filamentous fungus *Trichoderma reesei* has been proven to be a powerful expression system for the synthesis of various heterologous proteins and their secretion to the culture medium. In this study, we have used a low protease strain of *Trichoderma reesei* for the production of a variable domain (VNAR) of shark IgNAR (Immunoglobulin new antigen receptor) single domain antibody from the Wobbegong shark (*Orectolobus maculatus*). These single antibody variable domains are used as binding proteins and possess a number of applications in human diagnostics due to their relatively small structure and stability. The shark variable domain VNAR 14M-15 (339 bp) along with a dual FLAG tag was expressed under the strong *cbh1* promoter of *T. reesei*. The expression vector also featured a Kex-like protease cleavage site and the truncated terminator of the *cbh1* gene of *T. reesei* to assure cleavage of the shark gene product. Secretion of the VNAR-FLAG tag fusion protein into the culture medium has been confirmed by Western blotting. The yield of secreted antibody seemed to be affected by the pH of the culture medium. Analysis of the shark-antibody producing transformants, characterisation of the recombinant gene product and scaling up the production will be discussed.

S24PS1 - 0352

Isolation and characterization of genes related to growth of *Lentinula edodes* on lignocellulose

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The edible shiitake mushroom, *Lentinula edodes* (Berk.) Pegler, is a widely cultivated mushroom highly appreciated with good flavour, nutrition and medicinal values with anti-tumor and anti-hypertensive properties. In addition, owing to its efficient degradation of lignocellulosic materials, it has been extensively applied in bioconversion of biological wastes, such as wood and synthetic dyes. However, with only a few identified lignocellulytic enzymes, current knowledge on the lignocellulytic system of *L. edodes* is very limited and is a target of our research efforts. We are isolating genes related to the lignocellulytic pathway. Genes have been cloned with conventional methods, such as RNA arbitrarily primed PCR (RAP-PCR) and expressed sequence tags (ESTs). However, these approaches are time-consuming and low in efficiency. We adopted a new sequencing method to isolate more genes in relatively shorter period of time. A cDNA pool was prepared from mycelium grown on lignocellulose. A large number of cDNA were sequenced using the pico-titered plate based genome sequencer (Roche). A total of 5894 contigs were obtained, about a hundred of which have lengths longer than 500bp, the others are 100-500 bases long. Several putative ligninolytic enzymes, including chloroperoxidase and laccase, and cellulytic enzymes, such as a and b-glucosidases, endo-1,3(4)-b-glucanase were identified. Full-length cDNA of These genes will be obtained, cloned and expressed in *Kluyveromyces lactis* to further characterize their functions. Other genes like superoxide dismutase, catalase, radical-activating enzymes and cytochrome P450 were also identified. These enzymes may play some protective roles as lignin degradation involves the production of reactive oxidative agents, such as hydrogen peroxides and free radicals. In addition to gene isolations, we will have a better understanding on the biochemistry and physiology of *L. edodes* growing on lignocellulose.

S24PS2 - 0125

Detection of extracellular proteases produced by ectomycorrhizal fungi

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In northern forest ecosystems, most soil nitrogen (N) is in organic form and forest trees are dependent on symbiotic ectomycorrhizal (ECM) fungi and their degradative abilities for N uptake. The ability of ECM fungi to acquire N from organic substrates should, therefore, be a widespread trait given its ecological importance. However, little is known about the degradative abilities of the dominant ECM fungi as most taxa are considered intractable. Here, we present data on extracellular protease production by 26 species of ECM fungi, the majority belonging to this category.

Protease activity was detected using either a fluorescently labelled protein (FITC-BSA) as a substrate or visualized using gel electrophoresis (zymograms) or milk powder plates by the breakdown of insoluble proteins.

The majority (24/26) of species produced detectable protease activity. However, detection was method dependent. In general, activity was too low to be detected by the FITC-BSA assay. Zymograms detected proteases in *Amanita muscaria, Russula chloroides, Lactarius deterrimus* and *L. quieticolor*. Growth on milk powder plates was the most effective method for detecting protease production by intractable ECM species.

The study supports the hypothesis that protease production is a widespread physiological trait in ECM fungi and that this ability is of considerable significance for nitrogen uptake in forest ecosystems.

S25IS1 - 0441

Quantifying the species composition, density, spatial extent, and longevity of *Rhizopogon* spore banks in pine forests.

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Rhizopogon (Boletales, Suillineae) is a large genus of ectomycorrhizal fungi that that is almost exclusively associated with members of the Pinaceae. Spores are produced in truffle-like fruiting bodies and dispersed primarily by mammal mycophagy. *Rhizopogon* spp. are often the dominant or exclusive mycorrhizal associates of pine seedlings in settings where pine is expanding into non-forested areas, or where forests are regenerating following disturbance. *Rhizopogon* achieves this dominance by storing spores in the soil. These spore banks are so dense that one can dilute soils over 100-fold into sterile soil and *Rhizopogon* will often colonize half of the pine seedlings planted.
We used pine seedling bioassays coupled with direct sequence to identify ITS groups of *Rhizopogon* from over 20 widely separated pine forests. Most sites yield 5 to 8 ITS groups. Significant longevity of the spores is suggested by the fact that some of the groups found in the spore bank are not found in the mature forest except as spores, but are known to be common associates of young trees following disturbance.
To examine the density of *Rhizopogon* spore banks we have used serial dilutions of known quantities of spores to quantify the number needed for effective colonization. We found that 10 to 200 spores/ml of soil are needed to colonize half of the pine seedlings in such assays. Relating these numbers back to the observed colonization rate from diluted forest soils we estimate that a minimum of approximately 1 X109 *Rhizopogon* spores/m3 exist in typical pine forest soils.
To examine the spatial extent of these spore banks we have used pine seedling bioassays to sample soil and mammal fecal pellets at various distances from pine forest borders. We found that spore dispersal by mice and voles from a young pine forest decreases logarithmically from a forest edge and becomes undetectable between 100 and 200 meters from the edge. This margin coincides well with the detectable limits of the soil spore bank. However, further dispersal by deer occurs and may produce spottier and less predictable spore concentrations at greater distances.
To estimate longevity of spores we buried soil of known spore concentration and viability, and retrieved and assayed the samples annually. Over a three year period none of the three species showed any detectable lost in viability.
These results show that *Rhizopogon* species are well adapted to periodic disturbance cycles characteristic of pinaceous ecosystems, and that they play an integral role in pine reestablishment and expansion in these systems.

S25IS2 - 0185

Coevolution of plants and pathogens in a metapopulation context

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Plant-pathogen co-evolutionary processes occur across a range of spatial scales – from individual host plants, to the full geographic extent of associations. Across such spatial scales varying conditions generate a mosaic of environmental patches in which pathogen development is variously favoured or suppressed. This variation interacts with basic genetic processes to affect extinction, recolonization and selection thereby resulting in a complex patchwork of local host and pathogen populations with varying degrees of relatedness. At larger spatial scales encompassing multiple metapopulations, differences in pathogen selective pressures and host responses may lead to quite different coevolutionary trajectories. This may take the form of different resistance and/or pathogenicity mechanisms being favoured in different regions; or even the first steps in a process of speciation. The consequences of the interaction of these evolutionary processes are examined by reference to a range of studies covering a diversity of different associations with a range of different life-history features.

S25IS3 - 0654

Endobacteria and arbuscular mycorrhizal fungi: symbiosis in evolution?

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Arbuscular mycorrhizal fungi are obligate plant biotrophs which have been found to exist for more than 400 million years in symbiosis with land plants. A long coevolutionary history has therefore shaped this association which is the most widespread mycorrhizal type. By contrast, little is known on endobacteria which live inside some AM fungi. In *Gigaspora margarita* BEG 34 a homogenous bacterial population of about 20000 cells is hosted inside the fungal spore and is vertically transmitted through fungal spore generations. A protocol based on monosporal inocula both in pot and *in vitro* conditions caused a dilution of the population of *Candidatus* Glomeribacter gigasporarum, eventually leading to bacteria-cured spores. Molecular and morphological approaches demonstrate that the cured spores represent a stable variant of the original wild type genotype, showing differences in their cytoplasm and cell wall organization. Irrespectively of the phenotype, the cured spores were not affected in their mycorrhizal capacities both in confined and large scale experiments. A detailed analysis of many isolates belonging to Gigasporaceae and originating from diverse geographical areas consistently revealed the presence of endobacteria phylogenetically related to *Candidatus* G. gigasporarum. Analysis of ribosomal genes demonstrated that the bacterial and fungal phylogenetic trees are congruent, suggesting the presence of a coevolution mechanism. A cellular and molecular investigation of the bacterial cell cycle as well as the expression of the FtsZ gene revealed a relationship between the bacterial divisions and the symbiotic status of the fungus, suggesting an intimate symbiosis between the partners. In conclusion, our investigation demonstrates that – at least in some cases- mycorrhizal roots represent a tripartite association resulting from the interaction of plant, fungal and bacterial genomes. They offer an excellent example of multiple symbiotic events, and open many questions on the evolutionary dynamics of the three partners.

S25PS1 - 0632

Leaves and lichens are cradles of fungal diversification

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Fungal interactions with photosynthetic organisms are ancient and ubiquitous. Most fungi are known because of the fruit bodies and/or symptoms they produce (e.g., plant pathogens, mycorrhizal fungi, animal parasites, saprotrophs) or by their emergent properties as symbionts (lichen-forming fungi). However, the majority of fungal diversity occurs among cryptic fungi that rarely manifest their presence with reproductive structures or visual cues on the substrate they colonize, including endophytic fungi (fungi that live within plant tissues without causing disease). Despite their virtual ubiquity in symptomless leaves and growing evidence regarding their ecological importance, the diversity of endophytic fungi and their ecological distinctiveness relative to pathogens and saprotrophs has not been resolved. To examine the diversity of fungi capable of forming endophytic and endolichenic symbioses, we cultured microfungi from the interior of asymptomatic plant and lichen hosts in four bioclimatic zones. To assess the evolution and stability of major ecological modes in the Ascomycota, we reconstructed phylogenetic relationships of saprotrophs, pathogens, lichen-forming mycobionts, and previously unexamined fungi living within asymptomatic lichens (endolichenic fungi), and endophytic fungi based on the nuclear ribosomal small and large subunits (Bayesian methods) and ancestral states for each ecological mode using maximum likelihood (Mesquite). Our results demonstrate for the first time the tremendous diversity of foliar endophytic and endolichenic fungi, and the latitudinal structure underlying their diversity. Infection frequencies and diversity increased significantly from boreal and arctic sites to the tropics. We show that endolichenic and endophytic fungi represent phylogenetically diffuse symbioses that have diversified explosively relative to the better-known lichen-forming fungi. Among the euascomycetes (Pezizomycotina), transitions to saprotrophism appear largely inescapable, endolichenism is an incubator for transitions to endophytism, and endophytism represents a dynamic, transient state with frequent transitions to and from pathogenicity.

Wednesday 23rd August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	Discussion Group *MR 1 & 2* **Lichen-Fungal Genome Sequencing Project** Paul Dyer (UK)	*Hall C* **Australasian Mycological Society AGM**	Discussion Group *MR 3 - 5* **Fungal Genome Sequencing Programs at the DOE Joint Genome Institute** Scott Baker (USA)		
09:30	**Break**				
10.00	**Plenary 3:** James Galagan (USA) **Comparative Fungal Genomics** *Halls A & B*				
11:00	**Coffee Break** – *Hall 2*				
11:30	Symposium 26 *Halls A & B* **Lichen Symbiosis: Extraterrestrial Life, Evolution and Penguin Rookery** Francois Lutzoni (USA) Magdalena Pavlich (Peru)	Symposium 57 *MR 3 - 5* **Chytridiomycete Fungi** Gordon Beakes (UK) Peter McGee (Australia)	Symposium 28 *MR 1 & 2* **Enzymes and Infection Mechanisms** Michel Monod (Switzerland) Matt Templeton (New Zealand)	Symposium 29 *Hall D* **Fungal Physiology** Helena Nevalainen (Australia) Ken Hammel (USA)	Symposium 30 *Hal* **Asia-Pacific Fungal Biodiversity** Gareth Jones (Thailand) Sitti Aisyah Alias (Thailand)
13.30	**Lunch [pre purchase]** – *Hall 2*				
14.00	**Poster Session 3: Plant and Fungal Pathogens** **Poster Session 10: Animal Pathogens**				
15:00	**Coffee Break** – *Hall 2*				
15:30	Symposium 31 *MR 3 - 5* **Systematics and Ecology of Dimorphic Basidiomycetes** Alvaro Fonseca (Portugal) Jose Paulo Sampaio (Portugal)	Symposium 32 *MR 1 & 2* **Bioinformatics and Databases** Vincent Robert (Netherlands) Peter Dawyndt (Belgium)	Symposium 33 *Hall D* **Antifungal Resistance** Richard Cannon (New Zealand) Dominique Sanglard (Switzerland)	Symposium 34 *Hall C* **Importance of Small Non-Coding RNAs in Fungi** Carlo Cogoni (Italy) Rodolfo Aramayo (USA)	Symposium 35 *Halls A* **Gondwanan Fungi** Tom May (Australia) Peter Buchanan (New Zealand)
17:30	**"Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
18:00	*Hall 2* **Poster Session**	**Roundtable 2** *Hall C* **Access and benefit sharing in relation to the Biodiversity Convention** Lene Lange (Denmark)		*MR1&2* 17:45-19:00 **Meeting of the International Association Lichenology**	
20:00	**Wines of the World** *Hall 2*				

0800-0930 Meeting Room 1&2

Discussion Group: Lichen-Fungal Genome Sequencing Project

Paul S. Dyer, Peter D. Crittenden, David B. Archer (University of Nottingham, UK).

For pre-congress enquiries please contact: e-mail: paul.dyer@nottingham.ac.uk

Funding has recently been provided by the US Department of Energy (DoE) JGI scheme to allow genome sequencing of the lichen-forming fungus *Xanthoria parietina*. This will be the first lichen fungus to be sequenced. Forty two per cent of all known ascomycetes, and 19% of all known fungi, are lichen-forming and thus lichens constitute a major component of biodiversity in the fungal kingdom. *Xanthoria parietina* was chosen as a model organism to represent lichen-forming fungi because it has a wide distribution, a typical thallus morphology, is amenable to axenic cultivation and is one of the most commonly-studied lichenised fungi. It is anticipated that genome analysis will provide insights into the genetic basis of biological phenomena such as mutualistic symbiosis, adaptation to harsh environments, secondary metabolism and control of growth rate. The genome project is being co-ordinated by researchers at the University of Nottingham (UK) and the US DOE Joint Genome Institute.

A discussion group session will be held at IMC8 for interested researchers. Particular aims are to:

Provide an update of progress concerning the genome project with likely timelines.

We aim to establish an international consortium of scientists to help with genome annotation and contribute to an arising genome paper. Interested parties are invited to contribute ideas for areas to be investigated, and to become part of the consortium. Areas of expertise might include knowledge of genes involved in mutualistic symbioses, secondary metabolism, signalling, nutrient acquisition, control of growth rate and development etc.

Consider further exploitation of the genome sequence and possibility of future genomic technologies.

0800-0930 Meeting Room 1&2

Australian Mycological Society AGM

0800-0930 Meeting Room 3-5

Discussion group: Fungal Genome Sequencing Program of the DOE Joint Genome Institute

1000-1100 - 1017 Halls A&B

Plenary 3: Comparative Fungal Genomics

James Galagan (USA)

1130-1330 Halls A&B

Symposium 26: Lichen Symbiosis: Extraterrestrial life, Evolution and Penguin Rookery

Chairs: Francois Lutzoni (USA) / Magdalena Pavlich (Peru)

1130-1200 IS1 - 0713

Lichens Survive in Space

Leopoldo G. Sancho (Spain)

1200-1230 IS2 - 0743

Are symbionts less diverse than their hosts? - Insights from molecular systematics

Heath O'Brien (USA)

1230-1250 PS1 - 0292

Evolution of polyketides synthases in lichens

Lucia Muggia (Austria)

1250-1310 PS2 - 0722

Lichen nitrogen and phosphorus relationships in the vicinity of a penguin rookery

Peter D. Crittenden (UK)

1310-1330 PS3

European phylogeography of the epiphytic lichen Lobaria pulmonaria

Ivo Widmer (Switzerland)

1130-1330 Meeting Room 3-5

Symposium 57: Chytridiomycete Fungi

Chairs: Gordon Beakes (UK) / Peter McGee (Australia)

The uniflagellate chytrids are ancestral to the terrestrial fungal lineages. Molecular methodologies are leading to a fundamental re-evaluation of the phylogeny and taxonomy of these organisms. Traditionally they were considered to be benign saprotrophs of little economic importance. As well as providing the key to our understanding of the evolutionary origins of the all fungi, it is now realized that some species play a key role in the rumen ecosystem and at least one species, *Batrachochytrium* is a devastating pathogen of amphibians. This symposium will explore chytrid diversity and taxonomy and their roles of in the natural environment.

1130-1200 IS1 - 0950

A short history of nearly everything (about chytrids).

Gordon Beakes (UK)

1200-1230 IS2 - 0919

A new killer on the block - Batrachochytrium dendrobatidis***: a chytrid parasite of amphibians.***

Lee Berger (Australia)

1230-1300 IS3 -

Chytrid physiology – nutritional studies on soil chytrids.

David Midgely (Australia)

1300-1330 IS4 - 0914

Diversity and phylogeny of chytrids in Taiwan

Shu-Fen Chen (Taiwan)

1130-1330 Meeting Room 1&2

Symposium 28: Enzymes and Infection Mechanisms

Chairs: Michel Monod (Switzerland) / Matt Templeton (New Zealand)

Fungal pathogens of humans and plants face the same challenges. They must overcome various active and preformed defenses and extract nutrients from their hosts to be successful pathogens. Enzymes play crucial roles in these events. This symposium presents a rare opportunity to compare the mechanisms that plant and human pathogens use to successfully colonize and grow on host tissue.

1130-1200 IS1 - 0922

Secreted proteases from human pathogenic fungi

Michel Monod (Switzerland)

1200-1230 IS2- 0687

Licensed to kill: the role of phytotoxic proteins and pectinases in virulence of Botrytis cinerea

Jan van Kan (The Netherlands)

1230-1300 IS3- 1014

Key enzymes for iron uptake and hyphal morphogenesis in C. albicans infection

Yue Wang (Singapore)

1300-1315 PS1- 0455

Identification of effector genes in the apple scab fungus, Venturia inaequalis

C Mesarich (New Zealand)

1315-1330 PS2- 0458

Genomic approaches to isolating pathogenicity factors from the apple black spot pathogen Venturia inaequalis

Matt Templeton (New Zealand)

1130-1330 Hall D

Symposium 29: Fungal Physiology

Chairs: Helena Nevalainen (Australia) / Ken Hammel (USA)

The symposium will cover new physiological aspects of primary metabolism and its regulation in fungi from different habitats (endophytes, plant pathogens, saprophytes and basidiomycetes).

1130-1153 IS1 - 0768

A role for hydroquinone-driven Fenton chemistry in incipient wood decay by the brown rot basidiomycete Gloeophyllum trabeum

Ken Hammel (USA)

1153-1216 IS2- 1016

Extracellular enzymes involved in litter degradation by basidiomycetes

Kari Steffen (Germany)

1216-1239 IS3- 0149

Mannitol metabolism in Stagonospora nodorum

Peter Solomon (Australia)

1239-1302 PS1- 0670

Rapid, reversible motor response of fungi to blue, green and ultraviolet light

David Nehl (Australia)

1302-1325 PS2- 0808

Interactions between endophytic fungi and pests and pathogens of plants, a physiological view

Peter McGee (Australia)

1130-1330 **Hall C**

Symposium 30: Asian-Pacific Fungal Biodiversity

Chairs: Gareth Jones (Thailand) / Sitti Aisyah Alias (Malaysia)

Various estimates exist as to the world number of fungi, ranging from 1.5 to 9 million. While this may seem academic to many, the fact remains that only a small percentage of fungi has been so far documented. Many will challenge these figures as unrealistic. So what progress has been made since Hawksworth proposed the 1.5 million estimated number of fungi? Clearly different countries are further advanced than others in the documentation of their fungal diversity. Japan and China list 14,000 and 10,000 fungi respectively, while no figure is available for Indonesia. Figures for Malaysia and Thailand stand at 2,000 and 6,000 species, respectively (Jones and Hyde, 2004). What are the major problems facing Asian Pacific countries in the documentation of their fungal diversity? This symposium will focus on this issue.

1130-1200 IS1 - 0268

Progress in the documentation of Asian fungal diversity

Gareth Jones (Thailand)

E.B.Gareth Jones

1200-1230 IS2 - 0726

Advances in our understanding of fungal diversity - and Asian perspective

Lam Minh Duong (Thailand)

1230-1300 IS3 - 0689

Fungal diversity 'down under'

Eric H.C.McKenzie (New Zealand)

1300-1315 PS1 – 0265

On the diversity of Isaria species from Thailand

Jennifer Luangsa-ard (Thailand)

1315-1330 PS2 – 0503

Macrofungal diversity in the Cryptomerioid japonica plantations in Taiwan

Pi-Han Wang (Taiwan)

1400-1530

Poster Session 3: Plant and Fungal Pathogens

Poster Session 10: Animal Pathogens

1530-1730 **Meeting Room 3-5**

Symposium 31: Systematics and Ecology of Dimorphic Basidiomycetes

Chairs: Alvaro Fonseca (Portugal) and Jose Paulo Sampaio (Portugal)

Many fungi belonging to the three major lineages of the Basidiomycota have a unicellular vegetative growth form in their life cycles. These yeast-producing basidiomycetes are often called dimorphic. In sexual taxa, the yeast phase is generally haploid and gives rise to the dikaryotic hyphal phase upon conjugation of compatible cells. Sexual structures are formed in the mycelial stage, which may or may not be contained in a fruiting body, and germination of basidiospores restores the yeast stage. Many dimorphic basidiomycetes are plant parasites (e.g. most smuts such as *Ustilago maydis*), others are mycoparasites (e.g. members of the genera *Occultifur* and *Tremella*), and others appear to be exclusively saprobic (e.g. conventional basidiomycetous yeast genera such as *Rhodosporidium* or *Cystofilobasidium*). The widespread use of DNA sequence analyses in basidiomycete systematics over the last 10 years has led to a much desired coming together of fungal and yeast systematists. This symposium is intended to reveal some of the novel insights provided by recent research efforts in the areas of systematics and ecology of dimorphic basidiomycetes.

1530-1600 IS1 - 0755

Inter- and intra-species diversity of ballistoconidium-forming yeasts and related taxa

Masako Takashima (Japan)

1600-1630 IS2 - 0780

Mycosporine synthesis in dimorphic basidiomycetes - ecological and phylogenetic implications

Diego Libkind (Argentina)

1630-1700 IS3 - 0587

Are there eurybionts among the dimorphic basidiomycetes?

Andrey Yurkov (Russia)

1700-1715 PS1 - 0945

Dimorphic basidiomycetes: new perspectives for an old group

José Paulo Sampaio (Portugal)

1530-1730 **Meeting Room 1&2**

Symposium 32: Bioinformatics and Databases

Chairs: Vincent Robert (The Netherlands) / Peter Dawyndt (Belgium)

The symposium will host a series of talks related to some of the latest software and database developments in fungal genomics, identification or biodiversity.

1530-1600 IS1 - 0789

Bioinformatics for phylogenomics

Vincent A. Robert (The Netherlands)

1600-1630 IS2 - 1001

StrainInfo.net bioportal: an application of semantic web technologies for scaleable workflow management of microbial information"

Peter Dawyndt (Belgium)

1630-1700 IS3 - 0072

The Global Biodiversity Information Facility: data, products and services

Meredith A. Lane (Denmark)

1700-1715 PS1 - 0168

Approaching the taxonomic affiliation of unidentified sequences in public databases – an example from the mycorrhizal fungi

Henrik Nilsson (Sweden)

1715-1730 PS2 – 0374

Polyphasic identification of Phaeoacremonium species

Lizel Mostert (South Africa)

1530-1730 Hall D

Symposium 33: Antifungal Resistance

Chairs: Richard Cannon **(**New Zealand) / Dominique Sanglard (Switzerland)

The resistance of fungi to antifungal agents has important consequences for the treatment of humans with fungal infections and for the control of fungal agricultural pests. A major mechanism of antifungal resistance is the over-expression in fungal membranes of drug efflux pumps. This symposium will examine the role of efflux pumps in human fungal disease, the transcriptional control of drug efflux pump expression, structure/function analyses of ABC efflux pumps and the identification of efflux pump inhibitors.

1530-1600 IS1

Transcriptional regulation of drug resistance genes in yeast pathogens

Dominique Sanglard (Switzerland)

1600-1630 IS2 - 0783

Overcoming the efflux-mediated drug resistance of human fungal pathogens

Richard Cannon (New Zealand)

1630-1700 IS3 - 0908

The antifungal effect on human and plant pathogenic fungi by regulating stress response signaling

Kaihei Kojima (USA)

1700-1730 PS1 - 0644

Involvement of catalase activitiy and sensitivity to fungicides in Mycosphaerella fijiensis

Miguel Beltran-Garcia (Mexico)

1530-1730 Hall C

Symposium 34: Importance of Small Non-Coding RNAs in Fungi

Chairs: Carlo Cogoni (Italy) / Rodolfo Aramayo (USA)

Fungi, as other eukaryotes, react to double stranded RNA (dsRNA) molecules by processing them into short interfering RNAs that in turn are able to direct sequence sequence-specific mRNA degradation. The expression of dsRNA, from constructs containing an inverted repeat, is nowadays widely used for functional gene knock-out, especially in fungi in which gene disruption by homologous integration has proven to be inefficient. Interestingly, fungi appear to possess a variety of RNA silencing pathways that can be activated not only by the direct expression of dsRNA but also as a consequence of either the presence of transgenic repeats (quelling in Neurospora and related phenomena) or unpaired DNA during meiosis (MSUD, Meiotic Silencing by Unpaired DNA). This suggests that in fungi small RNAs can have an important role in the protection of the genome against invasive elements, in genome stability and speciation.

1530-1600 IS1 - 0761

Two dicer-like proteins in Magnaporthe oryzae

Hitoshi Nakayashiki (Japan)

1600-1630 IS2 - 0667

RNA interference machinery and function in the genus Aspergillus

Nancy P. Keller (USA)

1630-1700 IS3

Meiotic Silencing in Neurospora

Rodolfo Aramayo (USA)

1700-1715 PS1 – 0277

RNA Silencing Approach In The Rice Blast Fungus, Magnaporthe oryzae, Using An Opposing Promoter System

Bao Quoc Nguyen (Japan)

1530-1730 **Halls A&B**

Symposium 35: Gondwanan Fungi

Chairs: Tom May (Australia) / Peter Buchanan (New Zealand)

The supercontinent of Gondwana gave rise to South America, Africa, Antarctica, India, Australia, New Guinea and New Zealand. The shared geological history of these areas is reflected in the distributions of plants such as Nothofagus and the Proteaceae and Myrtaceae. This symposium explores the significance of geography and hosts for relationships among fungi occurring in the land masses derived from Gondwana. There is a focus on the Southern Hemisphere, but links between Southern Hemisphere and Northern Hemisphere fungi are also included.

1530-1600 IS1 - 0430

Biogeography and host preference of austral members of Laccaria – Hydnangium, a model clade of e ctomycorrhizal fungi

Gregory M. Mueller (USA)

1600-1630 IS2 - 0138

Where are New Zealand's ancient fungi?

Peter R. Johnston (New Zealand)

1630-1700 IS3 - 1015

Divergence time between Gondwana mushrooms and their northern hemisphere relatives

Jean-Marc Moncalvo (Canada)

1700-1715 PS1 – 0824

The rust mycobiota of South Africa: composition, relationships and distribution

Reinhard Berndt (Switzerland)

1715-1730 PS2 – 0241

Pacific boletes: implications for biogeographic relationships

Roy Halling (USA)

1800-2000

Poster Session

1800-2000 **Hall C**

Roundtable 2: Access and Benefit Sharing in Relation to the Biodiversity Convention - How do mycologists comply with the international Biodiversity Convention?

Chair: Lene Lange (Denmark)

Since 1993 all access and transfer of biological materials has been regulated by the international CBD charter: Biodiversity Convention. In order to be in compliance with CBD one must obtain prior informed consent (PIC) from the proper authority in the country from where the biological materials will be collected and transferred from. Such PIC should usually be accompanied by a contract under mutually agreed terms (MAT), in which is states the conditions under which the materials has been accessed and transferred. If any commercial or commercial purpose work is to take place the contract should include statements about how to implement the wording of CBD: equity in benefit sharing. The CBD has primarily been in focus for industries who wanted to access materials for their bioprospecting work. However, the CBD also includes transfer done for scientific purpose only. This is especially relevant when it happens that materials, collected for scientific purpose ends up in discovery of interesting compounds and such compounds end up being offered to industries for their evaluation. The many companies who are working hard to be in full compliance with CBD are saying no thank you to all materials which have not been accessed according to the ruels and regulations stated in the CBD Bonn declaration on access and benefit sharing. How can we in the scientific community do better? We must find a way which does not stop the scientific studies but which also are in compliance with international agreements of good, proper and fair behaviour.

Program:

1. Lene Lange, Denmark: CBD, current status on access and benefit sharing
2. Keith Seifert, US: how can mycologists be in compliance with CBD
3. Morakot, Thailand: how to establish scientific collaboration between universities, institutes and companies
4. Rob Samson, NL: International Culture Collections view on the Biodiversity Convention
5. Lene Lange: Industries approach to best practice regarding compliance with CBD

Discussion (all)

1745-1900 **Meeting Room 1&2**

Meeting of the International Association Lichenology

2000-2100 **Hall 2**

Wines of the World

1130-1330
SYMPOSIUM 26 - ***Lichen Symbiosis: Extraterrestrial life, Evolution and Penguin Rookery***

S26IS1 - 0713
Lichens survive in space
LG Sancho, R de la Torre, G Horneck, C Ascaso, A de los Rios, J Wierzchos, A Pintado
1 UCM, Madrid, Spain, 2 INTA, Madrid, Spain, 3 DLR, Köln, Germany, 4 CSIC, Madrid, Spain, 5 CSIC, Madrid, Spain, 6 University of Lleida, Lleida, Spain, 7 UCM, Madrid, Spain

Many lichen species are regarded as extremophiles in terms of temperature, radiation and desiccation. In high mountains and Polar Regions lichens are well adapted to long term desiccation, temperatures between -40ºC and 60ºC and high radiation including UV. It has been proved that stored at low temperatures dry lichens can recover after 10 years of inactivity and that they do survive after immersion in liquid nitrogen. Therefore, lichens have been previously proposed, together with unicellular algae and bacteria, as the living system most likely to resist the extreme conditions of outer space. This enables, following the "panspermia" theory, speculation about the possibility of life transfer between Earth and other planets. The experiment LICHENS was aimed at establishing, for the first time, the survival capability of these organisms exposed to space conditions. In particular, the damaging effect of solar UV was studied under various protecting conditions. The lichens used were the bipolar species *Rhizocarpon geographicum* and *Xanthoria elegans* that were collected above 2000m in Spanish mountains, and endolithic lichens inhabiting granite rocks in the Antarctic Dry Valleys. Lichens were exposed to space in the BIOPAN-5 facility of the European Space Agency located at the outer shell of the Russian Earth orbiting FOTON M2 satellite. Launching was the 31th of Mai 2005 from Baikonur, landing sixteen days later. After about two weeks in space their survival was tested. Chlorophyll fluorescence was used for the measurement of photosynthetic parameters. Scanning Electron Microscopy in back- scattered mode and Transmission Electronic Microscopy was applied to study the organization and composition of the both symbionts. Confocal Laser Scanning Microscope, in combination with the use of specific fluorescent probes allowed the assessment of the physiological state of cells. All exposed lichens, independently of the filters used, showed after the flight nearly the same photosynthetic activity as measured before the flight. Likewise, the multimicroscopy approach has revealed the lack of ultrastructural changes in the algal and fungal cells of these lichen thalli. These findings suggest that lichens could stay alive in space even completely exposed to massive UV and cosmic radiation, which have been proved being lethal for bacteria and other microorganisms. Lichen upper cortex seems to provide adequate shield against solar radiation UV. Moreover, after extreme dehydration induced by high vacuum, lichens proved to be able to recover their metabolic activity in a remarkable short time.

S26IS2 - 0743
Are symbionts less diverse than their hosts? Insights from molecular systematics.
H. E. O'Brien, F. M. Lutzoni
Department of Biology, Duke University, Durham, NC, United States

In a seminal 1983 paper (Biol. Jour. Linn. Soc. 20:249-76), R. Law and D. H. Lewis observed that organisms living enclosed within a mutualistic partner (inhabitant) tended to be less diverse then their hosts (exhabitant) and that genetic exchange was less common than in related free-living taxa. This was taken as evidence for asymmetric selective pressures between the partners. These asymmetries were particularly striking for fungal symbioses. In lichens, where the fungal partner forms the exhabitant, 370 genera of fungi were reported to associate with 30 algal and cyanobacterial genera, while 100 genera of mycorrhizal fungi, which were considered inhabitants, were associated with 11,000 genera of plants. It has been pointed out that these results are biased because the morphological complexity of exhabitants provides a much richer source of taxonomic characters and because sexual exchange is often cryptic in microorganisms. Recently, there has been a great deal of interest in using molecular systematics to reassess the diversity of inhabitants and to quantify the amount of cryptic sexuality in a wide range of mutualistic symbioses. The symbiosis between the lichen forming fungi and cyanobacteria provide an example of the power of this approach, as well as some of the limitations. The cyanobacterial partners in all lichens examined were members of a lineage that also includes symbionts of several plant groups and some free-living *Nostoc* strains, which is consistent with the patterns found by Law and Lewis. However, the diversity of cyanobacterial strains associated with each lichen species varied considerably, with some species always found with the same cyanobacterial genotype and others associated with strains throughout the lineage. There was also evidence for genetic exchange among cyanobacterial strains, though the population structures were predominantly clonal. These results will be presented in the context of studies of green algal lichens, mycorrhizal fungi, and other mutulistic symbioses to argue that the outcomes of evolution in a mutualistic environment vary on a case-by-case basis and cannot be predicted *apriori*.

S26PS1 - 0292

Evolution of polyketides synthases in lichens

Lucia Muggia1, Imke Schmitt 2, Juliane Blaha 1, Julia Rankl 1, Martin Grube 1
1 Institute for Plant Science, Karl-Franzens University, Graz, Styria, Austria, 2 The Field Museum, Department of Botany, S. Lake Shore Drive, Chicago, United States

The patterns of compounds produced by lichens have been used since long as chemical characters in systematics, but little effort has so far been made to characterise the corresponding genes. As polyketide synthases are responsible for the production of hundreds of secondary polyketide compounds in lichens, a phylogenetic analysis of the essential ketoacyl synthase domain of polyketide synthase (PKS) genes in different families of lichenized fungi is here presented, focusing on genes that are putatively responsible for non-reduced polyketides. The analysis reveals a high level of paralogy in mycobiont PKS genes. In many lichen species, more than one paralogous gene was detected in the genome by analyses of cloned PCR products, and further paralogs can also be detected using specifically designed primers. The application of codon substitution models in likelihood analyses reveal that most of the codon sites of paralogs undergo purifying selection, indicating functional genes, whereas the strength of purifying selection may vary among the paralog clades. We approach the question of the function of paralogs by isolation and analysis of the mRNA pool from either the lichen thallus or the cultivated mycobiont.

S26PS2 - 0722

Lichen nitrogen and phosphorus relationships in the vicinity of a penguin rookery

P.D. Crittenden 1, M.R. Theobald 2, Y.S. Tang 2, C.M. Scrimgeour 3
1 University of Nottingham, Nottingham, United Kingdom, 2 Centre for Ecology and Hydrology, Penicuik, Penicuik, Midlothian, United Kingdom, 3 Scottish Crops Research Institute, Dundee, United Kingdom

Penguin rookeries provide pathways by which nutrients of marine origin enrich oligotrophic Antarctic terrestrial environments and are associated with distinctive lichen communities. However, the spatial extent to which emissions from rookeries can influence the terrestrial biota remains unclear. At Cape Hallett (Borch Grevink Coast, Ross Sea, 72° 19´S 170° 16´E) c. 100000 Adélie penguins breed in a single rookery. The lichens *Usnea sphacelata*, *Umbilicaria decussata* and *Xanthoria mawsonii* were sampled at different distances from the rookery and analyzed for total N and P, ‰15N and surface phosphatase activity. Ammonia concentrations in air were also measured at different distances from the rookery and ‰15N values in NH3 compared with those in lichens to estimate N capture from penguin-derived N. The results suggest that nutrient relationships in *U. sphacelata* and *U. decissata* contrast markedly with those in *X. mawsonii* and also indicate that the rookery influences lichen physiology within a radius >10 km.

S25PS3

European phylogeography of the epiphytic lichen Lobaria pulmonaria

I Widmer
Switzerland

No abstract available.

1130-1330

SYMPOSIUM 57 - *Chytridiomycete Fungi*

S57IS1 - 0950

A brief history of nearly everything – about chytrids.

G.W. Beakes
University of Newcastle, Newcastle upon Tyne, United Kingdom

The posteriorly uniflagellate chytridiomycete fungi have always seemed at the periphery of mainstream mycology. At the time of the IMC1 in Exeter in 1971, most mycologists would have considered the holocarpic chytrids to be a group of entirely benign organisms, occurring as saprotrophs or at worst mild necrotrophic parasites of algae, plants and invertebrates. Thanks to Ed Cantino, *Blastocladiella emersonii*, had become chosen model organism in which to follow fungal development at the biochemical and cellular level, and was one of the first fungi on which molecular studies were undertaken. The zoosporic fungi were also much more amenable to conventional EM fixation than most terrestrial fungi, and some of this pioneering ultrastructural work was also show-cased at IMC1.
However at the last IMC meeting in Oslo there were no more than a handful of presentations on chytrid fungi. Yet, in the intervening three decades chytrids had been demonstrated to include the only true facultatively anaerobic fungi, which played a key role in the rumen ecosystem. Others were shown to be obligate biotrophic pathogens of algae which could significantly influence the course of algal blooms. Even more surprisingly, in the late 1990's a chytrid was identified as the mystery pathogen responsible for the death of frogs and other amphibians worldwide.
Recent molecular phylogenetic studies have clearly showed that chytrids are not protists, but true fungi which form the basal lineage to the whole Kingdom. The intention of this presentation is to give a brief overview of the diversity and importance of this fascinating ancestral group, drawing upon the authors own experiences working with chytrid parasites of planktonic diatoms and nematodes and soil and rumen chytrids.

S57IS2 - 0919

A new killer on the block - *Batrachochytrium dendrobatidis*: a chytrid parasite of amphibians

L Berger, R Speare, L Skerratt
James Cook University, Townsville, Queensland, Australia

Batrachochytrium dendrobatidis, the first chytrid to be found causing disease in vertebrates, causes the most devastating disease of wildlife on record. Amphibian chytridiomycosis has spread worldwide causing amphibian population declines and extinctions in pristine habitats since the 1970's, including the extinction of at least 4 species in Queensland's rainforest. The uniqueness of this pathogen added to the difficulty of investigating mortality in wild frogs, and chytridiomycosis was not reported until 1998. In the last 10 years there has been a huge increase in research effort. *B. dendrobatidis* lives within cells of the outer epidermal layers of frog skin, but grows well on tryptone agar. Resting spores have not been found and it is susceptible to heat and desiccation. Transmission experiments have confirmed its pathogenicity to a range of amphibian species, although many amphibians are resistant. Tadpoles do not die from infection but can be carriers. Cooler temperatures promote disease outbreaks. A sensitive PCR test has been developed for diagnosis. Amphibian surveys for *B. dendrobatidis* to map the distribution and understand its epidemiology are underway. We are also attempting to detect saprobic growth in the environment and to determine its pathogenesis and how it kills frogs. We would welcome collaborators who could assist with these topics.
For detailed information on chytridiomycosis see the Amphibian Disease Home Page:
http://www.jcu.edu.au/school/phtm/PHTM/frogs/batrachochytrium.htm

S57IS3

Chytrid physiology – nutritional studies on soil chytrids

D Midgely, *Australia*

No abstract available.

S57IS4 - 0914

Diversity and phylogeny of chytrids in Taiwan

Shu-Fen Chen, Hung-Du Lin, Tzen-Yuh Chiang, Chiu-Yuan Chien
1 Department of Health and Nutrition, Chia Nan University of Pharmacy and Science, Tainan, Taiwan, 2 Department of Life Science, Cheng-Kung University, Tainan, Taiwan, 3 Department of Life Science, Cheng-Kung University, Tainan, Taiwan, 4 Institute of Life Science, National Taiwan Normal University, Taipei, Taiwan

Studies on the fungal flora of chytrids in Taiwan have been carried out by authors since 1992. A microscopic exermination of more than 320 collections revealed 15 genera, *Allochytridium, Asterophlyctis, Catenochytridium, Chytriomyces, Cladochytrium, Diplochytridium, Entophlyctis, Gaertneriomyces, Phlyctochytrium, Polychytrium, Powellomyces, Rhizidium, Rhizophlyctis, Rhizophydium* and *Spizellomyces* – represented by 43 species. Among these 12 genera and 40 species of Chytridiales and Spizellomycetales have been reported for the first time in Taiwan. Zoospore ultrastructure are used as the principal characters of family and genus levels of uncertain species of chytrids. The isolated strains and pure cultures are offered to the phylogenetic studies. Small subunit ribosomal DNA (18S rDNA) sequence analysis has proven effective in resolving phylogenetic relations among Chytridiomycota. A molecular phylogeny of a group of *Rhizophydium* species is constructed utilising two independent gene, 18S rDNA and ITS rDNA. This phylogeny is used to 1) test if the traditionally used morphological species-characters actually characterize monophyletic group, and 2) estimate evolutionary relationships among the mechanisms of sporangial emptying.

1130-1330

SYMPOSIUM 28 - *Enzymes and Infection Mechanisms*

S28IS1 - 0922

Secreted proteases from human pathogenic fungi

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Many species of human pathogenic fungi secrete proteases *in vitro* or during the infection process. Secreted endoproteases belong to the aspartic proteases of the pepsin family, serine proteases of the subtilisin family, and metalloproteases of two different families. To these proteases has to be added the non-pepsin-type aspartic protease from *Aspergillus niger* and a unique chymotrypsin-like protease from *Coccidioides immitis*. Pathogenic fungi also secrete aminopeptidases, carboxypeptidases, dipeptidyl and tripeptidyl-peptidases. The function of fungal secreted proteases and their importance in infections vary. It is evident that secreted proteases are important for the virulence of dermatophytes since these fungi grow exclusively in the *stratum corneum*, nails or hair, which constitutes their sole nitrogen and carbon sources. The aspartic proteases secreted by *Candida albicans* are involved in the adherence process and penetration of tissues, and in interactions with the immune system of the infected host. For opportunistic fungi such as *Aspergillus fumigatus*, the role of proteolytic activity has not yet been proved. As a general rule, the genome of non opportunistic pathogenic fungi contains gene families encoding secreted proteases. Fungal pathogens apparently requires the use of combinations of different specific proteases suitable to each particular condition during the infection. Expansions of genes to form a gene family could reflect selection during evolution to allow organisms a better adaptation to different conditions of their environme

S28IS2 - 0687

Licensed to kill: the role of phytotoxic proteins and pectinases in virulence of *Botrytis cinerea*

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Botrytis cinerea is a ubiquitous pre- and post-harvest plant pathogen with a broad host range, causing a disease known as "grey mould". Infection of host plants by *B. cinerea* is mediated by numerous enzymes and metabolites that the pathogen secretes at the host-fungus interface. Each of these compounds may play a role in a different stage of the infection process, or it may be suitable for infecting a particular tissue type or host plant species. Two aspects are crucial for *B. cinerea* to succeed as a necrotroph:
1. the ability to kill cells from a wide spectrum of plant species and tissues, and 2. the ability to rapidly decompose plant tissue and convert it into fungal biomass.
Application of molecular-genetic approaches in recent years has unraveled novel and exciting insights into the infection mechanism. The genome sequences of two strains have been determined and are undergoing manual annotation. An overview will be presented of our current knowledge on the enzymes and metabolites that play a role in pathogenesis of *B. cinerea*. Emphasis will be on a class of phytotoxic proteins, not previously identified in *Botrytis,* and their possible role in causing host (programmed?) cell death. In addition, results will be presented about an endopolygalacturonase, BcPG2, which has extremely potent macerating and necrotizing properties. BcPG2 appears to be the most important virulence factor among the pectinases that *B. cinerea* produces.

S28IS3 - 1014

Key enzymes for iron uptake and hyphal morphogenesis in *C. albicans* infection

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Largely due to the AIDS pandemic of the past 25 years, the fungus *Candida albicans* has evolved from an almost harmless commensal to one of the most important microbial pathogens in humans. *C. albicans* has many traits that collectively contribute to its virulence in the host. In an effort to identify genes that are activated when the pathogen is exposed to serum, we have found, among many serum-induced genes, a number of genes encoding enzymes involved in iron metabolism. They include the iron permease Ftr1, several ferrous oxidases and an iron-regulated mannosyl-transferase Mnn5. We have characterized the cellular functions of these enzymes through genetic and biochemical means and evaluated their importance in infection. In addition to producing enzymes with specialized functions, *C. albicans* has also evolved mechanisms that make use of enzymes normally involved in the control of general cellular functions, such as the master cell cycle regulator cyclin-dependent kinase Cdc28, the key polarity regulator Cdc42 GTPase, and the septins, a group of filament-forming GTPases important for cytokinesis. I will present evidence that *C. albicans* hyphal morphogenesis directly involve the regulation of the activity of these enzymes

S28PS1 - 0455

Identification of effector genes in the apple scab fungus, *Venturia inaequalis*

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The most economically important disease of apples worldwide is scab, caused by the ascomycete fungus, *Venturia inaequalis*. Current control is by intensive fungicide use, however *V. inaequalis* is able to develop resistance to agrochemicals. Negative public perception towards the use of agrochemicals for disease control is also evident, and as a consequence, alternative control strategies are being sought. Apple cultivars with major gene resistance to scab are the focus of many breeding programmes. Scab resistance follows the classic gene-for-gene model, whereby resistance is governed by the presence of a resistance (*R*) gene in the host and an avirulence (*avr*) gene in the pathogen. On infection an *avr* gene product is thought to be recognised by the corresponding *R* gene product, which leads to a resistance reaction. This study involves the identification of novel effector genes from *V. inaequalis* that are involved in host specificity. Effectors may have a dual role in pathogenicity and avirulence, and these may be utilised in disease control strategies. Fungal effector/avirulence proteins are often secreted and are usually highly divergent in sequence, although many are rich in cysteine. Bioinformatics has been used to compare sequences from EST libraries from infected apple with those in all public sequence databases. Novel EST sequences (with no known function) have been screened for the presence of a signal peptide using SignalP and Sigcleave prediction software. Secreted proteins are most likely to be present at the plant pathogen interface, and therefore are likely targets for the development of control strategies. The expression of novel fungal genes *in vitro* versus various stages of infection of apple will be compared by quantitative RT-PCR. Full-length sequences of these genes will then be obtained. A collection of pathogenic races and broadly distributed geographical isolates of *V. inaequalis* will be screened for polymorphisms at these loci. These genes will then be tested in future functional studies (by gene silencing or gene knockouts).

S28PS2 - 0458

Genomic approaches to isolating pathogenicity factors from the apple black spot pathogen *Venturia inaequalis*

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Venturia inaequalis is the causal agent of blackspot of Apple (*Malus pumila*). The fungus enters the plant by penetrating the cuticle and forms a stroma between the host cuticle and epidermal cells. The morphology of the stroma is unique in that it resembles laterally dividing cells rather than hyphal filaments. Stroma can be produced *in vitro* if *V. inaequalis* is grown on agar plates covered with a layer of cellophane. We are particularly interested in identifying novel, secreted cysteine-rich proteins secreted by *V. inaequalis* that are responsible for the pathogenicity and specificity of this fungus. We have constructed two expressed sequence tag (EST) libraries as a resource for identifying these proteins. One has been constructed from apple leaves infected with *V. inaequalis*, the second is a suppression subtractive hybridisation library made from *V. inaequalis* grown *in vitro* on cellophane. The library from infected apple leaves contains 4000 ESTs and has been fully annotated. Putative ESTs of fungal origin were identified by comparing their nref BLAST expect values with the fungal refseqaa BLAST expect value. Full length *V. inaequalis* sequences were then examined for the presence of secretory leader sequences. Seventy ESTs coding for putative secreted proteins have been found and approximately one dozen putative pathogenicity factors identified. One of these (CIN-1) is predicted to be a highly unusual protein with an eight domain repeated motif, each of which contains four cysteine residues. Functional analysis of CIN-1 is being carried out using RNAi, real-time PCR, over-expression in yeast and immuno-localisation.

1130-1330

SYMPOSIUM 29 - *Fungal Physiology*

S29IS1 - 0808

Interactions between endophytic fungi and pests and pathogens of plants, a physiological view

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A diverse array of fungi colonise plant tissues without causing obvious signs of disease. We explored interactions between the endophyte *Chaetomium globosum* and the fungal plant pathogen *Pyrenophora tritici-repentis* in wheat, and the endophyte *Lecanicillium lecanii* and an insect pest *Aphis gossypii* in cotton.
Colonisation of the wheat under controlled conditions by the endophyte *C globosum* was uneven though not limited in extent. A strong host reaction was induced by the endophyte. Subsequent inoculation with the pathogen resulted in reduced disease, probably because of increased host resistance. Though *C globosum* releases a suite of secondary metabolites with antifungal activity *in vitro*, one major protein was not detected *in planta*. We suspect the protein from the endophyte is insufficient for direct interactions with the pathogen. The interaction appears to benefit the host plant by increasing host response to a deleterious microbe.
Cotton seedlings inoculated with *L lecanii* became moderately colonised, both on the plant surface and within leaf tissue under experimental conditions. A direct interaction with *A gossypii* was evident. Aphids were colonised by the fungus and were able to transfer the fungus from colonised leaf to uncolonised leaf. In these experiments, death of the aphid rapidly followed colonisation, and was not necessarily related to an induced plant response. The interaction appears to indirectly benefit the host plant by reducing herbivory by one pest.
Complex interactions between endophytes and other organisms associated with plants appear likely. The principles of fungal responses to plants and plant responses to fungi remain unclear.

S29IS2 - 0768

A role for hydroquinone-driven Fenton chemistry in incipient wood decay by the brown rot basidiomycete *Gloeophyllum trabeum*

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Wood decay fungi use a variety of low molecular weight oxidants and electrophiles to attack wood. This strategy presumably reflects the low porosity of lignified plant cell walls, which in their native condition are impermeable to biodegradative enzymes. The best known small agents that might disrupt lignocellulose and thus facilitate fungal colonization of wood are reactive oxygen species (ROS) such as hydroxyl radicals (•OH) and peroxyl radicals (ROO•). The best evidence for ROS involvement in incipient wood decay comes from work done with brown rot basidiomycetes. Some genera, including *Gloeophyllum* and *Postia*, have been shown on defined growth media to secrete methoxyhydroquinones that reduce extracellular Fe3+ and O2. The resulting Fe2+ and H2O2 undergo the well-known Fenton reaction to produce extracellular •OH, a powerful oxidant of both holocellulose and lignin. However, until now it has not been clear whether these fungi produce hydroquinones when they grow on wood. Even if they do, the question remains as to whether the extent of hydroquinone-driven Fenton chemistry in the wood would be large enough to influence the course of biodegradation.
To address this question, we grew *Gloeophyllum trabeum* on spruce blocks, crushed some of the blocks at intervals, and used a rapid HPLC method to monitor the levels of two reactive extracellular hydroquinones, 2,5-dimethoxyhydroquinone and 4,5-dimethoxycatechol, in the liquid that was pressed out. Both metabolites were produced at concentrations similar to those reported earlier for this fungus when it was grown on defined media. Additional experiments showed that the principal iron chelator in the liquid phase of the colonized wood was oxalate, and that the rate constants for the reaction of the hydroquinones with Fe3+ in physiological oxalate were significant– on the order of 50 M-1 sec-1.
From these values we estimated the number of hydroxyl radicals produced, and thus the number of oxidative hits on holocellulose that occurred, during the first week of fungal growth on the wood. These numbers were compared with viscosimetric determinations of the actual number of scissions that had occurred in the holocellulose after one week. The results showed that the two hydroquinones make a significant contribution to incipient wood decay by *G. trabeum*. However, the levels of 2,5-dimethoxyhydroquinone and 4,5-dimethoxycatechol found in vivo do not appear adequate to account for all of the early holocellulose depolymerization that occurs, and therefore additional decay mechanisms probably contribute.

S29IS3 - 0563

Extracellular enzymes involved in litter degradation by basidiomycetes

K Steffen 1

Litter-decomposing fungi (LDF) colonize the uppermost part of the soil (the so-called soil-litter layer) in forests and grasslands. Litter comprises dead animal and plant residues and consists mainly of lignocellulose (lignin, cellulose, hemicellulose), remainders of animals (e.g. chitin) and humic material. Of these compounds lignin is probably the most recalcitrant one and forms the limiting step in litter degradation. The heterogenic structure of lignin contains aromatic substructures and makes it extremely resistant to chemical and biological degradation. It also serves as a major parent material in the formation of humic substances (HS) in soils making HS equally recalcitrant.
Litter decomposing fungi, most prominently agaric basidiomycetes, are capable of attacking all components of the lignocellulosic complex including the recalcitrant lignin polymer to gain access to other carbohydrates, such as cellulose and hemicellulose. Furthermore, humic materials of different origin (e.g. humic and fulvic acids) are subject to fungal disintegration. Above all, agaric fungi, colloquially called "mushrooms" and "toadstools", produce powerful extracellular enzymes (oxidoreductases), mainly manganese peroxidase (MnP) and laccase, which are capable of attacking certain aromatic structures in lignin and humus. Several LDF of the families Bolbitiaceae, Strophariaceae and Tricholomataceae, namely *Agrocybe praecox*, *Stropharia coronilla* and *Collybia dryophila*, were found to secrete both enzymes in the laboratory under different growth conditions (liquid cultures, soil-litter microcosms). As the result, synthetic lignins and different humic acids were oxidized leading to the breakdown of high-molecular mass components and in their partial mineralization (CO2 formation). Newest results showed the production of ligninperoxidase like enzymes by Mycena epipterygia or heme-thiolate haloperoxidases by Agrocybe aegerita. The ligninolytic attack of LDF on litter is followed and assisted by the production of extracellular hydrolyzing enzymes to utilize the now unprotected cellulose and hemicellulose. Fungi such as *Marasmius spp*. or *Mycena spp*. typically produce laccase and some MnP followed by different sets of cellulolytic enzymes. Endo-1,4-,-xylanase and 1,4-,-glucosidase exhibited highest activities among these enzymes in litter degradation experiments.
All in all, litter-decomposing fungi can cause substantial litter transformation and degradation. A large arsenal of oxidizing and hydrolyzing extracellular enzymes is at hand to accomplish this. Furthermore, the conversion of lignin and humic materials is of utmost ecological importance: it drives the humus turnover that in turn is essential to maintain the global carbon cycle.

S29PS1 - 0149

Mannitol metabolism in Stagonospora nodorum

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The physiological role of the mannitol cycle in the wheat pathogen *Stagonospora nodorum* has been investigated by reverse genetics. A putative mannitol 2-dehydrogenase gene (*Mdh1*) was cloned by degenerate PCR and characterised by gene disruption. The resulting *mdh1* strains lacked all detectable NADPH-dependent mannitol dehydrogenase activity. The *mdh1* strains were unaffected for mannitol production and surprisingly, were able to utilize mannitol as a sole carbon source suggesting an alternative mechanism for mannitol catabolism. The mutant was not compromised in its ability to cause disease or sporulate. To further understand the mannitol cycle, a previously developed mannitol 1-phosphate dehydrogenase disruption construct was introduced into the mutated *mdh1* background, resulting in a strain lacking both mannitol dehydrogenase and mannitol 1-phosphate dehydrogenase activity. The *mpd1mdh1* strains were unable to grow on mannitol and produced only very low levels of mannitol. The double mutant strains were unable to sporulate *in vitro* when grown minimal medium although sporulation could be restored with the addition of mannitol. Pathogenicity of the double mutant was not compromised, although like the previously characterised *mpd1* mutants, the strains were unable to sporulate *in planta*. These findings question not only the currently hypothesized pathways of mannitol metabolism but also the role of mannitol metabolism during pathogenicity, particularly during sporulation.

S29PS2 - 0670

Rapid, reversible motor response of fungi to blue, green and ultraviolet light

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Many fungi exhibit responses to visible light and its components. Responses include phototropic growth, photo-induction of spore development and release, and regulation of circadian rhythms and metabolic processes, such as carotenoid synthesis. These responses involve stimulation of photoreceptors that activate signal transduction pathways. Perception of light and signal transduction occurs on the scale of a few seconds, with subsequent transcription responses occurring over minutes to hours and developmental and morphological responses occurring over days. There are no reports in the literature of motion (as distinct from growth) by hyphae and conidiophores in response to either light or any other stimulus. Here, I report observations of a rapid, reversible motor response by several hyphomycetes in culture.

Isolates of *Thielaviopsis basicola*, *Alternaria alternata*, *Aspergillus niger* and a *Trichoderma* sp. were cultured on potato dextrose agar, in polycarbonate Petri dishes incubated in the dark (22°C). Cultures were examined under a compound microscope. The mycelium was exposed to transmitted light (halogen lamp, 100W with a blue filter) which passed through the agar. Cultures were also irradiated with incident light from a mercury lamp with either blue, green or ultra violet light filters (Olympus filters U-MNIBA, U-MWG and U-MWU2, respectively) for repeated periods of either 2, 10 or 30 s exposure (according to the responses), alternating with similar intervals of no exposure.

A response to white light was not observed by any of the species examined. Aerial hyphae and conidiophores of all species exhibited flexuous movement in response to green light and, to a lesser extent, blue and UV light. The terminal end of individual aerial hyphae moved by up to 30% of their length. In general, most of this movement occurred during the first 1 to 5 sec of exposure, was reversed in approximately the same time after cessation of exposure, and occurred repeatedly with cycles of exposure. Similarly, aerial mycelium contracted and expanded rapidly in contorted motion with alternate exposures. The movement of aerial hyphae, of similar age, tended to be greatest in close proximity to conidiophores, whereas sterile mycelium did not move. Some conidia of *A. niger* were ejected rapidly during the first seconds of exposure.

The rapid, reversible motor response of the hyphae and conidiophores of these hyphomycetes represents a novel observation of behaviour in the Eumycota. The mechanisms underlying these motor responses and their ecological significance require further investigation.

S30IS1 - 0268

Progress in the documentation of Asian fungal diversity.

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Various estimates exist as to the world number of fungi ranging from 1.5 to 9 million and many will challenge these figures as being unrealistic. While this may seem academic to many, the fact remains that only a small percentage of fungi has been so far documented. So what progress has been made since Hawksworth proposed the figure of 1.5 million fungi in 1991? Clearly different countries are further advanced than others in the documentation of their fungal diversity. Japan and China list 14,000 and 10,000 fungi respectively, Taiwan lists 5,396 species, while no figure is available for Cambodia and Indonesia. The number recorded for Thailand is approximately 6,000, with the greatest activity in the period 1995 to 2005. The diverse habitats and substrata found in Thailand have yielded some 300 new taxa, in particular, lichens (72), basidiomycetes (68), Xylariales (16), insect fungi (15), peat swamp palm fungi (20), and yeasts (12) (Jones and Hyde, 2004). Other groups such as the discomycetes, Saprolegniales and hypogenous fungi have attracted little attention. In Malaysia approximately 2,000 fungi have been documented (Nawawi and Alias, unpublished), including those from mangrove decaying material, soil, dung, freshwater and terrestrial plants. The majority are plant pathogens. New species described include the Xylariales (5), Mucorales (10), those from soil (11), marine (15), freshwater habitats (17), leaf litter (87) and as the result of the studies of Corner some 173 basidiomycetes. What are the major problems facing Asian-Pacific countries in the documentation of their fungal diversity? 1. Lack of experienced taxonomist and parataxonomists to train and in capacity building of young mycologists. This exasperated by retiring experts and their non-replacement by mycologists, leading to a limited number of taxonomic and ecological groups under study. 2. The need to develop sampling protocols and deposition and conservation of material in national herbaria and culture collections. 3. Good library facilities for fungal characterization. 4. Wider use of sequence data for the identification and study of the phylogenetic relationship of collected taxa often because of lack of facilities, expense and trained staff.

S30IS2 - 0726

Advances in our understanding of fungal diversity - an Asian perspective

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The number of studies on taxonomic mycology in Asia has been in decline and many countries in the region lack active mycologists. The situation is worrisome considering the need for countries to have their in-house taxonomic expertise to fulfil the expectations of Convention of Biological Diversity and the Global Taxonomy Initiative. Some countries in the region are, however, doing particularly well. China, Hong Kong and Taiwan are carrying out extensive work on inventory and taxonomy of fungi and all are training young taxonomists. Thailand has also been training taxonomists, particularly in BIOTEC and some Universities. The Mushroom Research Centre in Association with The University of Hong Kong, Melbourne University, San Francisco State University and CBS and several Thai Universities are training mycologists from the region (Cambodia, Laos, Myanmar, Indonesia, Philippines, Vietnam). Some of the results of these projects will be discussed. Our understanding of fungal numbers is dependent on knowledge of whether fungi are host-specific on plants and insects. This is particularly true in tropical forests where there are large numbers of plant species and small number of individuals. We have therefore focused on forest litter and want to establish whether the fungi involved in litter decay are generalists or host-specific. We have therefore studied the diversity of fungi on several hosts contributing to rainforest litter in northern Thailand's forests. We have carried out succession studies on several hosts in order to reveal the changes in fungal communities during litter decay and have designed experiments to establish where fungi decaying leaf litter have originated. We have also supplemented our understanding by applying molecular techniques to identify endophytic mycelia sterilia and DGGE to establish whether non-culturable fungi occur in leaves. The talk will bring together our findings, discuss the implications on our understanding of fungal diversity and suggest areas needing future work.

S30IS3 - 0689

Fungal diversity 'down-under'

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The term 'down-under' is a colloquialism for both Australia and New Zealand, although sometimes the two countries are collectively referred to as 'Australasia'. Both countries were formerly part of Gondwana but New Zealand became isolated (about 80 mya) and Australia separated from Antarctica (about 35 mya). Each country has a separate evolutionary history with many unique plants, animals, and fungi. The fungi down-under comprise a complex array including cosmopolitan species, southern hemisphere species, bipolar species, pan-tropical species, Gondwanan species, and those endemic to Australia and/or New Zealand. Endemism is variable between groups of fungi; parasites and mycorrhizal fungi on native plants are usually endemic, but even among saprobes there are many endemic species. Endemism is usually at the species level, although the truffle-like fungi are particularly diverse with a high proportion of putatively endemic genera. 'Endemic' genera are often those described in recent years, and with time such genera tend to be found elsewhere. At the species level 25% of the approximately 5250 non-lichenised New Zealand species listed on the *NZFungi* website are regarded as endemic, a further 37% are indigenous and 38% introduced. Some saprobic microfungi, mainly described on litter of native plants have been later found widely distributed around the world. For instance, of 71 new hyphomycete species described from New Zealand by S.J. Hughes from 1964 onwards, 27 (38%) have been since recorded elsewhere including North and South America, Europe, southern Africa, and Asia. Conversely, approximately one-third of anamorph fungi recorded in studies in Cuba, Russia, and Thailand are also known from New Zealand. Some fungi, particularly exotic rusts, are regularly wind-blown from Australia to New Zealand, but further studies are needed to determine whether rust species native to Australia have spread naturally to New Zealand (or vice versa). No anamorph genera appear to be endemic to the Australasian region, and the two Australian endemic genera (*Catenophoropsis*, *Macrohilum*) that are also found in New Zealand have been introduced on eucalypts. Some down-under fungi have been inadvertently introduced to other regions, e.g., *Aseroe rubra*, *Clathrus archeri*, and *Poronia erici* to Europe, *Ileodictyon cibarium* to Europe and East Africa, *Puccinia lagenophorae* to Europe, and *Urocystis tritici* to North America. What is special about the down-under fungi? How many unique species are found down-under? How many more down-under species await discovery? What are the unique species?

S30PS1 - 0265

Fungal Succession On Dead Leaves of *Castanopsis diversifolia* In Doi Suthep-Pui National Park, Thailand

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Fungal succession is the sequential occupation of the same site by thalli either of different fungi or of different associations of fungi. There have been several fungal succession studies on leaf litter in temperate as well as in tropical forests. In this study, leaves of *Castanopsis diversifolia* (190), which were collected at Doi Suthep-Pui National Park in Thailand, were divided into 4 subsets and used as baits with different treatments to establish fungal succession. Sixty leaves were sterilized and hung 4 metres above the ground, under the *Castanopsis diversifolia* tree canopy. Sixty other leaves were sterilized and laid on the forest floor and 60 other unsterilized leaves were placed on the forest floor. The last ten leaves were sterilized and incubated with sterile tissue paper, in plastic containers to act as the control. Experiment was taken place from June to October 2004. The study yielded 112 taxa including 19 ascomycetes, 4 basidiomycetes, 1 myxomycete and 88 anamorphic fungi (10 coelomycetes and 78 hyphomycetes) during the 4-month incubation period. The sterile hanging leaves harbored the highest diversity with 65 taxa, while unsterile leaves on the forest floor yielded 55 taxa and sterile leaves on the forest floor with 53 taxa. The highest diversity during the treatment period differed for each treatment. Diversity indices were calculated by using ws2m software. The origins and functions of encountered fungi in the ecosystem are discussed in the relation to the experimental design.

S30PS2 - 0503

Macrofungal diversity in the *Cryptomerioid japonica* plantations in Taiwan

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Forest ecosystem is one of the most important ecosystems in Taiwan. According to the recently land survey, about 60% of the island's total landmass is still covered by forests. Among these area, 14% or 310,000 ha are plantation forests. In the past, the management goal of plantation was to produce large diameter trees with high timber value. This focus, however, has been gradually changed. The new goal is timber production with consideration of its impacts on climate changes, biodiversity losses, ecosystem functions and public acceptance. We (CTEB, TFRI, and TFB) organize a multi-disciplinary and multi-institutional research team that with integrated approaches to pursue our goals. We propose to monitor and quantify macrofungal community dynamics after 0%, 25% and 50% thinning practices from 2007. We have established 12 one hectare permanent plots in *Cryptomerioid japonica* plantation forest in central Taiwan. Within this plot, macrofungi are systematically survey and collect as baseline data in 2006. The base of our investigation is an assumption about affection of mycoflora structure by environmental changes and recruitment of native plant species.

1400-1530
POSTER SESSION 3: PLANT AND FUNGAL PATHOGENS

PS3-241-0015
Pathogenic variability of *Puccinia coronata* f. sp. avenae on oat in the Czech Republic

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Oat crown rust, caused by the fungus *Pucinia coronata* Cda. f. sp. *avenae* Erikson is the most damaging disease of oat (*Avena sativa* L.) also in the central Europe. The monitoring of changes of virulence in the pathogen population is an integral part of successful breeding for resistance. Totally forty-five samples of oat leaves infected with *Puccinia coronata* f. sp. *avenae* were obtained mainly from the trials of the Central Institute for Supervising and Testing in Agriculture in different areas of the Czech Republic during the 2004 and 2005. The isolates were analyzed to determine patterns of virulence on 26 single gene oat lines (Pc 38, 39, 40, 45, 46, 46, 48, 50-2, 50-4, 51, 52, 54, 54-1, 55, 56, 58, 59, 60, 61, 62, 67, 68, 94, 96, VIR 373-1, VIR 343-2). Disease reactions were evaluated 14 days after inoculation. The analyses confirmed high virulence variability of the pathogen and different virulence combinations occurred. The isolates were frequently virulent on Pc 40, 45, 46, 51, 54 and VIR 343-2. Detailed data are presented.
Selected isolates originated from the Czech Republic were also compared with isolates from various European countries and Israel using RAPD (Random Amplification Polymorphic DNA) to investigate feasibility of using molecular approach to elucidate genetic complexity of crown rust pathotypes. Isolated crown rust DNA was amplified using fifty random primers. Four associated groups and three detached isolates were identified from totally seventeen isolates tested. Two Czech isolates showed to belong to group with isolates originated from Belarus and Israel, two other ones were detached.
Despite of molecular similarity differences in virulence range were determined in these groups using phytopathological analyses.
It thus seems that the epidemic potential of a pathogen is based quantitatively and this polygenic system is manifested independently of oligogenic system which current cereal rust evaluation is based on.

PS3-242-0041
Antifungal activity of leaf extracts of six asteraceous plants against *Fusarium oxysporum*

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Species of *Fusarium* are commonly found in the soil. They cause diseases in a variety of cultivated plants ranging from tomato, plantain and bananna to several cereals, resulting in low crop yield or total plant damage. They are also one of the major causes of mycotoxicoses in man and animals. *Fusarium* is an agent of keratitis and corneal ulcers, septic arthritis, sinusitis and mycetoma. They produce toxins in infected plants which adversely affect man when such plants are consumed. Recent studies have focused on the general biology, ecology and natural means of controlling *Fusarium* diseases in plants since chemotherapy often has deleterious effect on the environment. Scientists have therefore been attracted to discover new herbal antimycotics. Documented information on herbal antimycotics is scarce in Nigeria. However, some Nigerian plant species have been reported to have antifungal activity.
Six plants belonging to the class Asteraceae were tested *in vitro* for antifungal activity against *F. oxysporum*. Water-extracted leaf active components of *Tridax procumbens, Tagetes erecta, Xanthium strumarium, Dahlia pinnata, Cichorium intybus* and *Carthamus tinctonus* were used. Glucose peptone broth was supplemented with the extracts. The test flasks were incubated at ambient temperature for 7 days. Mycelia were harvested, dry weights determined and percentage fungal inhibition was calculated.
The highest mycelial weight was recovered from *D. pinnata* culture (377.5mg) and the least produced by *T. erecta* (196.5mg). The percentage inhibition figures were 20.3 (*C.tinctonus*), 25.7 *(C.intybus*), 10.3 (*D. pinnata*), 53.2 (*T.erecta*), 14.7 (*T. procumbens*) and 26.0% (*X.strumarium*). All the tested plants showed some antifungal potential which should be further investigated and exploited.

PS3-243-0046
Study on foot rot and bakanae disease of rice in Mazandaran province, Iran

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Rice is a major crop in Mazandaran province in North of Iran. Bakanae is one of the most important rice seed-born diseases which occures in the nurserie as well as field. In order to investigate the disease, infected rice plants at different growth stages were collected from various localities in Mazandaran, during growing season from April to September, 2004. Sixty seven of *Fusarium* species belonging to section *Liseola* were established. Pathogenicity of isolated Fusaria were tested through two methods, injection of spore suspension into crown of 25 days old seedlings; and placing of inoculated seeds with spore suspension on sterile filter paper. In these tests, common symptoms were observed. Based on morphological characteristics, pathogenic isolates were identified as *Fusarium proliferatum, F. fujikuroi* and *F. verticillioides*, of which *F. proliferatum* was the most abundant (68.55%). Teleomorph of the fungi was found in most fields in the end of summer and identified as *Gibberella fujikuroi.* This is the first report of anamorph and teleomorph of causal agent of bakanae disease in this province. These fungi are widely distributed and affect on rice cultivars Tarom (a local cultivar), Fajr, Khazar and Neda (improved high yielding cultivars). Most of infected fields are located in central and eastern part of Mazandaran and refer to Tarom cultivar.

PS3-244-0047

Natural occurance of perithecia of Gibberella fujikuroi and its related *Fusarium* species in Mazandaran paddy fields, Iran

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During routine survey of foot rot of rice in Mazandaran province, some black and rough structure were observed on crown and stems of bakanae infected rice plants in 13 paddy fields located at different parts of the province, in August and September 2004. Investigation at laboratory revealed that superficial black structure were perithecia of *Gibberella fujikuroi*. Thirty nine isolates comprising three isolates from three perithecia representative of each field were obtained by single ascospore method and were identified based on morphological characteristics. All isolates from 11 and one fields were *Fusarium proliferatum* and *F. fujikuroi*, respectively. Both *F. proliferatum* and *F. fujikuroi* were found among one field isolates. Common symptoms of bakanae disease were observed in all isolates pathogenicity tests. These data indicated that *F. proliferatum* had widespread occurrence in Mazandaran paddy fields and was highly fertile.

PS3-245-0082

The interaction between Mycogone perniciosa, the causal agent of wet bubble disease, and Agaricus bisporus

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Agaricus bisporus (Lange) Imbach, the most commonly cultivated mushroom worldwide, can be infected by *Mycogone perniciosa* (Magnus) Delacroix, the fungal pathogen causing wet bubble disease. This contagious disease, known since 1888, results in malformed mushrooms, crop loss and subsequent economic losses. This work examined the *in vitro* and *in vivo* interaction between *M. perniciosa* and *A. bisporus* using light and scanning electron microscopy. Growth of the the fungi together in dual, paired and split agar plate cultures, on different media was measured to determine the effect *M. perniciosa* has on *A. bisporus* mycelium. In an effort to better understand the process of infection and subsequent disease manifestation, enzymes and volatiles produced by *M. perniciosa* were investigated. The effect of these volatiles on mushrooms cultivated under simulated commercial conditions was determined by attaching Petri plates with cultures of *M. perniciosa* to pots with developing mushrooms. Results showed no conclusive evidence of intrahyphal growth but *M. perniciosa* adhered to, coiled around and caused hyphal collapse of *A. bisporus* hyphae indicating that it is possibly an infective mycoparasite. *M. perniciosa* grew significantly more in the presence of *A. bisporus* in agar culture, while *A. bisporus* grew less than when cultured alone. The fungi influenced each other's growth when they had no contact within split plates or dual culture, indicating that a volatile compound may be produced when they grow together. Mushrooms subjected to possible volatile production by the pathogen developed disease. We were not able to verify the volatile constituents produced by *M. perniciosa*, but hydrolysates from liquid cultures of *A. bisporus* grown with *M. perniciosa* examined with thin layer chromatography revealed a compound that was not present when either fungus grew alone. This study was the first to investigate whether the dedifferentiation caused by *M. perniciosa* in fruit bodies of *A. bisporus* plays a role in the *in vitro* growth of both fungi and also the first attempt to elucidate the biochemical basis of the host-pathogen interaction in wet bubble disease.

PS3-246-0094

Diversity of fungal diseases in winter wheat fields under conditions of irrigated agriculture in Uzbekistan

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Study of wheat fields in Tashkent region of Uzbekistan had revealed presence of root rot infection in all areas. Mycological analysis of herbal material obtained from fields was performed on the following scheme: I)observation of infected plant tissues to determine the presence of the pathogen, II)damping camera, III)isolation and identification of the pathogen.
Mycological analysis of herbal samples has revealed 17 species of micromycetes from genera - *Alternaria, Aspergillus, Mucor, Penicillium, Cladosporium, Verticillium, Helmintosporium, Fusarium, Rhizoctonia*.
Preliminary investigation has shown possibility of increasing infection on irrigated wheat fields by the fungal phytopathogens which were not characteristic for the hot climate conditions of Uzbekistan.

PS3-247-0103

An Apparatus for Collecting Total Conidia of *Blumeria graminis* f. sp. hordei from Leaf Colonies using Electrostatic Attraction

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Conidia from living conidiophores of barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) on host leaves were consecutively collected using an electrostatic spore collector. The collector consists of an electrical conductor plate linked with an electrostatic voltage generator and insulator plates placed abreast on a timed conveyer. The conductor plate was negatively charged by the potential supplied from the voltage generator. The negatively charged conductor plate caused dielectric polarization of the insulator plate, and the surface charge on the insulator plate attracted mature conidia abstricted from conidiophores on colonies growing on leaves placed 2 cm from the insulator plate. The surface charge on the insulator plate was proportional to the voltage applied to the conductor plate. Under optimized conditions, abstricted conidia were attracted to the electrostatically activated insulator plates without any detriment to their survival. During a colony's lifespan of c. 460 h, conidia were released throughout the day and about 12 ? 104 conidia were collected during the lifetime of the colony. To our knowledge, this is the first report on the direct quantification of progeny conidia produced by powdery mildew infecting host leaves.

PS3-248-0104

Consecutive Monitoring of Lifelong Production of Conidia by Individual Conidiophores of *Blumeria graminis* f. sp. hordei on Barley Leaves by Digital Microscopic Techniques with Electrostatic Micromanipulation

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Conidial formation and secession by living conidiophores of *Blumeria graminis* f. sp. *hordei* on barley leaves were consecutively monitored using a high-fidelity digital microscopic technique combined with electrostatic micromanipulation to trap the released conidia. Conidial chains formed on conidiophores through a series of septum-mediated division and growth of generative cells. Apical conidial cells on the conidiophores were abstricted after the conidial chains developed 10 conidial cells. The conidia were electrically conductive, and a positive charge was induced in the cells by a negatively polarized insulator probe (ebonite). The electrostatic force between the conidia and the insulator was used to attract the abstricted conidia from the conidiophores on leaves. This conidium movement from the targeted conidiophore to the rod was directly viewed under the digital microscope, and the length of the interval between conidial septation and secession, the total number of the conidia produced by a single conidiophore, and the modes of conidiogenesis were clarified. During the stage of conidial secession, the generative cells pushed new conidial cells upwards by repeated division and growth. The successive release of two apical conidia was synchronized with the successive septation and growth of a generative cell. The release ceased after four to five conidia were released without division and growth of the generative cell. Thus, the life of an individual conidiophore (from the erection of the conidiophore to the release of the final conidium) was shown to be 107 hr and to produce an average of 33 conidia. To our knowledge, this is the first report on the direct estimation of lifelong conidial production by powdery mildew on host leaves.

PS3-248a-0105

Symptomatic Evidence for Differential Root Invasion by *Fusarium Crown* and Root Rot Pathogens Between Common tomato *Lycopersicon esculentum* and Its Varieties

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Symptomatic Evidence for Differential Root Invasion by *Fusarium Crown* and Root Rot Pathogens Between Common tomato *Lycopersicon esculentum* and Its Varieties
The pathogenic isolates (Kin2001a, Kin2001b and Kin2003) of *Fusarium oxysporum* f. sp. *radicis-lycopersici* were obtained from hydroponically cultured seedlings of pear tomato (*Lycopersicon esculentum* var. *pyriforme*) infected at different times, and their pathogenicity examined in an *in vitro* assay system on cotyledonal seedlings of pear tomato, cherry tomato (*L. esculentum* var. *cerasiforme*) and common tomato (*L. esculentum*). With the *in vitro* assay, infection and subsequent disease progress could be microscopically observed. Pear and cherry tomatoes suppressed invasion by all isolates at the junctions of epidermal cells along the root, comparable to the resistant cultivars of common tomato. The pathogen entered pear and cherry tomatoes at the tips of lateral roots and tap roots, in contrast to infection of susceptible cultivars of common tomato. In Kin2003-inoculated roots, the top of the lateral rootlets first became discolored, followed by the cortical parenchyma, central xylem vessel, and finally the crown. This dark-brown discoloration expanded rapidly, and severe rot developed in the discolored regions. In contrast, the dark-brown discoloration in Kin2001b-infected roots expanded into the cortical parenchyma cells abutting the originally infected lateral rootlets and at a much slower rate. Kin2001a was in a new group that entered via the cortical cleavage formed by the emergence of lateral rootlets, in addition to the tips of taproots and lateral roots. In this *in vitro* assay system, the Japanese pathogenic isolates collected from different districts of Japan were characterized and classified by the mode of host invasion. Of 13 isolates, four were placed with Kin2003, six with Kin2001a and three with Kin2001b.

PS3-249-0111

Evolution of Botrytis cinerea greenhouse population following introduction of marked selenate-resistant strains

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B.cinerea, the anamorph of *Botryotinia fuckeliana*, attacks a wide range of plant species, causing grey mould on many economically important crops. *B. cinerea* marked strains combining traits of fungicide resistance or sensitivity (carbendazim, iprodione) with resistance to selenate were created and used for studying the potential of the fungus to spread in the greenhouse from sources of infection; for studying the relative contribution of internal and external inoculum sources to *B. cinerea* epidemics; and for studying *B. cinerea* survival inside the plant tissue. Following up to 40-day-exposure to marked inoculum, no visible symptoms of disease appeared on the target plants (beans, pepper, strawberry); quiescent infection was visualized by exposing selected plants to high humidity at 20°C and then analyzed for wild-type or marked phenotype by transferring developed *B. cinerea* from the plants to selective media. The greenhouse air contained significantly more *B. cinerea* CFU in summer than in winter, although the overall infection incidence was about 90% in both seasons; its development into an epidemic depended mostly on weather conditions. The data on the proportion of marked *B. cinerea* in the air of the greenhouse match those on infection incidence: the higher the proportion of the marked strain in the air, the higher was the infection incidence caused by the marked strain. Only 24% of the infected plants showed marked *B. cinerea* infection in summer, whereas in winter this proportion reached 80%; the contributions of the marked phenotype to the airborne population in those periods were about 18 and 98%, respectively. This indicates that the indoor sources of infection could be significantly important in initiating disease in the cold season, when the conidia density in the air is the limiting factor for epidemic initiation. These results highlight the particular importance of sanitation in cold period, even though sanitation measures should always be applied. Mycelium of *B. cinerea* inside dried tissues lost viability within 3 to 4 months in the warm season whereas it did not lose viability during 4 months in the winter (December to March) but started to lose viability from April.

PS3-251-0129

Influence of Organic Acids on the Growth and Development of *Trichoderma aggressivum* a pathogen of *Agaricus bisporus*

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Previous research has shown that composted substrate for *Agaricus bisporus* prepared under low oxygen conditions resulted in earlier and more severe development of *Trichoderma aggressivum* (var. Th4*)*, the pathogen causing Green Mould disease on mushrooms. Organic acids are known to be produced under anaerobic conditions, and their residual compounds may be involved in the development of the pathogen in mushroom substrate. This research looked at the influence of several organic acids at different concentrations on the growth of *T. harzianum*. Results suggested that *in vitro* higher concentrations of most organic acids had a negative influence on the growth of the pathogen while lower concentrations of selected organic acids stimulated growth. *In vivo* assays suggested that when some of these acids were added to the substrate at low concentrations the substrate was predisposed to disease development. Disease development was quicker and more severe in composted substrate treated with several organic acids and at different concentrations of these acids. The results suggest that anaerobic conditions during the composting substrate preparation will predispose the mushroom crop to *Trichoderma* Green Mould disease.

PS3-252-0132

The use of a PCR diagnostic test to predict and control *Peronospora sparsa*, downy mildew of boysenberry

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Downy mildew (also called dryberry) is a major risk boysenberry in New Zealand. In 2002, yield losses in conventionally managed crops were as high as 45% and reached 100% in organic crops. Losses that year were estimated at NZ$1.8m. This has led growers to greatly increase the intensity of their spray programmes at a time when customers' food safety' requirements are moving towards less pesticide use. In order to develop better management strategies we need a better understanding of the disease cycle and the main pathways of infection. The causal agent, *Peronospora sparsa*, is a biotrophic and cannot be cultured. This has limited the monitoring of its presence and spread to observation of symptom expression, and the use of microscopy to determine the numbers of resting spores in plant tissues and debris. In 1998 researchers at the University of Helsinki developed a PCR diagnostic test for *P. sparsa* which allowed the detection of the pathogen in symptomless tissue containing small amounts of advancing pathogen mycelium. We have shown that that the PCR diagnostic test works for New Zealand isolates of the fungus, and modification of the test to a nested PCR test has significantly increased its sensitivity. Previous microscopical observations have detected the fungus mainly in the cortex tissues of the plant. PCR testing of various plant tissues during the 2005-2006 growing season has detected the fungus throughout the plant (ie root tips, root crown cortex, main root cortex, primocane cortex, primocane leaf, primocane tip, floricane cortex, floricane leaf, floricane buds and green fruit), the only exception being the very tip of the growing primocane. This PCR test will be used in the upcoming 2006/07growing season to determine the numbers of symptomless systemically infected plants in trial plots and commercial fields.

PS3-253-0164

Influence of water stress and wetness of open wound on lesion expansion in the xylem of *Cryptomeria japonica* seedlings inoculated with a canker fungus *Guignardia cryptomeriae*

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Guignardia dieback of *Cryptomeria japonica* (Japanese cedar) caused by *Guignardia cryptomeriae* (*Botryosphaeria* sp.) occurs under water stress. Influence of water stress and wetness of open wound, i.e. invasion of air from wound, on xylem lesion (discolored wood and dry zone) expansion in *C. japonica* seedlings inoculated with *G. cryptomeriae* was investigated by using neutron radiography. Two-year-old seedlings planted in the pots were wound inoculated with virulent isolate of *G. cryptomeriae*. Moisture content of soil (vermiculite – perlite) was adjusted to 300 % or 160-210 %. Wet wound inoculation was achieved by using moist cotton wool and dripping water into it, and the wound was covered with Parafilm. Seedlings were irradiated with thermal neutron beam (1.5?108 n/cm2/s) for 3 seconds one, 3 and 15 days after inoculation, and radiography images were obtained with an imaging plate or a CCD camera. Water potential of seedlings was different between two soil water regimes at the time of inoculation. No difference was observed in xylem lesion size between two regimes one day after inoculation. Xylem lesion expanded with the time after the inoculation in dry soil condition. In moist soil condition, however, xylem lesion hardly expanded. These results indicated that water stress accelerated the lesion expansion. No difference was recognized in the size of xylem lesion between wet wound and dry wound 3 and 15 days after inoculation. Invasion of air from open wound was not suggested to accelerate the expansion of xylem lesion. Wet wound, rather, induced larger dry zone on the 2nd day of the inoculation, suggesting suitable environment for the fungus. These results suggest that acceleration of xylem lesion expansion under drought stress occur via host-pathogen interactions, but not by tension itself.

PS3-254-0165

Occurrence of scab canker caused by *Scolecostigmina sp.* on five-needle pines in Japan

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Scab canker disease caused by *Scolecostigmina* sp. was found on five-needle pines in Japan. It induces gall and canker on stems and branches, death of twigs and seedlings. Inhibition of natural regeneration of refugee species *Pinus parviflora* by this disease becomes a serious problem in Boso peninsula, central Japan. Indigenous species *Pinus parviflora* and *P. parviflora* var. *pentaphylla* were found as hosts of the disease. In addition, it has been recorded on several exotic five-needle pines planted in arboretum. Spore formation, dispersion and lesion development were investigated to develop control measures. Spore traps were set up under or around infected tress to investigate the season and range of spore dispersion. Galls were marked and observed under a microscope in the field, and then the length was measured to determine lesion expansion. Newly formed minute swellings were sampled, and microtome sections were made for anatomical observation. Galls consisted of several layers of wound periderm, i.e. scab, which was quite different from the structure of other diseased gall tissues. Neither hypertrophy nor hyperplasia was not conspicuous in cortex / phloem, and was not observed in cambium / xylem. Conidia were produced in spring especially in April and May, and dispersed remarkably in May and early June. Then conidia dispersion decreased rapidly. They seemed to disperse with rainfall, and dispersed almost under infected crown. Conidia could germinate all the year around. Attachment of conidia was observed on the current shoots / branches for a long time until autumn. First visible symptom, minute swelling of bark surface under attached conidia, developed on current shoots in summer months. Inner tissue of such small gall necrosed and the pathogen was isolated from the necrosed tissue. The galls gradually enlarged for years.

PS3-255-0180

Evaluation of *Trichoderma* bio-inoculant for control of Specific Apple Replant Disease (SARD)

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Specific Apple Replant Disease (SARD) is a worldwide problem in replanted apple orchards, causing poor establishment and delayed productivity. Current control relies on soil fumigation, however, with the current international shift away from reliance on soil fumigation, alternative treatments are required. A commercial *Trichoderma* bio-inoculant was assessed for ability to control SARD in two pot trials.

Soil from a site with a history of SARD was used. For the first trial the treatments were:- i) untreated; ii) chloropicrin fumigation; iii) *Trichoderma* pellet (50g/planting hole, 106 cfu/g soil). In the second trial, to differentiate between *Trichoderma* and pellet formulation effects, the treatments were i) untreated; ii) chloropicrin fumigation; iii) *Trichoderma* pellet (106 cfu/g soil); iv) sterilised *Trichoderma* pellet; v) uninoculated pellet; vi) unformulated *Trichoderma* spores (106 cfu/g soil) vii) fertiliser (N:P:K) to the same levels as in the pellet. For both trials, rooted M26 apple rootstocks were planted in treated soil. Trunk diameter was measured at planting and at the end of the trial (7 months). At harvest, visual root health was assessed on a 0-4 scale (0=all roots healthy and 4=100% dead) and root and shoot dry weights measured.

In both trials, *Trichoderma* pellet treatment increased apple rootstock growth (trunk diameter, shoot and root dry weights) compared with the untreated control and comparable with the chloropicrin treatment. In the second trial, pellet alone (sterilised *Trichoderma* pellet and uninoculated pellet) and fertiliser treatment also increased growth comparable to the *Trichoderma* pellet, whilst unformulated *Trichoderma* spores had no effect. Visual root health was improved by both chloropicrin and to a lesser extent fungicide treatments, but not with any *Trichoderma* or pellet treatments.

Although no increase in apple root health was seen *Trichoderma* bio-inoculants increased apple root stock growth, probably due to the nutrients in the pellets and not *Trichoderma per se*. Nutrient addition using slow release fertilisers may help to alleviate SARD symptoms by increasing seedling growth and establishment.

PS3-256-0181

Biological control of *Sclerotinia minor* in lettuce using *Trichoderma hamatum*

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Sclerotinia species are major pathogens worldwide that cause economic losses to lettuce growers. Different application methods of a *Trichoderma hamatum* isolate, shown to have potential to control *Sclerotinia* spp. in biocontrol assays, were assessed for their ability to provide control *S. minor* in lettuce in three field experiments.

T. hamatum 6Sr4 was applied (a) as a transplant potting mix incorporation (transplant) where lettuce seedlings were grown in 6Sr4 inoculated potting mix,or (b) 6Sr4 colonised maizemeal:perlite (m:p) was incorporated into the soil at planting or (c) 6Sr4 as a spore suspension drench (drench) to the lettuce collar.

All field experiments were planted in sites naturally infested with *S. minor*. In Trial 1 there were three treatments:- i) untreated control; ii) 6Sr4 transplant and iii) 6Sr4 m:p. Trial 2 included :- i) untreated control; ii) carbendazim spray; iii) 6Sr4 transplant; iv) 6Sr4 m:p and v) 6Sr4 transplant plus 6Sr4 m:p plus 6Sr4 drench. Trial 3 included commercial formulations:- i) untreated control; ii) commercial 6Sr4 transplant plus m:p and iii) commercial transplant plus commercial drench. In all trials *S. minor* disease was assessed weekly until harvest.

In Trial 1, 6Sr4 applied as transplant controlled disease (44% disease control) under high disease pressure (77%). In Trial 2 all 6Sr4 treatments reduced disease (20-34% disease control) under high disease pressure (90%), and results were comparable with the carbendazim fungicide treatment (21% disease control). Under medium disease pressure (29%) in Trial 3, commercial applications of 6Sr4 also resulted in disease control (60-61% disease control).

T. hamatum consistently reduced *S. minor* disease in lettuce when applied as a spore suspension to the transplant potting mix or as a m:p soil incorporation at planting and control was comparable with the standard carbendazim fungicide treatment. Commercial formulations of 6Sr4 were seen to give consistently controlled *S. minor* and are being developed further.

PS3-257-0182

Regulation of gene expression in the interaction between *Fusarium* spp. and wheat

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Fusarium Head Blight is one of the most important diseases of wheat worldwide. In Australia, *F. graminearum* and the closely related fungus *F. pseudograminearum*, cause sporadic head blight epidemics and are also responsible for crown rot disease in wheat. These diseases can lead to substantial yield losses and accumulation of mycotoxins, particularly deoxynivalenol, which adversely affect human and animal health when consumed. We are investigating regulatory aspects of plant and fungal gene expression in the *Fusarium*-wheat interaction. Changes in wheat gene expression during early stages of crown rot infection have been analysed using Affymetrix microarrays and RT-qPCR. This has then been compared to gene expression changes after treatments with defence regulators as well as deoxynivalenol. Investigations into how the host may influence the expression of pathogen genes, such as the genes responsible for deoxynivalenol production, are also being carried out using green fluorescent protein expressing reporter strains and Affymetrix microarrays.

PS3-258-0200

PREVALENCE and pathogenic variability of *Pyrenophora teres f. maculata* from barley crops in Victoria.

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Pyrenophora teres f. *maculata*, the causal agent of the spot form of net blotch (SFNB), is a common disease of barley (*Hordeum vulgare*) that can cause significant yield loss. Its impact on the Victorian barley industry is, however, poorly understood. The majority of commercial barley cultivars are susceptible to SFNB. Some cultivars are resistant and rely on the single resistance gene Rpt4. Other resistance genes are known to exist but their ability to control SFNB is not well understood.
This study was undertaken to determine the prevalence and severity of SFNB and identify if there is pathogenic variability between Victorian isolates of *P. teres* f. *maculata*.
During 2003, 2004 and 2005, 130 barley crops in three cropping regions of Victoria were inspected to determine the prevalence and severity of SFNB.
A preliminary study was carried out to determine the pathogenic variability of *P. teres* f. *maculata*. Forty nine isolates collected from leaf tissue during the survey were each inoculated onto a set of 21 barley lines of various origins with unknown sources of resistances. The set included international and Victorian lines, some of which had the Rpt4 gene, while others had unidentified sources of resistance. Each line was rated for lesion type caused by *P. teres* f. *maculata*, allowing virulence and avirulence of isolates on each line to be determined.
SFNB was present within 95% of crops surveyed. Some crops had up to 18% leaf area affected by SFNB on the top four leaves, sufficient to significantly decrease grain quality and yield.
The preliminary inoculation of 49 isolates onto the differential set showed eight separate pathotypes based on variability in virulence and avirulence on six differential lines. No variation was detected on 15 lines, which included two cultivars with the Rpt4 gene that were resistant to all *P. teres* f. *maculata* isolates tested.
The survey showed that SFNB is a common disease and where conditions are suitable, and susceptible cultivars grown, there is potential for significant yield losses to Victorian barley crops. It is, therefore, important that resistant cultivars continue to be developed to protect the Victorian barley industry.
In the development of resistant cultivars, barley breeders need to consider pathogenic variability of *P. teres* f. *maculata*. Even though Rpt4 is currently effective against all Victorian isolates of *P. teres* f. *maculata* tested, the potential for the pathogen to acquire new virulence means new sources of resistance need to be identified and incorporated into future cultivars.

PS3-259-0207

Detection of hyphae of endophytic fungi in leaf tissue

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Almost all leaves of vascular plants contain endophytic fungi. Their presence is typically detected indirectly, through culturing studies. Small pieces of surface-sterilised leaf tissue are placed on agar plates to allow the endophytic fungi to grow out from inside the leaves. Direct observation of hyphae within the leaves is often difficult, although important for gaining an understanding of the biology of individual endophyte species. Although the fungi isolated from symptomless living leaves are commonly all referred to as "endophytes", they have a range of possible biologies, from latent pathogen (where hyphae may be restricted to a single cell or stomatal cavity), through to true symbiont (where hyphae ramify extensively through intracellular spaces within the leaf). We describe the use of a monoclonal antibody to (1?3)-,-glucan and a secondary antibody conjugated to Alexa 488 to visualise endophytic hyphae within leaves of *Leptospermum scoparium* (Myrtaceae).

PS3-260-0211

Disease Cycle of Japanese Cedar Sclerotial Dieback

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Sclerotial dieback is a serious disease of Japanese cedar (*Cryptomeria japonica*) caused by *Asteromassaria* sp. (the imperfect stage is *Scolicosporium* sp.) in northern Japan. The disease is characterized by the development of black sclerotia and mycelial strands on cedar needle surfaces. Periodicity of perithecia and acervuli formation, possible infection courts, and the disease cycle were investigated in this study.
Both the *Asteromassaria* sp. and *Scolicosporium* sp. states are observed in nature during June and July (summer) on twigs and branches which died during the previous year. The causal pathogen is also frequently isolated from incipient needle galls, containing larvae of the gall midge insect *Contarinia inouyei* Mani, which also develop during June and July. Hence it was hypothesized that the pathogen invades the host tissues through these galls at that time. New mycelial strands emerge in September (early autumn) from buds killed by *C. inouyei*. Sclerotia form with these mycelia on the needles in October and November (late autumn). New symptoms of necrosis subsequently develop around these sclerotia in late November and December (early winter).
These findings indicate that sclerotia play an important role in the development of new symptoms. After necrotic lesions expand, the most typical symptoms, dead twigs and dead branches, occur in the following January-to-April period. The infection cycle of Japanese cedar sclerotial dieback is presented diagrammatically.

PS3-261-0224

Patch canker disease of rubber trees on Hainan Island, China caused by *Pythium vexans*

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Since the early 50s, rubber tree production has played an important role in the economy of Hainan Island of South China. At present the total production of rubber on the island is ranked the fifth largest in the world. In recent years, a previously unknown disease occurred on rubber trees, *Hevea brasiliensis* (clone RRIM600) at some plantations. It is characterized by discrete, irregular patches of rotted, discolored bark and wood under the tapping cut, accompanied by a decrease in latex production and flow. It also affected the trunk, collar and first branch roots of the trees.

To isolate the pathogen, tissues were taken from diseased bark of the trunk or roots of the rubber trees and grown on selective carrot agar for Pythiaceous fungi. A total of seven isolates of *Pythium vexans* were obtained. Inoculating them into healthy, mature rubber trees resulted in symptoms similar to patch canker and the same fungal species was re-isolated from the diseased tissues. All isolates were similar in having radiate to finely petalloid, appressed growth pattern on V-8 agar plates, growth rate (25-27 mm per day at 26oC), growth/temperature relationships (min. 10, opt. 30 and max. 38oC) and morphology. They produced non-papillate, spherical , pyriform or ovoid, terminal or intercalary sporangia in sterile deionized water, av. 20.4 x 18.5 mm. Sex organs were produced readily on V-8 agar plates or in water. The oogonia were smooth, spherical, terminal or occasionally intercalary av. 20.9 mm diam. with single, aplerotic oospore av. 17.8mm diam. (wall av. 1.7 mm thick). Antheridia were bell-shaped or irregular, terminal, monoclinous, 1 per oogonium. The identification based on these characteristics was confirmed by the ITS I sequencing and a BLAST search with isolates from GenBank.

The cause of patch canker of rubber trees has been controversial for the past two decades. The disease was reported in Ceylon, Java, Summatra, Fiji, Malaya, Indonesia, India , Borneo, Congo and Costa Rica and was attributed primarily to *Phytophthora palmivora* , possibly associated with lightning damage. *Pythium vexans* was regarded as a weak parasite of *Hevea*, unlikely to be of any consequence. Present studies have proved that *Py. vexans* alone could cause patch canker of rubber trees in Hainan and neither *Ph. palmivora* nor lightning was involved. This is not only the first record of the disease in China but also the first report of the fungus in Hainan.

PS3-262-0251

Development of the species-specific primer for the identification and detection of *Alternaria panax* causing leaf spot on ginseng

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Alternaria leaf spot, caused by *Alternaira panax*, is one of the most important diseases of ginseng in Korea. A technique based on the polymerase chain reaction (PCR) was developed for the rapid detection of the *A. panax*. From the PCR bands of small-spored *Alternaria* isolates amplified with URP-PCR, a species-specific band of the *A. panax* was cloned and sequenced, and species-specific primer sets named PanaxF and PanaxR were designed for specific PCR detection of *A. panax*. These primer sets allowed the amplification of 470bp DNA fragment by PCR and were specific to the *A. panax,* as it did not amplify from genomic DNA of other *Alternaira* species. PCR assay using the species-specific primers should be detection and identification of *A. gaisen* from ginseng leaf where both *A. gaisen* and other pathogen are mixed.

PS3-263-0253

Molecular characterization of *Stemphylium* isolated from several host plants in Korea

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Molecular profiles of sequence analysis of ITS regions of rRNA, the translation elongation factor 1 alpha gene (EF-1alpha), glyceraldehyde-3-phosphate dehydrogenase gene (gpd), calmodulin gene and URP-PCR analysis were compared between morphologically distinguishable species of *Stemphylium* including *S. astragali, S. botryosum, S. lycopersici, S. sarciniforme, S. solani, S. vesicarium, Stemphylium* taxon 1 and 2. Sequence analysis of ITS rRNA, gpd, EF-1alpha, and calmodulin gene were able to distinguish each species of *Stemphylium* except morphologically similar *S. astragali, S. herbarum,* and *S. vesicarium*. The analysis of gpd, EF-1alpha, and calmodulin gene were found to be more useful for establishing phylogenetic relationship among *Stemphylium* isolates than of ITS. URP-PCR fingerprinting analysis of *Stemphylium* was found to be appropriate for taxonomic resolution of these species. Based on phylogenetic analysis and URP-PCR fingerprinting analysis of *Stemphylium,* the molecular species were well correlated with morphological species, and the *Stemphylium* taxon 1 and 2 were considered as new species.

PS3-264-0254

Management of *Phytophthora cinnamomi* in native vegetation in South Australia

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Phytophthora cinnamomi (Pc) is an introduced plant pathogen (Oomycete) carried in soil and water that causes root and lower stem rots in a wide variety of Australian native plant species. Infected plants show symptoms of drought and starvation and usually die within a month. Pc has been recognised by the Australian Government as a key threatening process. In South Australia, at least twenty-five percent of native vegetation found in areas with neutral to acidic soils and with 500 mm or more average yearly rainfall has been lost through Pc infestation.
Pc cannot be eradicated from natural ecosystems without undue disturbance. Rather, the risk of spread of Pc from infected areas is managed. Containment methods include temporary or permanent closure of tracks and trails. Where access to an infected area is allowed, hygiene procedures ensure that soil, water and plant material are removed from vehicles, machinery, equipment and footwear before leaving the area. Knowing the distribution of Pc enables the setting of appropriate management priorities and the deployment of containment methods.
Minimising the spread of Pc in South Australia is contingent upon information sharing and a collaborative approach to management by numerous stakeholders.
The SA Department for Environment and Heritage's Plant Dieback Ecologist has conducted at least one hundred workshops and presentations, and has published and disseminated extension material such as brochures, information sheets, booklets, posters and a bi-annual newsletter to increase the awareness and promote a "whole of community" approach to the management of Pc in South Australia. Statewide guidelines and standard operating procedures have been developed, and several government and non-government organisations and community groups, including Transport SA, National Parks and Wildlife SA, Local Councils, SA Water, Electricity Trust, Forestry SA and Trees for Life, are implementing hygiene strategies. The Country Fire Service has incorporated Pc management into fire fighting operations.
The SA-DEH will continue to be the lead agency in maintaining communcation links between the numerous stakeholders and encouraging a collaborative approach in the containment of Pc in South Australia.

PS3-265-0261

Fungus-arthropod mutualism and dispersal biology of *ophiostoma spp.* inhabiting *protea* flower-heads

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Ophiostoma represents a large genus of fungi, most of which are vectored by arthropods. One of the most unusual niches in which species of this fungal genus have been found is within the floral heads (infructescences) of *Protea* species in South Africa. Although it has been suspected that these fungi are transported by arthropods, their vectors have never been discovered. This is largely because the *Ophiostoma* spp. from *Protea* infructescenses are slow-growing, and isolations from insects are typically overgrown with saprophytic fungi.
The aim of this study was to identify putative vectors of the *Ophiostoma* spp. inhabiting *Protea i*nfructescences. This was facilitated by employing recently developed taxon-specific DNA-based primers. Application of this technique revealed the presence of *Ophiostoma* DNA on three insect species (*Genuchus hottentottus*: *Coleoptera*, *Oxycarenus maculates*: *Lygaeidae* and a *psocopteran* species) at low frequencies. Direct isolation methods revealed the presence of reproductive propagules of *Ophiostoma* spp. on four *Protea*-associated mite species (*Oodinychus* sp., two *Tarsonemus* spp. and *Proctolaelaps vandenbergi*) at high frequencies.
Isolations from freshly collected *Oodinychus* sp. individuals, which is the predominant species of these mites in the *Protea* infructescences, also yielded Ophio*stoma* spp. This mite also showed significantly higher reproductive rates when fed exclusively on *Ophiostoma splendens* than when it was fed on various other fungi. This suggests a mutualistic association between the *Oodinychus* sp. and *O. splendens*. These mites were also found to move over short distances between infructescences, but they were not found on sticky traps designed to collect wind-dispersed organisms. Microscopic examination revealed the presence of *Oodinychus* in large numbers on *G. hottentottus* emerging from *Protea* ssp. infructescences. Mites collected from these beetles were found to vector spores of various *Ophiostoma* spp. Based on these results, our view is that these mites act as primary vectors of the *Ophiostoma* spp. in *Protea* infructescences. The insects thus provide a mechanism for the long distance dispersal of the mites, together with their *Ophiostoma* symbionts.

PS3-266-0279

The Ultrastructural Morphology of *Eurychasma dicksonii*, An Oomycete Endoparasite of Filamentous Phaeophyte Algae

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Eurychasma dicksonii is one of the holocarpic oomycete endoparasites, which infects a broad host range of phaeophyte algae, including filamentous Ectocarpales to complex Laminariales. *E. dicksonii* is of particular interest because recent 18S rDNA phylogenetic studies show that this species occupies the most basal position of the monophyletic oomycete clade. Thus, this species may provide clues to the evolutionary origins and phylogenetic development of this important group. In this study we describe the cytology of E. dicksonii infecting Pylaiella littoralis and compare it with other oomycete groups.
A young unwalled vegetative cell of *E. dicksonii* is located in the cytoplasm of the host surrounded by what appears to be a double membrane envelope. Mitochondria with tubular cristae and dense body vesicles (DBV), both of which are the characteristic features of oomycete cytoplasm, are also observed. In more advanced walled stages, at least three different types of thallus have been observed. One is called "the net sporangium", whose zoospore cysts are peripherally located on the inside of the sporangium wall. These release zoospores into the central cavity of the sporangium. Another pattern of development was termed "direct discharge type sporangium", the zoospores of which directly swim out from the sporangium without the formation of a cyst net. Notable morphological differences were observed between the encysted zoospores formed from these two sporangial types. The final type has been described as "the cytoplasmic-mass thallus" in which a dense globular mass of cytoplasm is formed, surrounded by an empty space. The cytoplasm is multinucleate and contains zones that are rich in mitochondria. Typical oosporogenesis has never been observed in this species.
The characteristic features of peripherally produced zoospore initials with central vacuole and multiplanetic zoosporogenesis are features shared with saprolegnian oomycetes, which suggests that *E. dicksonii* has closer affinities with the Saprolegniales rather than the Peronosporales.

PS3-267-0298

Effect of green manure soil amendments on *Trichoderma spp.* population and diversity in the rhizosphere of onion

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The ecology (population size and diversity) of indigenous *Trichoderma* species at the rhizosphere level is likely to be influenced by farming practices such as green manure amendments. Increases in population size and diversity can promote the natural suppression of soil-borne plant pathogens in the field.
To quantify this effect, a pot experiment was set up in a glasshouse using a complete random design. Onions were planted in a volcanic clay loam soil amended with either dry onion residues (Treatment 2 = onion 1 t ha-1) or oat fragments as green manure (Treatment 3 = oat 4t ha-1 and Treatment 4 = oat 8 t ha-1). A control (Treatment 1) with no organic matter amendment was also set up. The plants were grown for 4 months and every month, 16 onions were harvested. The *Trichoderma* spp. population size in the rhizosphere soil was measured using soil dilutions plated on *Trichoderma* Specific Medium. Species diversity was determined through ITS-RFLP analysis (work currently underway) using *TaqI* and *MboI* restriction enzymes.
At the time of planting, there was no significant difference in *Trichoderma* colony forming units (cfu) numbers from the rhizosphere soil across the 4 treatments. However, the interaction between time and treatment revealed that at the end of the experiment, a significant difference ($P=0.007$) developed between *Trichoderma* cfu numbers in the rhizosphere soil amended with 1 t ha-1 of dry shredded onion scales (Treatment 2) and all the other treatments. Over the course of the whole experiment, *Trichoderma* cfu numbers in the rhizosphere soil of Treatment 2 were also significantly lower ($P<0.001$) than the other treatments. *Penicillium* cfu in the rhizosphere soil were also recorded. The number of *Penicillium* cfu was significantly higher ($P<0.001$) in Treatment 2 when compared with the other treatments. Preliminary results of species diversity indicate that *T. harzianum*, *T. hamatum*, *T. koningii* and *T. aureoviride* are the most prevalent species.
The results revealed that in the rhizosphere soil of onion, there was no difference in Trichoderma populations between Treatment 1, 3 and 4. Treatment 2, in contrast, had the lowest *Trichoderma* cfu and the highest *Penicillium* cfu counts. Therefore, onion residues appear to have a negative impact on the *Trichoderma* population in the rhizosphere soil of onion either directly by suppressing *Trichoderma* growth or indirectly by competition through the promotion of *Penicillium* growth. The addition of oat did not increase the *Trichoderma* population size.

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Novel single-stranded RNA mycoviruses in edible mushrooms

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Several novel ssRNA viruses were islated from cultivated *Agaricus bisphorus, Agaricus blazei, Flammulina velutips, Lentinula edodes, Pleurotus ostreatus, and Pleurotus eryngii* with the epidemic of mushroom malformation disease. These new viruses were spherical forms of 20nm to 40nm in diameter encapsidating monopartite or bipartite ssRNAs of approximately 8kb to 1.5kb. The partial nucleotide sequence of the viruses showed that there genomic RNA were different from each other. We made monoclonal and polyclonal antibodies to develop triple antibody sandwich ELISA kit for diagnosing the viruses. The antibodies of the viruses did not cross react with different viruses. This result revealed that there were several different spherical ssRNA viruses in cultivated mushrooms.

PS3-269-0353

Detection of Neotyphodium occultans – new methods to deal with this hard to find grass endophyte using DIC and PCR

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Neotyphodium occultans is an asexual fungal symbiont (endophyte) of *Lolium* grasses (sub-family Pooideae) and is closely related to the choke pathogen, *Epichloë* species. Detection and identification of this seedborne *Clavicipitaceous endophyte* is not easy due to the infection being symptomless and its limited localization in host plants; hyphae are confined to the basal few mm of leaves, the apical meristem, flowers and seeds. Although the distribution of hyphae of *N. occultans* in host plants is so restricted, recent surveys have revealed that it may have major ecological impacts, influencing associated flora and fauna. To make detection of this endophyte in *Lolium* grasses easier the feasibility of applying microscopic observations and PCR based methods to samples of flowers and seeds was examined and compared. Immature inflorescences of infected grasses were dissected under a dissecting microscope to isolate the ovaries. For microscopy examination the ovaries were soaked in a solution made by mixing lactic acid, glycerol, and distilled water in the ratio of 1 :2: 1 (v/v/v) (lactic acid in glycerol) for 4h to overnight at room temperature to clear the tissue following which they were observed with differential interference contrast microscopy(DIC). With the method, we were able to observe and identify the fungus with relative ease compared with conventional ways using seeds, since there is less possibility of confusion resulting from the presence of other parasitic and saprophytic fungi. As a PCR based method, amplification and direct sequencing of rDNA was tested. From the total DNA extracted from immature infected ovaries, we could easily amplify and sequence the rDNA of the fungi with conventional primers such as ITS1 and ITS4 (White et al. 1990). With total DNA from seeds the presence of other fungi caused difficulties so we made new primers specific for rDNA of *N. occultans*, which enabled us to sequence it directly. Since the morphology of flowers are a key to identify grass plants, and seeds are the easiest materials for collection and long time storage of the resident endophyte, a combination of the above methods is very useful to study *N. occultans* and other related symbionts.

PS3-270-0363

Puccinia striiformis (sensu lato) in Japan – long term fluctuations of occurrence on different hosts.

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Puccinia striiformis (sensu lato, Uredinales) is the cause of yellow rust or stripe rust on various species of grasses. As a serious pathogen of wheat and barley, its dispersal on a continental scale is of world wide interest. Recently, new nomenclature dividing the "species (s.l.)" into three was suggested, namely, *P. striiformis* (sensu stricto) on numerous hosts (mainly on Triticeae), *P. pseudostriiformis* on *Poa pratensis* (bluegrass, Poae), and *P. striiformioides* on *Dactylis glomerata* (orchardgrass, Poae), based on comparison of ribosomal RNA gene (rDNA) (Abbasi et al. 2004, also presented in IMC7). To examine the prevalence of these fungi in Japan, site studies and reference surveys have been carried out. *P. striiformis* (s.s.) had been recorded on wheat and barley since the beginning of the 20th century and serious epidemics were observed in the 1950-60s but had virtually disappeared by the 1970s. In contrast, it is still a major problem in neighbouring countries such as China. The reason of the change is not clear, but is assumed to be the introduction of resistant cultivars and fungicide applications. Bluegrass was introduced to Japan for pasture or turf use in the 19th century, and yellow rust has been widely observed on both naturalized and cultivated populations. Surveys in 2004-2005 in the northern two islands, Hokkaido and Honshu, indicated that yellow rust of orchard grass, which had not been recorded in the country, was present within both naturalized and cultivated populations. Since orchard grass was introduced to Japan in the 19th century, and diseases on this important pasture grass have been intensively recorded, the invasion of this rust is considered to be fairly recent. Records in Hokkaido indicate that total rust occurrence on orchardgrass has been increasing since the 1990s. This increase may correspond with the invasion of *P. striiformioides*. Interestingly, this rust first colonised orchard grass in North America and New Zealand in the late 1970s-1980s, as did yellow rust of wheat in Australia and New Zealand.

PS3-271-0405

Coprophilous fungal diversity in Thailand : Studies on antagonistic activity against plant pathogenic fungi and secondary metabolites of *Ascodesmis macrospora* and *Sordaria fimicola*

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Dung samples of wildlife and domestic animals, including barking deer, buffalo, camel, cow, deer, eld's deer, elephant, guar, goat, horse, rabbit, rat and toad, were collected from eighteen locations in Thailand. Identification of fungal isolates was based on morphological characteristics of colony growth on agar media and of spores and fruiting bodies using light and scanning electron microscopes. Nineteen genera and twenty-five species of coprophilous fungi were recorded in this study, including *Absidia corymbifera*, *Apiosordaria* sp., *Arthrobotrys oligospora*, *Ascobolus aldibus*, *Ascodesmis macrospora*, *Ascodesmis sphaerospora*, *Cercophora silvatica*, *Chaetomium crispatum*, *Chaetomium cupreum*, *Chaetomium globosum*, *Chaetomium* sp., *Coprinus* sp., *Cunninghamella elegans*, *Gelasinospora brevispora*, *Mucor* sp., *Pilobolus crystallinus*, *Podosordaria leporina*, *Podospora curvicolla*, *Podospora* sp., *Rhizopus oryzae*, *Rhizopus stolonifer*, *Saccobolus glaber*, *Sordaria fimicola*, *Sporormiella minima* and *Syncephalastrum racemosum*. Six genera, 7 species of coprophilous fungi comprising, *A. albidus*, *A. macrospora*, *A. sphaerospora*, *C. silvatica*, *G. brevispora*, *P. leporina*, and *S. minima* represent new records for Thailand.

Antagonistic activity tests for *A. macrospora* and *S. fimicola* against 9 species of plant pathogenic fungi including *Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Colletotrichum capsici, Pestalotiopsis guepinii, Lasiodiplodia theobromae, Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotium rolfsii* were conducted on potato dextrose agar. The results indicated that *S. fimicola* could effectively control 7 species of plant pathogenic fungi except *R. solani* and *S. rolfsii*, while *A. macrospora* showed moderate inhibition to 3 plant pathogenic fungi, including *A. alternata, F. oxysporum* and *C. capsici in vitro*.

The formation of secondary metabolites by *A. macrospora* and *S. fimicola* was determined by cultivating the fungi on potato dextrose broth for 28 days at 28-30oC. The fungal culture filtrates were extracted three times with equal volumes of ethyl acetate at room temperature. The organic phase was evaporated under reduced pressure by a rotary evaporator. The crude ethyl acetate extracts were applied to column chromatography and eluted with a gradient mixture of petroleum ether, chlorofrom and acetone. The compounds were purified by preparative TLC and their chemical structure was determined by spectroscopic methods as well as comparison of their NMR data. Ergosterol; 7,8- dehydroergosterol and a 3-keto derivative of ergosterol were isolated from *A. macrospora* and parahydroxy paradehyde was isolated from *S. fimicola*.

PS3-272-0410

Diversity of *Talaromyces species* with special emphasis on *T. flavus* for potential use in biological control of plant pathogenic fungi in vitro and in the greenhouse

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Soil samples were collected from 32 locations in Thailand from March 2002 to February 2005. The soil plate, dilution plate, alcohol and heat treatment methods and Gochenaur's glucose ammonium nitrate agar were used to isolate *Talaromyces* spp. Identification was based on macro-and microscopic characters when cultured on different media and after observation under stereo, light, and scanning electron microscopes. Camera lucida drawings were made. A total of 290 isolates of *Talaromyces* were recorded, comprising 9 species and 3 varieties including *Talaromyces austrocalifornicus*, *T. bacillisporus*, *T. flavus* var. *flavus*, *T. flavus* var. *macrospermus*, *T. helicus* var. *helicus*, *T. luteus*, *T. rotundus*, *T. stipitatus*, *T. trachyspermus*, and *T. wortmanii*. Among 170 isolates of *T. flavus*, 20 isolates were tested for antagonism to 9 plant pathogenic fungi *in vitro* and in the greenhouse. The tests for antagonism indicated that all isolates of *T. flavus* could effectively control *Phytophthora palmivora* and partially control *Phytophthora parasitica*, *Fusarium oxysporum*, *F. semitectum*, *Colletotrichum capsici*, and *C. gloeosporioides*, but did not control *Lasiodiplodia theobromae*, *Rhizoctonia oryzae* and *Sclerotium rolfsii in vitro*. The efficacy of *T. flavus* isolates to control stem rot of mungbean (*Vigna radiate* (L.) Wilczek), caused by *S. rolfsii*, was examined in the greenhouse. Dried mungbean seeds were immersed in a ascospore suspension (106 spores/ml) of *T. flavus* for 24 hours. Ten mungbean seeds were placed on the soil surface in each pot, and one sclerotium was placed 1 cm from each seed. The pots were incubated in a greenhouse at temperatures ranging from 25 to 30 oC. Symptoms of stem rot were recorded at 7 and 14 days. Treatments were arranged in a completely randomized design. The experimental was conducted 2 times, with 5 replicates, and the data were pooled. The greatest disease reduction was 82% when seeds were treated with ascospoes of *T. flavus* No.T193, followed by *T. flavus* No. T198, and *T. flavus* No.T4 at 79% and 75%, respectively. The remaining strains exhibited low to moderated capability to control the disease, ranging from 14% to 67 %. Pure cultures of all *Talaromyces* species are being maintained in the Culture Collection at Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand for secondary metabolites analysis and use in further studies of biological control.

PS3-273-0415

Compatibility assay of some antagonistic fungi of heterodera schachtii in in vitro.

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Fungal parasites of nematode eggs have great potential as biocontrol agents since they also infect females of sedentary nematodes and destroy the eggs they contain. They as any other fungi in soil, must have some capability to meet the fierce competition among themselves or from other nematophagous organism in soil. So, some nematophagous fungi may produce substances toxic to others in soil. Competition among fungi through nutritional depletion, space occupation and toxic activities. This study conducted to assessment of compatibility of five nematophagous fungi that include:two isolates of *Paecilomyces lilacinus* and one isolate each of *Pochonia chlamydosporia var. chlamydosporia, Cylindrocarpon sp., and Chaetomium sp*.They have been isolated from sugar beet fields in Iran.In this regard potato dextrose agar media were seeded with a plug of 5mm diameter of grown culture of fungus on corn meal agar , taken from a periphery colony of a 10 days old in all combinations ,two species in one plate. The treatments were replicated three times and incubated at 25° C in darkness. After 25 days, colony diameters and inhibition zones were measured.The area was calculated from the diameter measurements.The percent growth inhibition was calculated using the formula $n = (a - b)/a$?100 where *n* is the % growth inhibition; *a* is the colony area of control; and *b* is the colony area of isolates. *Chaetomium sp*. exhibited strongly inhibitation on mycelial growth of other fungi by producing green metabolite and decreased growth of *Pochonia chlamydosporia var. chlamydosporia, P. lilacinus* isolate P 8.1*, P. lilacinus* isolate 25.4 and *Cylindrocarpon sp.*, 52.3%, 61.8%, 68.9% and 65.1% ,respectively. The results reveal that *Chaetomium sp*. has antagonism interaction to others and is a candidate for biological control of pathogenic fungi . The other isolates have lower impact together that it may because of competition, so the combination of these isolate could be used for biological control of plant parasitic nematodes.

PS3-274-0416

Diseases of Weed in Vegetable Garden Plots and Their Potential Use for Their Biological Weed Control.

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Weeds showing leaf spot and leaf blight diseases were collected from vegetable garden plots in Kamphangsan, Nakhonpathom (*Zae mays* and *Asparagus officinalis*) and Sainoi, Nonthaburi (*Apium graveolens*, *Brassica alboglaba*, *Brassica campestris* and *Raphanus sativus*) during April, July and November 2003. Thirteen weed hosts used in this study included *Cyperus rotundus* (Cyperaceae), *Commelina benghalensis* (Commelinaceae), *Dactylotenium aegyptium*, *Echinochloa colona*, *Eleusine indica*, *Digitaria ciliaris*, *Brachiria reptans*, *Rottboellia cochinchinensis*, *Pennisetum polystachyon* (Poaceae), *Trianythema portulacastrum* (Azioaceae), *Amaranthus gracillis* (Amaranthaceae) and *Euphorbia hirta*, *Euphorbia heterophylla* (Euphorbiceae). Tissue transplanting, moist chamber method and potato dextrose agar were used to isolate microfungi. Identification was based on growth rate, colony colour and other microscope features as observed on artificial media. Microscopic characters were examined under stereo and light microscopes and with camera lucida drawings. A total of 642 fungal isolates, comprising 21 genera and 34 species were found. These included *Alternaria alternate*, *Aspergillus flavus*, *Bipolaris bicolor*, *Chaetomella raphigera*, *Colletotrichum capsici*, *C. gloeosporioides*, *Curvularia affinis*, *C. brachyspora*, *C. clavata*, *C. geniculata*, *C. inaequalis*, *C. intermedia*, *C. lunata*, *C. pallescens*, *C. penniseti*, *C. senegalensis*, *C. sorghina*, *Drechslera halodes*, *D. holmii*, *Eericella vareicolor*, *Exserohilum rostratum*, *Fusarium oxysporum*, *F. semitectum*, *F. solani*, *Myrothecium cinctum*, *M. verrucaria*, *Neosartorya fischeri*, *Nigrospora oryzae*, *Phaeotrichoconis crotalaria*, *Pestalotiopsis guepinii*, *phoma jolyana*, *P. tropica*, *Pyricularia grisea*, *Sordaria* sp., *Stemphylium sarciniforme*, *Talaromyces* sp. and unidentified species of Coelomycetes.
Pathogenicity tests of fungi isolated from weeds were conducted using *Drechslera holmii*, *Exserohilum rostratum* and *Stemphylium sarciniforme* by inoculating in the greenhouse to three healthy weed species, *Dactylotenium aegyptium*, *Cyperus rotundus*, *Trianthema portulacastrum* and two vegetable hosts, *Brassica alboglaba* and *Brassica campestris*. Spore suspension at106 spore/ml was sprayed on wounded leaves of the 4 week old seedlings. *E. rostratum* and *S. sarciniforme* caused severe damage only on their respective weed hosts (*D. aegyptium* and *T. portulacastrum*), whereas *D. holmii* caused mild damage only on *D. aegyptium*. Therefore, *E. rostratum* and *S. sarciniforme* are potentially useful as foras biological control of weed in vegetable garden plots.

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Appendaged coelomycetes on grapevines in Australia

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The occurrence of various fungi associated with infection of canes and trunks of grapevines *(*Vitis vinifera *L.)* in Australia has been reported recently by *Sergeeva* et al. (2001), *Castillo-Pando* et al.(2001) *and Edwards and Pascoe (2002)*. In general, those authors reported on a number of known woody tissue invading fungi such as *Botryosphaeria, Eutypa lata, Phaeomoniella* and *Phaeoacremonium*. However, little is known, in Australia, about incidence and distribution, or role in pathogenesis of various appendage-bearing coelomycetes on grapevines. In this paper we present descriptions of several appendaged *Coelomycetes* recently isolated from grapevines in Australia and infection studies on *Pestalotiopsis* uvicola.

Samples of dormant canes, leaves, flowers and darkened wood from trunk and arms of grapevines were used to obtain axenic cultures of the fungi. Established procedures for isolation, identification and storage of fungal cultures were used. Infection studies were carried out using P. uvicola *on grape berries of cv Chardonnay at pea size and* veraison stages. The surface sterilized berries were inoculated with spore suspension and examined after five days under a light microscope *(x400) for the presence of* P. uvicola.

Infection studies – P. uvicola *infected the berries at the veraison stage after 5 days incubation at 25*^{0}*C*. However we observed that infection of pea size berries occurred only when they were incubated for a further period of 14 days at 27^{0}C wrapped in wet cotton wool for increased humidity and wetness.

Descriptions of fungi - Five *morphologically distinct taxa of appendaged coelomycetes have been recognised as occurring on grapevines in eastern Australia. Morphological features of the fungi and symptoms caused by them are described:* Pestalotiopsis uvicola *(Spegazzini) Bissett* with its mostly concolorous cells and rugose cell walls has been isolated from bleached canes, internal wood rot, leaf spots, flower rachises and berries. P.menezesiana *(Bres.& Torr.) Bisset, distinguished from* P. uvicola *by* its opaque upper cells and darkened septum has been isolated from bleached canes and internal wood rot. Seimatosporium hysterioides *(Fuckel) Brockman was isolated from dead stems and from cankered canes.* Truncatella angustata *(Pers.: Link) Hughes has been isolated from dormant canes and roots.* Sporocadus rhodendri *(Schw.) Morelet was isolated from symptom less canes.*

The results from infection studies showed that berries of grapevines were infected by *P. uvicola* more readily at later stages of berry development than at the earlier stages. The delay in infection of berries at the pea size stage of growth may indicate that the fungus undergoes a quiescent phase after flower infection. Several appendaged coelomycetes are associated with grapevines in Australia and their role in pathogenesis is worth further investigation.

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Grapevine Wood Fungal Infection By Soil/Root Transmission

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Grapevine die-back by fungi such as *Botryosphaeria* and *Pestalotiopsis* is usually caused by infection of propagation material or by infection of wounds in aerial parts of the grapevine. However, this study shows that infection can also be initiated from soil borne inoculum.

The soil of mature potted Pinot Noir grape vines was inoculated with *Botryosphaeria stevensii*. Two-month-old rooted cuttings were also inoculated with 22 different grapevine fungal pathogens *via* the soil.

Shoots from two of three potted mature Pinot noir vines were found to be infected with *B. stevensii.* Many of the Chardonnay rootlings were also found to be infected by the soil inoculated fungi.

Infection from soil borne inoculum has serious implications for vineyard replanting after removal of diseased vines.

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Oxidative stress response of the fungus *Mycosphaerella fijiensis*, the black sigatoka pathogen of banana to hydrogen peroxide and other stress conditions.

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Mycosphaerella fijiensis (anamorph *Paracercopsora fijiensis*) is the causal agent of the foliar fungal disease black sigatoka, the major worldwide constraint to banana and plantain (*Musa sp.*) production. Leaf necrosis caused by black sigatoka results in yield losses estimated at 50-100%. This fungus presents an elevated aggressivity and highly resistance to fungicides. Plants produce ROS as response to pathogens, and also diverse fungicides convert into ROS to exert toxic effect on their targets. In spite of these facts, relatively little is know about the oxidative stress response of *M. fijiensis* for evading the effect of the ROS. If *M. fijiensis* can evade efficiently the toxicity of the H2O2 or other reactive oxygen species, it might explain why the fungus resists the hypersensitive reaction of plants or to some fungicides, where catalase and peroxidase could be a resistant factor that isolates fungal cells from detrimental environment favouring to infect the plants and to survive in vegetal tissue.
M. fijiensis mycelia totally decomposes 50 mM of H2O2 after 120 minutes and liberates oxygen bubbles intensely. Lethal concentration 50 (LC50) is close to 75 mM H2O2. In order to study the oxidative stress response to H2O2, chemical agents and heat shock, the intracellular catalase (CAT), superoxide dismutase (SOD) and total peroxidase (Pox) activities were evaluated. We found two different isozymes of SOD, where CuZnSOD is the major component when culture was exposed to paraquat (PQ) and hydroquinone (HQ), well known generators of superoxide anion. PQ and HQ enhances the synthesis of SOD three fold in relation with control, but not catalase. In contrast, H2O2, Rose Bengal (a singlet oxygen generator), and heat shock rapidly enhance the activities of two catalase activities (one typical catalase and catalase-peroxidase) and total peroxidase activity 3 to 5 fold. Variations of lipid peroxidation (LPO) products as oxidative stress biomarker depend on both the stress condition and the time of exposition.
The contribution to cell viability and fast H2O2 decomposition on *M. fijiensis* probably can be correlated with the catalase activities. There is growing evidence that some phytopathogenic fungi have the ability to insulate ROS during plant hypersensitive reaction or fungicide exposition. Further research is needed to understand if oxidative stress resistance in *M. fijiensis,* especially to H2O2, might predict the virulence or fungicide resistance of a particulate isolate.

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Silencing of pathogenicity genes in the apple scab fungus, *Venturia inaequalis*

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Apples (*Malus pumila*) are a major export for New Zealand. The pathogen *Venturia inaequalis* (apple scab fungus) causes apple scab disease (black spot), which reduces crop yield. Much study has been directed toward fungicide-mediated control and the breeding of resistant apple cultivars, however there has been little molecular investigation into the pathogen. This project will explore the functional properties of three expressed fungal genes; one with similarities to a polyketide synthetase, one similar to a phytoalexin (plant defence compound) detoxifying enzyme and another similar to a protein folding enzyme. These three enzymes were chosen from an EST library due to their similarity to pathogenicity genes in other fungi. Using Real Time RT-PCR, the expression of each gene will be analysed from *in vitro* growth and during a time course infection. The resultant expression profiles will give insight into their likely involvement in infection. Hairpin constructs have been shown to be effective at inducing post-transcriptional gene silencing in many biological systems, including *V. inaequalis*. Vectors containing hairpins have been constructed for these genes and will be introduced into *V. inaequalis* via *Agrobacterium*-mediated transformation. Expression of targeted genes in the resultant transformants will be monitored using Real Time RT-PCR. Silenced transformants will then be tested for any change in pathogenicity phenotype. This project will give insight into the role of the three enzymes of interest during the *V. inaequalis* life cycle. As these three enzymes contribute to pathogenicity in other fungi, they may therefore be targets for the control of *V. inaequalis* by acting as fungicide targets.

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Genetic variation for *Phymatotrichopsis omnivora* tolerance in Alfalfa (Medicago sativa L.)

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Phymatotrichopsis omnivora (Duggar) Hennebert is a destructive root pathogen of dicotyledonous plants. The disease, known as cotton root rot or Phymatotrichum root rot, caused by this pathogen is responsible for severe crop losses in Southern Oklahoma, Texas, New Mexico and Arizona. The production of Alfalfa, an important forage crop in Southern Oklahoma and Texas, is hindered by the presence of *P. omnivora* as no alfalfa varieties have shown resistant or tolerance to this fungus.

We have established a growth chamber screening procedure to identify tolerance to Phymatotrichum root rot in alfalfa. Twenty alfalfa cultivars and *Medicago truncatula* Jemalong A17 were planted in 96-cell trays containing Houston black clay and maintained in a growth chamber at 22-28oC. A *P. omnivora* culture was isolated from alfalfa, cultured on sterilized sorghum grains and used to inoculate each plant. Four weeks after inoculation the plants were scored for resistance based on a scale of 1 - 5 ranging from dead (1) to tall and healthy (5). One group of alfalfa cultivars, Enhancer, Magnum IV, OK 49, and Pioneer 5681 exhibited a low but consistent level of tolerance to Phymatotrichum root rot. Examination of the plants found the pathogen was associated with the roots of healthy plants. These plants were re-examined for tolerance to Phymatotrichum root rot with an infection assay using clonal plant material. Several tolerant alfalfa genotypes have been identified for further studies.

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PCR detection of *Mycosphaerella* species in early lesion developmental stages of Mycosphaerella leaf disease.

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Mycosphaerella Leaf Disease in eucalypts is associated with a suite of *Mycosphaerella* species that may co-occur on a single leaf or in a single lesion. Standard methods such as morphological analysis of spores and isolation reveal only a small fraction of the species present in advanced lesions. To assist elucidation of the disease aetiology and identification of the most aggressively pathogenic species, we used PCR detection of the five most commonly isolated Tasmanian *Mycosphaerella* taxa to determine which of those taxa were present in early lesion developmental stages. Leaf samples with lesions at a range of developmental stages were taken. Stage 0 – no visible lesion, Stage 1 – indistinct, barely discernible spot, Stage 2 – indistinct, purple-tinged spot. Thirty leaves were sampled for each of the lesion categories, the spot of interest was excised, DNA was extracted and nested PCRs for the detection of *Mycosphaerella cryptica*, *M. parva/grandis* (*M. grandis*), *M. nubilosa, M. tasmaniensis* and *M. vespa/ambiphylla/molleriana* (*M. vespa*) were performed.

Four species were detected in *Eucalyptus globulus* leaves with no visible lesions, three at low frequency and one, *M. grandis*, in 43% of samples. *M. grandis* frequency of detection increased to 57% and 90% for stages 1 and 2. *M. nubilosa* and *M. tasmaniensis* were detected at low frequency in all three stages and *M. vespa* was not detected in any of the leaves. *M. cryptica* was detected in 10% of stage 0, increasing to 63% and 83% of stage 1 and 2 lesions.

The high incidence of *M. grandis* in leaves with no visible lesion concurs with the hypothesis that *M. grandis* can survive as an endophyte, but this does not necessarily preclude a role in disease development. The increased incidence of *M. cryptica* in more advanced lesions indicates that this species is an aggressive competitor, but it is unable to eliminate *M. grandis,* even in advanced, necrotic lesions. Quantitative PCR may be able to shed further light on inter-species interactions and disease development.

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The distribution and impact of *Mycosphaerella cryptica* on regenerating Eucalyptus gomphocephala

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Mycosphaerella cryptica is one of the most destructive foliar pathogens of eucalypts in plantations in southern Australia. The majority of research on this pathogen has been conducted in plantations, with little focus on its distribution and impact on eucalypts in native forest. Tuart (*Eucalyptus gomphocephala*) is a magnificent woodland tree endemic to Western Australia. With almost 75% of its original area cleared and concerns for the health of those trees remaining, the recruitment of tuart is highly important. The study of pathogens and pests that pose a threat to tuart seedling survival will contribute to ensuring the regeneration of the remaining woodlands..

Mycosphaerella cryptica has been recorded on *E. marginata* (Jarrah), *E. diversicolor* (Karri) and *E. patens* (Blackbutt) in native forests. In the present study we carried out surveys of regenerating stands of tuart between 2003 and 2005, confirming the presence of *M. cryptica* throughout all stands surveyed, and in some cases contributing to mortality of seedlings. These findings are somewhat surprising given *M. cryptica* has not previously been recorded from tuart in native forests.

In addition to these surveys, trials were established in Yalgorup N.P. in the native forest to investigate how the presence/absence of ashbeds and competition with midstorey (mainly *Agonis flexuosa*) affects the survival and growth of planted seedlings. These trials were assessed seasonally over a 14 month period for phytophagous insect attack and fungal pathogen damage. Initial assessments at 12 weeks confirmed the presence of *M. cryptica* associated with leaf lesions (also known as Crinkle Leaf). Over the subsequent 12 month period the severity of Crinkle Leaf increased across the trial and in some cases resulted in seedling mortality. In comparison to phytophagous insect damage, at the final assessment Crinkle Leaf was by far the most dominant category of damage.

Comparisons were made between the climatic conditions, seasonal growth patterns, and severity of Crinkle Leaf at the study site and in eastern Victoria where previous studies on *M. cryptica* have been conducted. Seasonal periods of infection by *M. cryptica* differed between the two sites. We propose that this is due to optimal conditions for spread and infection of the pathogen, and growth of the seedlings occurring in winter in Yalgorup N.P. compared with summer in eastern Victoria.

Knowledge gained in this study provides important information for disease management and will benefit restoration/regeneration initiatives of this threatened species of eucalypt.

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Effects of winter hardening and winter temperature shifts on *Pinus sylvestris -Gremmeniella abietina* plant-pathogen interactions

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The pathogenic ascomycete *Gremmeniella abietina* (Lagerb.) Morelet causes shoot dieback in several genera of conifers, in Sweden mainly on *Pinus* species. The fungus is favoured by cold, wet summers and mild winters. *Gremmeniella abietina* infects the top shoots of its host in summer, and stays as a latent infection until winter, when it starts to grow in the inner bark and into the wood. It has been shown that *G. abietina* needs at least 44 conducive days of mild winter weather with temperatures near zero °C in order to be able to break latency.

Two experiments were conducted. In the first experiment 750 two-year-old *Pinus sylvestris* L. seedlings were pre-treated in three separate regimes (two winter-hardening regimes and one constant regime resembling Swedish autumn conditions) and subsequently inoculated with *G. abietina* mycelia in order to examine the relationship between the process of winter-hardening in the host and the growth of *G. abietina* within the host tissue during autumn and winter. Seedlings winter-hardened outdoors showed a significantly higher degree of disease incidence than seedlings winter-hardened in a phytotron climate chamber. Instead the latter showed about the same disease incidence as the seedlings pre-treated in the constant regime. However, all the plants that had visible necroses, showed the same disease severity, regardless of which pre-treatment they had been subjected to. This implies that the winter-hardening process itself doesn't predispose the host tree for *G. abietina* infection. Nor does it lead to severer infections. Instead, weather data indicated that the host may become prone to infection either when subjected to sudden large temperature shifts during winter or when its dormancy.

The second experiment looked at the effect of large temperature shifts during winter on the growth of *G. abietina* within the host tissue. Two-year-old seedlings of *P. sylvestris* were winter- and cold hardened in the phytotron and subsequently subjected to large temperature shifts, where after they were inoculated with *G. abietina* mycelia. Preliminary data analysis suggests that the effect of temperature shifts is minor. If anything, this kind of temperature stress may actually strengthen the host's ability to hamper the growth of *G. abietina* in the inner bark.

PS3-283-0552

High altitudinal plant ailments

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Northern areas of Pakistan are rich repository of natural vegetation and other bioresources. There are many tracks locally known as *Galliat* famous for their natural beauty and biodiversity. All tracks are full of coniferous trees with luxurious herbaceous flora. *Miranjani* is the top peak of this area with a height of 2920 m. The plants from the Miranjani Mountain trail were screened for the presence of diseases. A total of 12 types of pathogens were reported. Out of these 12 pathogens, 11 were rusts, while one was an unidentified member of *Erysiphales*. Rust on *Creatigus* and *Mellilotus* are new records for hosts while the Erysiphe on *Sibbaldia* sp., is a new record as regards fungal pathogen and host. It was found that there is a relationship between the prevalence of rust flora and plant with reference to altitude.

Key words: High altitude, plant diseases, rust flora, Pakistan.

PS3-284-0557

Biological control of *Pythium* and *Sclerotium* root rot of sunn-hemp and mungbean by *Gliocladium virens*

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Pythium aphanidermatum and *Sclerotium rolfsii* can cause serious root rot and damping-off in various economic plant crops. We investigated soil fungi producing enzyme capable of controlling plant pathogenic fungi *in vitro*. After the screening process, *Gliocladium virens* isolate no.119 obtained from rhizosphere soil of teak plantation in Uttaradit province, Northern Thailand was selected to test for antagonism to 2 plant pathogenic fungi, *Pythium aphanidermatum* and *Sclerotium roffsii* obtained from cucumber (*Cucumis sativus*) and mungbean (*Phaseolus aureus*) respectively. They were cultivated as dual cultures on potato dextrose agar for 4 days. The results showed that *G. virens* could effectively control *P. aphanidermatum* and *S. rolfsii in vitro*. Mechanisms of inhibition concerned with antibiosis, competition and mycoparasitism.

Liquid cultures of *G. virens* on various substrates were detected for enzyme activities. The results showed that *Gliocladium* sp. produced various enzyme activities, such as filter paperase, carboxymethyl cellulase, cellobiase, protease and chitinase at the highest amount of 53.71, 541.36, 42.17, 92.75 and 519.61 milli unit per millilitre within 3-6 days respectively.

The efficacy of *G. virens* to control damping-off and stem rot of sunn-hemp (*Crotalaria juncea*) and mungbean seedlings caused by *P. aphanidermatum* and *S. rolfsii* was examined *in vitro* . Dried seeds were immersed in conidia suspension (107 spores/ml) of *G. virens* for 15 minutes. Ten seeds were placed on damp filter paper in a petridish. One ml of *P. aphanidermatum* and *S. rolfsii* suspension was inoculated. They were incubated at 30 oC. The results indicated that the percentage of seed germination of sunnhemp and mungbean were increased from the infected seed at 54.0 and 41.6 to 98.6 and 96.4 respectively.

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Pathogenic fungi from banana, rambutan and rose apple

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Pathogenic fungi from banana, rambutan and rose apple
The purpose of this investigation was to isolate fungi from post harvest disease fruits, such as banana (Kluai Nam Wa), rambutan (Rong Rian) and rose apple (Tup Tim Chan). The samples of healthy fruits were collected from the orchards and markets in Suphanburi province and Bangkok. Tissue transplanting method and water agar were used to isolate fungi from three kinds of fruits after 1 week incubation at room temperature (28°C). Pathogenicity tests of all fungal isolates were conducted. Identification was based on growth rate, colony colour and other macroscopic features observed on potato dextrose agar. Microscopic feature was examined under light microscope. Six genera of fungi, including *Greeneria* sp., *Lasiodiplodia theobromae*, *Gliocephalotrichum bulbilium*, *Pestalotiopsis* sp., *Penicillium* sp. and *Aspergillus niger* were isolated from the rotten tissues of the rambutan fruits at 58.82%, 16.36%, 11.98%, 9.99%, 1.48% and 1.38% respectively. For the rose apples, seven genera of fungi including *Lasiodiplodia theobromae*, *Pestalotiopsis* sp., *Aspergillus niger*, *Penicillium* sp., *Colletotrichum gloeosporioides, Cylindrocladium* sp. and *Fusarium* sp. were observed at 33.24%, 20.56%, 18.62%, 11.71%, 9.78%, 4.65% and 1.45% respectively. For the banana fruits, three fungi including *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Fusarium* sp. were detected at 83.71%, 13.05% and 3.24% respectively from the diseased tissues. The results of pathogenicity tests of all fungal isolates were indicated that degree of infection was varied among the fungal isolates and kinds of fruits.The characteristics of isolate fungi; *Greeneria* sp.,waxy acervuli submerse in potato dextrose agar; conidia hyaline, 1-celled liked genus *Colletotrichum*. *Lasiodiplodia theobromae*, pycnidia black, erumpent; conidia dark and 2-celled at maturity, ovoid to elongate. *Gliocephalotrichum bulbilium*, conidiophores tall, simple, bearing at the apex a series of primary and secondary branches with terminate in phialide; fertire area subtended by a few long sterile divergent arms; conidia hyaline, 1-celled,oblong-elliptical, in mucouslike head. *Pestalotiopsis* sp., conidia dark, several-celled, with hyaline, pointed end cells, ellipsoid to fusoid, apical appendage. *Penicillium* sp., conidiophores arising from the mycelium singly, branched near apex, penicillate, ending the phialides; conidia hyaline, 1-celled, mostly globose, in dry basipetal chain. *Aspergillus niger*, conidiophore upright, simple, terminating in a globose swelling, bearing phialides at the apex; conidia 1-celled, globose, dark brown to black colour in mass, in dry basipetal chain. *Colletotrichum* sp., acervulus, waxy, subepidermal, setae at the edge or among the conidiophores; conidiophore simple; conidia hyaline, 1-celled, ovoid or oblong. *Cylindrocladium* sp., conidiophore upright, hyaline,regularly and repeatedly dichotomously branched; slender elongated sterile branch terminating in globose swelling; conidia hyaline, 2-celled, cylindrical. *Fusarium* sp., conidiophore slender and simple, or stout, short; conidia hyaline, two kinds, occured in small moist heads; macroconidia several-celled, curved; microconidia 1-celled, ovoid.

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In vitro Selection Techniques for *Fusarium* Head Blight Resistance in Wheat

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Fusarium graminiearum causes seedling blight, root rot and head blight in wheat. *Fusarium* head blight (FHB) disease is a serious disease in many wheat producing areas world-wide. This fungus produces a number of toxic compounds that can affect human health and animal productivity. Fusaric acid produced by this fungus which is the frequently detected mycotoxine worldwide and has been recently investigated. Most cultivars of wheat are susceptible to *F. graminiearum*. This study was conducted to develop an efficient *in vitro* selection system for FHB resistance by using in vitro selection method to culture filtrates of *F. graminiearum* and fusaric acid.
Calli of three genotypes of wheat (*Triticum aestivum* L.) Seri 82, Adana 99 and Genc 99 were used for selection of resistance against culture filtrate of *F. graminiearum* and fusaric acid. To obtain culture filtrate liquid potato dextrose medium was inoculated with *F. graminiearum* and incubated for 3 weeks. Culture filtrate and fusaric acid were added at different concentrations to MS callus growth medium, than 4 week-old calli were transferred on this medium for 4 weeks. It was observed that the culture filtrate and fusaric acid reduced callus growth *in vitro*. The phytotoxicity of culture filtrate was assessed for its inhibitory effect on callus, as well as on the viability of callus cells. MS containing 30% culture filtrate and 0,3 mM fusaric acid were found the optimum concentration to wheat calli for *in vitro* selection of wheat cell lines resistance against *F. graminiearum*. These results show the potential for culture filtrate and fusaric acid to provide a method of *in vitro* selection of wheat for resistance to the pathogen.
Keywords: wheat; *Fusarium graminiearum*; head blight, *in vitro* selection, fusaric acid, culture filtrate

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Effect of methyl jasmonate and salicylic acid on karnal bunt (Neovossia indica) resistance in wheat in vitro and In vivo conditions

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My principal project 'Genetic mapping and tagging of Karnal bunt (*Neovossia indica*) resistance genes in wheat using SSR markers' was to detect molecular markers for karnal bunt resistance in an Australian wheat variety Frame which had been phenotyped in CIMMYT, Mexico. Under this project I learnt many new techniques of molecular biology which are rarely practiced here. I have also collaborated with the cereal pathology group in projects like screening varieties for different diseases, fungicide efficacy tests etc.and benefited myself by having hands on experience on different methods and exchanging the ideas. The knowledge and skills gained in overseas laboratory can help me to carry on disease resistance programmes of my institute with more speed and better outcomes.

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Safety record of plant pathogens introduced for weed biocontrol in New Zealand

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Although biological control of both agricultural and environmental weeds is perceived as an environmentally benign alternative to chemicals, there have been recently reported cases of damage to non-target plants. This prompted research into the safety record of weed biocontrol in New Zealand. Internationally, systematic investigation of disease damage to non-target plants has rarely been reported for pathogens. With increased rigorous regulatory legislation in place worldwide, it is important that a good past safety record for weed biocontrol agents can be demonstrated, and that the methods used for host specificity testing deliver a reliable assessment of risk.

Disease surveys were conducted throughout New Zealand and focused on plants that are closely related to those target weeds most at risk of non-target attack. These surveys identified fungal pathogens associated with any disease symptoms observed on non-target hosts. There are four case studies in New Zealand where plant pathogens were deliberately, accidentally or autonomously introduced for weed biocontrol *Phragmidium violaceum* against blackberry (*Rubus fruticosus* aggregate), *Phoma clematidina* against old mans beard (*Clematis vitalba*), *Entyloma ageratinae* against mist flower (*Ageratina riparia*) and *Puccinia hieracii* var. *piloselloidarum* against mouse eared hawkweed, (*Hieracium pilosella*). Causative disease agents associated with any symptoms were identified either by direct examination or by cultural isolation methods.

Disease damage was observed on non target native hosts in two of the case studies. Pustules of the blackberry rust *Phragmidium violaceum* were found on an endemic species *Rubus cissoides.* Leaf pathogen *Phoma clematidina* was isolated from lesions found on two other endemic hosts, *Clematis foetida* and *C. paniculata*. Both results were predicted from original host range safety tests before to their introduction to New Zealand. No non-target damage was observed in the remaining two case studies, confirming both *P. hieracii* var. *piloselloidarum* and *E. ageratinae* as being highly host specific to their target weeds.

The accuracy of host range testing in weed biocontrol programmes using pathogens has often been questioned. This comprehensive field survey confirmed that host range pathogenicity tests before introduction were an accurate prediction of actual risk, after introduction. Investigation of non-target effects is an integral part of biological control practice in New Zealand.

PS3-290-0650

Pseudocercospora macadamiae, the causal agent of husk spot disease in macadamia

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Pseudocercospora macadamiae Beilharz, Mayers & Pascoe causes husk spot on macadamia. The pathogen infects the young nuts and after a long incubation period gives rise to chlorotic lesions which may lead to early drop of the macadamia nuts. The disease cycle of *P. macadamiae*, the causal agent of husk spot disease of macadamia has not been established. Although macadamia (*Macadamia integrifolia* and its hybrids with *M. tetraphylla*) are grown in many countries including Australia, Brazil, Costa Rica, Kenya, USA and South Africa, the husk spot disease has only been reported in Australia. Epidemiological and genetic studies on *P. macadamiae* in relation to macadamia were investigated in laboratory and field experiments. Naturally infected husks were assessed for *P. macadamiae* spore production and as a possible source of primary inoculum within the macadamia canopy. Putative life cycle of *P. macadamiae* showed that old infected husks served as a source of primary inoculum, new infections are formed from spores produced on necrotic lesions during the growing season and infection occur at high humidity or in free water on nuts. Incubation time (infection to symptoms expression) ranged from 4-5 weeks and time to sporulation is dependent on environmental conditions. Field experiments showed that infection occurred from four weeks after flowering, in fertilised ovary to matured green nuts. The severity of the disease was influenced by time of infection and not the number and severity of lesions produced on nuts. We have found that the fungus can survive for more than two years on infected husks. Further studies on infection process are currently underway. Preliminary analysis of the genetic relationship of *P. macadamiae* with other *Pseudocercospora* species showed that *P. macadamiae* isolates are closely related to *P. platybolii*, a very common species on eucalypts and *P. pseudoeucalyptorum*. Due to the need to ascertain the relatedness of *P. macadamiae* with other *Pseudocercospora* species, we are currently exploring the genetic, biological and pathogenic relationships using more isolates from different geographical regions and hosts.

PS3-291-0651

Using herbarium specimens to obtain DNA sequences and reveal genotypic diversity of two cereal rust pathogens

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The National Mycological Herbarium at Agriculture and Agri-Food Canada in Ottawa, Canada (DAOM) holds over 50,000 preserved specimens of rust fungi, including more than 500 type specimens. The collection is taxonomically diverse and embodies a valuable source of genetic data for many species of economically important crop pathogens but also for less-well-studied or rare species. Until now, the collection has been largely un-tapped for this data. In order to develop robust protocols for extracting and sequencing genomic DNA from herbarium material, two cereal rust species that are well-represented in DAOM, *Puccinia coronata* Corda (crown rust) and *P. graminis* Persoon (stem rust), were selected for experimental study.

Multiple specimens of both species were selected from a diverse number of crop and non-crop host plants, dating back about 50 years. Commercially available kits were used to extract DNA from spores excised from sori (aecia, uredinia, telia) or from infected plant tissue. Selected regions of the nuclear ribosomal rRNA gene (rDNA) were PCR amplified and sequenced. Various PCR reaction conditions, primer pairs, polymerase enzymes, and purification methods were tested to increase the specificity and final DNA concentration of PCR amplicons.

Successful PCR amplifications were obtained for 50 year-old specimens of *P. coronata*. Success was not correlated with age of the specimen; reactions for some that were only 25 years old failed. For *P. graminis*, success was restricted to younger specimens, less than 30 years old. Rust-specific primer(s) were used and initial PCR amplifications were equally successful regardless of harvesting method (spores vs infected plant material) or DNA extraction kit used. In many cases initial PCR products were extremely low in DNA concentration. These were subjected to a second round of PCR (re-amplification) before sequencing. Successful amplification for some individual specimens varied depending on the length of fragment targeted.

Developing reliable protocols for obtaining sequence data for dried herbarium specimens is challenging due to DNA degradation from poor initial drying methods and progressive degradation over time. Manipulation of PCR parameters appears to be the most important step in the process. *Puccinia graminis* and *P. coronata* are economically important pathogens, represented in DAOM by a large number of specimens dating back to the late 1800's. Our investigations are continuing on increasingly older specimens. Analyses of the rDNA sequences obtained indicated substantial genetic diversity among specimens accessioned under these two names, in part corresponding to *forma speciales* on different host plants.

PS3-292-0661

The multimechanisms of *Bacillus amyloliquefaciens* C06 in the biocontrol of peach brown rot caused by *Monilinia fructicola*

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Brown rot caused by *Monilinia fructicola* (Wint.) Honey is a major fungal disease on peaches (*Prunus persica* (L.) Batsch) in North America and often results in serious fruit decays both pre- and post-harvest. *Bacillus amyloliquefaciens* C06, a bacterial strain isolated from a cheese starter, has showed great inhibition toward *M. fructicola*; and postharvest sprays of its liquid culture to naturally infected peaches reduced brown rot by 80-100% as compared to non-treated peaches. The research presented here aimed to understand the mechanisms of this biocontrol system. Liquid cultures of strain C06 were separated into cell-free filtrate (CFF) and cells alone (CA) and then respectively sprayed onto peaches infected naturally with *M. fructicola*. The treatment of CFF reduced brown rot incidence to 0% from 100% in the water check after 7 days at room temperature while CA failed to control fruit rots. To determine the active components produced by strain C06, CFF was passed through an ion exchange column and all resulted fractions were assessed for their antifungal activities with a bioassay testing germination of *M. fructicola* conidia. The active fractions were further purified and identified with HPLC and LC-MS; and at least one of the antifungal fractions is a new compound. When CFF was autoclaved at 121?C for 20 min, it lost its inhibitory activity toward the fungal pathogen as accessed by spore germination tests. However, when it was sprayed onto *M. fructicola* infected peaches, the autoclaved CFF still reduced brown rot by 55%, about a half of the reduction by not-autoclaved CFF, indicating potential involvement of other mechanism(s). Except of potential of induced resistance, a new mechanism was proposed, that is presence of inhibitor(s) in autoclaved CFF against pathogenesis related enzymes such as pectinase. Potato dextrose broth (PDB) and medium that contained pectin as the sole carbon source (pectin medium) were used for growing *M. fructicola*. The fungus grew well on PDB with and without addition of autoclaved CFF. However, its growth in pectin medium treated with autoclaved CFF was significantly inhibited as compared to that in pectin medium without the treatment. These results indicated that *B. amyloliquefaciens* C06 produced antifungal compound(s), which played roles in suppressing fruit rot; and strain C06 might also produce compound(s) inhibiting pectinase activity of *M. fructicola*, which prevented peach fruits from infection by the fungal pathogen, resulting in reduced disease severity. The mechanism that biocontrol agents produce inhibitor(s) against pathogenesis related enzymes is a novel concept and will be further proved.

PS3-293-0664

Cylindrocarpon spp. associated with black foot disease of grapevines in New Zealand

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Black foot disease of grapevines (*Vitis* spp.) is a serious disease in most areas where grapevines are cultivated. Symptoms include weak shoot growth, abnormal root development, necrotic root crowns, dark discoloration of vascular tissue and internal necrosis extending from the bark to the pith of diseased rootstocks. Three species of *Cylindrocarpon*, (*Cy. destructans*, *Cy. liriodendri* and *Cy. macrodidymum*) and two species of *Campylocarpon* (*Ca. fasciculare* and *Ca. pseudofasciculare*) are reported to be associated with the disease. In New Zealand, *Cy. liriodendri* and *Cy. macrodidymum* have previously been reported from grapevines. However, this study involved only 4 isolates. The aim of the present study was to assess the *Cylindrocarpon* and/or *Campylocarpon* spp. associated with black foot diseased grapevines from all the grape growing regions in New Zealand. Isolations were made from typical black foot symptoms in declining grapevines that were sampled from Auckland, Hawke's Bay, Gisborne, and Martinborough in the North Island, and from Canterbury, Central Otago, Nelson and Marlborough in the South Island. A total of 208 *Cylindrocarpon*- and *Campylocarpon*-like isolates were obtained. By using morphological and cultural characters, a subset of 60 isolates was selected for molecular identification. These isolates were subjected to DNA phylogenetic analysis of the internal transcribed spacers 1 and 2 (ITS1 and ITS2) including the 5.8S ribosomal RNA gene as well as the partial ,-tubulin genes. All the isolates were identified as *Cylindrocarpon* spp., of which *Cy. destructans*, *Cy. liriodendri* and *Cy. macrodidymum* were most commonly isolated. A combination of these species occurred in most regions. The phylogenetic results furthermore revealed two cryptic species of *Cylindrocarpon* that are phylogenetically closely related to *Cy. macrodidymum*. Detailed cultural and morphological investigations are currently underway to further circumscribe these taxa.

PS3-294-0679

Natural occurrence of *Alternaria sp.* the casual agent of stem spots on *Berberis thunbergii*

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Approximately 150 *Berberis* sp. (*Berberis thunbergii* cv. Rose Glow) showing dark brown, eye-like elongated spots (7 to 22 mm long and 1 to 3 mm wide) on stems near the crown toward the top were observed in a park in Tehran, the capital of Iran. A total of 28 samples from 16 individual plants (sampling included all symptomatic parts of the plants) were collected and evaluated for the possible casual agent of the disease. Fungi with spores that are characteristic of the genus *Alternaria* (2), including dark mycelium and brief and simple conidiophores with catenulate dictyospores, were isolated from the detached leaf and stem pieces of diseased plants The pathogenicity of five isolates was investigated using Koch's postulate. Symptoms similar to those observed in the field appeared on inoculated stems in 6 to 7 days. inoculated stems died 3 to 4 weeks after inoculation.
The genus *Alternaria* (Deuteromycetes) caused leaf and stem spot diseases on different plant hosts (1).
References: (1) G. N. Agrios. Plant Pathology, 4th ed., Academic Press, NY, 1997. (2) M. B. Ellis. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, England, 1976.

PS3-295-0695

Biocontrol of root rot of chili caused by *Rhizoctonia solani* with a formulation of *Trichoderma harzianum* secreting extracellular chitinase

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Antifungal activity of chitinase was evaluated in a biological control formulation system which did not require sterile conditions during preparation, The formulation consisted of powdered wheat bran, sago powder, 1% colloidal chitin and biomass of solid state *Trichoderma harzianum* with superior chitinase activity. The viability of *T. harzianum* in the formulation was counted and more than 45% viability was recorded up to 240 days at 28 oC. Before application, the dry preparation was activated by remoistening with 0.05 N HCl. After 2-3 days of incubation at 23-25 oC, the development of young, actively growing hyphae was stimulated. Chitinase synthesis was found to be growth associated and maximum enzyme (6.2 U/g of dry substrate) and biomass production occurred at 96 h. The higher moisture content of significantly (P = 0.05) enhanced the chitinase production. Optimization of colloidal chitin concentration showed that improvements in chitinase yield and maximum activity were attained with a 2% (w/w) concentration. The formulation effectively reduced root rot of chili caused by *R. solani* , anastomosis group AG-4 under greenhouse and field trials.

PS3-296-0696

Anti-fungal properties of extracts of toxic *Microcystis aeruginosa:* potential to biocontrol the plant pathogens

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Aqueous and methanol extracts of four strains of *Microcystis aeruginosa* isolated from fresh water blooms of Surha lake of Ballia, India; the major source of irrigation, cultivation of wild rice and other aquatic crops, fisheries, and recreational waters; were examined for anti-fungal properties in different bioassays using plant pathogenic fungi: *Sclerotium rolfsii*, *Rhizoctonia solani, Fusarium oxysporum, Macrophomina phaseolina and Pythium.* Methanol extracts made from *M. aeruginosa* isolate MS-12 showed the most pronounced inhibitory effects. The fungal bioassays were based on agar diffusion tests and included pour-plate methods. The MIC value of diluted methanol extracts corresponded to 41mg/ml freeze-dried cyanobacteria *F. oxysporum* was more sensitive towards toxin and *R. solani* was found least affected at all concentrations. The crude methanol extract was also tested as soil drench to control the charcoal rot of chickpea caused by *M. phaseolina* in the greenhouse. 40% reduction in disease symptoms was observed till up to 28 days after seedling emergence. The findings suggests that toxic cyanobacterial species can be evaluated for antagonistic properties against plant pathogenic fungi and bacteria and may be tested as one of the integrated measures to control the diseases.

PS3-297-0697

Pathogenicity of *Ramularia pratensis* to *Rumex japonicus* and Methods of Its Artificial Reproduction and Long Term Preservation

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We found a pathogenic fungus to a weed, *Rumex japonicus* Houtt. in Zentsuji, Kagawa Pref. and Haha-jima island, Tokyo. It causes red leaf spot and leaf blight accompanied with white powdery molds on the lesions. The fungal isolates grow slowly and produce dense and gray colonies on artificial madia such as potato dextrose agar (PDA) and so on. Many conidiophores elongated from stromata formed beneath of stomata of the plant, and produced chained-conidia which were 1-2-celled, hyaline, cylindrical, with scars at both ends, smooth and 7-30?2-3 µm. We identified the fungus as *Ramularia pratensis* Sacc. In inoculation experiments with both isolates from Zentsuji (isolate OL9) and Haha-jima (isolate GR1) to potted *R. japonicus* leaves in a green house, leaf blight by GR1 were faster than that by OL9, though outbreak of the disease by OL9 was faster than that by GR1. From these results, the isolate GR1 was indicated to be more compatible with the host plant than the isolate OL9. For the first step to establish forms of dosage and long term preservation of isolate GR1, its conidia (1 ? 104/ml) were added into potato dextrose broth amended with 2% dextrose (PDB), PDB with 1% yeast extract and 1% malt extract (PDYMB), PDB with 1% peptone 1% malt extract (PDPMB), or V8 juice broth with 1% dextrose (V8B), and shaking-cultured at 25ºC for 10 days. In all of the liquid media except for in V8B, 1-2 ?107 conidia/ml were produced. The reproduced conidia were demonstrated to be pathogenic to the host plant. When conidia of GR1 (104/ml) were cultured in 50, 100, 200 or 400% of the PDYMB which was the easiest one to make, as same way as mentioned above, reproduction of conidia in the 50% PDYMB was the most vigorous. The reproduced conidia were then suspended (1 ? 108/ml) in the following three kinds of cryoprotectants to be stocked at -80ºC; i) 1.5% MSG and 10% skim milk, ii) 10% glycerol, iii) filtrate of the PDYMB. Conidia of each suspension thawed after 2 years storage were confirmed to survive, and their virulence to the host plant was also demonstrated. When conidia produced in 0.5% PDYMB were inoculated to the host plant in an experimental field, leaf blight began about 1 month after inoculation. We think that the isolate GR1 of *R. pratensis* is effective as one of myco-herbcide agents against *R. japonicus*.

PS3-298-0701

Stagonospora curtisii Causing Red Leafspot on Crinum

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Leafspot of crinum (*Crinum asiaticum* var. *sinicum*) was recognized in Tsukuba, Japan in September 2001. The developed lesions became red in color uniquely, resulting in early blight of leaves. Brown to black pycnidia of the causal fungus frequently appeared on their lesions. In culture of the fungal isolates on potato dextrose agar (PDA) under black light, pycnidia which resembled to those on host lesions were formed. Fungal isolates from the lesions grew on PDA at 5-35ºC and optimum at 23-25ºC. The isolates produced pale brown to white colonies consisted of cottony mycelia on PDA, and formed scattered and solitary pycnidia on the medium. Pycnidia were subspherical to ellipsoid, brown, unilocuted, 1-ostiolate, with papillate ostioles, with wall consisted of multi-layered cells, 200-560 µm in height and 192-460 µm in width. Conidia were holoblastic, percurrent on the tips of flask-shaped conidiogenous cells, which consisted the most inner layer of pycnidial wall, ellipsoid to cylindrical, hyaline to pale brown, 0-3 septate, constricted at the septa, truncate at their bases, smooth on their surfaces and 5-24 ? 3-7 µm in size. No teleomorph of the fungus was confirmed on the host or the artificial media, PDA. The fungus was identified *Stagonospora curtisii* based on its morphological and cultural characters. The symptoms were reproduced by inoculation with conidia of the isolates to wounded leaves of the host plant. The same fungus was repeatedly re-isolated from the lesions produced by the inoculations. We propose that the disease is called as red leafspot of crinum. In addition, the present fungus was also found to attack *Hippeastrum* spp.(amaryllis) and *Lycoris radiata* as well as *Crinum latifolium*.

PS3-299-0710

Identification of the causal agent of poplar (Populus nigra) rust disease in Maragheh, NW of Iran

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In a survey during summer of 2005, rust symptoms as yellow spot on leaves of *Populus nigra* was observed in Maragheh city and its suburbs. Uredinia hypophyllous, rarely epiphyllus. The urediniospores had a smooth area at the apex and were measured 29.38(±0.76) ? 17.17(±0.29) µm . The urediniospore walls were hyaline and finely echinulate and 3 µm thick. Paraphyses were clavate and measured 65.1(±3.10) ? 17.55(±0.79) µm. The walls of the paraphyses were evenly thick and measured 2 - 5 µm. Telia are formed on the under side of leaves in autumn. These sori were subepidermal and measured 400 - 800 µm in length. Teliospores were sessile, 45.5 (±1.27) ? 9.5(±0.46) µm, wall uniformly thick. Based on the key provided by Bagyanarayana (1998) and the species description by Pei and Shang (2005), studied fungus was identified as *Melampsora allii-populina* Kelb.

For molecular studies, DNA was extracted from urediniospores and the primers ITS1 and ITS4 (white et al. 1990) were used to amplify the internal transcribed spacer region of the rDNA. The ITS sequences, which covered the whole ITS1, ITS2 and 5.8S region, were identical to the sequences of *M. allii-populina* from the UK (GenBank AY444773). This result highlighted the effectiveness of using molecular tools in identification of rust fungi. *Melampsora* species are mainly determined based on the morphology of the uredinial and telial stages, alternate hosts and telial host range. However, in most cases, only one or two spore stages could be found at the time of observation and there is no information of the alternate hosts. Moreover, morphological characteristics can also be extremely variable with a high degree of overlap among species.

In this study, the rDNA sequence information provided a rapid and precise method for identification of the pathogen. This is the first the first report of *M. allii-populina* from E- Azarbaijan. The mentioned rust is probably the most important disease on poplar in this area. As poplar rust can cause severe damages to nursery plants and young trees, further research will help to elucidate the life cycle and epidemiology of the rust disease. The key question which must be answered is whether rust requires the alternate hosts (possibly *Allium* spp.) or goes through a short life and only uredinial and telial stages are present in the area.

PS3-300-0717

Passalora leaf and shoot blight disease and application of different micronutrient regimes in *Acacia crassicarpa* lowland plantation in Riau, Indonesia

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Since its first note in November 2002, Passalora leaf and shoot blight disease incited by *Passalora perplexa* on *Acacia crassicarpa* grown on lowland has increased in distribution. The disease infects shoots and young leaves, therefore affecting apical growth. However, its impact is less significant after tree is older than 12 months. There had been suggestions that nutrient deficiency, especially micronutrients, was among the determining factors for disease establishment and plant recovery. Investigation on whether application of micronutrients could lead to slower development of the disease was therefore worth pursuing. Such information is still very limited and very useful for acacia lowland plantation operation in the future.

To accommodate such a question, trials were established in two different lowland sites, Mandau and Pelalawan plantations, in the Riau Province, Indonesia. Treatments consisted of different regimes of micronutrients (B, Cu, and Zn) and/or boiler ash, i.e. CuSO4 10 g (treatment A), CuSO4 10 g and B 10 g (B), CuSO4 10 g, B 10 g, and ZnSO4 10 g (C), and CuSO4 10 g, B 10 g, and ZnSO4 10 g, and boiler ash 500 g (D) in addition to the macronutrient (NPK) fertilizers. The treatments were applied to *A. crassicarpa* plantations within one month after planting in a randomized complete block design. Each plot consisted of 10 rows, 15 trees each row. The spacing distance was 3 m x 2.5 m (Mandau site) or 3 m x 2 m (Pelalawan site). Disease severity of a given plot was determined using disease index. For this purpose, disease severity in each tree in the plot was assigned to the scores of 0 (no disease development detected), 1 (up to 25 % of the foliage infected), 2 (25 – 50 % of the foliage infected), or 3 (more than 50 % of the foliage infected).

Results indicated that at six months, no significant differences in disease index were detected ($p \leq 0.05$) among treatments in the Mandau trial. In contrast, disease index among treatments in Pelalawan trial was significantly different ($p \leq 0.05$). It is, however, interesting to note that treatment C (incorporation of CuSO4 10 g, B 10 g, and ZnSO4 10 g) consistently resulted in lower disease index compared to other treatments both in Mandau and Pelalawan trials. Treatment C is currently the operational practice in the Pelalawan plantation. One yet unknown possibility is to which extent the micronutrient application may affect disease development in older trees. This will be explored in the next months.

PS3-301-0733

The invasion and systemic transmission of *Aspergillus flavus* - a soybean seed pathogen

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Soybean seeds are susceptible to fungal invasion while still developing on the parent plant, and after harvest, by specific field- and storage-fungi. Pathogenic fungi are capable of invading and proliferating within tissues of stored seeds, developing plants, and floral structures. Transmission of such fungi through successive generations of soybean has the potential to cause significant economic losses.
This study sought to investigate possible routes of fungi into soybean seeds, and to monitor the systemic transmission of a known pathogenic fungus through successive generations of soybeans.
Soybean seeds were stored for one week under conditions that promoted the proliferation of the normal, seed-associated mycoflora. Seeds were subsequently processed for scanning electron microscopy, and seed structures were examined for the presence of fungal structures to establish possible routes of seed infection.
One of two batches of soybean seeds, previously microwave-irradiated to eliminate the inherent seed-associated mycoflora, was experimentally inoculated with conidia of *Aspergillus flavus* and planted. Various structures of the developing plant were examined to monitor systematic transmission of the fungus.
The second, uninfected seed batch was planted and grown to reproductive maturity, when flowers were experimentally inoculated with conidia of *A. flavus* to monitor systemic transmission of the fungus.
Fungi were observed to invade soybean seeds through the micropyle, the hilum, natural cracks, and pits on the seed coat.
The structures sampled from plants grown from experimentally inoculated seeds showed 100% infection by *Aspergillus flavus*. Plant structures sampled included stems, leaves, immature and mature pods, and seeds.
Inoculating soybean flowers with *A. flavus* resulted in a significantly reduced seed yield. The test fungus was isolated from all second-generation seeds.
The micropyle, hilum and natural cracks represent naturally occurring apertures, and these portals of entry pose minimal resistance to invading seed pathogens. The virulence and pathogenicity of the test fungus is clearly evident in the systemic studies. *Aspergillus flavus* was able to proliferate throughout all tissues of the developing plant, out-competing other less-aggressive fungi, permeating through to the second generation of seeds. Application of this fungus to floral structures was more pronounced with regard to seed production – reducing both the number and size of second-generation seeds.
The pathogenic and virulent nature of *Aspergillus flavus* is clearly evident here, necessitating the development of suitable techniques to eliminate the risk posed by systemically transmitted seed and plant pathogens.

PS3-302-0736

Simultaneous monitoring of both the host and pathogen using quantitative real-time PCR

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No abstract available

PS3-303-0737

Local and systemic host-response in Norway spruce to *Rhizoctonia sp.* compared to the effect of drought stress and to the combination of the two stresses

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Simultaneous monitoring of both the host Norway spruce and pathogen *Heterobasidion parviporum* using quantitative real-time PCR.
The root-rot causing fungus *Heterobasidion annosum* can attack both spruce and pine trees and is considered the most damaging pathogen in northern European forestry. We have monitored the *H. annosum* S-type (recently named *H. Parviporum*) colonization rate and expression of host transcripts in Norway spruce material with differing resistances. Ramets of two 33 -year-old clones differing in resistance were employed as host material and inoculation and wounding was performed. Multiplex real-time PCR detection of host and pathogen genomic DNA was also performed to follow the colonization of the host tissues by the pathogen and the collapse in host DNA levels in infected regions. Host defense transcript levels, as an indicator of the host defense response, were monitored with singleplex real-time PCR.
Three days after inoculation, comparable colonization levels were observed in both clones in the area immediately adjacent to inoculation. Fourteen days after infection, *H. Parviporum* colonization was restricted to the area immediately adjacent to the site of inoculation for the strong clone (589), but had progressed further into the host tissue in the weak clone (409). Transcript levels of class II and IV chitinases increased following wounding or inoculation, while the transcript level of a class I chitinase declined following these treatments. Transcript levels of the class II and class IV chitinases were higher in areas away from to the inoculation site in clone 589 compared to clone 409 at three days after inoculation, suggesting that the clones differ in the rate of pathogen perception and host defense signal transduction. At 14 days after inoculation the *H. Parviporum* was still spreading and transcript levels of the Class IV chitinase soared in clone 409 while in contrast for clone 589 the infection had been halted and host transcript levels returning toward basal levels at this later stage.
This an earlier experiments using mature spruce clones as substrate indicate that it is the temperospatial speed of the host response and not the maximum amplitude of the host response that is the most crucial component for an efficient defense in Norway spruce toward pathogenic fungi such as *H. Parviporum*.

PS3-304-0738

Fluorescent *Pseudomonas* Isolates Suppressing Chickpea Wilt and Promoting Plant Growth in India.

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A naturally-occuring bacterium fluorescent *Pseudomonas*, found on roots of chickpea plants grown in India, could protect roots from infection by *Fusarium oxysporum* f. sp. *ciceri*, which causes fusarium wilt of chickpea. Among the rhizobacteria, this is the most common in occurrence and effective biocontrol agents. Total 90 isolates of fluorescent *Pseudomonas* were collected from rhizosphere and rhizoplane of healthy, partially wilted and completely wilted chickpea plants. Isolate H-Pf5 showed maximum zone of inhibition (7mm) in dual culture with *F. oxysporum* f.sp. *ciceri* and was selected for green house and field bioassays along with isolate C7R12 originally isolated from wilt suppressive soils in France. Cell free culture filtrate of both the selected isolates inhibited 88-89.5% conidial germination as compared to100% conidial germination in control. Antagonists were applied in field as seed treatment while in green house it was applied as seed and soil treatments. Under green house conditions the seed treatment with isolate H-Pf5 and C7R12 showed 72.9- 73.9 per cent seed germination and 47.2-52.1% disease incidence after 120 days of sowing while in control 48.7 % seed germination and 100 % disease was observed after same interval. The isolate H-Pf5 of fluorescent *Pseudomonas,* selected from rhizosphere of chickpea plant significantly enhanced seed germination, reduced disease incidence and promoted plant growth of chickpea as compared to control.

PS3-305-0758

Viable teliosporogenous mycelia of *Neovossia indica* in infected grains of wheat

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Teliosporogenous mycelium of *Neovossia indica* (Mitra) Mundkur have been isolated from inoculated immature kernels, however isolation of teliosporogenous mycelia from stored bunted kernels has not been reported. In this investigation, survival of the teliosporogenous mycelia in 6, 7, 8 and 9 months old bunted kernels which had been infected in laboratory was examined. One infected kernel of susceptible variety WL711 with infection coefficient number 3 (CI = 3), which had relatively large and closed sorus, was disinfected using 0.5% sodium hypochlorite for 2 min., and rinsed with sterile distilled water for three times. The sorus was crashed in 10ml sterile distilled water by a scalpel. One ml of this suspension was placed on the surface of sterile distilled water in each plate. The rest of suspension was treated with a solution of 500ppm lactic acid for a night then were placed in plates in the same manner mentioned above as control. The plates were arranged in a completely randomized design with 5 replicates. Petri plates were incubated at 20C and 12hr. photoperiod. Ten to 15 days after incubation, floccose erected mycelia with the same characteristic of the teliosporogenous mycelia appeared on the surface of sterile distilled water in plates containing the suspension that had been prepared from 6-8 months old infected kernels. No teliosporogenous mycelia were grown in the control plates, indicating that the mycelia were not originated directly or indirectly from teliospores. Fifteen days after inoculation, colony numbers of teliosporogenous mycelia per plate were counted. According to dilution factor, colony forming units of the mycelia per each sorus were calculated (cfu/sorus). Two-three weeks after transferring the mycelia to fresh potato - dextrose agar amended with 0.1% yeast extract (YPDA), teliospores formed on this media. Mean teliospore formation on YPDA at 20C and under laboratory conditions, after 4 weeks, were 591.5 and 832 per plate, respectively. Also the teliosporogenous mycelial growth rate in YPDA at 20C and room temperature were 2.31 and 1.85 mm/day, respectively. Teliosporogenesis of the fungus was studied *in vitro* and *in situ* by light, fluorescent and scanning electron microscopy. During the teliosporogenesis, teliospore initials were formed in apical position in a lateral right-angled outgrowth of the teliosporogenous hyphae. The dikaryotic plasma was concentrated in apical portion of the hypha. Fusion of nuclei occurred during the early enlargement of teliospore initial. The swelling, pyriform to spherical protoplast of the teliospore initial was delimited from the empty part of the teliosporogenous hypha by a sheath, which was hyaline as observed by LM. The empty part of the hypha may formed appendages. Underneath the sheath, the exosporium with ornamented surface, and the smooth endosporium was deposited as seen in mature teliospores. This is the first report of survival of the teliosporogenous mycelia in Karnal bunt infected kernels after several months of storage. This may be of great importance in pathogen survival and disease epidemiology.

PS3-306-0759

Botryosphaeriaceae infecting Eucalyptus, Acacia and Pinus in Venezuela.

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Botryosphaeria spp. have been reported as pathogens of various forest plantation trees, world-wide. Some *Botryosphaeria* anamorphs have been reported from Venezuela, but their identification is not certain. Recent phylogenetic studies have identified several lineages within the *Botryosphaeriaceae*, which further influence the identification of these fungi. The aim of this study was to identify *Botryosphaeriaceae* affecting trees in plantations of *Eucalyptus*, *Acacia* and *Pinus* in Venezuela, and to compare them to those occurring in other regions of the world. Identifications were made using a combination of morphological characteristics and DNA based molecular techniques, namely DNA sequence data and restriction digestion (PCR-RFLP) patterns of ITS rDNA amplicons. From a total of 204 isolates from Venezuela, *B. mamane*, *B. dothidea*, the *Neofusicoccum ribis – N. parvum* (= *B. ribis / B. parva*) complex, *Lasiodiplodia theobromae* (= *B. rhodina*), and two previously unknown species, *Neofusicoccum andinum* and *Pseudofusicoccum stromaticum* were identified. To discriminate between isolates residing in the *N. ribis - N. parvum* complex, PCR-RFLP patterns that were characterised previously, were used. This technique showed that both these species are present in Venezuela. This study represents the first report of *B. mamane* outside Hawaii and the first records of *B. dothidea*, *N. parvum* and *N. ribis* in Venezuela.

PS3-308-0776

Colletotrichum species causing anthracnose of chilli (Capsicum annuum)

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Anthracnose of chilli pepper (*Capsicum annuum*) is a major disease in South East Asia. Several *Colletotrichum* sp. are known to cause the disease, with *C. capsici* being the most destructive. A survey of infected chilli fruit identified *C. capsici* and *C. gloeosporioides* as the main anthracnose causing pathogens in western Thailand, and *C. capsici* and *C. acutatum* in northern Thailand. In both cases *C. capsici* was the most prevalent species. Isolates of the three species were clearly discriminated on conidial shape, cultural characteristics and growth rate; and phylogenetic analyses of ITS and ,-tubulin gene sequences. In contrast, conidial size and appressoria shape and size were not correlated with the gene sequence clusters. Interestingly, only conidial length was able to discriminate *C. acutatum* isolates from chilli and strawberry since the strawberry conidia were significantly longer. In pathogenicity bioassays, chilli *C. acutatum* isolates were able to infect strawberry fruit however, strawberry *C. acutatum* isolates did not infect chilli fruit.
C. capsici infects a wide range of plant species including legumes, papaya, cucurbits, hibiscus, and chrysanthemum; but very little is known about the taxonomy and the ability of *C. capsici* to infect chilli. Isolates from culture collections in Australia showed large variation in culture characteristics, conidial size and appressoria size. Comparative analysis using a dominant marker system (ISSR) revealed a large range of genetic diversity among isolates. Less variation was detected among Thai chilli isolates than among Australian isolates collected from a range of hosts. Pathogenicity testing on chilli fruit revealed that most Thai isolates produced anthracnose symptoms within 3 days of inoculation. Isolates from *Manettia luteo-rubra* caused infection on chilli and several different pathotypes of *C. capsici* were identified, with some Thai isolates able to infect the resistant *Capsicum chinensis* genotype PBC 932. Conversely, isolates from *Glycine max* and *Annona squamos* were not able to infect chilli.

PS3-309-0790

First report of Alternaria leaf blight caused by *Alternaria alternata* on *Hevea brasiliensis* in India

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Alternaria leaf blight caused by *Alternaria alternata* was observed on rubber (*Hevea brasiliensis*) tree, the source of natural rubber latex of commercial value. In general, disease incidence of 10 to 80% is recorded in the affected fields in India. Initial symptoms appeared on young leaves as minute spots, which enlarged with the growth of the leaves. A characteristic browning and blackening of veins forming a 'fishbone' or 'railway track' symptom was noticed at later stage of the disease. This disease symptom appeared similar to that of the devastating Corynespora leaf disease, caused by *Corynespora cassiicola* on *Hevea*. Twenty six isolates from two states of India, Kerala and Karnataka, were characterized using random amplified polymorphic DNA (RAPD) analysis and two major profiles were detected. Genetic relationships among the isolates were analysed using genetic distance data based on RAPD profiles. Cluster analysis resolved the isolates into two major groups. Restriction analysis of the PCR amplified ribosomal DNA including the flanking internal transcribed spacers of representative isolates from both the groups also revealed two distinct RFLP patterns reflecting the same groupings as detected through RAPDs. The amplified rDNA from representative isolates of both the groups was cloned and sequenced. Sequencing results showed homology of one group of isolates with *A. alternata* and the other with *C. cassiicola*. The identification of the pathogen was subsequently confirmed by the International Mycological Institute (IMI), UK.
Pathogenicity tests were conducted on *Hevea* seedlings, by spraying the leaves with a spore suspension (5x104 spores per ml) followed by incubation in a glass house for two weeks. One week after inoculation, brown circular lesions of 2–5 mm diameter appeared on the challenged leaves. The pathogen was re-isolated from inoculated leaves. Similarly, leaf puncture bioassay was performed on detached leaflets of RRII 105, a *Corynespora* susceptible clone of *Hevea*. Twenty microliter spore suspension (5x104 spores per ml) of the pathogen was inoculated on the abaxial surface of the leaves on moist filter paper in petri plates. Symptoms were observed after 72 h. Dark brown spots, slowly spreading to the veins, produce a typical 'railway track' symptom. The study clearly revealed that *A. alternata* infects *Hevea* producing a similar symptom as that of *C. cassiicola*. *A. alternata* has been previously reported on *Hevea* in Mexico in 1947. This is the first report of this disease from India.

PS3-310-0801

Four *Fusarium species* causing diseases on orchids in Hawaii, including a member in the *Gibberella fujikuroi species* complex that is potentially undescribed

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Various diseases with possible *Fusarium* etiology were observed on commercially grown orchids in the Hawaiian Islands. These diseases were causing severe economic losses, in which an average of 50% of plants were diseased or dead. Symptoms included flower and leaf blight, root rot and damping-off. Fungi were isolated from diseased tissue samples representing 25 different orchid genera and hybrids, from nurseries on the islands of Maui, Oahu and Hawaii. *Fusarium* species were isolated from 70-100% of samples. Based on morphological characteristics and molecular analysis, using the translation elongation factor 1-alpha DNA sequence, *Fusarium proliferatum*, *F. solani*, *F. oxysporum*, and an uncharacterised group of *Fusarium* isolates were identified. The uncharacterised group of isolates closely resembled *F. subglutinans* morphologically, and phylogenetically divided into two groups, which had 99% homology with either NRRL 25204 or NRRL 25653, two closely related isolates in the *Gibberella fujikuroi* species complex (GFC). Each *Fusarium* species (or group) was tested for pathogenicity in greenhouse tests on *Dendrobium* orchid plants, symptoms were reproduced, re-isolation was successful and results were repeated. *F. proliferatum* caused leaf spot and blight, root rot and flower spots. Both *F. oxysporum* and *F. solani* formed minor leaf spot and root rot. The uncharacterised GFC isolates caused leaf spot and severe blight. Of significance, this work indicated the occurrence of one or more undescribed *F.* sp in the GFC, causing orchid disease, and the establishment of all four *Fusarium* species as orchid pathogens in Hawaii. Given the frequency of isolation of these pathogens, this work indicates that *Fusarium* species are having severe effects on commercially grown orchids in Hawaii. Additional *Fusarium* isolates are being identified and tested for pathogenicity, and further phylogenetic characterization of the GFC isolate groups are currently in progress. New findings will be presented.

PS3-311-0802

Development of Real-time PCR for the Rapid Detection of *Phytophthora* sojae Using TaqMan MGB Probe Assays

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Phytophthora sojae causes the destructive disease of soybean. The pathogen infects roots, stems, leaves and beanpods of soybean, resulting in root rot, stem rot, withering of plant. It is estimated that it brought an annual loss of 1billion USD to the soybean industry worldwide. The 14 species were studies by real-time PCR approach, including 37 isolates of *P. sojae* and its similar or related species, *P. sinesis,P . nicotianae, P. megasperma, P. citricola, P. capsici, P. syringae, P. parasitica, P. citrophthora, P. fragariae* var. rubi, *P. erythoseptia, P. bochmeniae, Peronospora manschurica, Pythium myriothlum, Py. aphanidermatum, Py. paroecandrum Py.* sp.. the primers and TaqMan MGB probes were designed for detection of *P. sojae* by analysis of nucleotide difference within the rDNA ITS sequences, mitochondrial sequences and the special protein sequences reported from GeneBank. The reaction conditions were optimized, The fluorescent monoplex PCR and doubleplex PCR were respectively developed. By the fluorescent doubleplex PCR, *P. sojae* and quality control DNA were specifically detected simultaneously through only 5 μL reaction mix. This procedure, with the cost being obviously lower, are rather specific, reliable, rapid and suitable for application in routine quarantine.

Key words: *Phytophthora sojae*; TaqMan MGB Probe; Real-time PCR

PS3-312-0804

The *Trichoderma/Hypocrea* from Russia (Tatarstan Republic) - interaction with microorganisms and plants.

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The genus *Trichoderma* is cosmopolitan in soil of all climate zones. Because of their characteristics, strains of *Trichoderma* have been exploited for production of enzymes and biofungicides. More than 500 Trichoderma isolates were selected from soil samples and mashes of Raif forest reserve, Tatarstan Republic agrocenosis, oil contaminating soils and oil slages; from soil samples of Bolshe-Klayrinsk city excavation dated as Early Period of Bulgar (XI century); from soils samples obtained in the human skulls dated as VIII-VI B.C. artifacts discovered in the Murzichinsk II Tomb (Mansur island in Kuibishev reservoir). The selected isolates were determined as *T. spirale*, *- í. Harzianum, - T.oblongisporum* (section Pachybasium), *í. longibraÒhiatum*, *- T. citrinoviride* (section Longibrachiatum),*- T. viride, - T. koningii, - T. atroviride, - T. saturnisporum, – T.asperellum* (section *Trichoderma*). The isolates selected from ancient tombs were determined as *T. atroviride, T. citrinoviride, T. viride, í. longibrachiatum*. The taxonomy of some isolates was verified by molecular analysis. The selected isolates were shown to be the aggregates of geterogenic populations. The values of compatibility reaction frequency and noncompatiblity reaction frequency were calculated as 63 and 37% respectively. The morphological analysis revealed the 2 types of colony (II and VI).

Growth rate at the different temperatures was analyzed to select the isolates with different life strategy: the r-strategists and k-strategists. The growth rates of slow grown isolates and fast grown isolates were calculated as 0,08-0,1 mm/h and 2 mm/h. The high competitive cold-tolerant (10-140) and mesophylic (25-280) species with high antagonistic activity against phytopatogens and growh stimulating activity for barley, corn, rye, wheat, potato, cucumber, tomates, pepper, decorative cultures and pine seedlings.

As a result of *Trichoderma* – plant interaction study host-specific and organotropic species with negative and positive influence on the plants were revealed.The species as *Azotobacter symbiotrophes* were detected. They were shown to incease the azotfixation in black soils at the 14 days after fungi introduction. Introduction of 2 isolates of T. viride was shown to increase to azotficsation at 2,7 times against control. The isolates of *T. koningii* and *T. harzianum* were able to raise respiration activity in soil at 2,5 times against control.

PS3-313-0826

Biology of leaf rust in *plumeria* spp. caused by*coleosporium sp.*

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The leaf rust disease in *Plumeria (*Apocynaceae*)* is wide spread in Sri Lanka. Development of rust on leaves of *Plumeria* caused by *Coleosporium sp*. has been reported in Hawaii islands. This study was undertaken to investigate the biology of *Plumeria* leaf rust.

Diseased leaves of *Plumeria rubra and Plumeria obtusa* at different maturity stages were observed visually, under dissecting microscope and stereomicroscope. Scrapings from rust lesions were examined under light microscope and the diameter of uredospores was measured.

To examine the development of the fungus within the leaf tissue, hand and microtome sections were taken across diseased sites. Several young healthy twigs were tagged and monitored for symptom development at weakly intervals. Needles of *Pinus* growing around infected *Plumeria* trees were observed for any symptoms to trace the possible secondary host relationship with *Plumeria* leaf rust fungus.

Young leaves down to about the fourth from the apical bud are resistant to rust infection. A spore germination assay was performed using latex from young and old leaves to ascertain the effect of latex on uredospore germination.

The infected leaves show numerous tiny, raised, orange, rusty pustules. The pathogen is restricted to the abaxial surface of the leaf while chlorotic areas were visible on the adaxial surface of the infected sites. Symptoms were absent on stem and flowers. The causal agent was identified as *Coleosporium sp.*

Two other fungi, *Absidia sp*. and *Verticillium sp*., colonized the rust spots of more mature leaves in succession, *Absidia sp*. appearing first followed by *Verticillium sp*. These two fungi grew on the rust pathogen and had no direct contact with the leaf tissue and are mycoparasites on *Coleosporium sp.* However, colonization by these two fungi caused some necrosis around the rust infections inflicting more damage to leaves.

Microscopic studies of rust pustules indicated the presence of uredium, formed from the transversing mycelium and emerged through ruptured lower epidermis. Any other fruiting structures, telium, aecium and spermatium were not encountered at any stage of the disease or in *Pinus.*

Young leaves contain more latex than mature leaves and the latex shows inhibitory action against germination of uredospores. Therefore latex may be playing a role in the resistance of young leaves against rust infection. The disease level was fairly constant throughout the study period (June 2003 – March 2004) but slightly increased during rainy weather as even younger leaves became rust infected and more mycoparasite colonization occurred.

PS3-314-0839

Detection of *Phytophthora nicotianae* from naturally infested soil, using soybean leaf disk baiting technique.

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A high detection rate of *Phytophthora nicotianae* was obtained from naturally infested soil of soybean fields. Air-dried soils were first moistened in a flask and then pre-incubated at 25?C for 2-4 weeks before flooding with distilled water and baiting with soybean leaf disks for 12 h. The baits were then thoroughly washed, flooded with 10-15 ml distilled water in petri-dishes and incubated under continuous fluorescent light for 72 h. Sporangia started to emerge from the margins of leaf disks which were easily observed under steromicroscope. Pure culture of the fungus was obtained by spreading zoospore suspension on a 1.5% water-agar containing anti-bacterial antibiotics (Ampicillin and Rifampicin). This is the first report of a high recovery of *P. nicotianae* from naturally infested of soybean soils using sensitive leaf disks of soybean (Williams cultivar) as a bait. All isolates were determined to be A2 mating type.

PS3-315-0843

Epiphytes on gorse and associated insects

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Gorse (*Ulex europaeus*) is a noxious spiny weed in North America, Hawaii and Australasia. In New Zealand, it was introduced a hedge plant from Europe over 100 years ago. It is currently New Zealand's most serious weed occupying > 700,000 ha (5%) of productive land (pasture & plantation forests). It is highly competitive under New Zealand's temperate climate conditions. It displaces native plants due to absence of natural enemies and prolific seed production (20,000 seeds/m2/year) with a viability of >30 years.
Gorse control is conducted by mechanical, chemical and/or biological means. Gorse hosts a variety of insects, such as gorse stem miner (*Anisoplaca ptyopte*ra), light brown apple moth (LBAM, *Epiphyas postvittana*), gorse seed weevil (*Exapion ulicis*), lemon tree borer (*Oemona hirta*), gorse pod moth (GPM, *Cydia ulicetana*), gorse soft shoot moth (*Agonopterix ulicetella*), gorse colonial hard shoot moth (*Pempelia genistella*), gorse spider mite (*Tetranychus lintearius*) and gorse thrips (*Sericothrips staphylinus*). The latter five insects were introduced deliberately as biological control agents. In order to employ these insects as potential vectors for fungal pathogens (particularly the gorse biological control agent *Fusarium tumidum*) we investigated the relationship between epiphyte loading on gorse, surrounding grassland and two insects with known attractants, LBAM and GPM. Both moths are abundant on gorse.
In the field, we quantified (colony forming unit counts, CFUs) and qualified (recognisable taxonomic units, RTUs) culturable epiphytes on gorse, grass and the insects. Studies were conducted on 10 replicate plots. Each plot was at least 500 m2. Insects were collected using sticky and live traps. To avoid trap competition, GPM and LBAM traps were on opposite sites of each plot. In the laboratory, gorse shoots were inoculated with fungal spores (1?106) using *Botrytis cinerea, Colletotrichum acutatum, Penicillium expansum,* and *F. tumidum.* Spore pick up by LBAM was assessed after 4 days exposure of the moths to the fungal spores.
In the field, grass (9.8?106 CFUs/g DW) supported more epiphytes than gorse (1.5?104 CFUs/g DW). The type of trap (sticky versus live) affected the number of organisms recovered from insects. Therefore only data from live traps was used. LBAM, although being the larger insect, carried similar amounts of fungi and yeasts (586 CFUs/insect) compared with GPM (527 CFUs/insect). In the laboratory, spore deposition onto the gorse shoots was very even between the different pathogen inocula, however uptake by LBAM was preferential according to spore size. A male moth picked up approximately 11 of the large *F. tumidum* spores compared with 45; 190 and 478 spores of *C. acutatum*, *B. cinerea* and *P. expansum*, respectively.
The work shows that LBAM can vector pathogen spores. However, its ability to carry sufficient quantities of the very large spores (123 µm2) of the gorse pathogen *F. tumidum* may be limited.

PS3-316-0846

Genetic Heterogeneity In The Ascomycete *Taphrina deformans*, Agent Of Peach Leaf Curl: Evidence For Two Distinct Forms Parasitic On Peach And Almond

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All species of the dimorphic ascomycete genus *Taphrina* have a biotrophic hyphal stage parasitic on different vascular plants and a saprobic yeast stage. Infection symptoms may develop on leaves, fruits, shoots or flowers. Most species parasitise a single host and/or cause a specific infection symptom. Genetic separation of *Taphrina* species has been confirmed by molecular studies1. Economically important species of the genus *Prunus* are potential *Taphrina* hosts, such as peach, almond, cherry and plum trees. The best known species is *Taphrina deformans*, the agent of peach leaf curl, a disease with a pronounced impact on peach growing areas worldwide. However, no epidemiological or population studies have been carried out for this species. To uncover the patterns of genetic variation within *T. deformans* we have studied about 40 isolates from peach and almond, collected mainly in Portugal. The following molecular techniques were used: PCR-fingerprinting with microsattelite primers (MSP-PCR), Amplified Fragment Length Polymorphism (AFLP), sequencing of rDNA (ITS region) and DNA-DNA hybridization experiments. Analyses of the results from each technique led to consistent clustering of the strains, which points to the existence of distinct genotypes or populations. Most strikingly, the strains isolated from almond leaves showed significant genetic divergence from those isolated from peach. This observation suggests that the two host forms are best considered as distinct varieties and lends credit to earlier proposals by other workers2.
1 Rodrigues & Fonseca 2003. IJSEM 53, 607-616
2 Schneider 1971. CR Acad Sci Paris (D) 273: 685-688

PS3-317-0854

Investigating the early stages of plant infection by the rice blast fungus *Magnaporthe grisea*.

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The rice blast fungus Magnaporthe grisea causes one of the most serious diseases of cultivated rice, and understanding the early events of the infection is of paramount importance if durable control measures are to be developed. *M. grisea* forms a specialised infection structure called an appressorium which is used to penetrate the tough outer cuticle of rice leaves, allowing the fungus entry to the underlying tissues Appressoria are melanin-pigmented, dome shaped cells, which form in response to the hydrophobic leaf surface and generate massive turgor pressure. We have been studying the attachment mechanism of *M. grisea* appressoria via the action of the Mpg1 hydrophobin and the role of camp and MAP kinase signalling in appressorium development. We have also investigated the cell biology of appressorium development and in particular, the importance of cell cycle control to appressorium morphogenesis. We have shown, by both pharmacological and genetic studies that appressorium formation requires the fungus to have undergone mitosis in the germ tube soon after spore germination. We engineered a temperature-sensitive mutation into the MgNimA kinase, that allowed us to study the significance of mitotic progression to appressorium development. This showed that completion of mitosis is an essential pre-requisite for plant infection by M. grisea. Furthermore, we found that following mitosis, conidia always undergo cell collapse and cell death, which appears to be a programmed, autophagic process. Deletion of MgATG8 prevented autophagy in *M. grisea* and rendered the fungus non-pathogenic. Appressoria formed by the mgatg8 mutant were able to form appressoria but these were completely non-functional. Taken together, our results indicate that appressorium morphogenesis requires genetic control by a cell cycle checkpoint and is always accompanied by autophagic cell death in the conidium. Progress towards understanding these events and their importance to plant disease will be presented.

Reference: Veneault-Fourrey, C., Barooah, M.K., Egan, M.J., Talbot, N.J. (2006) Autophagic fungal cell death is necessary for infection by the rice blast fungus *Science* 312:580-583

PS3-318-0865

Utilization of essential oil as herbal pesticide fight post harvest spoilage in fruits, Malus pumilo

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Fruits are among the most important foods of mankind. They are high-value commodities, offering good economic return even on small area of land. Harvested fruit and vegetables are attacked by microorganisms because of their high moisture content and rich nutrients. Usually synthetic pesticides are applied for the control of 'pest and disease' of the agricultural food commodities, as these are effective, dependable and economic. However, there indiscriminate use has resulted into several problems such as pest resistance to pesticides, resurgence of pests, toxic residues in water, air, soil and disruption of eco-system. Natural products are an alternative to the use of these synthetic pesticides.

We have tested the essential oil of *Mentha citrate* oil against post harvest losses fungi viz., *Alternaria alternata, Botrytis cinerea, Cladosporium cladosporioides, Colletotrichum capsici, C. falcatum, Fusarium cerealis, F. culmorum, Gloeosporium fructigenum, Penicillium digitatum, Penicillium expansum, P. italicum, P. implicatum, P. minio-luteum, Aspergillus flavus, A. fumigatus, A. niger, A. parasiticus, Curvularia lunata, Fusarium oxysporum, F. udum, Penicillium variable, Helminthosporium oryzae,H. maydis, Phoma violacea, and Rhizopus nigricans* in vitro. The oil was further studied for thermostability, storage durability and their effect on fruit skin. Antifungal activity of the oil was compared with some synthetic antifungals. Finally, the essential oil was formulated for field trial in the form of spray solution.

The minimum inhibitory concentration of the oil was found to be 0.5 ?l ml-1 with fungicidal action. The oil at MCCs showed heavy doses of inoculum potential and its activity did not expire even upto 72 months of storage. Moreover, oil did not exhibit any adverse effect on fruit skin up to 10% concentration. Further, the oil of *Mentha citrata* was formulated in the form of spray solition for field trial. The oil did not exhibit any phytotoxic effect upto 50 ?l ml-1 levels on fruit skin. Formulation of the oil prepared at different concentrations (10-50 ?l ml-1) in the form of fungicidal spray. The fungicidal spray, when tested in vivo on *Malus pumilo* for checking the rotting, it showed complete inhibition at 20 ?l ml-1 concentration by pre inoculation treatment while in post inoculation treatment 30 ?l ml-1 concentration of spray solution was required for the 100% control of rotting.

Thus, the essential oil of *Mentha citrata* could be used as potential sources of herbal pesticide against post harvest losses.

PS3-319-0866

A first report of *Phytophthora sojae* race1 from Moghan, Iran

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Phytophthora root and crown rot of soybean is a destructive disease of soybean in Iran. Races (1 and 3) of the pathogen have already been reported from the two major growing regions of the crop, Lorestan and Golestan provinces. In a survey during 2005, 30 isolates of *P. sojae* were recovered from infected plants and naturally infested soil samples using selective PARPH medium and leaf disk baiting techniques. Race identification was made possible by inoculating *Rps* differential soybean cultivars and lines.The majority of tested isolates belonged to race 1 of *P. sojae* and were highly virulent on Harosoy which carries the *Rps7* gene for resistance to certain races of *P. sojae*. This is the first report of *P. sojae* race 1 from Moghan, another major soybean growing area of the country.

PS3-320-0888

Reactive oxygen species play a role in regulating a fungus-grass mutualistic interaction

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The fungal endophyte, *Epichloë festucae*, forms a symbiotic association with perennial ryegrass, *Lolium perenne*. In wild-type associations, *E. festucae* grows systemically in the intercellular spaces of the leaves as infrequently branched hyphae parallel to the leaf axis. In a mutant screen to identify *E. festucae* symbiotic genes, we isolated a mutant in an NADPH oxidase gene, *noxA*, that disrupted the symbiosis. Plants infected with the *noxA* mutant become severely stunted, show precocious senescence, and eventually die. The fungal biomass in this association is dramatically increased. ROS accumulation was detected cytochemically in the fungal extracellular matrix (ECM) and at the interface between the ECM and host cell walls in wild-type but not in *nox*A mutant associations. These results demonstrate that fungal NoxA is responsible for the ROS production in the host plant, and is critical in maintaining a mutualistic fungal-plant interaction. We isolated a second *nox* gene from *E. festucae*, *noxB*, which encodes a Nox with an extra 50 amino acids at the N-terminus of the protein. In contrast to the *noxA* mutant, the *noxB* mutant has the same phenotype as wild type in the host plant. To further investigate the regulation of ROS production by Nox, the *E. festucae* gene for the small GTPase, *racA,* was isolated. Deletion of *racA* resulted in reduced ROS production and altered hyphal morphology in culture, including hyper-branching and cellular depolarization. Like the *noxA* mutant, the *racA* deletion mutant failed to maintain a mutualistic symbiotic interaction with the host, causing a stunted plant phenotype. Expression of constitutively active (CA) RacA resulted in an increase in ROS production in axenic culture. Cytochemical detection of ROS in plants infected with a CARacA strain revealed an increase in ROS accumulation on the fungal plasma membrane. These results indicate that *E. festucae* RacA plays a key role in maintaining a mutualistic association, as a regulatory factor for ROS production in the host plant.
Reference: Tanaka et al. (2006). Plant Cell 18: 1052-1066.

PS3-321-0889

Recruitment of a p67phox-like regulator controls hyphal branching in a fungal-plant mutualistic symbiosis.

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Endophytic fungi of the *Epichloë* group systemically colonize the intercellular spaces of grass plants to establish a symbiotic association. Maintenance of the symbiosis requires that the growth of the fungal endophyte be strictly controlled throughout the host plant, including branching and tip growth. Previously, we reported that this mutualistic interaction breaks down in associations between an NADPH oxidase A (NoxA) mutant of *Epichloë festucae* and perennial ryegrass, demonstrating that endophyte ROS production is crucial for maintenance of the symbiosis. Here we report that a fungal regulator of NoxA, designated NoxR (Nox regulator), which has similar motifs to those in the vertebrate p67phox, is crucial for symbiosis maintenance. An *E. festucae noxR* mutant grows normally in culture, but *in planta* is hyperbranched, and causes stunting and frequent death of the host plant. Consistent with these phenotypes, production of ROS by *noxR* in culture is comparable to wild-type but in the host is significantly reduced, indicating that *E. festucae* NoxR has been recruited to specifically regulate *in planta* production of ROS. Overexpression of NoxR increases hyphal branching in culture and compromises the localized production of ROS at the hyphal growing tips, suggesting that NoxR is spatially regulating the production of ROS to control hyphal branching. We propose that *E. fesucae* NoxR is a specific regulator of ROS production in *planta* to maintain the mutualistic association interaction between this fungus and its host plant.
Reference: Tanaka et al. (2006). Plant Cell 18: 1052-1066.

PS3-322-0890

Colonisation of the plant Gossypium hirsutum and the aphid Aphis gossypii by the fungus *Lecanicillium lecanii*

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The fungus *Lecanicillium lecanii*, a well known entomopathogen, was isolated as an endophyte from field grown cotton in Australia. We tested the potential for this fungus under laboratory conditions to colonize cotton and the aphid *Aphis gossypii*, a common pest in cotton production systems, and to transfer between these hosts.
Both cotton and the aphid were inoculated with a spore suspension of *L. lecanii* made by removing and macerating the aerial mycelia of cultures grown on potato dextrose agar, and suspending this in sterile distilled water and surfactant (Pulse 0.02% w/v). When inoculating cotton leaves, we also applied sucrose, chitin and mild leaf damage, and all combinations of these treatments to simulate the presence of an aphid population at the leaf surface. Colonisation was assessed by isolating the fungus from the surface or from within each host. *Lecanicillium lecanii* readily colonised and killed *A. gossypii*. Endophytic colonization of cotton was enhanced by leaf damage, such that the fungus was isolated from 100% of damaged leaves at 35 days after inoculation (dai). Epiphytic colonization of cotton leaves decreased linearly over time, from 100% at time zero to approximately 44% at 35 dai. The fungus transferred readily from inoculated cotton leaves to un-inoculated aphids and from inoculated aphids to un-inoculated cotton leaves under laboratory conditions.
This isolate of *L. lecanii* exhibits two life-cycle traits: 1) the ability to colonize a plant host endophytically, and 2) the ability to aggressively colonize and kill an insect host. In this case, the insect host and plant host are naturally associated. Thus there is potential for this isolate of *L. lecanii* to move between cotton and the aphid under field conditions when humidity is high and cause mortality in aphid populations. As cotton in Australia is an irrigated crop, "relative humidity in cotton canopies can be significantly higher than ambient conditions" (Pers. comm. M.P. Bange). The potential for this interaction to occur under field conditions and be exploited as part of integrated pest management strategies should be investigated.

PS3-323-0901

Infection of chilli pepper *(Capsicum spp.)* plants by *Colletotrichum capsici* at early stages of growth

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Anthracnose disease, caused by *Colletotrichum capsici*, is considered to be the most prevalent and destructive pathogen of chilli pepper *(Capsicum annuum)* in most of the chilli growing areas of the world. However, few studies have been carried out to understand its mode of invasion and colonization on the chilli plant and fruit. Therefore, this study is an initial step towards understanding host pathogen interactions involved in the infection of chilli plants with *C. capsici* isolates, particularly at early growth stages. Pre- and post emergence infection was studied in two *Capsicum* spp. cultivars with varying known levels of resistance (*C. annuum* "bang chang"; susceptible and *C. chinense* PBC 932; resistant). Seed were inoculated at 1x105 spores/ml with two isolates of *C. capsici* (F83B from Thailand and BRIP26974 from Australia).
The inoculated seed of both species had considerably reduced germination rates (*C. annuum* 72%; *C. chinense* 81%). Overall, the Thai isolate was more pathogenic than the Australian isolate and the *C. chinense* cultivar was more resistant to both isolates than the *C. annuum* cultivar. When *C. annuum* seedlings were recovered, after seed treatment with the F83B isolate, a 63% post emergence death rate was observed nine days after planting in sterile potting media. Prior to death, water-soaked necrotic lesions were observed on cotyledons, stems and in the collar region, and seedlings collapsed soon after these symptoms developed. No significant seedling death was observed for any of the other three seed treatments. Inoculation of wounded stems of four-week-old seedlings of *C. annuum* with the BRIP26974 isolate, resulted in no visible symptoms of infection although the pathogen was recovered four weeks later from the tissue collected 10 cm either side of the wound site.
Drop-inoculation of spores on 30-day-old detached leaves of *C. annuum* with isolate F83B resulted in the formation of melanised appressoria after 24 h followed by direct penetration of epidermal cells by hyphae and formation of intramural hyphae at 36 h. In contrast, with BRIP26974, necrotic lesions developed just beneath the inoculation drop 48 hours after inoculation without the formation of distinct intramural hypha and with a relatively higher frequency of stomatal penetration than for F83B.
These results indicate the presence of pathotypes of *C. capsici* in chilli.

PS3-324-0903

Biosecurity built on science

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Biosecurity has emerged as a major global issue. Emergency Plant Pests can impact on food safety, trade, market access, market development and, ultimately, the profitability and sustainability of plant industries.
Australia is relatively free from many of the plant pests and pathogens that seriously impact on agricultural and horticultural industries in other countries. This gives Australia a valuable competitive advantage in terms of securing market access and maintaining lower production costs through the absence of many plant pests commonly found overseas. To sustain that advantage into the future, Australian plant industries need the support of world class science and biosecurity technology.
The Cooperative Research Centre for National Plant Biosecurity (CRCNPB)
commenced in November 2005. The CRCNPB will play a vital role in enhancing the scientific effort to enable Australian plant industries to pre-empt and, therefore, diminish the economic, social and environmental impact of Emergency Plant Pests. The activities of the CRCNPB will cover the full biosecurity continuum, pre-border, border and post- border. The four CRCNPB scientific research programs are focused on innovative research and development, in key areas that will deliver benefits across commodity groups: Preparedness and Prevention, Diagnostics, Surveillance and Impact Management. These programs will also introduce new technologies that will meet Australia's plant biosecurity needs within the shortest possible timeframe and will provide long-term benefits.
The CRCNPB has a strong commitment to the training of high quality
Honours and PhD students and post-doctoral scientists to provide the nucleus of Australia's future plant biosecurity capacity. CRCNPB will also provide vocational training for scientists and other disciplines already working in the plant biosecurity field to enhance the core capacity of Australia. Other countries, such as the USA and New Zealand, are also addressing similar biosecurity issues and CRCNPB will be developing close linkages with key organisations in these and other countries.

PS3-325-0923

Fungal parasites associated with eggs of *Heterodera schachtii* from Iran.

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Soil-borne fungi have important role in parasitism and natural control of populations of the beet cyst nematode *Heterodera schachtii* Schmidt, a most serious pests of sugar beet in growing areas in Iran. Since fungal parasites of nematode eggs infect females of sedentary nematodes and destroy the eggs within them, have great potential as biological control agents.
Five isolates of nematophagous fungi were isolated from *Heterodera schachtii* eggs collected from sugar beet fields in Khorasan province of Iran and identified. Two isolate of *Paecilomyces lilacinus* grew 3.35 cm and 2.33 cm in diametere at 30 ° C on PDA after 10 days.Colonies choclate coloured and were flat without pigments.Conidiophores 420 µm, phialides 8.3 ? 1.87 µm, phialospores lemon shape to elliptical, 3 ? 2 µm. *Pochonia chlamydosporia var chlamydosporia* grew 2.63 cm in diameter at 20 ° C on PDA after 10 days.Colony white to cream colored, floccose. Conidiophores prostrate and branched from vegetative hyphae as single or 2-3 branches per node along the conidiophore. Phialides narrow subulate, 12 -22 ? 1-1.5 µm. Conidia formed ellipsoidal, 3 – 4.5 ? 1.5 - 2 µ.Dictyochlamydospores abundant on branches of mycelium, 17-20 ? 20-25 µ. *Cylindrocarpon sp.* grew 3.56 cm in diameter at 20 ° C on PDA after 10 days.Colony white coloured, floccose with yellow to light brown pigments.Conidia were hyaline, cylindrical with rounded ends, 16-26 ? 2-3 µm, 2-4 septate. Chlamydospores were absent. *Chaetomium sp*. grew 3.18 cm in diameter at 20 ° C on PDA after 10 days. Ascoma was an ostiolate perithecium, globose or vase-shaped, brown colour, clothed with hairs. Asci clavate, stalked, 4-8 -spored. Ascospores unicellular, light olive brown, almond-shaped and smooth.

PS3-326-0928

Understanding basic aspects of *Ustilago maydis* pathogenesis with the use of alternate hosts

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We have previously demonstrated that *Ustilago maydis*, the causal agent of corn smut, is capable of infecting a number of plants different from maize, including *Arabidopsis thaliana* (Leon-Ramirez et al, 2004; Mendez et al, 2005). The use of model systems is a useful approach to dissect complex phenomena, as it is the process of maize infection by *U. maydis*. In this pathosystem, it has been proposed that both, host and pathogen send and recognize characteristic signals that decide the development of infection. The use of alternate hosts has allowed us to begin the dissection of such signals at least during the initial infection stages. Accordingly we have analyzed the course of infection and the behavior of different *U. maydis* mutants in *A. thaliana* (Mendez et al, 2005). To confirm that in this model the infection process is similar to what occurs in the natural host, we have now demonstrated the expression of a host-induced *U. maydis* specific gene (*mig*; Basee et al, 2000) during the initial stages of infection of *A. thaliana*. Also, we have analyzed other aspects of pathogenesis and fungal development. Our previous work has demonstrated that polyamines play a role in the control of dimorphism in *U. maydis* (Guevara et al, 1997). Since the mycelial form is closely related to the pathogenic development of this fungus, the question whether they play a role during infection has now been addressed in the natural and model systems. In addition, we have identified Arabidopsis genes that are up-regulated upon infection, confirmed their expression, and analyzed their possible role during the pathogenic process. Finally, we have also analyzed the expression of a gene involved in leaf morphogenesis of *A. thaliana*, in plants infected with *U. maydis*.

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PS3-327-0932

Genetic Characterization Of *Peronospora sparsa* On Rose In Colombia And Its Relationship With Sensitivity To The Qoi Fungicide, Fenamidone

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Rose downy mildew caused by *Peronospora sparsa* is a disease of growing importance to the Colombian flower industry. The disease was first reported in the 1970's and during last five years, its agressiveness has increased dramatically. This has meant that an increasing and unacceptable number of fungicide applications are required to control it. This situation has a negative impact in the environment and public health, and requires a program to manage fungicide resistance, including the participation of flower growers, fungicide companies, govertment agencies and research institutions. This study to consider the genetic variability of *P. sparsa* in Colombia was undertaken in order to support chemical strategies used to control *P. sparsa* and specifically those directed at avoiding loss of sensitivity to the systemic QoI fungicides. A baseline of sensitivitiy of *P. sparsa* isolates to Fenamidone was developed. These results were linked to the presence of point mutations in the cytochrome b gene, which have previously been linked to resistance in these fungicides. PCR-RFLP of ITS regions, RAMs and RAPDs markers showed a low level of genetic variability amongst *P. sparsa* isolates in Colombia, with the ocurrence of one highly frequent genotype. Fungicide evalutions showed a high level of sensitivity of *P. sparsa* isolates to Fenamidone (EC50: 0.51 mg/L), which was linked to the absence of point mutations G143A and F129L in the Cytochrome b gene. A test is currently being dessigned to monitor QoI fungicide sensitivity in *P. sparsa* populations in the rose growing regions of Colombia.

PS3-328-0938

Investigation on toxin produced by *Alternaria alternata* f.sp. lycopersici, the causal agent of tomato stem canker.

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Tomato stem canker is one of the most common tomato diseases all over the world. One has also reported it from Iran and its causal agent is *Alternaria alternata* f.sp. *lycopersici*. The symptoms appeared in form of some injuries on lower parts of stem and on the other parts in oval and linear shapes along the stem in brown to black. On leaves, the symptoms are in form of midrib necrosis and black spots. On the fruits, there are some dispersed spots with alteration in color, which may lead to plant wilt and death in any states of plant growth. According to the researches, one has found the pathogen is able to produce a toxin called "AAL-toxin". Toxin production by pathogen is deliberated by bioassay , TLC and HPLC methods and it is also checked for case of isolates toxin production by completely randomized design. Toxin production by isolates was confirmed in all three methods above, so that formed symptoms during bioassay methods was the same as the symptoms caused by pathogen. Bioassay tests on these exudates showed they contain AAL-toxin and in absence of pathogen itself, this toxin could produce the disease. In addition, Rt and Rf values in TLC and HPLC methods respectively, were match by Rt and Rf values of standard toxin. One also surveyed toxin effects on pathogenesis according to bioassay methods. Results revealed AAL-toxin play a significant role in pathogenesis of stem canker.

PS3-329-0943

Identification of the physiological race of *Fusarium oxysporum* f. sp. melonis isolated from melon field in Varamin.

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Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis* is one of the most important diseases of melon in several province of Iran.The most effective and economic way of controlling this disease is the use of resistant cultivars. It is necessary to determine the races of pathogen for access to resistant cultivars. In this study, the following differential varieties of muskmelon were used: *Charentais* T, which lacks any genes for resistance, *Charentais* Fom-1 which is resistant to race 0 and 2, *Charentais* Fom-2 which is resistant to race 0 and 1, and *Vergos*. In February 2006, a isolate of *F. oxysporum* obtained from a wilted muskmelon plant in Varamin areas was cultured and single-spored. Seeds of Differential varieties were planted in autoclaved potting mix of peat and sand, after surface- disinfected with 1% NaOCL for 4 min. Seedlings in the stage of first-true-leaf (10 days old), were removed from seedling trays, roots were washed in tap water, pruned at a length of approximately 2.5 cm and inoculated by root- dipping in a suspension of 1.275 ? 106 conidia per ml in sterile distilled water for 1 min, following the method described by Zink (1983). The inoculated seedlings were transplanted in plastic growing trays and placed in greenhouse condition at 20°C night / 28°C day temperatures. The number of dead and healthy plants was recorded 21 days after inoculation. Symptoms were observed on susceptible plants as early as 3 to 5 days after inoculation and seedlings of susceptible genotypes were usually killed within 8 to 15 days. All plants were free of wilt symptoms in the control treatments. Based on the reaction of the inoculated differentials and according to the nomenclature proposed by Risser *et al*.(1976), the isolate was classified as *F. oxysporum* f. sp. *melonis* race 1 and the virulence of it was considered very high, because it induced the first symptoms in approximately 4 days. According to previous reports, this race is one of the most important races of Fusarium wilt in melon fields, in Iran. We can find resistant cultivars to management of this disease.

PS3-330-0946

Investigation on the antagonistic activities of fluorescent *Pseudomonads* on biological control of *Fusarium oxysporum* f.sp. melonis the casual agent of melon wilt

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Fusarium oxysporum f sp. *melonis* the causal agent of melon wilt, is one of the important soil born pathogens. In this study, the biological control of this disease was investigated. The pathogen was isolated from vascular tissues of infected plants from several melon fields in Varamin and Eyvankey areas and its pathogenicity was proven. 285 bacterial strains of fluorescent Pseudomonads were isolated from the Rhizosphere of healthy plants by using serial dilution method and their antagonistic activities were investigated against two isolates of *Fusarium oxysporum* f.sp. *melonis* in dual culture test. 96 strains had antagonistic activities against both of them. Six strains were selected for greenhouse experiments. On the basis of morphological, physiological and biochemical tests and bacterial protein electrophoresis, these strains were identified as: *Pseudomonas fluorescens* biov. III (I1), *Pseudomonas fluorescens* biov. V (G11, A13, D15), *Pseudomonas putida* biov. B (C4) and *Pseudomonas fluorescens* biov. I(H6). The ranges of inhibition of these strains were from 24.33% to 82.033% in dual culture, 33.7% to 86.76% in antibiosis test and 39.9% to 80.9% in production of antifungal volatile components. In greenhouse conditions, the antagonistic activities of these strains determinated by using two types of soil (sterile and non-sterile) and two methods of treatment (seed and soil treatment). In both methods, H6 and C4 strains were the most effective on biocontrol of disease. Also, these strains had good results in non-sterile soil to biocontrol of the agent of *Fusarium* wilt of melon.

PS3-331-0947

Postharvest biocontrol of Penicillium using yeasts of the genera Pseudozyma and Rhodosporidium

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Basidiomycetous yeasts belonging to the genera *Pseudozyma* and *Rhodosporidium* have been described as being able to act as biocontrol agents against different fungal pathogens. However, a detailed survey of the biological control potential of yeasts belonging to these genera has never been carried out. We tested the antagonistic activity of several species belonging to these genera, and other related species, against important postharvest fungal pathogens of the genus *Penicillium*. The potential of different yeast strains as biocontrol agents was assessed in experiments performed on artificially wounded apples and oranges inoculated with suspensions of yeast cells and conidia of a postharvest pathogen. The severity and incidence of infection were measured. Several of the yeasts that we have tested seemed to have some ability to reduce the decay caused by *Penicillium expansum* on apples. Four isolates related to the genus *Rhodosporidium* were the most effective against this posthavest pathogen. A strain belonging to the genus *Pseudozyma* was able to control the decay caused by *Penicillium italicum* and *Penicillium digitatum* on oranges. The possible mechanisms of antagonism are presently being studied. Preliminary experiments showed that these strains are able to inhibit the germination of *Penicillium* conidia in liquid fruit juice medium. The separation of yeast cells and conidia by a membrane seemed to compromise this inhibition, suggesting that the production of a substance with antifungal activity may be involved. The addition of nitrogen sources in experiments performed on apples also seemed to compromise the protective ability of some of the selected yeasts, suggesting that competition for nitrogen sources may also be playing a role in their antagonistic ability.
The results obtained in the experiments performed so far suggest that more than one mode of action is present. We hypothesize that inhibitory compounds and competition for nutrients may act simultaneously. This work was financially supported by grant POCTI/AGR44310/2002.

PS3-332-0948

Biological control of *Gaeumannomyces graminis* on wheat with *Bacillus spp.*

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Bacillus pumilus(4km), *B.pumilus* (7km), *B.subtilis*(1j), *B.licheniformis*(b3n) were evaluated as potential biological agent for wheat take-all caused by *Gaeumannomyces graminis var.tritici* in vitro and vivo. Dual culture, volatile metabolite and cell free culture test showed that all isolates of *Bacillus* tested inhibited growth of the pathogen. Inhibition varied from 41 to 87% in dual culture, from 85 to 96% in volatile metabolite and from 95 to 98% in cell free culture test. The seed soaking treatment with Subtilin(commercial antagonist formulated from *Bacillus subtilis*) and *Bacillus pumilus* (7km) were the most effective in reducing disease index and also promoted root and shoot weight in glasshouse and field experiment. The weight of 100 grains from plants treated with pathogen+Subtilin, *Bacillus pumilus* (7km) and *B. licheniformis* (b3n) were significantly greater than in controls inoculated with pathogen alone in micorplot field test. These results indicate that *Bacillus pumilus* (7km) could be an important new biological control for take-all of wheat

PS3-333-0949

First report of *Ardisia japonica, Prunus lusitanica, Euonymus kiaut, Gaultheria shallon* and *Osmanthus decorus* as hosts for Sudden Oak Death caused by *Phytophthora ramorum*

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Sudden Oak Death (SOD), caused by the Stramenopile *Phytophthora ramorum*, was first detected on five previously unreported host plants during the Canadian Food Inspection Agency's 2005 annual SOD survey. The new SOD hosts identified were *Ardisia japonica* Blume (Myrsinaceae), *Prunus lusitanica* L. (Rosaceae), *Euonymus kiaut* L. (Celastraceae), *Gaultheria shallon* Pursh (Ericaceae), and *Osmanthus decorus* (Boiss. & Bal.) Kasapligil. (syn. *Phyllirea vilmoriana*) (Oleaceae). Foliage samples were removed from suspect plants located at a nursery in mainland British Columbia, Canada and sent to the Quarantine Phytopathology laboratory in Ottawa, Ontario for official testing for *P. ramorum*. Symptomatic leaf tissue was excised and initially tested with the Adgia® Phytophthora ELISA assay as a preliminary screen for SOD. Symptomatic tissue from samples that tested positive by ELISA were plated onto PARP selective medium and incubated in the dark at 20C for 7 days. Suspect mycelia were then plated onto 5 percent V-8 medium and incubated at 20C and a 12hr photoperiod for further morphological determination. Morphological identification of *P. ramorum* was performed and confirmed with the Phyto 1&4 PCR assay. Work is being completed to prove Koch's postulates for each of the new host species. Both non-wounded and wounded inoculations will be performed to determine the pathogenicity and susceptibility of each host species to *P. ramorum*. Preliminary results have confirmed Koch's postulates for the host plant *Gaultheria shallon* on both unwounded and wounded inoculations with *P. ramorum*.

PS3-334-0952

Pathogenic Factors Involved in Infecting Nematode by Nematophagous Fungi

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Plant-parasitic nematodes, important agricultural pests, have been reported to cause serious loses throughout the world. In recent years, nematophagous fungi, one of the most important natural enemies of nematodes, have been employed in biological control for their unique ability to capture and kill nematodes. To explore the mechanisms involved in the host infection, a series of related pathogenic factors, including mechanical activities, nematicidal metabolites and hydrolytic enzymes, have been analyzed.

Efficient killing of nematodes was observed of *Stropharia rugosoannulate* Farlow ex Murrill cultures and a newly identified *stropharia* sp.. These fungi were confirmed to have the ability to immobilize the free-living nematode *Panagrellus redivivus* in minutes and the pine wilt nematodes *Bursaphelenchus xylophilus* within hours on agar plates. The *Stropharia* cultures studied, share the characteristic of abundantly producing cells with finger-like projections called acanthocytes. We showed that the nematode-attacking and paralyzing activities were carried out by these spiny acanthocytes, which were believed to have a similar role with the hyphae appendages found in other nematode-trapping fungi. Additionally, we also performed scanning electron microscopy and transmission electron microscopy to elucidate the structural details of the acanthocytes.

Except for the mechanical activities, nematicidal metabolites, another important pathogenic factor involved in the infection, are tried to isolated and identified in nematophagous fungi. In the survey for the metabolites against *B xylophilus*, four hundred fungi strains were assayed, and the further phytochemical studies undertaken have resulted in the isolation of various diketopiperazines, terpenoids, peptides as well as polyketides. For example, a strain of *C rosea,* a fungus commonly used as biochontrol agent, showed significant ability to kill nematodes, and then five new verticillin-type epipolysulfanyldioxopiperazine were isolated from wheat solid-substrate fermentation, along with four known compounds. In vitro immersion tests showed that all nine compounds exhibited nematocidal activity.

In the molecular mechanisms of nematophagous fungi infecting their hosts, it has been suggested that hydrolytic enzymes participate in several steps of host infection. Thus we designed to clone and characterize the virulence enzymes in the representative fungi of nematode-trapping fungi as well as endo-parasitic fungi. Seventeen genes encoding cuticle-degrading enzymes including serine proteases and chitinases were cloned. Moreover, the bioassay experiments showed that these enzymes were involved in the processes to penetrate the cuticle and eventually digest them.

PS10-335-0032

Sera analysis of asthmatic patients for specific IgE to *Candida albicans* from Sari city- Iran

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Candida albicans (C. albicans) as a micro flora of the human is known to be one of causative allergens inducing asthma. The formation of IgE to *C. albicans* has an important role in triggering asthma. The aim of this study was determination of specific IgE to *C. albicans* in asthmatic patients.
A total of 84 asthmatic patients (male 41 and female 43; range age 5-65years) were studied. Solid phase capture sandwich ELISA assay using a micro well format for determination of specific IgE to *C. albicans* was used according the manufacturer instruction (ALerCHEK Allergen specific human IgE Cat. No. A10400**).**
Of the 84 patients, 22.61% had total IgE less than 100, 5.95 % 100-144, 14.28% 144-188 and 57.14% higher than 188 IU/ml. 46.43% of the patients had specific IgE against C. albicans. Among the patients who were positive for specific IgE to C. albicans, 61.54% were women.
Our study showed 57.14% of the asthmatic patients had total IgE upper than normal range. About half of the patients were positive for specific IgE to *C. albicans.* Therefore we think the presence of specific IgE against *C. albicans* can play an important role in inducing asthma, especially in women because they showed high frequency of this specific IgE in their serum.
Key word: Asthma, *C. albicans,* Specific IgE.

PS10-336-0053

Antifungal activity of Thuja occidentalis extracts on Candida albicans

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The increasing occurrence of opportunistic systemic mycoses associated with the use of immunosuppressive drugs and AIDS has led to new efforts in the search of novel antifungal compounds. Candidiasis is a fungal infection, commonly observed in the immuno-compromised host, predominantly caused by *Candida albicans.* It is a commensal organism and part of the normal microbial flora in about 30-50% of the population and capable of producing opportunistic infections with body system when appropriate predisposing factors exist. In the present preliminary study, *Candida albicans* were isolated from infections of the oral cavity of human races and maintained in culture were tested for their sensitivity with the extracts of *Thuja occidentalis*. The needles of the plants were collected and extracted with different solvents viz., Ethanol, Methanol, Chloroform, Diethyl ether and Ethyl acetate. Different concentrations of extracts were tested for their anticandidal activity by using agar diffusion method. Percent of inhibition in growth of the fungus were calculated by radial growth analysis. Apart from growth, the efficacy of the extract on the total protein, carbohydrates and nucleic acid content were also analyzed by using standard methods. The ethanolic and methanolic extracts of *Thuja* showed higher percentage of growth inhibition when compared with other solvent extracts. TLC analysis also showed a clear band of the bioactive compound having the property of anticandidal activity. Hence the extract of *T. occidentalis* may be considered as a potential species for the bio-control of *Candida albicans* in an eco-friendly manner. The results and significance of the findings will be discussed in detail.

PS10-337-0055

Evaluation of the Effects of Incubation Temperature and pH on the In Vitro susceptibility Test for ketoconazole Against *Candida albicans* Isolates from Women with Recurrent Vaginities

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Candidiasis, as an opportunistic infection, is created by the *Candida* species. Although *Candida albicans* is classified in the body as endogen flora, it plays an important role in creating *Candida* related diseases. *Candida Vulvovaginitis* in pregnant women, diabetic mellitus and the patients using multiple antibiotics and contraceptive drugs, demonstrates high resistance against the conventional medication. On the other hand, the recurrent Vaginites disintegrates the long-term process of treatment in majority of the patients. At the present research, which is aiming at determining the optimum conditions for the susceptibility testing before the retreatment of the patients. 10 isolates of *Candida albicans* obtained from 31 patients suffering from recurrent *Candida vaginitis* and drug of ketoconazole, were used at two pH 7.2 and 5.5, and at two temperature 35°C and 27°C. The Microdilution broth test technique was performed to do this. The RPMI 1640 medium within the 96 well microplates with range of 12 tests was used to determine the MIC50 , MIC90 and MFC ketoconazole drug. The obtained MIC50, MIC90 and MFC for ketoconazole at these conditions (T=35°C and pH=7.2) were 0. 25 to 1 µg/ml, 1 to 4 µg/ml and 64 to ? 512 µg/ml respectively, while these values at temperature 27°C and pH 5.5 were 1 to 8 µg/ml, 8 to 64 µg/ml, 512 to ? 512 µg/ml and at temperature 35°C and pH 5.5 were 1 to 8 µg/ml, 4 to 32 µg/ml, 256 to ? 512 µg/ml and at temperature 27°C and pH 7.2 were 1 to 2 µg/ml, 8 to 32 µg/ml, 128 to ? 512 µg/ml, respectively. The obtained results confirmed that the temperature of 35°C and pH 7.2 in comparison to the other conditions, produced better treatment outcomes.
Key Words: *Candida albicans*, ketoconazole , Temperature, pH

PS10-338-0056

Comparison of the effect of D-glucose different concentrations on germ tube formation in *Candida albicans*

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Candida species are the most important agents of fungal infection in human. The most common cause of candidosis is the polymorphic species *Candida albicans*, which can grow as yeast cells, pseudohyphae and hyphae. As a lot of yeasts like *Candida* species are either normal flora and infectious agent in human and alive beings, determination and differentiation of these species is based on colony morphology, microscopic figures, assimilation of carbon and nitrogen, fermentation of sugers, different enzymes and PCR. Detection of *Candida albicans* is also possible by germ tube formation in serum.
As D-Glucose is one of the most important inducer for germ tube formation, at the present research, which is dim at determining the best concentration of D-Glucose for germ tube formation in *Candida albicans*. For this purpose, a clinical isolate of *Candida albicans* was tested. For this procedure, we evaluated the effects different concentrations of D-Glucose (0.001 - 1000 mM), different times (20, 40, 60, 80, 100 and 120 minutes) and pH (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 and 10). Testing was carried out by adding 1*106 yeasts per ml to different concentrations of D-Glucose and tubes were incubated at 37, then germ tube formation was counted. This process was repeated 5 times. The level of germ tube formation significantly induced by D-Glucose in the range 7-8 mM after 2 hours incubation in pH 7, that this concentration is the same in diabetic patients.

PS10-340-0085

A population-based threshold model shows that manipulation of endogenous reserves increases virulence of insect pathogenic fungi

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Entomopathogenic fungi are being used as biocontrol agents of insect pests, but their efficacy can be poor in environments where water availability is reduced. In this study, the potential to improve biocontrol by physiologically manipulating fungal inoculum was investigated.
Cultures of *Beauveria bassiana*, *Lecanicillium muscarium, Lecanicillium longisporum, Metarhizium anisopliae* and *Paecilomyces fumosoroseus* were manipulated by growing them under conditions of water stress. The endogenous sugar alcohols were analysed by HPLC. The germination was assessed on media of different water availability conditions and the rates of germination quality assessed. Bioassays with *M. anisopliae* were carried out with the melon cotton aphid *Aphis gossypii* under different relative humidity regimes.
Conidia produced under modified water activity conditions contained increased concentrations of erythritol. The time course of conidial germination at different aw levels described using a generalized linear model. Germination of *M. anisopliae*, *L. muscarium*, *L. longisporum* and *P. fumosoroseus* was accelerated over a range of aw levels as a result of physiological manipulation. However, this relationship varied with fungal species. There was a linear relationship between germination rate, expressed as the reciprocal of germination time, and aw of the germination medium. In bioassays with *M. anisopliae*, physiologically manipulated conidia germinated more rapidly on the surface of the insect host and fungal virulence was increased even when relative humidity was reduced after an initial high period. Physiological manipulation may lead to improvements in biocontrol in the field, but choice of fungal species/isolate will be critical. In addition, the population-based threshold model used in this study (germination in terms of physiological time), also called hydrotime, could have general applications in mycology and environmental microbiology.

PS10-341-0088

Use of volatile fingerprints (electronic nose) for early detection and discrimination between Trichophyton species (dermatophytes).

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There has been interest in developing methods for the early diagnoses and discrimination between the three-four important dermatophytes isolated from skin and scalps of patients (*T.mentagrophytes*, *T.rubrum*, *T.verrucosum* and *T.violaceum*) There is knowledge to show that there are different volatile fingerprints produced by different species of fungi. We have used two different sensor array systems to examine the volatile fingerprints produced by these species in vitro to evaluate the potential for early discrimination and differentiation and the sensitivity of detection of individual species.
Initial studies were carried out with two electronic nose systems based on conducting polymer and metal oxide/ion sensor arrays. The latter was found to perform much better and was subsequently used in all studies reported. Spread-plate spore cultures of the four species were used in different solid and liquid broth cultures and the head space analysed over periods of 24-120 hrs. For threshold limits of detection log1-log7 CFUs ml were used. Data was analysed using PCA, and Cluster analyses to determine the relative capacity for using volatile fingerprints for discriminating between species and between concentrations of a single species.
This has shown that it is possible to differentiate between all four species after about 96 hrs in both solid and liquid culture. Cluster analyses showed that there was a clear separation between the species. For the two fastest growing species successful discrimination was achieved in 72 hrs (*T.mentagrophytes*, *T. rubrum*). The sensitivity of detection was examined for *T.mentagrophytes* and *T.rubrum*. It was found that it was possible to differentiate between log3-4, log6 and log8 inoculum levels within 96 hrs. However, the blank controls and the Log1 concentrations could not be discriminated based on qualitative volatile fingerprints.
This is the first study to show that volatile fingerprints will discriminate between dermatophytic related species. This approach could be used for patient samples and for drug resistance evaluation.

PS10-342-0120

Identification and estimation of fungi in the ocular tear film and the contact lens biofilm

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The occurrence of opportunistic pathogenic filamentous fungi and yeasts in the ocular tear film has been well documented. It is difficult however, to determine the biomass and activity of fungi in the ocular tear film and in the biofilm which forms over a contact lens. We report on fungi isolated from the ocular tear film and contact lenses and the measurement of ergosterol as an indicator not only of the presence of fungi but also as an estimate of fungal biomass.

The ocular surface of non contact lens wearers (NCLW) and contact lens wearers (CLW) was sampled for the presence of fungi. Samples were incubated at room temperature on Sabouroud's agar and examined periodiocally. Contact lenses that had been worn continuously for periods of 1-2, 7, 14 and 30 days were removed from the eye rinsed in sterile double distilled water and transferred to 100% methanol. In addition, a sterile, unworn control lens was treated in the same manner. Measured volumes were placed in 200 ml round bottom flasks along with an additional 15 ml of methanol per sample. Known quantities of pure ergosterol were added to replicate samples and used as a spike sample. Ergosterol levels were obtained in the following way. Lipids were extracted first using a reflux apparatus followed by sterol release and full separation by mixing with pentane and dry bath evaporation. Samples were analyzed via HPLC with a Perkin Elmer 785 A UV/VIS Absorbance Programmable Detector, Series 200 LC pump, Chromatography Interface 600 Series Link and PE Nelson 900 Series Interface. Injection standards were prepared with pure ergosterol in methanol with known values of 25 ug/ml, 50 ug/ml, and 100 ug/ml. All absorbance peaks were recorded at a wavelength of 282 nm.

The fungi isolated from the eye included *Aspergillus sp, Cladosporium sp., Fusarium sp., Paecilomyces sp., Penicillium sp., Candida sp*. and *Rhodotorula sp*. A total of 92 eyes were sampled for fungi. Fifteen eyes were positive for fungi which were more commonly isolated from the eyes of NCLW than CLW. The mean level of ergosterol was found to be 0.2487 ug/ml in lenses worn continuously for 1-2 days, 0.2833ug/ml after 7 days, 0.3693ug/ml after 14 days and 0.4284 in lenses worn for 30 continuous days. A considerable amount of interpatient variabilty was noted. Ergosterol was not detected in the control lenses.

The species of fungi identified from the ocular tear film are not unlike those found in the ambient environment nor unlike those previously reported from the ocular tear film. Ergosterol levels may be a useful measure of fungal biomass in the contact lens biofilm. The observed increase in fungal biomass on contact lenses over time coincides with the presence of certain cell wall constituents such as mannans that are able to activate the alternative pathway of complement. In some cases, fungi in the biofilm may be a catalyst for the ocurrence of contact lens induced red eye (CLARE). Whether fungal adherence to the contact lens surface is material dependent, species dependent or subject dependent is not fully understood.

PS10-343-0124

Characterization of two morphologically different Phoma species isolated from the same host using Somatic Incompatibility Test (SIT) and Isoenzyme analysis

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Two isolates of *Phoma* isolated from frond of *Borassus flabellifer* showed varied morphological characters like colony margin, colony texture and pigmentation. Hence a molecular (Isoenzyme analysis) and a non-molecular approach (SIT) were employed to find out the differences among the isolates. In Somatic Incompatibility Test (SIT), the mycelial interactions between the two isolates of *Phoma* were studied. Intermingling of mycelium was observed between the isolates suggesting that they are somatically compatible. In Isoenzyme analysis study, one enzyme system was used i.e., Esterases. Both the isolates exhibited similar band pattern in electrophoresis. This study shows that both the isolates are similar even though they show variation in few morphological characters.

PS10-344-0136

First Report of *Candida dubliniensis* in Iran Using Specific Primers

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Candida dubliniensis is described species that is a close phylogenic relative of *Candida albicans*. Although previously associated with oral candidiasis in HIV infected patients, it has more recently been recognized as a cause of superficial and systemic diseases in HIV negative and HIV positive patients. The goal of this study was to identify *Candida dubliniensis* obtained from clinical samples from patients, suspected having Candida infection, referred to Medical Center, University of Tehran, Tehran, Iran.
Six hundred & twenty five fresh isolates were collected from different patients referred to Medical Center. The yeasts were cultured on Sabouraud Dextrose Agar and incubated at 45°C for 48h, also they were subcultured on Casein agar (at 25°C) and CHROMagar (at 37°C) respectively. Assimilation test and Intracellular beta glucosidase activity were carried out for suspected *Candida dubliniensis* isolates.
All Putative isolate of *Candida dubliniansis* were final identified by specific primers.
From 625 isolate, only 5 cases produced dark green colonies on CHROMagar and had negative beta glucosidase activity.
According to these results, these isolates were diagnosed as *Candida dubliniensis*, in which four (3 from vagina and one from urine) were reacted by specific primers and they identified as *Candida dubliniensis*.
According to this finding it seems to identify *C. dubliniensis* from *Candida albicans* molecular methods should be used as a gold test. We isolated this organism from patients with out predisposing factors. This is the firs report of isolation of *Candida dubliniensis* in Iran by molecular method.
Keyword: Candida dubliniensis, Candida albicans, PCR, CHROMagar

PS10-345-0153

Menstrual Cycle and Ovarian Function in Obese Polycystic Ovary Syndrome Women Treated with Auricularia polytricha Formula through a Randomized Double Blind Placebo-Controlled Trial

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A number of reports showed that Chinese herbs improves menstrual function, however, only few of them have been studied by placebo-controlled. The aim of our study was to use a double blind approach, placebo-controlled, with detailed assessment of menstrual and ovarian function to assess the validity of the group. Ninety-six patients of Chinese women with obese polycystic ovary syndrome (PCOS) were randomized to receive "Black Wood Ear (*Auricularia polytricha*) Formula" (BWEF), metformin, or placebo with three times daily for 6 months. Anthropometric, endocrine, and ovarian ultrasound assessments were effected for 6 months. BWEF treatment resulted in a significant decrease in menstrual index ($p<0.001$), triglycerides, and low-density lipoprotein (LDL), as well as in a significant increase in progesterone. The results suggest that BWEF treatment can induce ovulation and improve metabolic factors, thus it is well tolerated by the majority of patients and may be clinically useful as an alternative treatment for metformin, particularly in patients having obese PCOS and menstrual disturbances.

PS10-346-0231

Antifungal/antagonistic activity of medicinal mushroom *Pleurotus tuberregium* against filamentous fungi

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A tropical mushroom *Pleurotus tuberregium* (Fr.) Singer is a white-rot fungus that produces sclerotia and fruiting bodies. It is an excellent source of proteins, carbohydrates and minerals. Reported antibacterial and antitumor properties of *P. tuberregium* makes it valuable for medicinal purposes. Antifungal/antagonistic activity of *P. tuberregium* was studied in dual culture experiment at 26 and 30?C on potato-dextrose-agar (pH 5.5, Difco) against filamentous fungi that are pathogenic on humans and animals *(Chrysosporium keratinophilum, Microsporium gypseum)*, pathogenic on plants (*Fusarium culmorum, Bipolaris sorokiniana)* and their antagonists *(Trichoderma harzianum, T. asperellum)*. Antagonistic activity against testorganisms were estimated by 3 types and 4 subtypes of reactions: *deadlock* at the distance or at the contact (A, B types), *overgrowth* (C) and partial or complete overgrowth after deadlock (CA1, CA2, CB1, CB2) (Badalyan et al., 2002). Linear (mm) and radial (mm/day) mycelial growth rates (GR) were calculated before and at the contact of colonies (Buchalo, 1988).
The control GRs of tested fungi decreased twice at 30oC in comparison with data received at 26oC, except for *P. tuberregium* and *B. sorokiniana* the GRs of which increased up to 20 and 14.6%, respectively. At 26oC, in dual cultures, the GRs of *P. tuberregium* and filamentous fungi decreased more in comparison with control data to 27% and 22%, whereas at 30oC increased to 17.2% and 25%, respectively.
In our experiments A, CA1, CA2 and CB1 types of interactions were described. Strong deadlock was observed after mutual overgrowth between *P. tuberregium* and *F. culmorum* at 26oC, whereas *P. tuberregium* overgrew *F. culmorium* by rhizomorphic mycelium at 30oC. At the contact zone, pigmentation and exudate droplets were detected. After initial deadlock, *M. gypseum* (CA1) and *B. sorokiniana* were partially (CB1) and *Ch. keratinophilum* was completely (CA2) overgrown by *P. tuberregium*. At 26 and 30oC, *T. asperellum* was suppressed by 11.1 and 23.7%, respectively and completely overgrown by mushroom. At 26oC, *P. tuberregium* was remarkably inhibited (30%) and overgrown by *T. harzianum* (CA2), whereas at 30oC strong deadlock (A) with pigmentation and primordia formation at the contact reactions were described.
High temperature stimulates the combative ability of *P. tuberregium*, particularly against *F. culmorium* and *T. harzianum*. Against keratinophilic species antagonism was expressed by the intensity of described reactions. Revealed antifungal activity makes further studies of *P. tuberregium* perspective, particularly as a biocontrol agent towards phytopathogens and for obtaining novel antimycotic drugs.
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PS10-347-0272

Immunomodulating action of edible mushrooms, *Pleurotus cornucopiae* var. *citrinopileatus* and *Pholiota nameko*

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In Oriental traditional medicine, it has been considered that medicine and food have the same origin, which was called "Ishoku – Dougen" in Japanese. Many edible mushrooms have already become attractive as health beneficent foods, and as source materials for immunomodulators, antitumor agents, antibiotics, and antihypertensive etc. Therefore, the medical and pharmaceutical interest in the mushrooms has become increasingly strong worldwide. The basidiomycete fungi *Pleurotus cornucopiae* var. *citrinopileatus* and *Pholiota nameko* are delicious and popular edible mushrooms in Japan. They grow on stumps of tree and fallen trees such as elms in Japan, Korea, the north-eastern part of China and Taiwan. We investigated whether these mushrooms functioned as an immunomodulator, and could be expected to become functional foods, and sources of chemotherapeutical agents. To investigate the effect of the two mushrooms, *P. cornucopiae* and *P. nameko*, on immunomodulating actions, cytokines productions secreted from macrophage cell lines, RAW264 (mouse) and U937 (human), treated with water soluble extracts from these fungi were measured. Both extracts showed high activities of productions of tumor necrosis factor (TNF) -? and nitric oxide (NO) from treated human monocytes (U937) as well as other mammal?s (RAW264). And, it was shown that TNF-? was secreted significantly earlier (at 1 hr) than NO at the time course experiment. Moreover, the levels of some kinds of cytokines mRNA in the macrophages, which were promoters of type 1 response in immune system (Th1 type), such as TNF-?, interleukin (IL)-12 and 18, and type 2 (Th2 type), IL-4 and 10, were evaluated by RT-PCR. Then, it was shown that both extracts from *P. cornucopiae* and *P. nameko* induced the mRNA expression of Th1 type cytokines in the stimulated macrophages. These data might suggest that these edible mushrooms could prevent Th2-biased immune responses, such as allergy. We furthermore chromatographically (ion exchange, gel filtration) separated and purified polysaccharides from the water soluble extracts of these mushrooms. And, their immunomodulating actions were investigated as described above. Then, purified polysaccharides showed high activities of cytokine productions. These data provided that the polysaccharides from the two mushrooms possessed potent immunomodulating activities of cytokines productions from macrophages. Therefore, it could be suggested that they were available as source materials for the development of a novel immunomodulator. Moreover, these findings suggested that *P. cornucopiae* and *P. nameko* fruiting bodies were expected to become functional, or medicinal, foods.

PS10-348-0278

Presence of fungi in the nose, throat and ear of Kurdish refugees in Southern Italy

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The geographic position of Southern Italy has favoured, in recent years, many migratory flows, not always under a sanitary control. The Authors wanted to evaluate if the illegal arrival of refugees may increase the importation of pathogenic microorganism that are endemic in the origin countries. In particular, fungi colonizing nose, throat and ear were studied because the mycosis in otorhinolaryngology (ORL) have increased and some cases due to dimorphic fungi have been reported in Europe too.
Through a simple sampling, 2970 refugees, who had arrived in Apulia from Kurdistan, were enrolled and asked to complete a questionnaire regarding demographic aspects; then they underwent one throat, two nose and two ear swabs to isolate yeasts and moulds. The nose and throat swabs were also tested for dimorphic fungi.
The Kurdish were of Iraqi (36%), Turkish (33.7%) and Iranian origin (30.3%). The mean age was 23 (range: 13-48), of which 84.2% were males. 680 people (22.9%) tested positive for fungi: 430 (63.2%) for yeasts and 250 (36.8%) for moulds.
Among the people positive for yeasts, in the throat 290 (67.4%) resulted colonized by *Candida* (*C.*) *albicans* and 10 (2.3%) by *C.krusei*; in the nose 40 (9.3%) by *C.famata*, 30 (6.9%) by *C.parapsilosis,* 20 (4.7%) by *Cryptococcus albidus*, 10 (2.3%) by *Cryptococcus luteolus* and 10 (2.3%) by *C.pelliculosa;* in the ear 20 (4.7%) by *C.parapsilosis.*
Among the people positive for moulds, in the nose 190 (76%) resulted colonized by *Penicillium* spp, 20 (8%) by *Aspergillus (A.) niger,* 10 (4%) by *A.fumigatus* and 10 (4%) by *Alternaria alternate*; in the ear 20 (8%) by *A.niger*. No dimorphic fungi were found.
The ORL mycotic colonization among refugees is different from the Italians only with respect to the prevalence of *Penicillium* in the nose. The lack of dimorphic fungi rules out the hypothesis of exotic diseases importation from Kurdistan.

PS10-349-0281

Where is the origin of the *Cryptococcus gattii* Vancouver Island outbreak?

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The pathogenic basidiomycetous yeast *Cryptococcus gattii* may cause a life-threatening disease of the central nervous system, lungs and skin in humans and animals. *C. gattii* is found mainly in tropical and sub-tropical regions of South America, Africa, Asia and Australia where it is endemic. Recently, a cryptococcosis outbreak in both humans and animals occurred on Vancouver Island (British Columbia, Canada) (Kidd *et al.*, PNAS 101, 2004). This outbreak was shown to be caused by a rare genotype of *C. gattii* (AFLP6A or RAPD VGIIa) using Amplified Fragment Length Polymorphism (AFLP) and sequence analyses. The objective of this study was to find the origin of the outbreak isolates. A selection of thirty-four *C. gattii* outbreak isolates and ninety *C. gattii* reference strains were analyzed by AFLP. The AFLP fingerprint analyses were carried out with six different primer combinations in duplicate. Reproducible marker fragments were used for population genetic analysis. In addition, polymorphic fragments from the AFLP analyses were used to develop a multilocus sequence typing (MLST) approach.
Fraser *et al.* (Nature 437, 2005) suggested that the Vancouver Island outbreak isolates originated from Australia. However, our results based on AFLP and MLST analyses show that the outbreak isolates originated from South America. South American isolates were found to be ancestral to Australian and Asian isolates as well.

PS10-351-0371

Immunomodulatory and Antitumor Effects of Crude Polysaccharides Extracted from Korean Wild Medicinal Mushrooms

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This study was initiated to evaluate immuno-modulatory and antitumor effects of mushroom extracts prepared from Korean wild medicinal mushrooms such as *Elfvingia applanata, Paecilomyces sinclairii* and *Lepista nuda*. Neutral salt soluble (0.9% NaCl), hot water soluble and methanol soluble substances (hereinafter referred to Fr. NaCl, Fr. HW and Fr. MeOH, respectively) were extracted from each of the mushrooms. *In vitro*, cytotoxicity tests showed that none of Fr. HW had cytotoxicity against cancer cell lines such as Sarcoma 180, HepG2 and HT-29 at the concentration of 0~2,000?/?, while Fr. NaCl and Fr. MeOH showed slight cytotoxicity against the cell lines. Fr. HW from *E. applanata*, Fr. NaCl and Fr. MeOH from *P. sinclarii*, and Fr. HW from *L. nuda* exhibited antitumor effects with life prolongation effect of 32.3%~48.4% in mice inoculated with Sarcoma 180. Fr. HW from *E. applanata*, and Fr. NaCl from *P. sinclairii* improved proliferation of spleen cells and the immunopotentiation activity. The polysaccharide and protein contents of the extract were 93.89% and 3.10%, respectively.

PS10-352-0389

Possible impacts of fungi in sow-bedding for abortions and reproductive disorders

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Problems with fertility and early abortions are unfortunately well known in swine herds, and can from time to time be considerable. In Norway sows commonly are kept on a particular type of beddings called '*talle*'. *Talle* is made of wood chippings or straw or a mixture of those and the depth of the *talle* in the pig houses commonly exceed one meter. The main advantage of *talle* is a positive impact on the environment offered the pigs. The aim of the present pilot study is to increase the knowledge of the mycobiota in *talle*.
In order to determine the occurrence and significance of filamentous fungi in *talle* in Norway, sampling from 6 big herds was performed. Two of the herds had reproductive problems. Frequencies (cfu/g) of the most common fungal species and the species diversity in samples from *talle* and air were determined. The moulds were phenotypically identified to species level. In addition, a few non-identifiable isolates that were molecularly identified by ITS sequencing.
Talle from the problem-herds has shown high mould counts and presence of fungi known to give reproductive problems. *Aspergillus fumigatus* constitutes the main problem as it is the species most often isolated from *talle,* quantitatively as well as qualitatively.
Several of the species identified are known to be able to cause reproductive problems. Our results show the need for further studies on the quality of talle in terms of mycology.

PS10-353-0422

Botanical antifungal drug from lichen metabolites fights fungal infections

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Dermatophytoses occur more frequently than subcutaneous and systemic mycoses and remain a therapeutic problem in tropical and subtropical countries, despite the availability of a number of ointments, lotion, paints and powders. Sources of these agents are largely petro-products that are non-biodegradable and causes adverse effects and residual toxicity and other hand these products are thermolabile and expire in short duration (maximum 35 month).
We have tested the lichen metabolites (LM) of *Usnea longissima* Ach against dermatophytes viz., *Epidermophyton floccosum, Microsporum audouinii, M. nanum, M. canis, M. gypseum, Trichophyton mentagrophytes, T. rubrum, T. violaceum* and *T. tonsurance* in vitro. The lichen metabolites were further studied for thermostability, storage durability and their effect on human skin. Antifungal activity of the lichen metabolites was compared with some synthetic antifungals. Finally, the lichen metabolites were formulated for clinical trial in the form of ointment.
The minimum inhibitory concentration of the LM was found to be 30 ?l ml-1 at which LM showed fungistatic action. The minimum fungicidal concentrations (MCCs) of the LM were found to be 50 ?l ml-1 against human pathogenic fungi. The LM at MCCs showed heavy doses of inoculum potential and its activity did not expire even upto 36 months of storage. Moreover, LM did not exhibit any adverse effect on mammalian skin up to 10% concentration. Further, the LM of *Usnea longissima* was formulated in the form of ointment and trialed clinicaly in MLN Medical college, Allahabad. 30 patients were selected, showing positive potassium hydroxide (KOH) results at the start of the trial. Patients were diagnosed as tinea corporis, tinea cruris or tinea pedis. All patients were treated with ointment twice in a day for 3 weeks. At the end of medication, 30.0% of patient's recovered complete cure, 45.0% showed significant improvement from the disease. No KOH negative cases of relapse were observed when patients were reexamined after two month following the end of treatment thereby, denoting the absence of relapse. The ointment was found cost effective, had long shelf life and absence of any adverse effects.
Thus, the lichen metabolites (LM) of *Usnea longissima* could be used as potential sources of antifungal agent after undergoing successful multicentre clinical trial.

PS10-354-0462

Screening for growth inhibition of animal-diseased bacteria in natural thalli of lichens.

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Lichens have been used as medicines from ancient times all over the world. Metabolites from natural lichen thalli and cultured mycobionts were shown to have biological activities, such as inhibition of enzymes and growth inhibition of virus, bacteria, fungi, plants, invertebrates and tumours. Lichens grow slowly in nature, but they are healthy in overcoming attacks of microorganisms. Therefore, lichens have been expected as important sources of antibiotics, so many papers on antibacterial activities of natural lichens have been reported since about sixty years ago. Burkholders et al. (1947) first tested 100 lichens on growth inhibition of two bacteria. After his work many papers have been reported on antibiotics from natural thalli. This presentation shows the screening results of antibacterial activity in natural thalli of lichens and active compounds in them.
Natural thalli of 117 species collected in Japan and other countries were tested. Tested bacteria were 15 species; *Actinomyces pyrogenes*, *Bacillus subtilis*, *Bifidobacterium pseudolongum*, *Clostridium perfringens*, *Erysipelothrix shusiopathiae*, *Escherichia coli*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *Pasteurella multocida*, *Propionibacterium acnes*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus faecalis*, *S. mutans*, *S. pyogenes*. Each dry material of natural thalli was powdered and submerged in acetone overnight. Acetone solution was obtained by filtration, and evaporated to dryness in vacuo. Acetone extract was weighed and dissolved in acetone (0.1 % w/v). Acetone solution (50 Ìl) of each extract was absorbed onto a paper disk (8 mm diam.). Each of tested bacteria subcultured on agar-medium on 37 °C in aerobic or anaerobic condition was picked up and swabbed on agar plate in a 9 cm Petri dish and prepared disks were put on the agar plate. These cultured on 37 °C for 20 hrs. Growth inhibition of bacteria was estimated from the diameters of the clear or turbid zones that developed after incubation. *Micrococcus luteus* was cultured on an agar plate in the Petri dish in where TLCs of extracts and authentic usnic acid was laid at the bottom and spots showing no growth of this bacterium were detected.
In the screening result, 82 extracts (70 %) from tested species inhibited the growth of at least one bacterium, and 27 extracts inhibited the growth of more than 8 bacteria. Among them, 10 extracts contained no usnic acid known as an antibiotic agent. *Heterodermia pseudospeciosa* and *Anaptychia isidiza* containing no usnic acid were strong inhibitory activity against the growth of several bacteria.

PS10-355-0511

Antifungal activity of components isolated from *Epicoccum nigrum* MET0425

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Watermelon is the most popular fruit in the summer time among the cucurbits in Korea, and now produced all the year round by greenhouse cultivation. Since the greenhouse culture of watermelon as well as other cucurbits was widespread, incidences of major diseases in the past such as *Fusarium* wilt, gummy stem rot and anthracnose, have been decreased. On the other hand, a group of new borne diseases have been prevalent, causing serious economic losses, one of which is a root rot/vine decline disease that has recently occurred on cucurbits worldwide (Martyn and Miller, 1996; Mertely *et al*., 1991, 1992, 1993; Park *et al*., 1994; Stanghellini *et al*., 1996). This study was carried out to isolate antagonistic fungi against *Monosporacus cannonballus* causal agent of a sever root rot/vine decline disease of muskmelon and watermelon. *M. cannonballus* infects young secondary and tertiary roots early in the season, colonizes in the cortical tissue, ultimately killing most of the feeder roots. By mid to late season, most of the root system is affected and the vines begin to collapse, typically beginning with the crown leaves and progressing distally.
We isolated potent antagonistic fungi against the pathogen from soil of Chonnam area and investigated antifungal activity against various plant and animal pathogen. The isolate strain MET0425 was identified based on morphological, biochemical and 18S rDNA, ITS1, 5.8S rDNA and ITS2 sequence analysis. The antagonistic fungus was submerged cultured in the medium Czapack-Dox at 25? for 6 days. An antifungal component was extracted with ethyl acetate from culture filtrates and partial purified using Sephadex LH-20 column and YMC-Pack ODS-A HPLC. The purified agent takes an accurate measurement of antifungal activity by paper disc. The pH and thermal stability, and structure of this antifungal agent were determined.
Based on nuclear ribosomal 18S-ITS1-5.8S-ITS2 sequence analysis, the strain MET0425 was identified as *Epicoccum nigrum*. The antifungal activity was observed maximum level after 6days shaking culture at 25?. The antifungal agent that purified using Sephadex LH-20 column and YMC-Pack ODS-A HPLC was exhibited broad spectrum activity against the fungi *Phytophthora capside*, *Botrytis cinerea*, *Candida albicans, Aspergillus awamori*, and *Aspergillu nidulans*.
We can expect that the *Epicoccum nigrum* MET0425 will provide for a powerful resource in studying the biology and in developing cucurbitaceous crops disease biocontrol agent.

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Does indoor mycobiota reflect outdoor one?

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The indoor mycobiota is formed primarily by the fungal propagules introduced into the environment by anemo-, zoo- and anthropochoric routes. The aim of the investigation was to compare indoor (our data) and outdoor (literature data) microfungal communities of Moscow.
The indoor mycobiota of Moscow, as that of other regions, is a xerotolerant community (in contrast to outdoor mycobiota), which is manifested by a great species diversity (up to 82% of all revealed species), high frequency and considerable proportion of xerotolerant and xerophilic species, as well as *Aspergillus* and *Eurotium* species. *Penicillium* and *Aspergillus* dominate indoors in Moscow. Their frequency is about 95% and the abundance is up to 80%. In contrast, *Cladosporium* species dominate in the outdoor air of Moscow. A number of species most frequent in sod-podzolic soil or in the urban soils in Moscow, such as *Fusarium moniliforme, Penicillium funiculosum*, *P. vulpinum* were not revealed in dwellings. It was shown that the seasonal dynamics of hygrophilic, phylloplane and stenotropic species is similar to that outdoors. The seasonal dynamics of xerotolerant and xerophilic species depends mainly on the indoor environment conditions.
Specific mycobiota, differing from outdoor ones, was formed indoors under the conditions of a relatively closed space, constant temperature and humidity, influenced by a row of anthropogenic factors.

PS10-357-0583

Determination of Biological Efficiency of Entomopathogenic Fungus *Fusarium subglutinans* Against *Aphis gossypii* in Greenhouse

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In this study, biological efficiency of entomopathogenic fungus *Fusarium subglutinans* (Wollens &Reinking) Nelson, Toussan&Marasas that is attacking to *Aphis gossypii* Glover (Hom:Aphididae) in cotton fields in east Mediterranean Region of Turkey was evaluated against *A. gossypii* on pepper and cotton plants in climatic room and greenhouse condition.
It was found that temperature had an effect on colony growth *F. subglutinans* and fungus grew more rapid in higher temperatures, but no statistical differences was observed in colony growth at 25 and 300C. Slowest colony growth of *F. subglutinans* was observed on Sabouraud Agar. The higher conidiospores production reached in Potato Dextrose liquid medium as 2X106 conidospores/ml.
Three different concentration of 19 isolates of *F. subglutinans* showed significant differences in aphid mortality at 25 0C. While highest mortality rate was calculated in 1X106 conidiospores/ml, there was no differences in mortality rates between 1X108-1X1010 conidospores/ml. Pathogenicity test were carried out with three different concentration of Fs 1 and Fs 4 isolates at three different temperatures to determine effect of temperature and concentration of the fungus on aphid mortality. While increasing temperatures resulted in high aphid mortality increasing concentration did not show same result. In greenhouse experiment with these two isolates, aphid population was controlled 2 weeks later after application.

PS10-358-0599

Comparison of two methods for quantification of viable *Saprolegnia* sp. propagules in water samples

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Infections with oomycetes from the genus *Saprolegnia* (saprolegniasis) are a major disease problem in wild and farmed fish worldwide. In aquaculture the disease has previously been controlled with malachite green treatment, but after this chemical was banned due to health risks, new ways to prevent infection have to be established. Knowledge of zoospore concentrations in different water sources is valuable in epidemiological studies of the organism, as well as in testing of water treatments aiming to reduce saprolegniasis in aquaculture facilities.
The aim of the present study was to evaluate two methods for isolation and quantification of viable *Saprolegnia* sp. propagules in water samples.
Each method was applied with samples from three different water qualities with presumed differences in zoospore concentration; a) untreated lake water, b) UV-treated lake water and c) lake water treated with malachite green. Three identical treatment units were established for each water quality and the analysis was performed in parallel for each unit.
In method 1 water samples were filtered through 45 µm Millipore® filters, with subsequent incubation of the filters for 48 hours on a selective medium. The total number of fungal colony forming units was recorded, and the proportion of *Saprolegnia* sp. was determined.
In method 2 sampled water was inoculated in liquid selective medium in 96 well microtitre plates with subsequent incubation for 48 hours, before the total number of fungal colony forming was recorded and the *Saprolegnia* sp. proportion was determined.
For confirmation of genus *Saprolegnia*, colonies were transferred from filters and microtitre plate wells, respectively, to liquid medium and allowed to grow for 48 hours before the mycelium was washed and left in autoclaved tap water for 24 hours. Genus was then confirmed from zoospore-release morphology.
The total number of fungal colony forming units, and the proportion of *Saprolegnia* sp., yielded in both methods were compared for each water quality. Further, the applicability of the methods for quantification of *Saprolegnia* zoospore concentration in water samples is discussed.

PS10-359-0669

Phyllosphere fungi of perennial pastures associated with ill thrift syndrome of livestock in New Zealand

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Animal production dependent on intensive and extensive grazing of summer and autumn pastures can be reduced through slower than expected animal growth or live weight gain (LWG). Livestock affected by this phenomenon are commonly referred to as suffering from "ill-thrift". The causes of ill-thrift can be difficult to diagnose due to interactions between the effect of pasture quality, animal health or other animal and environmental factors. Previously reported animal health issues that can suppress LWG include viral pneumonia, gastrointestinal parasitism, and trace element deficiencies. Effects of fungal toxins and metabolites known to be produced by phyllosphere and endophytic fungi have also been implicated in poor LWG. Mycological studies were undertaken to further elucidate populations of phyllosphere fungi potentially associated with ill-thrift incidents in New Zealand pastures.

Surveys were conducted over three growing seasons throughout the main pastoral regions in New Zealand, as well as sampled pastures where incidents of poor LWG were recorded. Cultural isolation methods were used to process 700 pasture plant samples. Post-serial dilution plating, fungi were enumerated as colony forming units (CFU) and recognisable taxonomic units (RTU) that included the identification of fungi capable of producing mycotoxins.

Hyphomycete species from the genera *Acremonium*, *Alternaria, Aureobasidium, Cladosporium, Cylindrocarpon, Fusarium, Gliocladium, Myrothecium, Penicillium, Phoma,* and *Phomopsis* were the most frequently identified fungi. Other ubiquitous fungi in the survey were species of yeasts, Zygomycetes and non-sporing forms. Results from this survey showed that 20% of phyllosphere fungi (RTUs) are potentially toxic. They contributed approximately 8% towards the total fungal biomass based on CFU counts. Following the assumption that not all potentially toxin-producing fungi are indeed toxic, the chronic toxic fungal abundance was estimated. We conclude that approximately 4% of the fungal biomass present in New Zealand pasture is chronic toxin producers as measured by CFUs. This equates to approximately 11% of RTUs. The total amount of fungi consumed per animal per day was estimated at approximately 705 x 106 CFU per 7.5 kg of fresh grass or 1.5 kg dry matter. Lambs may therefore ingest 28 x 106 CFU of chronic toxic fungi per day.

The diversity and distribution of fungi present on the phyllosphere of pasture plants New Zealand was characterised, and research directly linking poor LWG to specific fungal toxins produced by pastoral fungi is ongoing.

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Molecular diversity of *Cryptococcus neoformans* isolates from cats

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Incidence of human cryptococcosis has been increasing and remains an opportunistic infection especially in immunocompromised hosts. The actual sources of infection are still unclear. One of the most possible sources aside from soil and pigeon droppings are animals. We aimed to examine the molecular diversity *of Cryptococcus neoformans*, the causative agent of cryptococcosis, by investigating isolates from one of favorite domestic pets, cats. Eight C. neoformans isolates from lesions of eight cats were randomly selected. These cats were brought to the Small Animal Hospital at the Faculty of Veterinary Science, Chulalongkorn University, Thailand, with lesions in the nasal and face regions. The disease was diagnosed based on the presence of encapsulated yeast cells, positive urease and phenoloxidase reactions. DNA from these isolates was prepared and subjected to orotidine monophosphate pyrophosphorylase (URA5) gene restriction fragment length polymorphisms (RFLP) analysis via double digestion with the enzyme HhaI and Sau96I. PCR fingerprinting with the microsatellite primer M13 was carried to determine their molecular type via comparison to molecular type standard strains. Based on URA5-RFLP analysis, type VNI and type VNII of *C. neoformans* var. *grubii* were found in 6 out of 8 (75%) and 2 out of 8 (25%) cats respectively. This was confirmed by and a variation among strains was demonstrated via PCR fingerprinting. Strain variation within and between cats was revealed in 1 out of 6 which had infections with *C. neoformans* var. *grubii* molecular type VNI and 1 out of 2 which had infections with the molecular type VNII. In conclusion, molecular diversity was firstly found in *C. neoformans* isolates from cats with varying underlying diseases. The relation of those strains to strains from human, animal and environment sources should be further investigated.

PS10-362-0732

Hydrolytic enzymes as the virulence factors of dermatophytes

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Dermatophytoses results due to infection by a group of keratinophilic fungi called dermatophytes. These fungi have developed several virulence traits enabling invasion of the outer keratinized skin layer-the stratum corneum, hair and nails and avoidance of host defence mechanisms. Virulence factors that contribute to this process are the hydrolytic enzymes, most of which are secreted extracellularly. Of these extracellularly produced enzymes, only the role of proteases for several *Trichophyton* and *Microsporum* infections has been described in score of papers, whereas, only little is known about the role of secreted lipases and almost nothing about the involvement of the phospholipases, esterases and DNAses in virulence. They may play an important role in the pathogenicity of dermatophytoses and their hydrolytic activity probably have a number of possible functions in addition to the simple role of digesting molecules for nutrition.
Among proteases, presence of keratinase activity has been considered particularly relevant as keratin constitutes a major proportion of skin and other cornified tissues. The often described *in vitro* hydrolysis of other soluble and insoluble proteins (i.e. elastin, collagen etc.) is probably only a consequence of the broad substrate-specificity of the cleavage and does not prove the presence of specialized proteases in dermatophytes. Correlations between keratinase activity of several dermatophytes and their virulence *in vivo* or in animal models would be highlighted in this presentation. However, presence of gene families encoding extracellular hydrolytic enzymes in several species of dermatophytes further prove the dermatophytic pathogenicity to be a multifactorial process. An exact knowledge of the highly complex interaction between these distict virulence attributes of the dermatophytes and the host's defence mechanisms may lead to the development of new prophylactic and therapeutic strategies targeting factors that may be essential for virulence for cutaneous infections.

PS10-363-0745

Growth inhibition of *Candida* species and *Aspergillus fumigatus* by statins

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There is a major need for novel antifungal therapies but the high costs of testing new drugs can slow developments immensely. We have found that a major currently-used drug class, the statins, have potent antifungal properties. Statins are a class of drugs widely used for lowering high cholesterol levels through their action on HMG-CoA reductase, a key enzyme in the synthesis of cholesterol. Our studies addressed the effects of two major statins, simvastatin and atorvastatin, on five *Candida* species and *Aspergillus fumigatus*. The statins strongly inhibited the growth of all species, except *C. krusei*. Statin-inhibited cells had their levels of ergosterol levels lowered two two-thirds of normal levels. Supplementation with ergosterol or cholesterol in aerobic culture led to substantial recovery from the inhibition by statins, indicating a possible specificity for the mevalonate synthesis pathway. We also found that in the petite-positive yeast, *C. glabrata*, statins caused an elevated petite frequency and the total loss of mitochondrial DNA. This finding may be relate to the occasional muscle myopathy, seen as a side-effect of statins, and suggests that *C. glabrata* is a good host for examining such issues. Our findings suggest that the statins could have utility as antifungal agents.

PS10-364-0766

Antifungal activity of the medicinal mushroom *Flammulina velutipes* (Curt.: Fr.) P. Karst. in vitro

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There is growing interesting in the use of complementary medicine for the prevention and treatment of various diseases. The recent increase of human and animal fungal diseases makes useful the development of new antifungal drugs, especially from Higher fungi. The systematic myco-pharmacological experiences of medicinal mushrooms and their biological active compounds are not sufficiently investigated.

In present study we reported results on screening of antifungal activity of medicinal mushroom *Flammulina velutipes.* The 6 strains of this mushroom from different geographical regions (III-2, 99 (Armenia), F-1, F-2, F-3 (France), R-9 (Russia)) were tested against 12 pathogenic for humans and animals filamentous fungi in dual culture on agarized malt extract medium during 30 days at 24°C. The microfungi isolates were separated from Armenia soil samples. The antifungal activity of *F. velutipes* strains was determined by index of antagonism. The rating scale, which containing 3 types and 4 subtypes of reactions, was used for the estimation of relative combative ability of tested organisms. During the study the pigmentation of mycelia, development of pseudosclerotial lines on the deadlock, production of exudates droplets, fruit-body formation was also described.

The results of experimental pairings showed that *F. velutipes* markedly inhibited and suppressed the growth of most of the tested microfungi. The strains of *F. velutipes* was active against *Verticillium lecanii, Paecilomyces lilacinus, Alternaria alternata, Aspergillus wentii, Chrysosporium keratinophilum, Penicillium aurantiogriseum, Penicillium griseofulvum, Stachybotris chartarum, Fusarium tricinctum, Acremonium alternatum*; moderate active against *Aspergillus candidus.* In our investigation *F. velutipes* was not active against *Geotrichum candidum.*

During our experiment the mutual inhibition (15.2%) at the contact (A) or on distance (B) amounted around 1.4% and 13.8%, respectively. The microfungi were overgrown by *F. velutipes* – 75.0%: without initial deadlock barrier (C) – 11.1%, partial overgrown (CA1`, CB1) – 40.2%, 11.1%, respectively and complete overgrown (CA2) – 12.5%. In all pairings only 9.8% of strains *F. velutipes* were overgrown by microfungi.

The pigmentation of *F. velutipes* mycelia and production of exudates droplets with different colors on the mycelia and at the interaction zone, as well as the development of pseudosclerotial lines on the deadlock were observed between some test-organisms. The strains F-1 and F-2 developed the fruit bodies at the contact zone with *S. chartarum* and *A. candidus.*

Antifungal activity depended from bio-ecological properties (geographical origin, mycelial growth rate, etc.) of *F. velutipes.* The fast growing strains from *Armenia* (III-2 and 99) were more active (91.7% of overgrown on microfungi), than the strains from France (F-1, F-2, F-3) (75.0% of overgrown on microfungi) and Russia (R-9) (58.3% of overgrown on microfungi). Thus, the antifungal activity of *F. velutipes* may be modified by the ecological/environmental conditions, and it is useful to take these properties in account in the screening of strains for biotechnological purposes and to obtain the new antifungal drugs.

PS10-365-0778

Antibacterial activity of extracts of selected indigenous edible and medicinal tropical mushrooms

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Antibacterial resistance is a world wide growing problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world. One of the measures to combat the increasing rate of resistance is to have a continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with no-effective ones. In this study the antibacterial activity of extracts of selected mushrooms of the Teso region of Uganda against common pathogenic bacteria was investigated. Antibacterial assays (agar well diffusion and Paper disc assays) were done using methanol and petroleum ether extracts from mushrooms. *E. coli* was the least sensitive organism to most petroleum ether and methanol extracts. *S. aureus* was significantly inhibited by petroleum ether extracts while *P. aeruginosa*, was also sensitive to the methanol extracts. The demonstration of bioactive potential is of importance since some of the mushrooms are reportedly used as ethno medicines. Further studies are planned to isolate and characterize the active compounds.

Key words: Antibacterial, mushroom extracts, Edible and Medicinal mushrooms, *S. aureus, E. coli and P. aeruginosa*.

PS10-366-0785

Fungal biopesticides for tick and buffalo fly control

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Both the cattle tick (*Boophilus microplus*) and the buffalo fly (*Haemotobia irritans exigua*) are serious pests of cattle in Northern Australia. Current control strategies for ticks and buffalo flies rely on extensive chemical usage which is fraught with negative issues. Fungal biopesticides have emerged as realistic non-chemical control options for a range of pests. DPI&F researchers have investigated the potential of a *Metarhizium* based fungal biopesticide to control ticks and buffalo flies. Thirty - one isolates of *Metarhizium anisopliae* were screened in the laboratory. Two isolates were selected for animal trials based on their high optimal growth temperature (30oC); good spore production characteristics and ability to kill adult ticks in the laboratory in minimum time. Formulation studies showed that fungal spores formulated in an emulsified oil - water mix halved the time to 100% tick mortality compared to spores suspended in aqueous mixtures. Three field trials were then conducted over 12 months to assess the virulence of formulated *Metarhizium* spores to the parasitic stages of ticks on dairy cattle. In the last trial the effect of the fungal biopesticide on buffalo flies was also assessed. Fungal spores formulated in an oil emulsion were applied with a motor driven spray unit to dairy heifers. Surface temperatures of selected animals were monitored as were the ambient temperature and relative humidity. Unengorged ticks sampled from each animal immediately after treatment were incubated under laboratory conditions to assess the efficacy of the formulation and application. Side counts of adult female ticks 4 – 8 mm were conducted daily before and after treatment to assess the performance of the fungus against all tick stages on the animals. At each trial the formulation caused 100% mortality in unengorged ticks under laboratory conditions. In the field, the fungal formulation was able to reduce ticks on animals by up to 70%. However the results varied between trials and appeared to be affected by the environmental conditions, particularly the temperature. In the last trial the fungal biopesticide also caused a significant reduction in the number of buffalo flies on the animals. This research has shown that a biopesticide based on *M. anisoplae* can offer a non-chemical control option for cattle ticks and have the added benefit of controlling buffalo flies.

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Fungal biocontrol of sheep lice *(Bovicola ovis)*

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The annual cost of lice to the Australian wool industry has been estimated at $169 million. Currently the only options for lice control involve the application of chemicals to the fleece. However the loss of effectiveness of some chemicals due to resistance and the unacceptably high levels of chemical residues left in the fleece by others mean that alternative methods of control are needed. One area of potential is the use of entomopathogenic fungi to control lice. A DPI&F research programme has carried out extensive laboratory and some on-sheep investigations into the control of the sheep body louse (*Bovicola ovis*) with endemic isolates of the fungi *Metarhizum anisopliae* and *Beauveria bassiana*. Scanning electron microscopy and light microscopy were used to investigate the uptake of spores from the wool by lice, and bioassay methodologies using a wool substrate were developed to test pathogenicity. Twenty – four isolates of *M. anisopliae* and eight isolates of *B. bassiana* were screened for optimal growth temperatures, sporulation characteristics and virulence to different stages of *B. ovis.* Several of these isolates caused 100% mortality within 7 days to all stages of lice. Both the level of grease in the wool and stage of louse development had some effect on mortality rates. Scanning electron microscopy showed that fungal spores applied to the wool grease adhered easily to the surface of lice. However no germinating spores were observed on the surface of the louse, while light microscopy showed a large number of spores in the gut within 48 hours and extensive hyphal growth in louse tissues within 72 hours. Three isolates of *B. bassiana* and nine isolates of *M. anisopliae* were selected for dose mortality studies and their toxicity statistics determined. Preliminary studies with one of the most toxic strains of *M. anisopliae* applied to sheep indicated that the lipophilic spores adhered well to wool grease and were still viable and highly virulent to lice in laboratory tests after 14 weeks. Formulated *Metarhizium* spores applied by hand jetting to the fleece of lousy sheep caused a 96% reduction in louse numbers for three weeks after application decreasing to 80% after nine weeks. This research is the first to show that fungal biocontrol of sheep lice shows potential as an alternative control strategy to chemicals.

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Identification of genomic differences between *Cryptococcus gattii* and *Cryptococcus neoformans* var. grubii by Representational Difference Analysis

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Cryptococcus neoformans is an opportunistic basidiomycete that causes cryptococcosis predominantly in immune-compromised individuals. The varieties in the *C. neoformans* and *C. gattii* species complex present biochemical, immunological, molecular and epidemiological differences. In this study, Representational Difference Analysis (RDA) was carried out to isolate genomic differences between *C. neoformans* var. *grubii* and *C. gattii*. Sequence determination of approximately 200 clones and comparison analysis using public databases (BLASTN and tBLASTX) led to identification of 69 sequences with significant similarities (*E*value< 10-5) to 13 different sequences from completely sequenced genome strain of *C. neoformans* var. *neoformans* JEC21. Results indicated that most of the sequences identified, as expected, are hypothetical proteins or proteins of unknown function in *Cryptococcus* species. Hybridization experiments from five of these subtracted clones confirmed the presence of polymorphism, for four sequences, or specificity to *C. gattii,* for one sequence. One of the five tested products hybridized only with total DNA of tester *C. gattii*. BLASTN and tBLASTX searches of the public database revealed a significant similarity between this sequence and a region of the conserved hypothetical protein CNL05140 described in the genome sequence of *C. neoformans* var. *neoformans* JEC21. The cloned DNA fragment of 987 bp of this ORF strongly hybridized to *C. gattii* genomic DNA but not *C. neoformans* var. *grubii*. One other sequence, corresponding to a putative endoplasmic reticulum protein (CND02670) revealed also difference in hybridization patterns of digested genomic DNAs of *C. gattii* and *C. neoformans* var. *grubii*. The comparison between hibridization patterns of the sequences from the putative insulin degrading enzyme (CNL05710) and chitin syntase (CNC00050) against three isolates of the species complex *C. neoformans* demonstrated polymorphism in these sequences. The product of locus CNL05710, coding for a putative insulin degrading enzyme, has a metallopeptidase activity involved in the maturation of peptide pheromone, proteolysis and peptidolysis reactions. The product of locus CNC00050, a putative chitin synthase, is involved in cell budding according with *C. neoformans* var. *neoformans* JEC21 genome information. However, the hybridization patterns of the sequence corresponding to a putative proteasome subunit alpha type 5 (CNH01360) gave similar patterns for *C. neoformans* var *grubii*, *C. neoformans* var. *neoformans* and *C. gattii* genomic digested DNAs, therefore it is not a *C. gattii* specific sequence or PCR-based marker candidate. Although only one product is apparently unique to *C. gattii* at this level of analysis, the other sequences with EcoRI polymorphisms may also be considered as containing potentially informative markers. This is the first report of RDA application in pathogenic yeast and may lead to the study of new genes in *C. gattii*. Furthermore, our study suggests that RDA is applicable to identify genome-specific sequences from yeasts.

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The automated DNA sequencing was performed at the facilities of the Brazilian Genome Network at CBiot-UFRGS-RS.

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Isolation and characterization of cDNA encoding for heat shock protein 30 from *Penicillium marneffei*

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Penicillium marneffei is a dimorphic pathogenic fungus that can cause disseminated mycosis, especially in AIDS patients. The organism has a saprobic mycelial form at 25oC and a parasitic yeast form at 37oC. The role of heat shock proteins and stress response-related proteins of *Penicillium marneffei* remains unknown. In this study, we isolated, sequenced and characterized the cDNA encoding for heat shock protein 30 (*Pmhsp30*) from *Penicillium marneffei.* The deduced amino acid sequence and DNA sequence analysis showed high homology to fungal *hsp30* gene. Expression of *Pmhsp30* during phase transition or at the temperature increase was determined by Northern blot analysis. The high level of *Pmhsp30* transcript occurred in the yeast phase or in culture cells grown at 37oC whereas very low or undetectable transcript was observed in the mold phase or at 25oC. The recombinant protein was produced by using glutathione S-transferase (GST) fusion system in *Escherichia coli.* The fusion protein was purified by affinity chromatography and tested preliminary for its immunoreactivity with sera from *Penicillium marneffei*-infected AIDS patients by using Western blot analysis. The results showed positive reactions with 2 out of 10 cases. In conclusion, the *Pmhsp30* encoding cDNA was cloned and characterized from *Penicillium marneffei*. As a high response to temperature increase, the PmHsp30 may play a role in stress response and in cell differentiation process of this fungus. The produced recombinant protein exhibited immunoreactive property. It could react specifically with IgG antibody in some serum samples derived from AIDS patients infected with *Penicillium marneffei.*

PS10-370-0820

THE ULTRASTRUCTURE OF *ASPERGILLUS* SPECIES ISOLATED FROM PATIENTS AND THE SOIL

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Before this time we know little about the ultrastructural bases of hyphal cells of vegetative mycelium (HCVM) morphogenesis in species from genus *Aspergillus*.
A. niger van Thiegh., *A. fumigatus* Fres., *A. versicolor* (Vuill.) Tiraboschi., *A. flavus* Link isolated from patients and *A. nidulans* (Eidam) Wint., *A. silvaticus* Fennell et Raper, *A. wentii* Wehmer, *A. terreus* Thom, isolated from soil and cultivated on Czapek agar have been used in our study. For TEM the samples were fixed in 2% glutaraldehyde in 0,1 M sodium cacodilate (pH 7,2) and postfixed in 1% osmium tetroxide. Samples were then dehydrated in a graded ethanol series and embedded in epon-araldite.
The mature HCVM in *A. niger*, *A. nidulans*, *A. silvaticus*, *A. wentii*, *A. terreus* contained two nuclei and in all others species - four. The hyphal cells of the aerial mycelium in all investigated species had the similar structure and characterized by low level of vacuolization and the presence of dense cytosole which conceals all cellular components. Differences among the pathogenic strains in the morphogenesis types of the HC in submerged mycelium of one the same strain, on the one hand, and in different species, on another hand, are revealed. The several types of morphogenesis in submerged HCVM are recognized . That is four types for *A. niger*, five types for *A. fumigatus* and *A. flavus* and six types for *A. versicolor* according to: (1) sizes and shape of nuclei; (2) number and structure of mitochondria; (3) level of vacuolization; (4) presence or absence of endoplasmic reticulum and its structure; (5) presence or absence of microbodies; (6) presence or absence of storage substances and their combinations. In comparison with the pathogenic strains, the mature HC in submerged mycelium of the soil isolates had only one pattern of morphogenesis. Only differences in the types of accumulated storage substances were demonstrated.
According to our observations of the hyphal cells in submerged mycelium, in comparison with aerial one, are at the higher level of metabolism independence on the type of substrate from which strains were isolated. The variability in types in submerged HCVM for pathogenic strains of the species morphogenesis in comparison with soil isolated once, have been shown.

PS10-371-0832

EFFECTS OF DRUGS AND CHEMICAL AGENTS ON CELL-SURFACE-HYDROPHOBICITY AND ADHESION OF *Cryptococcus neoformans* TO VERO EPITHELIAL CELLS

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This is the first study on drugs and chemical agents affectingthe cell-surface-hydrophobicity (CSH), the capsule and adhesion of *Cryptococcus neoformans* (*C. neoformans*) to cultured Vero cell lines in order to explore the relationship among CSH, the capsule, cell wall and adhesion of *C. neoformans* to host cells.
CHS of *C. neoformans* was determined by the test, microbial adhesion to hexadecane (MATH), and the adherent rate (percent) was tested with radial count of 3H-leucine labeling adherent yeast to Vero cells of a confluent mono-layer which grew in 96-well containing 1640 medium in advance. A series of formula drugs and regulative chemical agents were used to pre-treat the yeast.
The anti-fungal drugs, Amphotericin B (AmB) and Fluconazole (FCZ), decreased significantly the adherent rate, and produced a thinner capsule as well as a lower level of CSH inducing the special varieties of the yeast cell wall ultra-structure. Of other drugs, the antibiotic Ampicillin (AMP) affected little both CSH and adherent rate of *C. neoformans* to Vero cells, but it produced the same characteristic ultra-structural change of the cell wall with a reversible disappearing of the capsule as that of the varieties produced by FCZ.
On the other hand, these chemical agents, PHA, ConA, Fucose (FC), Mercaptoethanol (ME), decreased the their items of CSH levels and adherent rates of *C. neoformans* to Vero cell lines while the chemical agent lectin (LC) increased otherwise them without the alternative capsule of *C. neoformans.*
These above data implied that the capsule of *C. neoformans* isn't obviously associated with CSH level and the relative adherent rate of *C. neoformans* to host cells, and that there are no significant relationship between adhesion and CSH level of *C. neoformans*. Although this changeable association among CSH, capsule and wall structure is still undetermined in the current study, the typical three traits of capsule, CSH and adhesion embedded on the surface of *C. neoformans* play an important role in keeping its invasive growth on the surfaces of host tissues, which is very different from that of other yeast.

PS10-372-0835

ANTIFUNGAL ACTIVITY OF SOME LABIATAE SPECIES AGAINST SOME FILAMENTOUS FUNGI

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In this study, some species known as "kekik" (thyme),*Thymbra spicata* L.,*Satureja hortensis L., Origanum onites* L., *Origanum vulgare* spp *hirtum* (Link.) Ietswaart, *Origanum vulgare* L. ssp *vulgare*, *Origanum minutiflorum,* and known as "da€çay›" (herbal tea), *Sideritis Vuralii* H.Duman & Bafler and also *S.caeserea* H.Duman & Aytaç have been tested:
Many different species including the genus of *Origanum, Satureja, Thymbra* and *Thymus* known as "kekik" in Anatolia, are widely used for several purposes (Sat›l et al., 2005). Apart from their culinary usage, they are used to cure stomachaches, respiratory track infections, cold and diabet. They are also known to prevent the fungus growth in dried food. For example, fig, which is one of the most important export products of West Anatolia, Turkey, is left to dry after being soaked into boiled water with kekik (Tümen & Sekendiz, 1989). Under the light of the ethnobotany, it was decided to search the antifungal activity of some "kekik" species. The genus *Sideritis* is quite wide spread in the world with 150 species, 46 of which are in Anatolia. 42 of 55 taxons are endemic. Known as "da€çay›" in Anatolia, the *Sideritis* is used as antienflammatuar, antirheumatism, digestive, antimicrobial and antispasmodic. In herbal tea form, it is used to cure cold as a folk medicine (Kirimer 1999; Davis, 1982).
To determine the antifungal activity, the plant methanol extracts were tested against four fungal species. The results of antifungal activity of these plants were given and evaluated in the presentation.

Key words: Antifungal, *Origanum, Satureja, Sideritis, Thymus, Thymbra,*

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PS10-373-0844

Histopathological and immunological changes in mice experimentally infected with *Candida albicans*

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Candida spp. are the most common fungal pathogen of systemic candidiasis. *C. albicans* had been frequently isolated from blood of patients with systemic candidiasis, followed by *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*. The objectives of the study were to identify any pathological changes and to evaluate the immune responses of mice experimentally infected with a clinical isolate of *C. albicans*. *C. albicans* was cultured in YPD broth (2% bacteriological peptone, 2% D-glucose and 1% yeast extract) at 37∞C for 3 days on a shaking incubator (100rpm). The culture was supplemented with 10% foetal bovine serum for 3 hours for germ tubes induction. The yeast cells were harvested, washed, re-suspended with phosphate buffered saline (PBS) and counted with a haemocytometer. Eighteen female inbred Balb-c mice (15 – 20 g) were inoculated intravenously with 4 x 106 to 5 x 107 attenuated yeast cells and PBS (control group). The animals were observed daily until sacrifice 34 days post-infection. The blood samples were collected via cardiac puncture and the sera were obtained, stored and used for antibody and antigen detection using ELISA methods developed with monoclonal and polyclonal antibodies. The remaining blood was cultured on Sabouraud dextrose agar at 37∞C. The liver, spleen, kidneys, brain, heart and lungs were collected and processed for histopathological examination. Cystic lesions with intense chronic inflammatory cellular response were observed in haematoxylin and eosin (H & E) stained sections from kidneys of animals infected with 1 x 107 and 5 x 107 yeast cells. Cystic lesions of various sizes with destruction of renal parenchyma and chronic inflammatory reaction were seen in kidneys of animals infected with 4 x 106 and 8 x 106 yeast cells. Cystic lesions with large clumps of fungus consisting mainly hyphae and some yeast were observed in the PAS stained sections from kidneys of mice infected with 1 x 107 and 5 x 107 yeast cells. Clusters of yeast cells were detected in spleens and lungs. Two isolates were obtained after culturing the blood from the infected mice and were confirmed to be the same species with DNA sequencing and sequence alignment with ATCC and other strains. The antibody levels of the infected groups (4 x 106, 8 x 106 and 5 x 107 yeast cells) were significantly higher compared with the control group. The levels of antigens detected were significantly higher in mice infected with 4 x 106, 6 x 106, 8 x 106 and 5 x 107 yeast cells per mouse compared with control group. The antibody and antigen levels in infected mice did not correlate significantly with the infective doses. In conclusion, fungal lesions were mainly seen in the kidneys and spleens of animals experimentally infected with this isolate of *C. albicans*. The antibody and antigen levels in infected mice were elevated during the period of infection.

PS10-374-0857

Sex, virulence, and homeodomains in the fungal pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen that affects primarily immunocompromised individuals such as people with AIDS, chemotherapy patients, and transplant recipients. Infections with *C. neoformans* are thought to be caused by spores, which result from sexual development. During sexual development haploid a and a cells fuse and initiate a process controlled by the homeodomain proteins, Sex Inducer 1a (Sxi1a) and Sex Inducer 2a (Sxi2a) that includes cell fusion, dikaryotic growth, and spore formation.

Our current experiments are focused on determining the molecular mechanisms by which Sxi1a and Sxi2a control sexual development and ultimately, spore production. Our hypothesis is that Sxi1a and Sxi2a control sexual development by directly regulating the transcription of key targets to specify the dikaryotic state. Our hypothesis is supported by the observations that 1) both Sxi1a and Sxi2a are predicted members of the homeodomain family of DNA binding transcription factors and bind DNA directly in vitro, 2) mating gene transcript levels are significantly altered in crosses in which *SXI1a* or *SXI2a* has been deleted, and 3) Sxi1a and Sxi2a interact with one another in a manner similar to previously described cell type-specific homeodomain transcriptional regulators.

To identify targets of Sxi1a and Sxi2a, we are taking several integrated approaches. In a bioinformatics approach, we are analyzing the regulation by Sxi1a and Sxi2a of genes in *C. neoformans* similar to genes involved in sexual development in other fungi. At the same time, we are using *C. neoformans* microarrays to identify genes regulated by Sxi1a and Sxi2a. Targets of interest are being tested for direct regulation by Sxi1a and/or Sxi2a using chromatin immunoprecipitations. Promoter sequences from both bioinformatic and microarray targets are being tested using in vitro DNA binding experiments with purified Sxi1a and Sxi2a proteins.

Preliminary DNA binding studies show that the homeodomain regions of Sxi2a and Sxi1a bind specifically to the promoter sequences of many microarray targets, including a putative homolog of the *clampless* (*CLP1*) gene (first identified in *Coprinus cinereus*). We have found that the *C. neoformans CLP1* homolog is required for proper sexual development. We are currently refining the determinants of binding in vitro and elucidating the specific role that Clp1 plays in the developmental regulatory circuit controlled by *SXI1a* and *SXI2a*. *SXI1a* and *SXI2a* are the first identified sexual cycle regulators in *C. neoformans*, and the characterization of the pathway they control will reveal how sexual development and spore formation occur in *C. neoformans.*

PS10-375-0873

Effect of *Pholiota adiposa* Extract in Hyperlipidemic Mice

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The purpose of this study was to investigate the effect of *Pholiota adiposa* extract on the serum and hepatic lipids in hyperlipidemic mice fed with high-fat diet. The *Pholiota adiposa* extract decreased significantly in total serum cholesterol, serum triacylglycerol and hepatic triacylglycerol but not in the total hepatic cholesterol. The *Pholiota adiposa* extract also decreased in very low-density lipoprotein increased by the high fat diet without affecting high-density lipoprotein cholesterol. These results suggest that *Pholiota adiposa* extract may be beneficial for the regulation of hyperlipidemia.

PS10-376-0874

Molecular characterization of the Cryptococcus neoformans species complex from Brazil

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Cryptococcosis, is a life-threatening infection of the lung and central nervous systems, which is caused by *Cryptococcus neoformans* (serotypes A, D and AD) and *C. gattii* (serotypes B and C), and occurs in immunocompromised and immunocompetent hosts. PCR-fingerprinting and RFLP analysis have been used to determine the molecular types of clinical and environmental isolates, with VNI, VNII, VNIII and VNIV corresponding to *C. neoformans* and VGI, VGII, VGIII and VGIV to *C. gattii*. Previous studies have shown genetic and phenotypic instability among strains of the *C. neoformans* species complex obtained from clinical and environmental sources. Among Brazilian clinical and environmental samples, we observed 7 isolates expressing phenotypes of both species, which may represent more than a mixed colony, resulting in un-typeable strains such as serotype BD or AB, correlating with dubious or instable results on CGB media and serotyping in successive subcultures. The aim of this study is to determine the distribution of the molecular types in Brazil and correlate PCR fingerprinting, *URA5*-RFLP analysis, microsatellites, and MSLT analysis. Besides, the origin of the phenotypic/genotypic variability of the atypical clinical isolates the possible presence of recombination or hybridisation between the different molecular types will be studied as has been previously described for serotype AD. Fifty seven *C. gattii* and 30 *C. neoformans* isolated from clinical and environmental sources in Brazil were typed by PCR fingerprinting (M13 primer) and *URA5*-RFLP analysis. Two isolates representing each subgroup and 7 isolates that have showed unusual phenotypic/genotypic patterns have been analysed by MLST using the LAC1, CAP59, ACT1, PLB and URA5 genes. The genotypic variability of 20 single colonies from each atypical isolate was screened by *URA5*-RFLP analysis. Three different molecular types were observed in a single strain (LMM 868), which were cloned and sequenced. The molecular type distribution in Brazil seems to be regionally related. Preliminary results show VGI and VNII occur only in the S and SE regions of Brazil, while VGII and VNI, the most prevalent types of *C. gattii* and *C. neoformans* respectively, were found in all Brazilian regions. The few VGII infections diagnosed in the SE region are from natives of NE region, thus suggesting imported cases.

PS10-377-0891

Establishment of a quality controlled internal transcribed spacer (ITS) sequence database as basis for routine clinical diagnostics of human fungal pathogens

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In the study, we sequenced internal transcribed spacer regions (ITS) of rDNA from 225 strains comprising ca. 100 medical fungal species. We also setup a in house ITS sequence database based on our own (225) and assumed "correct' GenBank sequences using BIOLOMICS. The database will provide us a useful tool to identify medical relevant fungus species, given the valuable bio-resources available in the GenBank. Our ITS sequence database will be an ongoing database – we will sequence additional clinical strains and also regularly download the latest "assumed correct" GenBank sequences to improve our collections for robust and reliable identification of medical fungi.

PS10-378-0902

Clinical utility of a panfungal PCR assay on tissue specimens for the rapid diagnosis of invasive fungal infection

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Invasive fungal infection (IFI) is caused by an increasing diversity of pathogens. Current culture-based diagnostic methods are insensitive and slow. Timely and accurate detection and identification of the etiologic agent is critical for directing clinical management. We developed a panfungal PCR assay targeting the internal transcribed spacer 1 (ITS1) region of the rDNA gene complex, to detect fungal DNA in fresh and paraffin-embedded (PE) tissue specimens. PCR products were sequenced and analysed against the GenBank database. The feasibility of using the PCR to diagnose IFI is illustrated in the following 3 cases.

Case 1: A 21 year old male underwent allogeneic hematopoetic stem cell transplant (HSCT) for aplastic anemia. He developed early (day 3) graft versus host disease affecting the skin and gastrointestinal tract. Increasing abdominal distension ensued, followed by multi-organ failure and death. Post mortem studies showed fungal hyphae in multiple organs including heart, kidney, adrenal and lung but the organism was not recovered by culture. PCR analysis of PE sections from heart and kidney revealed the causative agent to be *Trichosporon asahii* (99-100% identity with GenBank sequences).

Cases 2: A 51 year old male with acute lymphocytic leukaemia underwent induction chemotherapy, complicated by the development of multiple nodules in both lung fields. He received intensive antifungal therapy with incomplete response. Repeated sputum cultures and an aspiration biopsy of the residual lung nodule were culture-negative but histology positive; PCR analysis identified the pathogen to be *Aspergillus fumigatus* (99% identity with GenBank sequences).

Case 3: A 34 year old male developed fever, cough and haemoptysis following an autologous HSCT for lymphoma. A chest CT showed diffuse infiltrates, and *A. fumigatus* was grown from sputum. Examination of lung biopsy tissue (PE) showed numerous branching hyphae but was culture-negative. PCR revealed the pathogen to be *A. fumigatus* (100% identity with GenBank sequences).

Panfungal PCR analysis correlated well with clinical and histological findings. The assay successfully detected and identified fungal DNA from both fresh and PE tissue from patients infected with two important fungal pathogens. In all cases, the pathogen was not detected by culture.

PS10-380-0924

Fungal keratitis in patients with corneal ulcer refered to a tertiary care hospital.

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Fungal keratitis is a suppurative, ulcerative and sight-threatening infection of the cornea that sometime leads to loss of the eye. The objectives of this study were to improve facilities for Laboratory diagnosis, to determine the predominant causative micro-organisms and to identify the predisposing factor.

Patients who presented with clinically suspected corneal ulcer at the eye unit of Boo-Ali Sina University Hospital in Sari (Iran) between May 2004 and March 2005 were included in the study. The corneal scraping smeared on two slides, which were stained with Gram stain (for bacterial keratitis) and 10% potassium hydroxide(KOH) with or without calcofluor white (KOH+CFW) stain (for fungal keratitis) for microscopic examination. The specimens were also inoculated directly onto blood agar, Sabouraud dextrose agar, and Potato dextrose agar in C-shaped streaks.

A total of 22 patients met the inclusion criteria of this study, among whom 10 (45.5%) were female and 12 (54.5%) were male. The mean± SD age of the patients was 61.5 ± 17.7 years (range: 15-83) years. In direct microscopy branching, and septate hyphae was identified in 7(31.8%) patients. Two (28.6%) fungi (*Aspergillus fumigatus* and *Fusarium* Spp) isolated. Five (71.4%) Patients with fungal keratitis were male and 2 (28.6%) female. The mean± SD age of with fungal keratitis was 60.4 ±12.1 years (range: 39 to 73) years. Three (42.85%) of patients with fungal keratitis were farmer. The mean interval between the onset of symptom and diagnosis was 26.4 (range: 1 – 93) days. Trauma with plant debris and straws were noted in two (28.6%) patients with fungal keratitis. Five (71.4%) patients received topical antibiotics. Analyses using KOH + CFW as the gold standard revealed a sensitivity of 71.4% for KOH, and 42.9% for Gram stain.

The direct microscopy method is an essential tool in the diagnosis of fungal keratitis. Therefore, wet mount with KOH+CFW or only KOH can be relied upon as the single most important screening test for rapid diagnosis of fungal corneal ulcer.

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Killer fungi attract springtails to their doom

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Wood decay basidiomycete fungi are vital for releasing inaccessible carbon and mineral nutrients from wood into the decomposer invertebrate community. They do not grow alone but in communities in which they interact aggressicely. Such interactions are associated with chemical production and biochemical change. The aggressive interaction of two cord-forming saprotrophic basidiomycete fungi (*Resinicium bicolor* and *Hypholoma fasciculare*) was investigated to determine its effect on fungal grazer (collembola or springtails) behaviour. Initially, collembola aggregated on *R. bicolor* but later moved to *H. fasciculare*. Collembola are know to graze far more heavily on *R. bicolor* than on *H. fasciculare* yet their movement to the latter from the former occurred when there was still *R. bicolor* mycelium available to graze upon. Collembola movement correlated with the production of interaction specific volatile organic compounds (VOCs) and the significant up-regulation of non-interaction specific VOCs. Ten volatiles were produced during interactions that were not detected in single species controls. In general, most (18) fungal volatiles were sesquiterpenes; a benzoic acid methyl ester, a benzyl alcohol, and a quinolinum type compound with a distinctive fragmentation pattern at m/z 203, 204, 206, 207 were also identified; three volatiles with m/z maxima of 163, 159 and 206-208 respectively, remained not identified. Collembola movement was also linked to death of springtails on both species but in higher levels on *H. fasciculare*. As well as the production of VOCs, diffusible organic chemicals (DOCs) were produced as deep pigmentation by both species, and the deepening colour in *H. fasciculare* with time was correlated with an accelerated death rate of collembola on *H. fasciculare* mycelium. It is hypothesised that the production of such chemicals may be involved in the attraction of springtails to their death. Their high nitrogen content could be utilised by the fungi as an important resource in enzyme synthesis, which is vital during such aggressive fungal interactions.

PS10-382-0939

Propective typing of *candida* species in oncology patients, A mulicenter study- preliminary results

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Early detection of *candida* species in body site could improve the survival of the immunosuppressed patients by allowing the initiation of specific antifungal treatments while the fungal biomass is still low.
The objective of the study was to determine the predominant *Candida* species in patients with malignancies.
A total of 39 (25 male and 14 female) oncology patients in three university hospital met the inclusion criteria. Oral, pharyngeal and tongue swabs, midstream urine, sputum and blood sample and aspirates from wounds were collected and cultured for identification of fungi in Sabouraud Dextrose Agar and CHROMagar *Candida*. The latter medium is intended to rapidly identify *C.albicans* and to detect mixture of different *Candida* species.
The strains also identified by germ tube test, morphological characteristics on corn meal agar tween 80. The results were read according to the colours and morphology of colony.
Acute lymphoid leukaemia was the dominant type of malignancies (38.5%). A total 23 *Candida* isolates were obtained. More than one *Candida* species were determined in 5 patients. Nineteen of 23 isolates that grew as distinctive light green colonies and produced germ tube identified as *Candida albicans*. In this study four of 23 isolates produced blue violate colony of *C.tropicalis.*
This study is being done at the present time and we plan to use RAPD_PCR for differentiation of *Candida* species

PS10-383-0940

Phagocytosis and killing of human pathogenic *Penicillium marneffei* and *Penicillium* sp. by mouse macrophage J774.1 cells

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Penicillium marneffei, a dimorphic fungus endemic in Southeast Asia, can cause disease in those with impaired cell-mediated immunity, especially in people infected with human immunodeficiency virus. The types of exposure and the route of entry of this fungus leading to infection in humans are still unclear. By analogy with other opportunistic fungal pathogens, it seems quite likely that conidia may be inhaled from a contaminated environment and subsequently disseminated from the lungs when immunosuppression occurs in host's condition. During infection, non-specific immunity plays a major role in the clearance of this pathogen. In this study, we examined phagocytosis and killing activity of mouse macrophage J774.1 to conidia of *Penicillium marneffei* and non-pathogenic *Penicillium* sp. *in vitro*. Conidia of *Penicillium marneffei* and *Penicillium* sp. were collected and co-incubated with adhered mouse macrophage J774.1 cells in a ratio of 1:10. Phagocytosis and killing assays were determined by using microscopy and viable colony plate counting method. The results indicated a high efficiency in phagocytosis effect of mouse macrophage J774.1 cells against the conidia of both fungi. The percentage of phagocytosis (PP) of *Penicillium marneffei* at 30 min of incubation was 48% and reached to the maximum of 93% at 2 h and phagocytic index (PI) was 1.6 and 4, respectively. The PP of *Penicillium* sp. at 30 min of incubation was 47% and reached to the maximum of 88% at 4 h and the PI was 1.4 and 3, respectively. In the killing assay, about 8% of *Penicillium marneffei* conidia were killed by phagocytes after 30 min of incubation. The killing rates increased to approximately 21, 37 and 60% after 1, 2 and 4 h of incubation, respectively. In non pathogenic *Penicillium* sp., 42% of conidia were killed after 30 min of incubation and reached to the maximum of 68% after 1 h of incubation. The collective results of *in vitro* phagocytosis study and anti-fungal activity of macrophage to the conidia of both *Penicllium marneffei* and *Penicillium* sp. showed that macrophage plays a crucial role in the immune response to the fungi. However, conidia of *Penicillium marneffei* seem to be more resistant to the killing by macrophages than non-pathogenic fungus, *Penicillium* sp. The mechanism of intracellular infection and survival of *Penillium marneffei* inside macrophage will need further investigations.

PS10-384-0942

Efficiency of immunoblot assay for rapid diagnosis of human pythiosis

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Human pythiosis, a life-threatening infectious disease, has been occasionally reported in tropical and subtropical area. *Pythium insidiosum*, an aquatic fungus-like organism, is known as a cause of the disease. Most patients develop chronic infection, which is hardly identified by culturing in both early and late state without losing infected-organs. From this reason, an effective diagnostic tool as immunoblotting technique has been developed. The Western immunoblotting using culture filtrate antigens was performed with serum samples. We have reported the use of immunoblot assay to diagnose pythiosis in one Thai thalassemic patient. In the study described here, we demonstrated the usefulness of the immunoblot assay in the rapid diagnosis of eight cases of human pythiosis, in comparison with the other tests using culturing and PCR. The sera of candidosis, cryptococcosis, aspergillosis, penicilliosis marneffei, and histoplasmosis patients were also tested for specificity of the immunoblot assay. Moreover, *Pythium insidiosum-, Cryptococcus neoformans-, Penicillium marneffei-, Histoplasma capsulatum*-immunized rabbit antisera and pooled normal human sera were compared. All seven pythiosis serum samples showed dominant reactive bands at 110, 73, 56, 42 to 35, 30 to 28, 26 and 23 kDa but one serum showed bands at 73, 42 to 35, and 30 to 28 kDa. In control group, *C.neoformans*-immunized rabbit antisera, sera of cryptococcosis, aspergillosis, penicilliosis marneffei patients and pooled normal human sera showed weak reactive band at 73 kDa only but *Penicillium marneffei-, Histoplasma capsulatum*-immunized rabbit antisera, candidosis and histoplasmosis patient's sera showed no reactive band. The PCR with specific primers for *Pythium insidiosum* and the fungus culture were positive in 4 and 3 cases, respectively. The results revealed that immunoblot assay is specific and applicable as a rapid laboratorial tool for the diagnosis of human pythiosis.

PS10-385-0965

Human and Animal Isolates of *Pseudallescheria boydii* and *Scedosporium species* Identified from 1977 to 1995

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The identification of isolates of *Pseudallescheria boydii*, *Scedosporium apiospermum* and *Scedosporium prolificans* are recorded from 1977 to 1995. The cultures had been isolated from 21 human patients and three animals. Typical macro and micro morphology on potato dextrose agar incubated at 28deg.C was used for identification. Selected isolates were referred to appropriate culture collections for confirmation of their identities. Nine human isolates were identified as *P. boydii*, ten as *S. apiospermum* and three as *S. prolificans*. Isolates were from localised infections in immunocompetent patients, colonisation in diseased lung and from immunocompromised patients with haematological malignancy or after organ transplantation. Isolates of *S. prolificans* were from immuno-compromised patients. An isolate from the vaginal discharge of a horse was identified as *S. apiospermum* and isolates from the milk of a goat and the milk of a cow were identified as *P. boydii*. Both the goat and the cow had a history of mastitis and the horse had an infected uterus.

S31IS1 – 0755
Inter- and intra-species diversity of ballistoconidium-forming yeasts and related taxa
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In the classification of fungi, the morphology of teleomorph is considered the most important criterion, and the mode of conidiogenesis is thought to be important for the anamorphic species. Although the morphological characteristics of yeasts are poor compared with filamentous fungi, the production of arthroconidium, stalked-conidium or ballistoconidium has been used as a taxonomic criterion for genus or higher level; however, based on the sequence analyses of rDNA and other genes, the present taxonomic system does not necessarily reflect the phylogenetic relationship among fungi. Since most isolates obtained from the natural environment seem to belong to anamorphic species, reconstruction of the taxonomic system is most important to correctly identify these strains.
We have carried out isolation and identification studies of yeasts from various plants in many countries focusing on ballistoconidium-forming yeasts. Our intention is to clarify the relationship between species diversity and their niches, and to discuss the intraspecies diversity of ballistoconidium-forming activity. First, each phyllosphere seems to have its own microbiota. For example, in the genus *Dioszegia*, *D. crocea* has been isolated from England, Canada, Hungary and Tasmania; *D. zsoltii* only from China and Japan; and another distinct species only from Thailand. Another good example was shown in the genus *Udeniomyces*. In *U. pyricola*, which has been found in various countries, only one base difference was found in the sequence of the D1D2 region of European strains and Japanese, and in the ITS region some insertion/deletion was recognized. Regarding the intraspecies diversity of ballistoconidium-forming activity, the strength of this ability varied among strains even within the same species. We have also found that the sequences of D1D2 and ITS regions of a ballistoconidium-forming isolate were identical to those of *Cryptococcus carnescens*. The ballistoconidium-forming ability seems to be a naturally possessed characteristic of basidiomycetous yeasts, and thus is not appropriate as a taxonomic criterion.

S31IS2 - 0780
Mycosporine synthesis in dimorphic basidiomycetes - Ecological and phylogenetic implications
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Mycosporines (MYC) are UV absorbing compounds that are accumulated by several basidiomycetous yeasts when they are exposed to light (Libkind et al., 2004). In the present study we evaluated the distribution of the ability to synthesize MYC among dimorphic basidiomycetes and the ecological relevance of the formation of this compound. 327 wild and collection strains corresponding to 144 species representing the various phylogenetic groups (Pucciniomycotina = Urediniomycetes: 64 species, 200 strains; Agaricomycotina = Hymenomycetes: 53 species, 100 strains; and Ustilaginomycotina = Ustilaginomycetes: 27 species, 27 strains) were screened for MYC synthesis. MYC production was restricted to certain taxonomic groups. Among Ustilaginomycetes, MYC occurred only within the Microstromatales and in the Cryptobasidiaceae. For the Pucciniomycotina MYC were produced in the Mixiomycetes, Agaricostilbomycetes and Cystobasidiomycetes. Among the dimorphic Agaricomycotina MYC were found in *Auriculibuller-Papiliotrema*, *Bulleribasidium*, *Dioszegia* and in the Filobasidiales. In the order Cystofilobasidiales, MYC production was normally negative with the exception of *Udeniomyces* and *Phaffia*. A single type of molecule, identified as mycosporine-glutaminol-glucoside (MGG), was so far detected in the studied strains. In Patagonian aquatic environments we observed a higher proportion of MGG producing yeast species at high altitude lakes (increased UV exposure). Several strains isolated in high altitude lakes produced larger quantities of MGG than those isolated in lowland aquatic reservoirs. Our results indicate that MGG production occurs in specific lineages of basidiomycetous yeasts. Therefore the study of the distribution of this trait might have taxonomic implications. Environmental factors like exposure to UV radiation may select for MGG-producing yeasts thus contributing to the distribution of mycosporinogenic species in nature.
- Libkind et al. 2004. Constitutive and UV-inducible synthesis of photoprotective compounds (carotenoids and mycosporines) by freshwater yeasts. *Photochem. Photobiol. Sci.* 3: 281-286.

S31IS3 - 0587

Are there eurybionts among the dimorphic basidiomycetes?

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For more than 50 years of ecological studies it has been reported that different plants and soils of various types are populated with the similar yeasts, e.g. *Cr.albidus*, *Cr.laurentii*, *Cr.diffluens*, *L.scottii*, *Rh.mucilaginosa*, *Sp.roseus*. These species were found to be very polymorphic and to have large ecological lability. Thus they appeared being good candidates to be eurybionts. In the last decades criteria of species differentiation has been significantly changed from the using of phenotypic characteristics to the monophyletic species concept. It turned out to be that these species previously supposed to be widespread represent a number of phylogenetically distinct yeast species, e.g. *Cr.laurentii* (Takashima), *Sp.roseus* (Bai), 'red colored' *Rhodotorula* species (Sampaio). At the same time determination of the species distribution patterns needs to be supported by the large amount of isolations. For the recently described and re-identified species the amount data seems to be not sufficient for any speculations about their distribution.
The present report is based on the observations and experimental data obtained in the Soil yeast laboratory founded by Dr.Babjeva. The comparison of phenotypic identification result with the genetic approach has been done on standardized conditions. Yeasts belong to widespread phenotypical species isolated from the identical biotopes spatially separated on 3500 km. All the strains have been identified using polyphasic approach (conventional identification, MSP-PCR, sequence).
The obtained results demonstrate different level of intraspecific genetic variability among the taxa studied. Some yeast appears to form geographical populations, e.g. *Curv.cygneicollum* and *Cr.podzolicus*. Others being identical irrespectively to region of isolation (*Cr.terricola*, *Rh.pinicola*) or characterized with the high variability did not correlate with ecological factors (Fillobasidiales). Thus, good candidates to be eurybionts are again the most variable species: *Cr.albidus*, *Cr.magnus* and *Cr.victoriae*. The latter have been isolated in different regions of the world. In our study the continuous change of the genetic characteristics of the strains isolated in temperate zone have been shown.
Discrete or continuous variability are specific in dependence of the taxa studied. Moreover, the criteria of species differentiation should differ in these two cases. The continuous change of the species characteristic should be more common for eurybionts. But this could be shown only on the unified consequence investigation of large amount of samples. The other aspect of ecological research is the problem of the choosing taxonomical level. Distribution patterns of different scale assume using different scale of taxonomic differentiation. Using of species level only is not strictly obligate.

S31PS1 - 0945

Dimorphic Basidiomycetes: New perspectives for an old group.

Jose Paulo Sampio (Portugal)

1530-1730

SYMPOSIUM 32 - *Bioinformatics and Databases*

S32PS1 – 0945

Dimorphic basidiomycetes: new perspectives for an old group

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Basidiomycetous yeasts constitute an extremely diverse assemblage of fungi and, considering the entire Basidiomycota, include evolutionary old forms. In many cases the unicellular stage alternates with a filamentous phase and consequently these fungi are also referred to as dimorphic basidiomycetes. Presently, several lineages of basidiomycetous yeasts are known. In several cases unsuspected relationships with fungi traditionally excluded from the yeast domain have been revealed by sequence analysis of rDNA. The discovery of such connections is helping to solve the puzzle of the evolutionary history of basidiomycetes and has contributed to transform the way we look at basidiomycetous yeasts. In this presentation several issues pertaining to the phylogenetic study of this group of yeasts will be addressed. Current problems in the systematics of basidiomycetous yeasts involve their integration in a unified system for the Basidiomycota, the polyphyletic nature of some asexual genera like *Cryptococcus* and *Rhodotorula,* and a revision of the criteria and procedures adopted for species descriptions. With respect to ecological studies, molecular methods have allowed much faster and more accurate species identifications. These advances are contributing for a more precise definition of the habitat of each species and have allowed the exploration of new and extreme ecological niches such as deep-sea hydrothermal vents and acidic mine waters. The next major revolution will be probably fostered by the results of whole genome comparisons of basidiomycetes. The wealth of data that soon will be available could benefit the study of the evolution of sexual behaviour and asexuality, the investigation of the molecular causes of parasitic and saprobic lifestyles, the study of the evolution of forcibly discharged meio- and mitospores, the molecular causes of unicellular or filamentous development and many other fascinating questions. Are we ready for the challenge?

S32IS1 - 0789

Bioformatics for phylogenomics

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Most of modern biological science could hardly be envisaged without (bio-)informatics. Phylogenomics is an emerging field where the latter statement is particularly true. In this presentation, we'll present a new method to select the most suitable genes to provide phylogenies that are as close as possible to the "truth". We used 25 available complete genomes data (plants, animals including humans and fungi) and the assignment of their proteins to KOGs (euKaryote Orthologous Groups). We found that among the thousands of existing KOGs, only 531 could be aligned and used for phylogenetic analyzes in such a heterogeneous group of organisms. For each KOG, a multiple alignment was produced and transformed in a distance matrix. The 531 distance matrices were compared by correlation analysis and classified using the Principal Coordinate Analysis method (PCoA). The "gravity" centre (i.e. the KOG that showed the minimum average Euclidean distance with the others KOGs) was selected as being the potential best candidate to produce a good phylogenetic tree. The remaining KOGs were then selected in increasing distance order to the "gravity" centre and were included in multiple KOGs – concatenated alignments. With the limited group of organisms used in this study, 190 KOGs phylogenies provided the "best" possible tree. However, 20 to 40 KOGs seemed already sufficient to reach very high levels of correlation with the supposed "best" tree. Our method shows that few (1 to 5) genes phylogenies (based on accurately selected genes) can be quite reliable as well.

S32IS2 - 1001

StrainInfo.net bioportal: an application of semantic web technologies for scaleable workflow management of microbial information*

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With the advent and rapid emergence of the Internet, world wide access to multiple public microbial information sources has given a strong impetus to research in the field of microbiology, by instantly disseminating the latest breakthroughs and insights within the problem domain and establishing in the long term a pool of the microbiologist's collective knowledge. The online catalogues of biological resource centres (BRCs) provide basic information on the isolation, identification and availability of many important and well-characterized micro-organisms. Empirical databases contain information on many of the genotypic and phenotypic traits of these microbial strains for a broad range of experimental techniques, which seriously vary in their inter-laboratory standardization and reproducibility. As such, the International Nucleotide Sequence Database3 has emerged as one of the greatest successes in the accumulation of reproducible experimental information, providing parts or the whole genetic map of many of the life forms on earth. Completely new branches of research, such as computational genomics, have been established on the foundations of these sequence databases. Finally, probably the largest contributions to microbial research are at present only published in the scientific literature, which in itself forms a heterogeneous knowledge base that is progressively accessible in electronic form6.
The bewildering proliferation of these massive amounts of information urges the establishment of solid cross-references between different autonomous and heterogeneous data sources, in order to reduce the amount of data duplication between the information providers, assist the researchers in the navigating this data-rich environment by merging all relevant information into a uniform view, discover new insights through knowledge discovery in databases and monitor the overall data quality provided by different web services through continuous quality control of the integrated information. Primordial to the establishment of durable cross-reference scenarios is the assignment of globally unique and persistent object identifiers for unequivocal discrimination, persistent localisation and autonomous integration of the different entities in the problem domain. As an example, the assignment of accession numbers as the unique object identifiers for genetic sequence data and the assignment of digital object identifiers (DOI4) that act as proxies to scientific publications, have enabled a cross-referencing scheme that maintains mutual links between the International Nucleotide Sequence Database and the large PubMed literature repository collecting scientific publications from the life sciences. However, as a consequence of the lack of globally unique identifiers for micro-organisms kept in BRCs, these living biological resources have only started to become involved in similar information cross-reference scenarios5. Instead, the necessary information about microbial resources is partially copied into the peripheral data sources, which perturbs the management of this information that is subjected to dynamic changes.
The StrainInfo.net portal (www.straininfo.net) envisions to overcome some of the problems related to the integration of basic information on biological resources as disseminated by hundreds of BRCs worldwide with the dynamically growing amount of downstream information that is generated on these organisms. A key issue in the philosophy of the portal is the compilation of the Integrated Strain Database1,2, a central knowledge base that accumulatively learns about the equivalence relation that exists amongst the strain numbers assigned to biological resources in a global research context, by means of the calculation of the transitive closure. Currently, information is gathered from 42 microbial culture collections that cover all earth's continents and range from small niche specific research collections to large general-purpose service collections. In addition, the information extracted from two lists of bacterial type strains is equally incorporated. This integration process has currently lumped over 600.000 strain numbers into some 250.000 equivalence classes that represent different strains of bacteria, archaea, filamentous fungi and yeasts. Special attention has been paid to error detection and correction within the equivalence classes due to irregularities in the data provided by the underlying information sources, through the design of novel intelligent tools that enable the automatic discovery of intrusions in the consistency of the integrated information. Without profound quality control of

the integrated information, at least 719 (11.89%) of the bacterial type strains would have been affected by illegitimate merges into single equivalence classes2.
While incrementally calculating the strain equivalence classes, new unique identifiers are assigned to strain numbers that were not previously encountered during the integration procedure. This helps to resolve some of the ambiguities that are a logical consequence of the local nature of the strain number assignment process and enables to set down context-dependant resolution of ambiguous strain numbers that often require some form of human-intervention. The latter is important to secure the tedious disambiguation procedure of existing cross-references for correct machine interpretation in the future. Moreover, it turns out that the information content of the Integrated Strain Database offers the perfect semantic context to guide the disambiguation process in a number of ways. To demonstrate the potential of the StrainInfo.net portal to fill the gap where there is no universally adopted system for assigning and recognizing persistent and unique identifiers for biological resources, we have consolidated the strain information captured within the Integrated Strain Database with relevant sequences and literature references assembled within public repositories. Not only does this offer a de-duplicated view on the downstream information that is available on the micro-organisms worldwide, but also allows for the execution of all sorts of dynamic queries that can automatically bridge over multiple web services that were physically separated before the integration process. The presented cross-reference model will however only show its full dynamic strength when the reverse references to the Integrated Strain Database are included in third party databases, thus establishing a true divide and conquer strategy for tracking related information within autonomously operating biological information sources.

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S32IS3 - 0072

The Global Biodiversity Information Facility: data, products and services

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As a major international organisation (currently with 47 countries and 31 organisations as members), GBIF's mission is to make the world's biodiversity data freely and universally available via the Internet, for science, society and a sustainable future. As of January 2006, over 85.6 million species-occurrence records are available via the GBIF data portal. These data are being shared by 616 collections from around the world, and represent animals, plants, microbes and of course fungi. Species Fungorum is an essential component of the Electronic Catalogue of Names of Known Organisms (ECAT) that is a central feature of the GBIF information architecture. By 2008, the ECAT will contain 65% of known species concepts, and 95% by 2011. To facilitate this, GBIF will provide open-source, free, supported tools for web-enabled taxonomy, and is of course working in partnership with many initiatives to accomplish common goals. Other plans now underway include the development of infrastructures to electronically assign globally unique identifiers (GUIDs) to publications of names and concepts, which work is being carried out in partnership with the various codes of nomenclature. Already available through GBIF are data cleansing tools, a website toolkit, and simple-to-install software to enable data sharing. GBIF demonstration projects that are also available via the portal show the utility of georeferenced species-occurrence data not only in research but also in land use planning, decision-making, etc. New demonstration projects now underway will result in an application of the PYXIS global grid for use with GBIF data, which will enable in-common visualisation and analysis of disparately georeferenced datasets, and two GIS-based tools to facilitate selection of locations for new survey efforts and analysis of survey intensity and biological dissimilarity across sites. GBIF plans for the next five years include continuing to increase data content, both of the types of data already served and of new data types (e.g. images, video, identification keys etc.) and strengthening and extending the information infrastructure to link easily and quickly across levels of biological organisation. Steps are already being made in this regard by GBIF and other efforts; one of GBIF's major roles is as a coordinating entity.

S32PS1 - 0168

Approaching the taxonomic affiliation of unidentified sequences in public databases – an example from the mycorrhizal fungi

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Background: During the last few years, DNA sequence analysis has become one of the primary means of taxonomic identification of species, particularly so for species that are minute or otherwise lack distinct, readily obtainable morphological characters. Although the number of sequences available for comparison in public databases such as GenBank increases exponentially, only a minuscule fraction of all organisms have been sequenced, leaving taxon sampling a momentous problem for sequence-based taxonomic identification. When querying GenBank with a set of unidentified sequences, a considerable proportion typically lack fully identified matches, forming an ever-mounting pile of sequences that the researcher will have to monitor manually in the hope that new, clarifying sequences have been submitted by other researchers. To alleviate these concerns, a project to automatically monitor select unidentified sequences in GenBank for taxonomic progress through repeated local BLAST searches was initiated. Mycorrhizal fungi – a field where species identification often is prohibitively complex – and the much used ITS locus were chosen as test bed.

Results: A Perl script package called emerencia is presented. On a regular basis, it downloads select sequences from GenBank, separates the identified sequences from those insufficiently identified, and performs BLAST searches between these two datasets, storing all results in an SQL database. On the accompanying web-service http://emerencia.math.chalmers.se, users can monitor the taxonomic progress of insufficiently identified sequences over time, either through active searches or by signing up for e-mail notification upon disclosure of better matches. Other search categories, such as listing all insufficiently identified sequences (and their present best fully identified matches) publication-wise, are also available. This tool takes away much of the manual work associated with sequence-based identification of mycorrhizal fungi.

S32PS2 - 0374

Polyphasic identification of *Phaeoacremonium* species

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Phaeoacremonium species are known to cause dieback diseases of woody hosts. These species have been extensively isolated from grapevines where they are involved in Petri disease and esca. Petri disease and esca are serious diseases of young and old vines in most areas where grapevines are cultivated. Several species of *Phaeoacremonium* have also been isolated from phaeohyphomycosis in humans. *Phaeoacremonium* species occurring on humans are opportunistic pathogens and need either a predisposed host or traumatic subcutaneous injection to be able to infect and cause disease. During the course of this study 15 new species of *Phaeoacremonium* were identified on the basis of cultural and morphological characters as well as DNA phylogeny of the partial b-tubulin and actin genes. In total 22 species of *Phaeoacremonium* are currently known. The species that are of pathological importance include 13 species that have been isolated from grapevines and nine species that have been isolated from humans. The identification of these species is important for the grapevine industry as well as in the medical field. A polyphasic online electronic key based on micromorphological, cultural and b-tubulin sequence data of 22 *Phaeoacremonium* species was developed with BioloMICS database manager to facilitate routine identifications. This *Phaeoacremonium* identification database is available on the CBS website at http://www.cbs.knaw.nl/phaeoacremonium.htm. Any convenient number of characters can be entered and, through pairwise comparison, the *Phaeoacremonium* species most similar to the query strain can be identified. The similarity of each character of the unknown species to those of the known *Phaeoacremonium* species can also be seen in the output file.

1530-1730

SYMPOSIUM 33 - *Antifungal Resistance*

S33IS1

Transcriptional regulation of drug resistance genes in yeast pathogens

D Sanglard *Switzerland*

No abstract available

S33IS2 - 0783

Overcoming the efflux-mediated drug resistance of human fungal pathogens

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Fungi can cause life-threatening diseases in immunosuppressed individuals and often patients with fungal infections are treated with azole drugs. This treatment is sometimes compromised by the fungi developing drug resistance. The most common cause of high-level azole resistance in fungi is drug efflux mediated by ATP-binding cassette (ABC) membrane proteins. It may be possible to overcome this resistance by developing efflux pump inhibitors. The objective of this study was to over-express fungal ABC efflux pump proteins in *Saccharomyces cerevisiae* in order to investigate pump function and to screen for pump inhibitors. Genes encoding ABC proteins were cloned from *Candida albicans, Candida glabrata, Candida krusei, Cryptococcus neoformans* and *Aspergillus fumigatus* and expressed in *S. cerevisiae* AD1-8u-, which has had seven endogenous ABC genes deleted. The susceptibility of *S. cerevisiae* strains expressing efflux pumps to xenobiotics, and their ability to efflux the fluorescent pump substrate rhodamine 6G (R6G), was measured. The ATPase activities of the ABC proteins in membrane preparations was also determined.
Expression of different ABC proteins in *S. cerevisiae* conferred different levels of resistance to azole, but not to polyene, antifungal drugs. There was also pump-specific variation in ATPase activities and the abilities of strains to efflux R6G. For *C. albicans* Cdr2p, and to a lesser extent for Cdr1p, there was extensive allelic heterozygosity due to single nucleotide polymorphisms. Strains were found to usually contain two different *CDR2* alleles which, when expressed in *S. cerevisiae*, showed functional variation. Site-directed mutagenesis indicated that a G1473A substitution in Cdr2p transmembrane span 12 affected pump activity. *S. cerevisiae* AD/CDR1 (expressing *C. albicans* Cdr1p) was used to screen a combinatorial peptide library for pump inhibitors. A D-octapeptide from the library, RC21, was identified that chemosensitised AD/CDR1 to fluconazole and inhibited Cdr1p ATPase activity (IC50 = 1.25 μM). RC21 is a specific Cdr1p inhibitor and did not inhibit the closely related pump Cdr2p, the plasma membrane proton pump, the major facilitator superfamily transporter Mdr1p or ABC pumps from *Candida glabrata* or *Candida krusei*. Despite its specificity RC21 did chemosensitize azole-resistant *C. albicans* clinical isolates to fluconazole. Conclusions: We have discovered a modified D-octapeptide that is a potent specific inhibitor of the major drug efflux pump in *C. albicans*. This compound may help in the development of a pump inhibitor that renders azole-resistant clinical isolates sensitive to azole therapy. This work was supported by NIH grants DE015075 and DE016885 and the Japan Health Sciences Foundation.

S33IS3 - 0908

The antifungal effect on human and plant pathogenic fungi by regulating stress response signaling

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Fludioxonil is a phenylpyrrole fungicide derived from the antibiotic pyrrolnitrin, and is used to control a variety of important plant-pathogenic fungi. Commonly, antifungal compounds inhibit enzymatic reactions involved in fungal cellular biosynthesis. In contrast, here we demonstrate an example of antifungal effects through hyperactivation of the Hog1 MAP kinase (MAPK) pathway caused by fludioxonil treatment in both plant and human pathogenic fungi. Furthermore, we demonstrate that calcineurin and Mpk1 MAPK pathways also regulate fungal sensitivity to fludioxonil.

To investigate the roles of fungal Hog1 pathway for antifungal resistance, homologs of the *S. cerevisiae HOG1* gene were isolated from the plant fungal pathogen *Colletotrichum lagenarium* (*OSC1*), which causes cucumber anthracnose, and the human fungal pathogen *Cryptococcus neoformans* (*HOG1*), which causes fatal disease in immunocompromised hosts. To investigate the activation of the Hog1 pathway in presence of fludioxonil, the phosphorylation patterns of *C. lagenarium* Osc1 and *C. neoformans* Hog1 were monitored by western blot with phosphorylated Hog1 specific antibody.

In *C. lagenarium*, the *osc1* knockout mutants were sensitive to high osmotic stress and showed increased resistance to fludioxonil. The Osc1 MAPK was activated via phosphorylation following fludioxonil treatment, suggesting that improper activation of Osc1 by fludioxonil has negative effects on growth in this species. In the presence of fludioxonil, the wild-type strain was unable to infect the host plant because of a failure of appressorium-mediated penetration, whereas *osc1* mutants successfully infected plants in the presence of the drug.

In *C. neoformans*, fludioxonil inhibited growth of the serotype A strain H99 but not that of the serotype D strain JEC21, and the *hog1* knockout mutants of serotype A displayed increased resistance to fludioxonil. The Hog1 MAPK of serotype A was activated in presence of fludioxonil but not in resistant strain, suggesting a conserved inhibitory effect of fludioxonil by activation of the Hog1 pathway in both plant and human pathogenic fungi. Interestingly, in the sensitive strain H99, calcineurin deletion mutants and *mpk1* deletion mutants exhibited hypersensitivity to fludioxonil, indicating that the calcineurin and Mpk1 MAPK pathways contribute to drug resistance in this fungus.

These studies provide evidence that the broad-spectrum antifungal drug fludioxonil exerts its action via activation of the Hog1 MAPK pathway in both plant and human fungal pathogens. Combination of fludioxonil with the calcineurin inhibitor FK506 synergistically inhibited growth of *C. neoformans*, providing insight into novel targets for synergistic antifungal drug combinations.

S33PS1 - 0644

Involvement of catalase activitiy and sensitivity to fungicides in *Mycosphaerella fijiensis*.

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The ascomycete *Mycosphaerella fijiensis* (anamorph *Paracercospora fijiensis*) causes black leaf streak (black sigatoka disease), the most economically important disease of banana and plantain. Black sigatoka disease causes leaf necrosis and plant defoliation, resulting in yield losses and poor-quality fruit which is prone to premature ripening. The chemical control is the main strategy used for disease treatment. Approximately 35-50 applications of fungicides are sprayed each year. *M. fijiensis* rapidly acquires a highly resistance to fungicides. A Fungitoxic effect is mediated by reactive oxygen species (ROS), and accumulation of these by products can cause lethal damage to cellular proteins, membranes and nucleic acids. Specialized enzymes can neutralize toxic oxygen metabolites. Catalase and catalase-peroxidase are well known examples of antioxidant enzymes utilized by fungi. Little is know about mechanism of resistance to fungicide in *M. fijiensis*. We examine the possibility that defensive enzyme systems against ROS are related to the fungicide sensitivity.

We evaluated the cell viability against fungicides such as mancozeb and thiram (dithiocarbamate), Azoxystrobin (strobilurin), Vinclozolin (dicarboximide) and chlorotalonil by determining effective concentration 50 (EC50) by biomass increase during 7 days of incubation at 27°C and 100 rpm. The specific catalase activity during exposition to fungicide spectrophotometrically by following the disappearance of H2O2 at 240 nm by 1 minute, upon addition of cell free extracts and PAGE.

The EC50 for mancozeb and thiram was 25µg/ml and 15 µg/ml; 1 µg/ml for azoxystrobin, 50 µg/ml for vinclozolin and 125 µg/ml for chlorothalonil. A 30-240 min pulse of fungicides at EC50 induced the activities of catalase and catalase peroxidase. At 2 h after the fungicide treatment the catalase activities were enhanced 2 to 5 fold compared with respective control. The use of catalase specific inhibitor (3-aminotriazole or 3-ATZ) increases the sensitivity to fungicides up to 35 %. These results suggest the role of catalase in sensitivity and probable resistance to fungicides in *M. fijiensis*. We are currently analyzing the role of alternative oxidase and catalase in fungicide resistance, genetic polymorphism between different *M. fijiensis* isolates and the relation between fungicide resistance and catalase biosynthesis.

1530-1730

SYMPOSIUM 34 - *Importance of Small Non-Coding RNAs in Fungi*

S34IS1 - 0761

Two dicer-like proteins in *Magnaporthe oryzae*

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Dicer is an RNase III-like enzyme playing a crucial role in various RNA-mediated gene silencing pathways. The Dicer protein processes a long dsRNA precursor into shorter units that then act as guides for either cognate mRNA degradation, translation inhibition or DNA methylation. Two Dicer-like genes, *Magnaporthe* Dicer-like (MDL)-1 and -2, have been identified in the genome of the rice blast fungus *Magnaporthe oryzae*. By constructing knockout (KO) mutants, we previously showed that MDL-2 was solely responsible for hairpin RNA-triggered RNA silencing and related siRNA accumulation during the vegetative stage of this fungus. RT-PCR analysis revealed that the expression level of MDL-1 mRNA was much lower than that of MDL-2 mRNA during the vegetative stage. Over-expression of MDL-1 cDNA under the trpC promoter partially compensated for the RNA silencing deficient phenotype of the MDL-2 KO mutant. Therefore, in addition to the protein structure itself, transcriptional control likely plays an important role in the functional diversification of *Magnaporthe* Dicer proteins *in vivo*.
In plants and fission yeast, siRNAs have been shown to direct nuclear chromatin modifications such as DNA methylation or histone H3 K9 methylation. We previously showed that the LTR retrotransposon MAGGY is targeted for cytosine methylation when introduced in the genome of MAGGY-free *M. oryzae* isolates. The MAGGY element was methylated normally even when the MDL-2 KO mutant, in which no MAGGY siRNAs were detected, was used as a transformation recipient. Additionally, expression of hairpin GFP RNA in a GFP-expressing *M. oryzae* strain resulted in GFP silencing and GFP siRNA accumulation but did not induce methylation of the GFP gene in the genome, indicating that DNA methylation was not directed by siRNAs in *M. oryzae*. Further, we also examined if DNA methylation affected the level of siRNA accumulation in *M. oryzae*. However, no significant difference was observed in MAGGY siRNA accumulation between a DNA methylation deficient mutant (dim-2 mutant) and wild type. These results indicated that DNA methylation and RNA silencing are independent gene silencing mechanisms in *M. oryzae*.

S34IS2 - 0667

RNA interference machinery: use and function in the genus *Aspergillus*

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Genetic transformation with inverted repeat transgenes can be used to experimentally activate RNA silencing in *Aspergillus* species. Currently, we are investigating the possibility of using double-stranded RNA to silence genes without the need for genetic transformation. Silencing by exogenous RNA molecules could be very useful in efforts to control medically or agriculturally important fungi. In addition to this avenue of research, we are also investigating the natural roles of RNA silencing in *Aspergillus nidulans*. We have deleted all of the Dicer, Argonaute, and RNA dependent RNA polymerase homologs from this species and are continually assessing the effects of these gene deletions on various biological processes. We have also identified two truncated loci encoding the remnants of Dicer and Argonaute homologs in the commonly studied FGSC A4 lineage of *A. nidulans*. A modest sampling of wild *A. nidulans* isolates suggests that this phenomenon is not specific to the FGSC A4 lineage. Future experiments will determine how widespread these truncated alleles are in wild populations of *A. nidulans* and other closely related *Aspergillus* species.

S34IS3 -

Meiotic Silencing in Neurospora

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No abstract available.

S34PS1 - 0277

RNA Silencing Approach In The Rice Blast Fungus, *Magnaporthe oryzae*, Using An Opposing Promoter System

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The phytopathogenic fungus *Magnaporthe oryzae* (formerly *Magnaporthe grisea)* causes blast diseases on many gramineous plants including major crops such as rice, wheat and oat. The rice-rice blast system is supposed to be a model for understanding plant-fungus interactions since the genome projects of both the host and pathogen have been completed. We previously reported that hairpin RNA expressing vectors efficiently induced RNA silencing in *M. oryzae*. However, since the hairpin vectors require two steps of orientated cloning, they are not feasible on a global scale. Here we report the construction of pSilent-Dual with two opposing Pol II promoters, *Aspergillus nidulans* trpC (PtrpC) and gpdA (Pgpd) promoters. This system allows one-step cloning of a target gene for silencing, therefore providing a high throughput method for constructing a RNA silencing library. The model gene eGFP as well as one *M. oryzae* endogenous genes, polyketide synthase-like gene (PKS) were targeted for silencing by pSilent-dual based vectors. Silencing of the target genes was induced in wild type but not in the dicer mutant, indicating that pSilent-dual induces RNA silencing. Consistently, siRNAs were detected in GFP-silenced *M. oryzae* transformants but not non-silenced ones. The size of the target fragment to be inserted in pSilent-dual did not affect much the efficiency of silencing within the range examined (200bp to 700bp). However, introduction of a target gene in "sense" orientation with reference to Pgpd gave a slightly higher silencing efficiency than that in the opposite orientation. Over all, pSilent-dual should offer a high throughput system for genome-wide gene function analysis in *M. oryzae* as a feasible alternative to other systems.

1530-1730

SYMPOSIUM 35 - *Gondwanan Fungi*

S35IS1 - 0430

Biogeography and host preference of austral members of *Laccaria – Hydnangium*, a model clade of ectomycorrhizal fungi

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Laccaria forms a well-supported monophyletic clade with the false-truffle genus *Hydnangium* based both on morphological and molecular characters. Species of *Laccaria* form ectomycorrhizas with a wide diversity of plant hosts, but individual species show varying degrees of host specificity. *Hydnangium* species are restricted to *Eucalyptus*, the dominant group of forest trees in Australia. Because of the clade's importance, it has been the subject of many studies on the biology of ectomycorrhizas and relatively much is now known about various aspects of the group's diversity and biology. However, phylogenetic relationships, distribution patterns, and information on host preference of species in the group remain unknown. Preliminary phylogenetic hypotheses indicated that basal members of the clade are restricted to Australasia, but taxon sampling was insufficient to confirm this. Our recent collecting efforts have yielded new collections of the clade from Australia (Queensland, Tasmania, and Victoria), New Zealand (South Island), and Papua New Guinea (Eastern Highlands) to add to material collected by our collaborators in each of these countries. The goals of the study are to: (1) develop a rigorous phylogeny of the group based on multi-gene sequence data, (2) examine biogeographic and diversity patterns among members of the clade, and (3) investigate potential historical host specificity of the group by looking for evidence of biogeographic (e.g., Australia versus New Zealand) or host associated (e.g., Myrtaceae versus *Nothofagus*) radiations at the base of the resulting phylogeny.

S35IS2 - 0138

Where are New Zealand's ancient fungi?

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Ancient geological changes associated with the break-up of the super-continent Gondwana are often invoked to explain the distribution of Southern Hemisphere fungi. This is especially true of those fungi associated with *Nothofagus*. However, in contrast to South America and Australia, it is now thought that the history of *Nothofagus* in New Zealand comprises an ongoing series of extinctions, followed by reintroduction through long-distance dispersal. Although the genus *Nothofagus* has been present in New Zealand since before the split from Gondwana, palynological and molecular evidence show that the ancestors of New Zealand's modern-day *Nothofagus* species arrived within the last 30–40 million years, following trans-oceanic dispersal. Is the same true for the *Nothofagus*-associated fungi of New Zealand? Does New Zealand have any truely ancient fungi? What are the origins of New Zealand's putatively endemic fungal species? These questions will be addressed using examples from the Cyttariales, Helotiales, and Pezizales.

S35IS3 - 1015

Divergence time between Gondwana mushrooms and their northern hemisphere relatives

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The Southern Hemisphere endemism of many organisms including fungi has been largely interpreted as a consequence of the separation between the Gondwana and Laurasia landmasses that took place about 100 million years ago (vicariance hypothesis). However, this classic view is becoming increasingly questioned by molecular phylogenies, which often favour dispersal biases rather than the ancient vicariance hypothesis. We used molecular phylogenetic data to infer divergence times between Southern Hemisphere agarics and polypores (homobasidiomycetes) and their Northern Hemisphere counterparts. We examined diverse taxonomic groups with cosmopolitan distribution and different ecology and life strategies, including saprophytes (e.g., Ganoderma applanatum group and Pleurotus) and ectomycorrhizal (e.g., Cortinarius and Russula) taxa.
Gene phylogenies were constructed from LSU and ITS rDNA sequence data. Sequences from Southern Hemisphere taxa were either newly produced or downloaded from the GenBank database. Northern Hemisphere relatives of these taxa were identified using BLAST searches in GenBank. Phylogenetic trees were constructed using maximum-likelihood. Divergence times were estimated under non-molecular clock models of evolution. A variety of techniques were used to calibrate time at divergent nodes.
Direct calibration of divergent nodes (e.g., at the animal/fungi and ascomycetes/basidiomycetes splits as well as at various nodes within the basidiomycetes) strongly suggests that divergence times in most of the study groups are significantly < 100 mya. Reverse calibration (by dating nodes that segregate Southern and Northern Hemisphere taxa at 100 mya) often results in dates for the animal/fungi or ascomycetes /basidiomycetes splits that are older than the currently assumed dates of the prokaryotes/eukaryotes split.
Although several difficulties are associated with both time calibration at divergent nodes and the calculation of divergence times, our results strongly indicate that dispersal plays a much more important role than generally admitted to explain the Southern Hemisphere distribution of many fungi. This is particularly evident for non-specialist saprobes, such for instance in Ganoderma or Stereum, in which identical ITS alleles were found in both the Southern and Northern Hemispheres. This could be attributed to recent dispersal due to human activities, however, the exclusion of these alleles from the analyses still results in inferred divergence times much younger than 100 mya. In contrast, within ectomycorrhizal taxa (that have much narrower ecological niches than generalist saprobes), taxonomic distinction between Southern and Northern Hemispheres taxa is generally much easier, and in some cases inferred divergence time > 100 mya cannot be excluded, in agreement with the vicariance hypothesis following the Gondwana/Laurasia landmasses separation. In summary, our results indicate that the natural history of Southern Hemisphere fungi varies among fungal groups with different life style and can only be comprehended when both dispersal and vicariance theories are considered.

S35PS1 - 0824

The rust mycobiota of South Africa: composition, relationships and distribution

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The South African rust mycobiota comprises c. 450 species. Because of the geographic and floristic diversity of the country also the rust fungi are very diverse.
The present contribution attempts to point out mycogeographic relationships of the South African rust mycobiota, to identify members of mycogeographic entities and to discuss arising patterns of species richness of rust fungi in South Africa.
Mycogeographic relationships and elements. - South African rusts can be assigned to different mycogeographic entities: (1) a broad tropical element, (2) a paleotropical one, (3) an African one, (4) an indigenous or endemic element and (5) an element with affinity to the temperate zone of the northern hemisphere. The last one is well represented by several members of Pucciniastraceae: e.g., *Pucciniastrum agrimoniae*, *Milesina*- and *Uredinopsis* species and related *Milesia* anamorphs. It is assumed that these rusts or their progenitors migrated to southern Africa via "step stones" with appropriate vegetation and host plants.
Paleotropical affinities are shown by the occurrence of species like *Crossopsora zizyphi*, *Hamaspora longissima*, *Hemileia* spp., *Masseeëlla flueggeae* and *Ravenelia atrides*. There is also a relationship between the rusts of southern Africa and the Indian subcontinent exemplified by the disjunct occurrence of, for example, members of *Hemileia*, *Puccinia*, *Ravenelia* and *Uredopeltis*.
Identifying autochthonous species of rust in South Africa is difficult because of the paucity of data. Members of *Puccinia* occurring on Cucurbits, *Helichrysum* (Asteraceae) and *Lycium* (Solanaceae) are presented to highlight the problems.
Species richness and distribution. - Little is known about the distribution of rust fungi in South Africa and their species richness in different parts of the country. It is becoming evident, however, that species richness of the rust fungi needs not coincide with species richness of vascular plants in a given area. The Cape Floristic Region (CFR) is a prominent example. Though the region is famous for its very rich flora, it is relatively poor in rust fungi, and some of its most typical plant families and clades are not known to bear any rust in the CFR (e.g., Restionaceae, Ericaceae [*Erica*] and Proteaceae).

S35PS2 - 0241

Pacific boletes: implications for biogeographic relationships

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The obligate association of boletes with their plant partners is critical to understanding biogeographic distribution of these fungi. It is only a very rare instance that boletes are NOT obligatory associates with plants. In the southern hemisphere, a few species of Boletaceae (less than 10) have distributions that overlap with *Nothofagus* forests in Australia, Papua New Guinea, New Caledonia, and New Zealand. However, these are not the same species that are associated with *Nothofagus* along the Pacific coast of South America (five species). Most Australian species of Boletaceae appear to be associated with *Acacia*, Myrtaceae and/or Casuarinaceae. However, the Australian Boletaceae mycota remains poorly known. Similarly, accounts of the southeast Asian bolete fares somewhat better with only preliminary documentation and scattered reports. These latter taxa appear to be associated with Fagaceae, Pinaceae, and Dipterocarpaceae. Knowledge of the African bolete mycota is similarly quite fragmentary, and is based mostly on material collected in former European colonies. The presumed angiosperm associates are predominately members of the Caesalpinoid legumes. Boreal boletes are better known with a long documented period of study. Primary plant associates include members of the Pinaceae, Fagaceae, Ericaceae, Salicaceae, and Betulaceae. The broad array of obligate plant associate distributions provides a potential handle for evaluating bolete distribution on a global scale.

Recent explorations for austral boletes have uncovered many novel entities; not an unexpected result in any under surveyed geographic region. Interestingly, fresh material gathered in Australia/SE Asia within the last decade suggests the occurrence of boreal (often New World) morphotypes in regions bordering the SW Pacific. Such representatives include species of *Gyroporus*, *Tylopilus*, *Pulveroboletus*, *Boletus*, and *Boletellus*.

Possible hypotheses to explain such disjunction range from: (1) original Pangaean distribution with no change since the Cretaceous; (2) change or shift to different symbiotic partners; (3) migrations over post-Cretaceous land bridges during glacial epochs; (4) long distance dispersal of spores.

Based on morphological and molecular evidence, hypotheses one and two appear more likely to support apparent disjunctions and the global distribution of most bolete taxa. Hypotheses three and four lack credibility for global bolete distribution based on paleoclimates and population genetic studies.

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Abdel-Wahab	M A	**0199**	S49IS2	Documentation of marine fungal diversity: classical vs. molecular techniques	Pang L K	343
Andrianopoulos	A	**0997**	S4IS3	Control of dimorphic switching in *Penicillium marneffei*		13
		0998	S46IS3	Comparative genomic analysis of hypoxic stress response in *Aspergillus fumigatus* and *Aspergillus nidulans*	Borneman A, Han K-H	336
Aramayo	R		S34IS3	Meiotic Silencing in *Neurospora*		243
Archer	D	**0675**	S24IS1	Genome-wide analysis of secretion stress in *Aspergillus niger*	van Peij N, Lanthaler K, Robson G, Stam H, Goosen T, van den Hondel C, Guillemette T	156
Arzanlou	M	**0390**	P3PS7	A phylogenetic approach to accommodate *Ramichloridium orphans*	Groenewald J Z, Crous P W	331
Atkinson	T J	**0744**	S47PS2	Unusual new species, exciting relationships – expecting the unexpected among woody decay pyrenomycetes from New Zealand	Orlovich D A	339
Avery	S V	**0739**	S42IS2	Functions determining yeast fitness during stress, derived from genome wide haploinsufficiency tests	Lodwig E, Holland S L, Sideri T, Clarke I, Gkargkas K, Hoyle D C, Delneri D, Oliver S G	253
Avrova	A	**0523**	S7PS1	Transient gene silencing in the oomycete, *Phytophthora infestans*, for determination of gene function	Grenville-Briggs L, van West P, Boevink P, Birch PRJ, Whisson SC	93
Baerlocher	F J	**0381**	S14IS2	Reproduction and dispersal in aquatic hyphomycetes		114
Bagagli	E	**0879**	S53IS1	*Paracoccidioides brasiliensis*: ecological and evolutionary aspects	Bosco S MG, Theodoro R C, Macoris S A, Richini V B	410
Baker	S	**0988**	S121S1	Non-isotope-based quantitative proteomics in the absence of genomic sequence information		110
Banke	S	**0413**	P3PS5	High level of gene flow and origin from native soil characterize Scandinavian populations of the soil borne fungus *Penicillium scabrosum*	Rosendahl S	330
		0437	S56PS2	Migration in space and time for 14 worldwide populations of *Mycosphaerella graminicola*		313
Basiri Jahromi	S	**0068**	P2PS2	Aspergillosis in High Risk Patients	Khaksar A S	258
		0069	P2PS5	Outbreak of Tinea Corporis Gladiatorum in Tehran	Khaksar A S	259
Beakes	G W	**0284**	S36IS2	The diversity of oomycete pathogens of nematodes and its implications to our understanding of oomycete phylogeny.	Glockling S L	261
		0950	S57IS1	A brief history of nearly everything - about chytrids		167
Bebber	D L	**0727**	S37IS3	Network structure and dynamics of fungal mycelia		263
Beltran-Garcia	M J	**0644**	S33PS1	Involvement of catalase activitiy and sensitivity to fungicides in *Mycosphaerella fijiensis*	Vargas-Prieto F, Ravelo-Soto Z, Garcia-Torres E, Ogura T, Manzo-Sanchez G, Esqueda M	242
Berger	L	**0919**	S57IS2	A new killer on the block - *Batrachochytrium dendrobatidis*: a chytrid parasite of amphibians	Speare R, Skerratt L	168

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Bergerow	D	**0613**	S56IS3	Hitchhiking through the botanic realm: Ustilaginales in time and space	Stoll M, Bauer R	313
Berndt	R	**0824**	S35PS1	The rust mycobiota of South Africa: composition, relationships and distribution		245
Bills	G F	**0408**	S51PS1	High throughput fungal culturing from plant litter by dilution-to-extinction	Collado J, Platas G, Paulus B	407
Binder	M B	**0296**	S1IS4	Molecular systematics and evolution of Boletales.	Hibbett D S	7
Blackwell	M	**1005**	S3IS1	Microbial communities in the gut of wood-ingesting beetles		10
Boddy	L	**0619**	S14IS3	Mycelia foraging strategies of saprotrophic cord-forming basidiomycetes		115
		0618	S19IS2	Interspecific mycelial interactions: major drivers of colonization and succession of wood-inhabiting fungi		145
Bonfante	P	**0517**	S48PS1	Pre-penetration apparatus: an arbuscular mycorrhiza-specific cell response in root epidermis	Genre A, Chabaud M, Timmers T, Barker D	341
	P	**0654**	S25IS3	Endobacteria and arbuscular mycorrhizal fungi: symbiosis in evolution?	Lumini E, Anca I, Ghignone So, Bianciotto V	159
Bougher	N L	**0469**	S52IS2	Mycorrhizal fungi and eucalypts - fungal significance in conservation and land management		408
Bouvet	G B	**0792**	S42PS2	Exploring the mobility of DNA transposons in the Dutch elm disease fungi.	Jacobil V, Bernier L	354
Bovers	M	**0407**	S6PS1	Multi-locus sequence typing of the *Cryptococcus neoformans – Cryptococcus gattii* species complex	Hagen F, Kuramae E, Boekhout T	91
Boyce	K J	**0812**	S17PS1	Conidial germination in the dimorphic pathogen *Penicillium marneffei*	Andrianopoulos A	141
Brasier	C M	**0657**	S42PS1	FITNESS effects of interspecific gene transfer in *Ophiostoma*	Paoletti M, Buck K W, Et-Touil A, Bernier L, Kirk SA	254
Braus	G	**0752**	S4IS2	Signalosome and development in the filamentous fungus *Aspergillus nidulans*		13
Bruns	T D	**0441**	S25IS1	Quantifying the species composition, density, spatial extent, and longevity of *Rhizopogon* spore banks in pine forests.	Boynton P J, Hynson N A, Kennedy P G	158
Burdon	J J	**0185**	S25IS2	Coevolution of plants and pathogens in a metapopulation context	Thrall P H	158
Burgess	T I	**0320**	S45PS1	Movement of the devastating Eucalytpus leaf and shoot pathogen *Phaeophleospora destructans*, throughout Asia	Andjic V, Hardy GEStJ, Dell B, Xu D, Wingfield MJ	320
Burgess	T I	**0322**	S52PS1	A reassessment of *Phaeophleospora* species on eucalypts.	Andjic V, Barber PA, Hardy GEStJ, Wingfield MJ	409
Butchko	R A E	**0401**	S39IS3	Fumonisin mycotoxin biosynthesis, genetics and genomics in *Fusarium verticillioides*	Brown D W, Proctor R H	266
Cannon	R D	**0787**	S23IS1	Oral adhesion of *Candida albicans* – a cellular and molecular view	Rodrigues EM, Van der Wielen PA, Zhang N, Schmid J, Dawes PJD, Holmes AR	154

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Cannon	R D	**0783**	S33IS2	Overcoming the efflux-mediated drug resistance of human fungal pathogens	Lamping E, Holmes AR, Niimi K, Niimi M, Monk BC	241
Carroll	G C	**0605**	S41IS2	Host specificity among endophytes in transient plant communities		310
Chadha	B	**0026**	S44IS1	Diversity of Xylanase and Plant Cell Wall Esterases in thermophilic and thermotolerant Fungi		317
Chapraisert	A	**1004**	S15IS3	An update on human pythoisis		117
Chen	S C A	**0760**	S53IS4	National, population-based surveillance of candidemia in Australia with emphasis on disease acquired outside of hospitals	Slavin M, Nguyen Q, Marriott D, Playford EG, Ellis D, Sorrell TC	411
Chen	S-F	**0914**	S57IS4	Diversity and phylogeny of chytrids in Taiwan	Lin H-D, Chiang T-Y, Chien C-Y	168
Chouksey	R	**0115**	S20PS1	Effect Of Physico-Chemical Parameter On Fungus In Water bodies Of Jabalpur (M.P)-India	Shukla R, Raipuria N	148
Christensen	M	**0160**	S20IS3	Diversity of ecto-mycorrhiza fungi in Nepal - relation to forest types and management		148
Co	DLV	**0453**	P1PS2	The molecular phylogeny of the genus *Entoloma*	Noordeloos ME	255
Coetzee	MPA	**0512**	P1PS5	Phylogeny of *Armillaria* species based on combined DNA sequence and phenotypic data	Wingfield BD, Maphosa L, Mwenje E, Wingfield MJ	256
Coloe	S	**1019**	S15IS5	Dermomatophyte demographics downunder: Melbouren Australia		update
Cooper	C R	**0584**	S12PS2	Protein profiling of the dimorphism in the fungal pathogen, *Penicillium marneffei*	Chandler J M, Treece E R, Kim T D, Walker G R	111
Crittenden	P D	**0722**	S26PS2	Lichen nitrogen and phosphorus relationships in the vicinity of a penguin rookery	Theobald M R, Tang Y S, Scrimgeour C M	167
Crous	P W	**0676**	S52IS1	How host specific are *Mycosphaerella* spp. infecting eucalypts?	Groenewald JZ	408
Daniel	H M	**0382**	P4PS1	Yeasts associated with flowers in Cuba	Jiménez AF, Evrard P, Decock C	332
Dawyndt	P	**1001**	S32IS2	StrainInfo.net bioportal: an application of semantic web technologies for scaleable workflow management of microbial information		238
de Beer	Z W	**0582**	S3PS1	A new phylogenetic lineage of *Ophiostoma* spp., discovered on termites and termite combs in South Africa	de Fine Licht HH, Aanen DK, Wingfield MJ	11
Dearnaley	J D W	**0023**	S48PS2	Molecular identification of fungal endophytes in australian myco-heterotrophic orchids		342
dela Cruz	T E	**0027**	S49PS1	Metabolic profiles support species concept of two marine *Dendryphiella* species: *D. arenaria* and *D. salina*	Druzhinina I S, Kubicek C P, Schulz B E	343
Dianese	J C	**0757**	S8IS4	Microfungi of the Brazilian Cerrado: example of Neotropical mycodiversity	Carvalho R C P	75
Druzhinina	I S	**0357**	S21IS2	An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*	Kopchinskiy A G, Komon M, Kubicek C P	150
Dujon	B	**0986**	S16IS1	Comparative genomics of yeasts illustrates eukaryotic genome evolution		138

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Dunk	C W	**0480**	S13PS1	The ectomycorrhizae of *Nothofagus cunninghamii* and host shifting by the exotic fungus *Amanita muscaria*	Lebel T, Keane PJ	113
Duong	L M	**0726**	S30IS2	Advances in our understanding of fungal diversity - an Asian perspective	Hyde K D	173
Ellis	D	**0708**	S52IS3	Eucalypts as the natural host for the human pathogenic fungus *Cryptococcus gattii*		409
Ezawa	T	**0706**	S48IS3	Acquisition and long distance translocation of phosphorus in the symbiotic phase of arbuscular mycorrhizal fungi	Kuga Y, Ohtomo R	341
Fischer	R	**0753**	S9IS3	The MTOC-associated protein ApsB interacts with the peroxisomal Woronin body protein HexA in *Aspergillus nidulans*	Veith D	96
Francis	A A	**0173**	P1PS4	Partial harmony: agreement between morphological and molecular data for the sequestrate cortinarioid fungi	Bougher N.L., O'Brien P A	256
Fraser	J A	**0907**	S46ISI	The *Cryptococcus neoformans* mating-type locus: evolutionary insights from related species	Findley K M, Hall C, Dietrich F S, Heitman J	335
Frisvad	J C	**0849**	S22IS2	Chemical diversity in *Penicillium* and *Aspergillus*: do all species produce terpene, non ribosomal peptide and polyketide secondary metabolites?	Larsen T O	152
Fukasawa	Y F	**0159**	S19PS2	Small-scale variation in chemical property within logs of Japanese beech in relation to spatial distribution and decay ability of fungi	Osono T, Takeda H	146
Gadd	G		S37PS1	Advanced microscopic imaging coupled with X-ray absorption spectroscopy to characterise fungall metal and mineral transformations		263
Galagan	J	**1017**	PLEN 3	Comparative Fungal Genomics		Update
Garbelotto	M	**0638**	S45IS3	Microsatellite analysis documents worldwide and regional spread routes of the sudden oak death pathogen	Ivors K, Prospero S, Vettraino A, Rosensweig N	319
Garnica	S	**0192**	P3PS6	Phylogenetic classification and geographical patterns of species distribution in the ectomycorrhizal genus *Cortinarius*	Weiss M, Oberwinkler F	331
Garrill	A	**0137**	S23PS1	Invasive hyphal growth: an F-actin depleted zone is associated with invasive oomycete hyphae	Chitcholtan K, Walker S, Yu Y, Christenhusz G	156
Glen	M	**0484**	S40PS1	The utility and limitations of positive and negative controls for PCR detection of quarantine pathogens	Alfenas AC, Zauza EAV, Langrell SRH	268
Göker	M	**0622**	P3PS3	Evolution of downy mildews		330
Gold	S E		S17IS2	An earful of corn smut: Dimorphism and disease in the *Ustilago maydis*-maize interaction		
Gomez	B L	**1009**	S38IS2	The darker side of *Candida albicans* and *Paracoccidioides brasiliensis*		264
Gonthier	P	**0270**	S45IS4	Invasion of an exotic root pathogen of forest trees: the case of *Heterobasidion annosum*	Linzer R, Nicolotti G, Garbelotto M	320
Griffith	G W	**0774**	S41IS1	Competition of heterozygous deletant *Saccharomyces cerevisiae* strains in a grape juice environment	Cross E J M, Davey H M, Delneri D, Hoyle D C, Kell D B, Oliver S G	253
Gueidan	C	**0751**	S11IS3	Phylogenetic relationships and evolution of lifestyles within the Eurotiomycetes (Fungi, Ascomycota)	Ruibal C, de Hoog GS, Lutzoni F	109

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Gurr	S	**0993**	S23IS2	Sticking, Sensing, Starting Signal Relay and Stress in the Cereal pathogens *Blumeria graminis* and *Magnaporthe grisea*	Skamnioti P	155
Gusmao	L	**0898**	S8IS1	Diversity of microfungi of the brazilian semi-arid northeastern region		94
Halling	R E	**0241**	S35PS2	Pacific boletes: implications for biogeographic relationships	Neves M A, Osmundson T W	245
Halmschlager	E	**0411**	S41PS1	Endophytic fungi in non-mycorrhizal oak roots	Kowalski T	311
Hambleton	S	**0649**	S47PS1	*Geomyces pannorum*, a cosmopolitan soil fungus: phylogenetic relationships and species concepts	Sigler L	339
Hammel	K E	**0768**	S29IS2	A role for hydroquinone-driven Fenton chemistry in incipient wood decay by the brown rot basidiomycete *Gloeophyllum trabeum*	Suzuki M R, Hunt C G, Houtman C J, Dalebroux Z D	171
Hansen	K H	**0440**	S11IS1	Phylogenetics in Pezizales emphasizing Pyronemataceae	Perry B A, Dranginis A W, Pfister D H	108
Hawksworth	D L	**0899**	HONLEC	Mycology and mycologists		100
Hernández	J R	**0983**	S8IS2	Rust fungi from Northwest Argentina		94
Higuchi	Y	**0486**	S37IS2	Visualization of the endocytic pathway and endosomal structures in the filamentous fungus *Aspergillus oryzae*	Nakahama T, Shoji JY, Arioka M, Kitamoto K	263
Himmelreich	U	**0624**	PS4PS2	NMR spectroscopy: a tool for rapid yeast characterization and screening		333
Hocking	A	**0872**	S5IS1	Biogeography and ecology of *Aspergillus* in Australia		15
Hoffmeister	D	**0240**	S39IS2	Terrequinone biosynthesis in *Aspergillus nidulans*		265
Holst-Jensen	A	**0427**	S54PS1	Assays for rapid multiplex detection of toxigenic *Fusarium* spp. in cereals and derived products applying DNA array hybridisation and capillary SNP analysis, respectively	Kristensen R, Berdal K G, Gauthier G, Hamels S, Remacle J	414
Horiuchi	H	**0513**	S9PS2	Polarized localization of chitin synthases in *Aspergillus nidulans*	chinomiya M, Takeshita N, Fukuda K, Ohta A	97
Hosaka	K	**0921**	S56IS2	Biogeography of the Hysterangiales		312
Howlett	B J	**0725**	S39IS1	The sirodesmin biosynthetic gene cluster of the plant pathogen, *Leptosphaeria maculans*	Elliott CE, Fox EM, Gardiner DM, Cozijnsen AJ	265
Huhndorf	S M	**0767**	S47IS2	Phylogenetic relationships within the Helminthosphaeriaceae and Chaetosphaeriales	Miller A N, Fournier J	338
Inaba	S	**0504**	P3PS4	The phylogenetic studies on the genus *Cornumyces* (Oomycetes) based on the nucleotide sequences of the nuclear large subunit ribosomal RNA and the mitochondrially- encoded cox2 genes	Harayama S	330
Iturriaga	T	**0992**	S8IS3	Diversity of Discomycetes in Venezuela	Mardones M	95
		0633	S19IS3	Wood inhabiting fungi on decomposing logs in three Venezuelan forests		145

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Jedd	G	**0729**	S9IS1	Woronin bodies: Crystalline peroxisomes close the door at the septal pore	Kitamoto K	95
Jeewon	R	**0647**	S11PS2	Multigene phylogenies in the systematics of Sordariomycetes and Loculoascomycetes	Cai L, Tang A M C, Shenoy B D, Kodsueb R, Thongkantha S, Hyde K D	110
		0813	S41IS3	Endophytes: lifestyle and phylogenetic diversity	Hyde KD, Promputtha I, Yeung SY	310
Johnson	L J	**0303**	S22PS1	An endophyte nonribosomal peptide synthetase in siderophore biosynthesis is essential for mutualistic interactions with grasses	Bryan G, Christensen M, Johnson RD, Koulman A, Rasmussen S	153
Johnston	P R	**0138**	S35IS2	Where are New Zealand's ancient fungi?	Peterson K	244
Jones	E B G	**0268**	S30IS1	Progress in the documentation of Asian fungal diversity	Alias S A	173
		0121	S49IS1	Biodiversity of marine filamentous fungi and their phylogenetic relationships	Sakayaroj J	342
Kahmann	R	**0762**	PLEN 4	Mating in fungi		259
		0682	S2IS1	The establishment of biotrophy in the *Ustilago maydis*/maize pathosystem.	Brefort T, Schipper K, Mueller O, Macek B, Mann M	8
		0763	S7IS4	Microarrays meet pathogenicity: gene regulation during the early infection phase of *Ustilago maydis*	Vranes M, Scherer M, Kaemper J	93
Kao	R Y	**0668**	S46PS1	Identification of novel small molecule compounds that differentially inhibit the yeast form of *Penicillium marneffei*	Lee S W, Madar J C S, Yuen K Y	336
Keller	N	**0667**	S34IS2	RNA interference machinery: use and function in the genus *Aspergillus*		243
Kjoller	R	**0591**	S21PS1	UNITE – reliable identification of ectomycorrhizal fungi - DNA barcoding in action	Kõljalg U, Abarenkov K, Alexander IJ, Anderson I, Eberhardt U, Erland S, Larsson E, Larsson KH, Nilsson HR, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T	151
Klepzig	K D	**0018**	S3PS2	Interactions of fungi and tree killing bark beetles: geographic variation and interspecific competition.	Hofstetter RW, Ayres MP, Six DL	12
Kohl	J	**0842**	S43IS2	Screening of biocontrol agents against fungal leaf diseases		314
Koide	R T	**0221**	S13IS1	Structuring of Mycorrhizal Fungal Communities	Lekberg Y K, Rohr J R	112
Kojima	K K	**0908**	S33IS3	The antifungal effect on human and plant pathogenic fungi by regulating stress response signaling	Bahn YB, Takano YT, Yoshimi AY, Tanaka CT, Cox GC, Okuno TO, Heitman JH	241
Kronstad	J		S17IS1	A SAGE approach to investigate cAMP signaling in basidiomcyete pathogens		140
Kuramae	E E	**0589**	S16IS2	Fungal Phylogenomics: from Kingdom to Species	Robert V, Snel B, Weiss M, Theelen B, Boekhout T	139
Kurne	A	**0671**	S36PS2	Study Of Mechanism Of Zoospore Release In Stramenopiles Through Videoclips	Gandhe R V, Gandhe K R	262
Kurtzman	C P	**0179**	S6IS1	Phylogeny and systematics of the yeast genus *Pichia* from multigene sequence analysis		89

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Kwok	I S W	**0352**	S24PS1	Isolation and characterization of genes related to growth of *Lentinula edodes* on lignocellulose	Chum Winnie W Y, Kwan H S	157
Lacey	E		S5IS3	Is the novelty of morphological species in Trichocomaceae reflected in metabolic diversity?		15
Lachance	M A	**0066**	S6IS2	Sex, endemism, and gene flow in natural yeast populations	Lawrie D, Dobson J	90
Landolt	J C	**0155**	S50IS1	Global diversity of cellular slime molds	Stephenson S L, Cavender J C	344
		0144	S50PS2	Dictyostelid cellular slime molds from caves		345
Lane	M A	**0072**	S32IS3	The Global Biodiversity Information Facility: data, products and services		239
Lange	L	**1007**	S44IS2	Enzyme discovery for industrial biotechnology, focusing specifically on novel enzymes for biofuel and biomass conversion		318
Lawrie	A C	**0564**	S13PS3	Distribution And Function Of The Mycorrhizal Fungus Associated With *Caladenia fulva* G.W. Carr	Ong M F, Raleigh R E	114
Lebrun	M-H	**0999**	S2IS2	A secondary metabolite is involved in recognition of the blast fungus *Magnaporthe grisea* by resistant rice cultivars		8
Leslie	J F	**0637**	S54IS1	Genetic Diversity in *Fusarium* from *Sorghum* and *Millet*	Leslie J F	412
Levesque	C A	**0716**	S54IS2	Development of an oligonucleotide array for detection of *Fusarium* species by hybridization of PCR products	Barasubiye T, Seifert K A	413
Li	G	**0154**	S43PS3	Nematicidal Metabolites From Fungi	Zhang Keqin	316
Libkind	D	**0780**	S31IS2	Mycosporine synthesis in dimorphic basidiomycetes - Ecological and phylogenetic implications	van Broock M, Sampaio J P	236
Lichtwardt	R W	**0178**	S1IS2	Trichomycetes: major taxonomic revisions based on molelcular phylogenies	White M M	6
Lindner Czederpiltz	D L	**0641**	S19PS3	Community analysis of wood-inhabiting fungi using fruiting bodies, culturing, and rDNA	Allmer J, Banik M, Glaeser J, Stenlid J, Trummer L, Vasiliauskas R	147
Luangsa-ard	J J	**0265**	S30PS2	On the diversity of *Isaria* species from Thailand		174
		0501	S20PS2	Tracking down beauvericin-production in the genus *Isaria* using molecular phylogenetics	Hywel-Jones NL, Isaka M	149
Lumbsch	H T	**0038**	P3PS1	Lichen-forming pyrenomycetes are highly polyphyletic and not related to Sordariomycetes	Schmitt I, del Prado R, Kautz S, Grube M	329
Machida	M M	**0985**	S16IS3	Genome structure, gene redundancy and gene expression of *A. oryzae*		139
Magan	N	**0822**	S43IS1	Production and formulation of antagonists for improved competitiveness and biocontrol		314
Malloch	D	**0707**	S51IS1	Fungi associated with marine wrack		405
Manoch	L	**0418**	S5PS2	*Aspergillus* and *Penicillium* Teleomorphs from Thailand and Application of *Talaromyces flavus* against Plant Pathogenic Fungi *in vitro*	Jeamjitt O, Dethoup T, Kokaew J	16
Mantle	P G	**0058**	S49IS3	Recognition of a caribbean marine fungus as a new genus by classical and molecular characters		343

Oral Abstract Authors (list is according to presenting author)

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Naqvi	N I	**0324**	S9PS1	Septal seal: certified and secure host invasion	Soundararajan S, Jedd G, Ramos-Pamplona M, Chua NH	96
		0014	S17PS2	Hard-surface dependent or thigmotropic cue regulates the G-protein cascade during blast-disease initiation	Liu Hao	141
		0340	S38PS2	Peroxisomal acetyl-CoA is essential for appressorial melanization, and virulence in *Magnaporthe*	Ramos-Pamplona M	265
Nara	K	**0256**	S13IS2	Ectomycorrhizal symbioses and vegetation development in the primary successional volcanic desert on Mount Fuji		112
Nehl	D B	**0670**	S29PS2	Rapid, reversible motor response of fungi to blue, green and ultraviolet light		172
Nevalainen	H	**0834**	S24IS3	Expression of a shark antibody using *Trichoderma reesei* as a heterologous host	Mohammed S, Te'o V, Nuttall S	157
Ngamskulrungroj	P	**0784**	S46PS2	Microarray analysis reveals genes responsible for the high virulence of the *Cryptococcus gattii* VGIIa Vancouver Island outbreak strain	Perfect JR, Meyer W	337
Nguyen	B Q	**0277**	S34PS1	RNA Silencing Approach In The Rice Blast Fungus, *Magnaporthe oryzae*, Using An Opposing Promoter System	Kadotani N, Tosa Y, Mayama S, Nakayashiki H	244
Nilsson	R H	**0168**	S32PS1	Approaching the taxonomic affiliation of unidentified sequences in public databases – an example from the mycorrhizal fungi		240
Nosanchuk	J	**1006**	S38IS1	Clinical impact of fungal melanization		264
Nygren	C	**0125**	S24PS2	Detection of extracellular proteases produced by ectomycorrhizal fungi	Edqvist J, Taylor AFS	157
Oberwinkler	F	**0995**	PLEN 1	The Fungal Tree of Life		6
O'Brien	C R	**0915**	S18IS3	Comparative aspects of aspergillosis in dogs, cats and people	Barrs VR, Beatty JA, Lindgard A, Malik R	143
O'Brien	H E	**0743**	S26IS2	Are symbionts less diverse than their hosts? Insights from molecular systematics	Lutzoni F M	166
Oide	S O	**0672**	S22IS1	Biological roles of fungal non-ribosomal peptide synthetases: elemental and diverse	Turgeon G	152
Ormsby	M D	**0917**	S40IS1	The Importance of Mycology in Biosecurity: The NZ Experience		266
Osmani	S A	**0677**	S4IS1	New insights into the mitotic regulation of the nuclear pore complex during the partially open mitosis of *Aspergillus nidulans*	Davies J, Espeso EA, Martínez JF, Liu H-L, Osmani AH	12
Osono	T	**0127**	S20IS2	Fungal decomposition of lignin in leaf litter: comparison between tropical and temperate forest soils		148
Padgett	D E	**0678**	S1IS1	The Saprolegniaceae -- new species concepts	Bailey J Craig	6
Palm	M E	**0796**	S40IS2	Biosecurity: latest developments in systems and tools for fungal identification and disease diagnostics		267
Pavlic	D	**0558**	S10PS2	Speciation And Gene Flow In The *Botryosphaeria parva-B. ribis* Complex On Native And Introduced Hosts In South Africa	Slippers B, Coutinho T, Wingfield M J	99

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Penttila	M		S24IS2	Genome-wide analysis and physiology of protein production		156
Perumal	K	**0803**	S38PS1	Production and utilization of fungal pigment in textile dyeing	Sumathi E, Chanrasekarentheran S	264
Peterson	S W	**0291**	S5PS1	Using GCPSR to resolve synonymies in *Penicillium toxicarium*		16
		0765	S21PS2	Barcoding identification of *Penicillium* species occurring in cork bark of Quercus suber trees using calmodulin, B-tubulin and ITS and LSU rDNA sequences	Serra R, Venâncio A	151
Pitt	J	**0883**	S5IS2	The astonishing biodiversity of *Penicillium* in Australia		15
Read	N		S37PS2	Optical tweezer micromaniplulation of filamentous fungi		264
Rep	M	**0851**	S12IS2	The mixed xylem sap proteome of Fusarium oxysporum-infected tomato plants	Houterman P M, Speijer D, Dekker H L, van der Does H C, Meijer M, de Koster C G, Cornelissen B J C	110
Requena	N	**0754**	S48IS1	Molecular signaling at early stages of the arbuscular mycorrhizal symbiosis	Serrano E, Aurora O, Magdalene B, Hannah K	340
Riquelme	M	**0740**	S4PS2	Localization and traffic of secretory vesicles in living hyphae of *Neurospora crassa* by laser scanning confocal microscopy.	Sanchez-Leon E, Bartnicki-Garcia S, Freitag M	14
Robbertse	B	**1003**	S121S3	Evolution of the Ascomycotan Proteome		111
		0215	S16PS1	A Phylogenomic Analysis Of The Ascomycota	Reeves J B, Schoch C L, Spatafora J W	140
Robert	V	**0789**	S32IS1	Bioinformatics for phylogenomics	Kuramae E, Boekhout T	238
		0788	S55IS2	MycoBank: linking names to genomes		415
Robinson	R M	**0210**	S52PS2	Fire and Fungi: survival, succession and composition of macro fungal community following fire in eucalypt forest in Western Australia.	Mellican A, Smith R H	409
Rossman	A	**0775**	S47IS1	Phylogeny and biodiversity of the Hypocreales and Diaporthales	Castlebury L A, Samuels G J	337
		0773	S55IS1	The value of herbaria in the DNA age	Farr D F	415
Balesdent	M-H	**0705**	S2IS3	Are A+T-rich isochores niches for pathogenicity genes in the genome of *Leptosphaeria maculans*?	Balesdent M H, Profotova B, Fudal I, Eckert M, Ross S, Gout L, Rouxel T	9
Sampaio	J P	**0945**	S31PS1	Dimorphic basidiomycetes: new perspectives for an old group		237
Sancho	L G	**0713**	S26IS1	Lichens survive in space	de la Torre R, Horneck G, Ascaso C, de los Rios A, Wierzchos J, Pintado A	166
Sanglard	D		S33IS1	Transcriptional regulation of drug resistance genes in yeast pathogens		240
Sano	A	**0266**	P2PS3	Isolation of *Ochroconis gallopava* from Hot Springs in Japan and its pathogenicity	Yarita K, Murata Y, Takayama A, Yaguchi T, Takahashi Y, Kamei K, Nishimura K	258
		0666	S18IS1	Emerging Fungal Infections in Animals in Japan.		142
Saul	N S	**0850**	S18IS2	"Koala cracks *Cryptococcus* story wide open" The importance of molecular typing in understanding cryptococcosis in Australia: the WA connection	Carter D C, Meyer W M, Malik R M, Krockenberger M K	142

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Schmitt	I	**0436**	S39PS1	Evolution of polyketide synthase genes in lichenized Ascomycetes	Lumbsch H T	266
Schoch	C L	**0540**	S11IS2	Investigating Dothideomycete evolution using multi-gene sequence data	Kohlmeyer J, Volkmann-Kohlmeyer B, Spatafora JW	108
Schrank	A	**0987**	S43IS3	Strategies to improve Metarhizium control of arthropod pests	Butt TM	315
Schwelm	A	**0459**	S2PS1	Early onset of toxin biosynthesis in a forest pathogen	Bradshaw RE	9
Scott	B	**0800**	S22IS3	The genetic basis for indole-diterpene chemical diversity in filamentous fungi	Saikia S, Monahan B J, Young C A, Takemoto D, Parker E J	153
Scott	J	**0799**	S51IS2	Lessons learned from fungi associated with alcoholic beverage production	Untereiner W, Ewaze Jt, Wong B	406
Seifert	K A	**0606**	S21IS1	Canadian barcode of life network and COI barcoding of *Penicillium*	Samson R A, de Waard J, Houbracken J, Lévesque C A, Moncalvo J M, Hebert P D N	149
Sekimoto	S	**0280**	S36IS4	Molecular phylogeny and comparative ultrastructural morphology of marine oomycete endoparasites	Beakes GW, Honda D	262
Shearer	C A	**0798**	S47IS3	Phylogeny and biodiversity of freshwater euascomycetes	Campbell J, Raja HA, Ferrer A, Marvanová L, Miller AN	338
Simon	UK	**0756**	P1PS1	Demystifying Dothideomycetes - combining ultrastructure and molecular tools to study phylogenetic relationships of fungi	Bauer R, Begerow D, Rioux D, Simard M	255
Sipiczki	M	**0718**	S43PS2	Effect of antagonistic fruit-borne yeasts on pathogenic and saprophytic fungi	Csoma H	316
Sivichai	S	**0524**	S14PS1	Propagation strategies patterns of Thai freshwater fung	Boonyene N	115
Six	D L	**0659**	S3IS2	Temperature driven symbiont shifting in a bark beetle-fungus ectosymbiosis: a mechanism of stability?	Bentz B J	10
Sjamsuridzal	W	**0506**	S6PS2	Diversity of yeasts from gastropods in Gunung Halimun National Park, West Java, Indonesia		91
Skinner	S J	**0639**	S41PS2	Metabolic and taxonomic approaches to investigating the effects of plant function on communities of root and nodule-associated fungi	Currah RS	311
Slippers	B	**0665**	S3IS3	Comparison of molecular ecological patterns in populations of different woodwasp fungal mutualists	Vasiliauskas R, van der Nest MA, Stenlid J, Wingfield MJ	11
Smith	J A	**0429**	P1PS3	Rust of Salix species in North America caused by *Melampsora epitea* s. lat.	Blanchette R A	256
Solomon	P S	**0148**	S17IS3	Dissecting the role of signal transduction in *Stagonospora nodorum* during infection on wheat	Tan K-C, Waters ODC, Oliver RP	140
Solomon	P S	**0149**	S29PS1	Determining the role of the mannitol cycle in *Stagonospora nodorum*	Waters ODC, Trengove RD, Oliver RP	172
Spanu	P D	**0862**	S7IS3	Transcriptome dynamics in barley powdery mildew: insights into development and pathogenicity of an obligate biotroph	Both M, Andras C, Stumpf MPH	92

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Spatafora	J W	**0433**	S1IS5	Assembling the fungal tree of life: evolution of the ascomycota	Schoch C L, Consortium AFTOL	7
Spencer-Phillips	P T N	**0297**	S12PS1	The proteome of *Peronospora viciae*: from spore to endophytic hyphae	Amey R C, Smith R, Schleicher T, Lewis M, Macdonald H, Neill S	111
Spiegel	F W	**0293**	S1IS3	An up to date assessment of the place of the mycetozoans among the eukaryotes	Lindley L A, Silberman J D	6
		0294	S50IS3	Global distribution of the protostelids with particular emphasis on the deep southern hemisphere	Shadwick J D, Stephenson S L	345
Stadler	M	**0379**	P1PS5	A polythetic approach to the taxonomy and phylogeny of the Hypoxyloideae	Fournier J, Læssøe T, Asakawa Y, Quang DN	257
Stadler	M	**0361**	S22PS2	Polyketide and cytochalasin production during stromatal ontogeny of the Hypoxyloideae	Asakawa Y, Hashimoto T, Quang DN, Fournier J	154
Stalpers	J A	**0570**	S55IS3	A global network of genetic resource centres to preserve fungal biodiversity	Smith D, Crous P W, Nakagiri A	416
Steadman	J R	**0156**	S10PS1	Within and between bean field phenotypic variation of a fungal biotroph – estimation of populations	Jochua C N, Xue X, Eskridge K M, Amane M I V	99
Steenkamp	E T	**0585**	S45IS2	Global distribution and evolution of the pine pitch canker fungus, *Fusarium circinatum*	Wright J, Ganley RJ, Iturritxa E, Ahumada R, Wingfield BD, Marasas WFO, Wingfield MJ	319
Steffen	K	**1016**	S29IS3	Extracellular enzymes involved in litter degradation by basidiomycetes		171
Stenlid	J	**0715**	S19IS1	Fungal dispersal and succession in boreal forests		144
Stephenson	S L	**0142**	S50IS2	A global perspective on myxomycete biodiversity		344
		0146	S50PS1	Dictyostelid cellular slime molds of Australia		345
Stone	J K	**0452**	S45PS2	*Phaeocryptopus gaeumannii* and Swiss needle cast disease in New Zealand	Hood I A, Ramsfield T, Kerrigan J L, Kriticos D	321
Sudhadham	M	**0593**	P2PS4	Genetic diversity and detection of the neurotropic black yeast *Exophiala dermatitidis*	de Hoog G S, Gerrits van den Ende A H G, Prakitsin S	259
Suetrong	S	**0128**	S49PS2	Morphological and molecular observations of *Manglicola guatemalensis*, a poorly known ascomycete	Sakayaroj Jariya, Phongpaichit Souwalak, Jones E B G	344
Sugita	T	**0771**	S6IS3	Molecular taxonomy of the medically relevant yeasts *Malassezia* and *Trichosporon*		90
Summerbell	R C	**0836**	S21IS3	DNA barcoding, 'accelerated ecology' and the acremonioid fungi	de Hoog G S	150
Suzuki	A	**0334**	S14IS1	Propagation strategy of ammonia fungi		114
Svegaarden	I B	**0370**	S56IS1	Phylogeography of *Serpula lacrymans* reveals global migration events and multiple transitions to an indoor lifestyle	Kauserud H, Knudsen H, Stensrud Ø, Högberg N	312
Takamatsu	S	**0233**	S11PS1	Molecular phylogenetic analyses reveal adaptive evolution occurred in the powdery mildew fungi (Ascomycota: Erysiphales)	Sato Y	109
Takao	Y	**0473**	S36IS3	The viral impact on thraustochytrids	Tomaru Y, Sasakura Y, Yamane K, Nagasaki K, Honda D	261

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Takashima	M	**0755**	S31IS1	Inter- and intra-species diversity of ballistoconidium-forming yeasts and related taxa		236
Talbot	N J	**0253**	S42IS3	Functional genomics of pathogenicity in *Magnaporthe grisea*	Richards T A, Soanes D M, Gilbert M J, Wilson R A, Bhambra G K, Wang Z Y, Caracuel-Rios Z	
Taylor	J W	**0819**	PLEN 2	Species of fungi: their recognition, maintenance and utility		117
Templeton	M D	**0867**	S23IS3	Structural basis for rodlet assembly in fungal hydrophobins	Winefield RD, Kwan AHY, Sunde M, Haverkamp RG, Mackay JP	155
		0458	S28PS2	Genomic approaches to isolating pathogenicity factors from the apple black spot pathogen *Venturia inaequalis*	Sutherland PW, Rikkerink EHA, Crowhurst RN, Hill G, Cui w, Bowen JK, Rees-George J, Jones WT, Al-Sammarrai T, Plummer KM, Hahn M	170
Thrall	P H	**0214**	S10IS3	The impact of plant population structure on disease epidemiology and pathogen evolution in the *Linum marginale* – Melampsora lini interaction	Burdon J J	98
Thrane	U	**0300**	S54IS3	Secondary metabolome – the bridge between phenetics and phylogenetics in *Fusarium*		413
Tokumasu	S	**0331**	S20IS1	Why is the species diversity of fungi in tropical monsoon Asia high?		147
Trilles	L	**1011**	S15IS4	Emerging *Coccidioidomycosis* and *Cryptococcosis gattii* in Brazil		
Tsui	K M	**0772**	S36IS1	Evolution and phylogeny of the Labyrinthulomycetes inferred from protein-coding genes	Marshall W, Yokoyama R, Honda D, Lippmeier J C, Craven K D, Berbee M L	260
Tunlid	A	**0690**	S48IS2	Transcriptional responses of *Paxillus involutus* and *Betula pendula* during formation of ectomycorrhizal root tissue	Johansson T, Le Quéré A, Wright D P, Schutzendubel A, Ahren D, Canbäck B, Rajashekar B, Erland S, Hedh J, Söderström B	340
Untereiner	W A	**0719**	S51IS3	Vertebrate-associated and keratin-degrading fungi from northern Canada		406
Unterseher	M	**0034**	S19PS1	Diversity and ecological patterns of wood decay fungi in a temperate, deciduous forest canopy	Otto P	146
van den Hondel	C		S44IS3	Fungal Cell wall biosynthesis and discovery of antifungals		
van der Merwe	M M	**0305**	P3PS2	Tackling phylogenetics in the large and diverse group of rusts in the family Pucciniaceae	Thrall P H, Burdon J J, Ericson L, Maier W, Walker J	329
van der Nest	M A	**0571**	S14PS2	The effect of selection against sexual recombination on the diversity of *A. areolatum* mating-type genes	Slippers B, Wilkens M, Stenlid J, Wingfield MJ, Wingfield BD	115
van Driel	K G A	**0684**	S9IS2	Structure and biochemical characterization of septal pore caps in basidiomycetes	van Peer AF, Wösten HAB, Verkleij AJ, Müller WH, Boekhout T	96
van Kan	J A L	**0687**	S28IS2	Licensed to kill: the role of phytotoxic proteins and pectinases in virulence of *Botrytis cinerea*		169

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Vanittanakom	N	**0951**	S15IS2	*Penicilliam marneffei* infection and current knowledge on it s potent virulence genes		116
Vánky	K	**0117**	P4PS2	Australian smut fungi (Ustilaginomycetes), as surprising and diverse as the continent itself	Shivas R G	334
Verkley	G J M	**0691**	S41IS1	Foliar endophytes versus leaf litter saprobes: annual cycle of an ascomycete community associated with oak leaves	van Kempen I	309
Vilgalys	R	**0748**	S1IS6	Evolution of basal lineages in Fungi: deconstructing Chytridiomycota and Zygomycota	James T Y, Kauff F, Schoch C, Matheny P B, Hofstetter V, Cox C, Celio G, Longcore J E, Hibbett D S, Lutzoni F, Mclaughlin D, Spatafora J Others many	8
Villarreal-Ruiz	L	**0795**	S13IS3	Dynamics of ectomycorrhizal fungal communities in a Scots pine chronosequence L		113
Vismer	H	**0994**	S15IS1	*Sporothrix schenckii* infections in South Africa - a clinical, epidemiological, ecological and molecular taxonomic overview		116
Vrålstad	T	**0425**	S18PS2	Molecular detection of *Aphanomyces astaci* from Norwegian crayfish plague outbreaks in the time span from 1971 to 2005	Knutsen AK, Taugbøl T, Håstein T, Holst-Jensen A, Dale OB, Kvellestad A, Tengs T, Cudjoe K, Skaar I	144
Wachtler	V	**0189**	S4PS1	Fission yeast cytokinesis: two paths to one destination	Karagiannis J, Balasubramanian MK	14
Wang	Y	**1013**	S7IS2	Transcriptome study of C. *albicans* genes important for infection and virulence?		92
		1014	S28IS3	Key enzymes for iron uptake and hyphal morphogenesis in C. *albicans* infection		169
Wang	P-H	**0503**	S30PS2	Macrofungal diversity in the *Cryptomerioid japonica* plantations in Taiwan	Wang Y-T, Cheng W-C, Lin W-R, Chen M-I, Kao M-S, Guu T-Y	174
Wang	B	**0311**	S54PS1	Origin and Diversity of *Fusarium oxysporum* f.sp. *vasinfectum* (Fov) in Australia	Brubaker C L, Burdon J J	414
Webber	J F	**0634**	S40PS2	Dissemination of aerial and soilborne *Phytophthoras* by human vectors	Rose J	268
Weiß	M	**0217**	P4PS3	The expanding realm of the Sebacinales: basidiomycetes involved in a uniquely wide spectrum of mycorrhizal associations	Setaro S, Selosse M-A, Oberwinkler F	334
Whisson	S	**0519**	S2PS1	Defining the role of the Avr3a avirulence gene in *Phytophthora infestans* – potato interactions	Armstrong MR, Morales J, Moleleki L, Boevink P, Birch PRJ	10
Whittle	P	**0961**	S40IS3	A major exotic disease outbreak, emergency response and eradication: banana black Sigatoka, Tully, Australia, 2001		267
Widmer	I		S26PS3	European phylogeography of the epiphytic lichen *Lobaria pulmonaria*		167
Wingfield	M	**0750**	PLEN 5	Emerging fungal diseases threaten world forests		417

Oral Abstract Authors (list is according to presenting author)

Last name	Initial	Abstract No.	Prog No.	Title	Co-authors	Page No.
Wingfield	B D	**0569**	S45IS1	*Cryphonectria* canker of Eucalyptus: A little-known disease caused by an assemblage of fungi of extreme quarantine relevance	Gryzenhout M, Wingfield MJ	318
Wirtz	N	**0304**	S56PS1	A phylogenetic and phylogeographic approach to delimit Antarctic and bipolar species of the genus *Usnea*, *Neuropogon*	Printzen C, Lumbsch HT	313
Xu	J-R		S17IS2	Signalling pathways and infection-related morphogenesis in Magnaporthe grisea		140
Yokoyama	R	**0481**	S36PS1	Taxonomical reinvestigation of the genus *Schizochytrium* (Thraustochytriaceae, Labyrinthulomycetes)	Kon W L, Salleh B, Honda D	260
Yurkov	A M	**0587**	S31IS3	Are there eurybionts among the dimorphic basidiomycetes?		237
Zancope-Olivera	R M	**0860**	S53IS3	Molecular epidemiology of histoplasmosis: An update	Tavares P M S, Muniz M M	411
Zare	R	**0093**	P4PS5	Molecular phylogeny of *Verticillium fungicola* reveals its affinity with the genus *Lecanicillium*	Gams W	334
Zarrinfar	Hosse in	**0054**	P2PS1	Evaluation of the Effects of Incubation Temperature and pH on the Antifungal susceptibility Test Against *Candida albicans* PTCC 5027 Strain	Yadegari M H, Riazipoor M, Farahnejad Z, Katiraee F, Nasrollahi Z	257
Zhang	H		S7IS1	DNA microarray analysis of signal interference of the dimorphic transition of *Candida albicans*		91

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Abdel-Raheem	A	**0024**	PS5-483	Myxomycetes From Delta Region in Egypt		346
Abdullah	F	**0831**	PS1-135	Molecular Studies Support The Interfertility Data In Solving Taxonomic Problems Of *Ganoderma boninense*	Hussin Husrita, Hassan Nuddin Noor Fatihah, Zainuddin Zabitah	65
Abell	S E	**0475**	PS5-539	Seasonality of hypogeous fungi availability – implications of global climate-change	Gadek P A, Pearce C A, Congdon B C	367
Aghayeva	D N	**0031**	PS5-485	Identification of fungal associates of some deciduous tree species in Azerbaijan	Harrington TC	346
Akinsanmi	O A	**0650**	PS3-290	*Pseudocercospora macadamiae*, the causal agent of husk spot disease in macadamia	Miles AK, Drenth A	195
Alian	S A	**0046**	PS3-243	Study on foot rot and bakanae disease of rice in Mazandaran province, Iran	Aminian H, Javan-Nikkhah M, Khosravi V, Bahrami M	175
		0047	PS3-244	Natural occurance of perithecia of *Gibberella fujikuroi* and its related *Fusarium* species in Mazandaran paddy fields, Iran	Aminian H, Javan-Nikkhah M, Khosravi V	176
		0048	PS8-450	Population diversity of *Fusarium proliferatum*, as the major causal agent of rice bakanae disease in Mazandaran province, using vegetative compatibility groups	Alian SA, Aminian H, Javan-Nikkhah M, Khosravi V	297
Alimova	F	**0804**	PS3-312	The *Trichoderma/Hypocrea* from Russia (Tatarstan Republic) - interaction with microorganisms and plants	Alimova FK, Tukhbatova RI, Cabrera FHA, Tazetdinova DYu, Karimova LYu	204
Al-Sa'di	A M S	**0126**	PS1-18	Phylogenetic data and morphological characteristics provide evidence for *Pythium kunmingense* to be a synonym to *P. spinosum*	Aitken EAB, Drenth A, Deadman ML, de Cock AWAM	22
Aminian	H A	**0938**	PS3-328	Investigation on toxin produced by *Alternaria alternata* fsp *lycopersici*, the causal agent of tomato stem canker	Zad J, Okhovat S M, Sharifi-Tehrani A, Talebi Kh, Safari M	211
Anderson	C M T	**0890**	PS3-322	Colonisation of the plant *Gossypium hirsutum* and the aphid *Aphis gossypii* by the fungus *Lecanicillium lecanii*	McGee PA, Nehl DB, Mensah RK	209
Ando	K	**0502**	PS5-542	A list of fungi recorded in Japan	Katumoto K	368
Andrianova	T V	**0423**	PS5-534	Worldwide movement of horse chestnut (*Aesculus hippocastanum* L) anamorphic leaf pathogens: monitoring in Ukraine		365
		0424	PS5-535	The Altai mountains as an area of missing and rare anamorphic fungi	Minter DW	366
Annett	K	**0209**	PS5-510	Small mammal mycophagy within *Phytophthora cinnamomi*-affected heathland at Anglesea, Victoria, Australia	Wilson BA	355
Antropova	A B	**0522**	PS10-356	Does indoor mycobiota reflect outdoor one	Bilanenko EN, Mokeeva VL, Chekunova LN	223
Aoki	T	**0188**	PS1-31	Systematics and evolution of the soybean sudden death syndrome and dry bean root-rot *Fusaria*	Scandiani María Mercedes, Starkey David E, O'Donnell Kerry L	28

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Aono	T	**0393**	PS9-167	Relation between alkaline phosphatase and polyphosphate in arbuscules of arbuscular mycorrhizal fungi	Funamoto R, Kizawa K, Oyaizu H	77
Arici	E	**0576**	PS3-285	In vitro Selection Techniques for *Fusarium* Head Blight Resistance in Wheat	Koc NKemal	193
		0583	PS10-357	Determination of Biological Efficiency of Entomopathogenic Fungus *Fusarium subglutinans* Against *Aphis gossypii* in Greenhouse	Yuce Satar Hatice, Koc NKemal	223
Arzanlou	M	**0399**	PS1-86	Molecular-based detection and quantification of causal agents of Sigatoka disease complex of banana	Abeln ECA, Kema GHJ, Waalwijk C, Carlier J, Crous PW	48
Asef	M R	**0037**	PS8-449	Intersterility Groups Of *Pleurotus* In Iran		296
Askun	T	**0835**	PS10-372	Antifungal activity of some labiatae species against some filamentous fungi	Tümen G, Agaoglu Y, Aktar V, Kilic T	230
Austwick	P K C	**0643**	PS5-564	Structures of some white polypores		376
Aveskamp	M M	**0177**	PS1-28	A Phylogenetic Re-evaluation Of The Sections Within The Genus *Phoma*	de Gruyter J, Crous PW	27
Ayatollahy	E	**0415**	PS3-273	Compatibility assay of some antagonistic fungi of *Heterodera schachtii in vitro*	Fatemy S, Roustaei A, Etebarian H R, Aminian H	187
		0923	PS3-325	Fungal parasites associated with eggs of *Heterodera schachtii* from Iran	Fatemy S, Roustaei A, Etebarian H R, Aminain H	210
Badalyan	S M	**0274**	PS4-407	Growth parameters, morphological and genetic variability of the medicinal mushroom *Flammulina velutipes* (Curt : Fr) Sing	Hughes KW, Sakeyan CZ, Helmbrecht E	279
		0568	PS4-428	Proteolytic activity of several Coprinoid mushrooms	Kües U, Avetisyan HK	289
		0231	PS10-346	Antifungal/antagonistic activity of medicinal mushroom *Pleurotus tuberregium* against filamentous fungi	Isikhuemhen OS, Gharibyan NG	219
Bagyanarayana	G	**0829**	PS1-134	Possible evolution of pedicillate teliospored rust fungi		65
		0830	PS5-573	The diversity and distribution of rust fungi of India		380
Baldrian	P	**0218**	PS4-399	Fungal Ligninolytic Enzymes in the Forest Soil Environment: Occurrence, Distribution and Role in Soil Organic Matter Transformation	Šnajdr J, Valášková V	275
Banke	S	**0403**	PS8-466	Congruence found among recombination rates and population ages for different populations of *Mycosphaerella graminicola*		303
		0434	PS8-468	Evolution of microsatellites in the mitochondrial genome of *Rhynchosporium secalis*	Torriani S, Linde C, MacDonald BA	304
		0439	PS8-469	Population expansion-migration scenarios explain the demographic history of the fungal pathogen *Mycosphaerella graminicola*		304

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Barber	P A	**0507**	PS3-281	The distribution and impact of *Mycosphaerella cryptica* on regenerating *Eucalyptus gomphocephala*	Archibald RD, Bowen B, Calver M, Hardy GStJ	191
Barrett	L G	**0235**	PS8-456	Geographic variation in the genetic structure of Australian populations of *Melampsora lini*: implications for regional coevolutionary dynamics	Thrall PH, Burdon JJ	299
Bayliss	K L	**0903**	PS3-324	Biosecurity built on science	McKell S, McKirdy S	210
Beaulieu	M E B	**0793**	PS1-131	Phylogenetic relationships among ophiostomatoid fungi associated with bark beetles colonizing white spruce in Eastern Canada	Bernier L	64
Beltran-Garcia	M J	**0446**	PS3-277	Oxidative stress response of the fungus *Mycosphaerella fijiensis*, the black sigatoka pathogen of banana to hydrogen peroxide and other stress conditions	Ogura Tetsuya, Manzo-Sanchez Gilberto, Arias-Castro Carlos, Esqueda Martin	189
Bermek	H	**0409**	PS7-610	Efficient immobilization of & increased manganese peroxidase production by the white-rot fungus LSK-27	Catal Tunc, Tamerler Candan	395
Beyer	D M	**0129**	PS3-251	Influence of organic acids on the growth and development of Trichoderma aggressivum a pathogen of *Agaricus bisporus*	Paley K, Wilkinson V, Pecchia J	178
Bilanenko	E N	**0426**	PS5-536	Micromycetes inhabiting soda solonchaks and halophytic plants in Central Asia, Micromycetes inhabiting soda solonchaks and halophytic plants in Central Asia	Georgieva ML	366
Bills	G F	**0365**	PS7-607	MORINIAFUNGIN, a potent antifungal sordarin derivative produced by the endophytic fungus *Morinia pestalozzioides*	Collado J, Basilio A, Justice M, Harris G, De la Cruz M, Díez MT, Hernández P, Liberator P, Nielsen Kahn J, Peláez F, Platas G, Schmatz D, Tormo JR, Vicente F	393
Boberg	J B	**0400**	PS4-414	Carbon and nitrogen dynamics of *Mycena epipterygia* decomposing pine needles	Finlay RD, Stenlid J, Lindahl BD	283
Boddy	L	**0926**	PS1-148	Molecular and morphological discrimination of stipitate hydnoids in the genera *Hydnellum* and *Phellodon*	Ainsworth AM, Parfitt D, Simpson D, Rogers HJ	69
		0925	PS10-381	Killer fungi attract springtails to their doom	Rotheray TD, Hynes J Müller C T Jones T H	234
Bodensteiner	P	**0216**	PS1-41	Phylogenetic relationships and evolution of cyphelloid homobasidiomycetes	Binder M, Hibbett DS, Agerer R	32
Boekhout	T	**0539**	PS5-550	Macrofungal diversity, variation in time and space in tropical lowland forests in the Colombian Amazon	Lopez Quintero Carlos, Franco Molano A Esperanza	371
		0541	PS5-551	Macromycetes from the middle Caquetá region (Colombia, Amazon)	Vasco-P Aída Marcela, Franco-Molano Ana Esperanza, Lopez Quintero Carlos	371
		0543	PS5-552	Ethnomicological studies among indigenous Uitoto from Colombia Amazon	Vasco-P Aída Marcela, Franco-Molano Ana Esperanza	371

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Boonyuen	N	**0870**	PS1-139	The Significance of the Anamorph in the Mega genus *Hymenoscyphus*	Sivichai Somsak, Hywel-Jones Nigel	66
Bourguignon	E L T	**0298**	PS3-267	Effect of green manure soil amendments on *Trichoderma* spp population and diversity in the rhizosphere of onion	Stewart A , McLean K, Condron L M, Ridgway H J, Jones E E	184
Branco	S	**0463**	PS9-171	Mediterranean serpentine and non-serpentine ectomycorrhizal fungal communities		79
Briere	S C	**0949**	PS3-333	First report of *Ardisia japonica*, *Prunus lusitanica*, *Euonymus kiaut*, *Gaultheria shallon* and *Osmanthus decorus* as hosts for sudden oak death caused by *Phytophthora ramorum*	Llewellyn S , Kumor L , Grant S	213
Bulman	S R	**0827**	PS2-206	Investigating gene structure and transcription in the intracellular plant pathogen, *Plasmodiophora brassicae*	Siemens J, Ridgway H J , Eady C, Conner A J	123
Capelari	M	**0162**	PS1-24	Two new species of *Marasmius* (Basidiomycota, Marasmiaceae) from Brazil	Puccinelli Carla	25
Catal	T	**0404**	PS4-415	Selenium induces manganese peroxidase production by the white-rot fungus LSK-27	Tamerler Candan, Bermek Hakan	283
Chadha	B S	**0030**	PS1-3	Molecular Characterization of Thermophilic Fungi using Internal Transcribed Spacer (ITS) region primers	Sharma M, Kaur M, Saini HS, Manhas RK	17
Chaichi Nosraty	A	**0109**	PS7-587	A Qualitative Investigation On Tea Garden Air Fungal Pollution In The North Of Iran , Gilan Province Estern Region	Modiri Leila, Khosravi Alireza, Mirhosseini Moghaddam Seid Abdol, Majid Khsoshkholgh Pahlaviani Mohammad Reza, Taghi Shokr gozar Sied Amir, Koochaki Vahid	386
Chalannavar	R K	**0252**	PS5-517	Fungal Diversity On Leaf Litter	Rames, C H	358
		0271	PS5-519	Studies On Litter Fungi: Fungal Colonization Of *Ficus benjamina* L and *Gliricidia maculata* HBK	Ramesh, C H	359
Chang	F-M	**0337**	PS7-604	Isolation of monokaryon from asexual spore of *Taiwanofugus camphoratus* and breeding of high triterpenoid strains	Su Ching-Hua	392
Chawla	V	**0628**	PS3-288	Effect of methyl jasmonate and salicylic acid on karnal bunt (*Neovossia indica*) resistance in wheat *in vitro* and *in vivo* conditions	Kaushik Subhash, Yadav Neelam	194
Chen	L-C	**0545**	PS1-105	Study on the double-stranded RNA elements in different isolates of *Rhizoctonia solani* AG-1	Lin Yi-Chen, Yang Hsiang-che, Chan Sz-hau	55
Chen	C-J	**0152**	PS7-592	Bioactivities of *Phellinus linteus* Extracts Growing in Chinese Herbal Formula Substrates	Li Jiay-An, Chen Jian-Chyi	387
		0153	PS10-345	Menstrual Cycle and Ovarian Function in Obese Polycystic Ovary Syndrome Women Treated with *Auricularia polytricha* Formula through a Randomized Double Blind Placebo-Controlled Trial	Chen Yen- Lin	218

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Chikballapur	N	**0990**	PS2-215	A proteomic approach into biological control of sugar canegrubs	Te'o V, Braithwaite K, Brumbley S, Samson P, Nevalainen H	126
Chin	Y-M	**0492**	PS1-99	Phylogenetic analysis & identification of Antarctic microfungi by PCR of the ITS1, ITS2, mitochondrial small subunit rDNA & beta-tubulin gene	Ng Ching-Ching, Alias Siti Aisyah	53
Cho	D-H	**0228**	PS1-44	Some New Species of *Amanita*, *Boletus* and *Cantharellus* (Agaricales) from Korea		33
		0868	PS1-138	Some New Species of *Boletus* and *Cantharellus* from Korea		66
Cho	S M	**0873**	PS10-375	Effect of *Pholiota adiposa* Extract in Hyperlipidemic Mice	Lee Young-Meen, Chun Hae-Kyoung, Lee Jong-Soo	231
Choi	D S	**0327**	PS7-601	New cropping system to improve productivity of *Gastrodia elata*	Jung J K J, Choi C H G, Park P I J	391
		0328	PS7-602	Reduction effect the production cost of *Flammulina velutipes* by re-using of the used media	Jung J K J, Choi C H G, Park P I J, Chung C K C	391
Chooi	Y H	**0497**	PS4-422	The search for polyketide synthase genes producing beta-orsellinic acid and methylphloroacetophenone as precursors for beta-orcinol depsidones and usnic acids in the lichen *Chondropsis semiviridis*	Stalker D M , Louwhoff S H J J , Lawrie A C	286
Christensen	M	**0161**	PS5-505	Collection of wild edible fungi in Nepal	Larsen Helle O , Bhattarai Sanjeeb, Devkota Shiva	354
Chum	W W Y	**0351**	PS2-197	Transcriptome Analysis and Expressed Sequence Tags of Differentially Expressed Genes in Sporulating Shiitake mushroom *Lentinula edodes*	Kwok I S W , Ng K T P , Bian X L , Kwan H S	119
Chung	K-C	**0511**	PS10-355	Antifungal activity of components isolated from *Epicoccum nigrum* MET0425	Lee Yoon-Gyo, Ryu Jae-Won, Lee Jae-Chang	222
Coetzee	M P A	**0509**	PS8-471	Genotypic diversity of *Armillaria fuscipes* in South African Pine plantations	Wingfield BD, Wingfield MJ	305
Crittenden	P D	**0837**	PS4-440	Nitrogen enrichment promotes phosphatase activity in *Cladonia portentosa*	Hogan E J , Sheppard L J , Crossley A , Leith I D , Ancion P	293
Crockatt	M	**0060**	PS5-488	Ecology of the rare tooth fungi *Hericium cirrhatum*, *H. coralloides* and *H. erinaceus* in Britain	Parfitt D, Hynes J, Wald PM, Boddy L, Rogers HJ	348
Cuero	R G	**0635**	PS5-562	Effect of Toxic Metals on Gene Expression of Fungi in Relation with Bioremediation	Shnyreva A , Terekhova V A	374
Cunnington	J H	**0471**	PS2-202	Distribution of optional mitochondrial introns encoding putative homing endonuclease genes in the *Fusarium oxysporum* complex		121
da Silva	M	**0197**	PS5-508	Marine-derived Fungi Isolated from Corals from Brazilian Coast for Bioprospection	Passarini M R Z , Thompson F L , Migotto A E , Sette L D	355
		0198	PS5-509	Fungi Isolated from Sediment from Northern Region of Brazil and their Ability to Degrade Industrial Dyes	Nascimento C R S , Bergsten L R , Magalhães D P , Nishikawa M M	355

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Dahlberg	A	**0546**	PS4-426	THE relative importance of different groups saprophytic and mycorrhizal fungi revealed by a flux model of dead plant matter and belowground assimilate allocation in Norway spruce forests	Hyvönen R	288
Dai	Y H	**0150**	PS5-502	Pathogenic wood-decaying fungi in China		353
Damadi	S M	**0710**	PS3-299	Identification of the causal agent of poplar (*Populus nigra*) rust disease in Maragheh, NW of Iran	Pei M H	199
Davydov	E A	**0191**	PS1-33	The family Umbilicariaceae (lichenized Ascomycota) in Russia: systematic, phylogeny and geography	Peršoh D , Rambold G	29
de Beer	Z W	**0615**	PS1-114	*Ceratocystiopsis* and *Grosmannia* distinguished from *Ophiostoma* sensu stricto based on multigene phylogenies	Zipfel RD, Jacobs K, Wingfield BD, Wingfield MJ	58
		0616	PS1-115	Identification of *Paecilomyces* spp from wooden utility poles in South Africa	de Meyer EM, Samson RA, Wingfield MJ	58
		0617	PS1-116	*Ophiostoma* spp associated with *Scolytus ratzeburgii* infesting birch in Finland and Russia	Linnakoski R, Rousi M, Niemela P, Pappinen A, Wingfield MJ	58
Degawa	Y	**0378**	PS1-78	A new gall-forming facultative mycoparasite of the Mucorales from Japan		45
dela Cruz	T E	**0028**	PS1-2	Conidial morphology: homology or homoplasy? The analysis of molecular data shows that marine *Dendryphiella* species do not belong to the genus *Scolecobasidium*	Schulz B E , Komon M , Druzhinina I S	17
Deshmukh	S	**0734**	PS7-621	Biotechnological Potential of Keratinophilic Fungi and their Secondary Secondary Metabolites	Verekar Shilpa	400
Dethoup	T	**0410**	PS3-272	Diversity of *Talaromyces* species with special emphasis on *T. flavus* for potential use in biological control of plant pathogenic fungi *in vitro* and in the greenhouse	Manoch Leka, Visarathanonth Niphon, Chamswarng Chiradej	186
Dianese	J C	**0969**	PS1-151	A new *Anhelia* (Myriangiales, Dothideomycetidae) species from the Brazilian cerrado	Inacia C A	70
		0973	PS1-153	A new trichomatous hyphomycete on *Emmotum nitens* (Icanaceae) from the Brazilian cerrado	Carvalho R C P	71
Diaz-Godinez	G	**0816**	PS7-623	Decolourising of textile dyes by laccases of *Pleurotus ostreatus* grown in submerged fermentation	Juarez-Hernandez J, Sanchez C, Montiel-Gonzalez AM, Bibbins M	400
		0817	PS7-624	Increased production of laccases in submerged cultures of *Pleurotus ostreatus* in the presence of copper	Juarez-Hernandez J, Sanchez C, Montiel-Gonzalez AM, Bibbins M	401
		0818	PS7-625	Production of laccases of *Pleurotus ostreatus* in solid-state and liquid-state fermentation	Tellez-Tellez M, Sanchez C	401

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Dijksterhuis	J	**0019**	PS4-386	Becoming less protected; germination of highly stress-resistant ascospores of *Talaromyces macrosporus* includes unique cellular features	Dijksterhuis J , Samson R A, Wösten H A B, Golovina E, Hoekstra F A , Lugones L	269
Djoyobisono	H	**0438**	PS2-201	Characterization of *Leptosphaeria maculans* gene that enables this fungus to Infect *Arabidopsis thaliana* ecotype Col-0	Elliott C E , Howlett B J	121
Dodd	S L	**0132**	PS3-252	The use of a PCR diagnostic test to predict and control *Peronospora sparsa*, downy mildew of boysenberry	Boyd-Wilson K, Shanmuganathan D, Walter M	179
		0139	PS5-498	Non-target impacts of an introduced *Trichoderma* biocontrol agent on soil microbes	McLean K L, Stewart A	352
		0140	PS7-591	The use of denaturing gradient gel electrophoresis (DGGE) to identify key fungi involved in the commercial mushroom composting process	Butler R C, Visnovsky S B, Khan M I, Shah F A, Marshall J W	387
Dorner	J W	**0099**	PS6-218	Manipulation of the toxigenicity of *Aspergillus flavus* soil populations to control aflatoxin contamination in peanuts		127
Driessen	S A	**0662**	PS8-477	Investigating the genetic diversity of *Puccinia boroniae* in Western Australia	O'Brien P A , Hardy G E St J	307
Esqueda	M	**0466**	PS1-94	Macromycetes of Pinacate and Great Altar Desert biosphere reserve, Sonora, Mexico	Coronado M, Encinas I, Pérez-Silva E, Herrera T	51
		0468	PS7-611	Effect of lignin on biomass and the activity profile of principal ligninolytic enzymes in submerged cultures of *Lentinula edodes*	Harris-Valle C, Valenzuela-Soto E, Beltrán-García MJ, Gaitán-Hernández R	395
Etcheverry	M G	**0623**	PS6-226	Potential Inhibition Effect Of Essential Oils On *Aspergillus* Sección Flavi Growth In Maize Grain	Bluma RV, Amaidén MR	131
		0625	PS6-227	Effect of Osmotic Potential on Growth and Endogenous Polyols and Sugars Accumulation by Toxigenic Strains of *Aspergillus* section Flavi from Peanut	Ferrari L , Marioli J M , Nesci A V	131
		0627	PS6-228	Effect of Natural Maize Phytochemicals, Nutrients And Water Activity on Sclerotium Formation of *Aspergillus flavus* and *A. parasiticus*	Morales M E , Nesci A V	132
		0629	PS6-229	Impact of Competitive Mycoflora on *Aspergillus flavus* and *A. parasiticus* Populations in Stored Peanut Pods	Passone M A , Ponzio V , Nesci A V	132
		0630	PS6-230	Fungal Population Succession in Stored Peanut Seeds	Passone M A , Ruffino M , Nesci A V	133
Eyre	C	**0581**	PS4-430	Interspecific interactions between saprotrophic basidiomycetes: effect on gene expression and chemical activity of mycelia	Boddy L, Rogers H J	290
Fechner	N A	**0885**	PS1-144	*Ramaria* in australia	Young A M	68

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Fonseca	A	**0847**	PS1-137	Species diversity of phylloplane yeasts: do nitrate-negative *Cryptococcus* spp belong to *Cr. laurentii*?	Inácio J , Santos M , Spencer-Martins I	66
		0846	PS3-316	Genetic heterogeneity in the ascomycete *Taphrina deformans*, agent of Peach Leaf Curl: Evidence for two distinct forms parasitic on Peach and Almond	Rodrigues M G	206
Fossdal	C G	**0736**	PS3-302	Simultaneous monitoring of both the host and pathogen using quantitative real-time PCR	Hietala A , Kvaalen H , Solheim H	200
		0737	PS3-303	Local and systemic host-response in Norway spruce to *Rhizoctonia* sp compared to the effect of drought stress and to the combination of the two stresses	Nagy Nina, Johnsen Øystein, Dalen Lars	201
Friedl	M A	**0944**	PS2-211	Carbon source depending interaction of cAMP signalling pathway and light in *Hypocrea atroviridis*	Schmoll M, Kubicek C P, Druzhinina I S	124
Fukiharu	T	**0548**	PS1-107	Two new ammonia fungi from Japan	Suzuki A	55
Fungaro	M H P	**0953**	PS6-239	Real-time PCR assay to quantify *Aspergillus westerdijkiae* in coffee beans	Morello Luis Gustavo, Sartori Daniele, Taniwaki Marta Hiromi, Iamanaka Beatriz, Oliveira André Luiz Martinez, Watanabe Maria Angélica Ehara	137
Gafur	A	**0717**	PS3-300	*Passalora* leaf and shoot blight disease and application of different micronutrient regimes in *Acacia crassicarpa* lowland plantation in Riau, Indonesia	Tjahjono B , Tarigan M , Mulawarman, Golani G D	199
Gaitán-Hernández	R	**0608**	PS7-616	Bioconversion of agro-wastes by *Lentinula edodes*: the high potential of viticulture residues	Esqueda M, Gutiérrez A, Beltrán-García M	397
Gams	K W	**0368**	PS1-72	An old forgotten genus for the "glomerellalean" anamorphs *Verticillium nigrescens* and *Acremonium furcatum*	Zare R , Summerbell R C	43
Gardiner	D M	**0182**	PS3-257	Regulation of gene expression in the interaction between *Fusarium* spp and wheat	Desmond O, Rusu A, Bursle J, Edgar C, Mudge A, Kazan K, Manners J	180
Garrill	A	**0312**	PS4-411	An F-actin depleted zone is present at the hyphal tip of invasive hyphae of the ascomycete *Neurospora crassa*	Suei S	281
Ghadiri	M	**0943**	PS3-329	Identification of the physiological race of *Fusarium oxysporum* f sp *melonis* isolated from melon field in Varamin	Etebarian H R, Ruostaei A, Aminian H, Shahriari D	212
Ghadiri	M	**0946**	PS3-330	Investigation on the antagonistic activities of fluorescent *Pseudomonads* on biological control of *Fusarium oxysporum* fsp *melonis* the casual agent of melon wilt	Etebarian H R, Ruostaei A, Aminian H	212
Ghobadnejhad	M	**0158**	PS5-504	Contribution to the knowledge of the biodiversity of wood-inhabiting Aphyllophorales (Basidiomycetes) in the Caucasus hotspot	Hallenberg Nils	353

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Gholampour Azizi	I	**0730**	PS6-233	Occurrence of aflatoxin M1 in raw milk at cattle farms by ELISA in Babol, Iran	Khosravi Alireza, Hashemi Sayid Gamal, Sefidgar Sayid Aliasqar, Azimy Hassan	134
		0731	PS6-234	Survey of the occurrence of Aflatoxin M1 in pasteurized milk consumption primary schools in Babol, Iran	Khoushnevis Seyed Hasan, Norozi Mehdi	135
Gibertoni	T B	**0061**	PS5-489	Aphyllophorales (Basidiomycota) from the Floresta Nacional de Caxiuanã, State of Pará, Brazilian Amazonia – preliminary results	Martins-Junior AS, Sotão HMP	348
		0163	PS5-506	Aphyllophorales (Basidiomycota) from coastal ecosystems in the state of Amapá, Brazilian Amazonia	Sotão HMP	354
Gilbert	R L	**0451**	PS1-92	Another teleomorph for Nimbya - the first from Amaranthaceae	Priest MJ, Gurr G	50
Glen	M	**0489**	PS1-97	Molecular identification of fungi in rot types from logs in *Eucalyptus obliqua* forest in Tasmania	Trang TT, Mohammed CL	52
		0487	PS3-280	PCR detection of Mycosphaerella species in early lesion developmental stages of Mycosphaerella leaf disease	Smith AH, Mohammed CL	190
		0485	PS9-174	Ectomycorrhizal fungal communities in rehabilitated bauxite mine sites and adjacent forest	Bougher NL, Colquhoun IJ, Vlahos S, Loneragan WA, O'Brien PA, Hardy GEStJ	80
Gonzalez	M C	**0302**	PS1-58	A new record of *Emericella* from a Mexican coral reef	Medina-Ortiz C, Glenn AE, Cifuentes J, Hanlin RT	39
		0313	PS1-59	A new record of *Aniptodera* from a freshwater habitat of Mexico City	Rosique E, Chavarria A	39
Goodwin	S B	**0595**	PS1-112	Genomic and EST sequencing of *Mycosphaerella* species will permit comparative analyses with related fungi and anamorphs	Dunkle L D, Churchill A C L, Carlier J, James A, Souza M T, Crous P, Roux N, van der Lee T A J, Waalwijk C, Lindquist E, Bristow J, Kema G H J	57
Gowland	K M	**0448**	PS9-169	Fungal discrimination amongst three epiphytic Aeridinae orchids of south-eastern Australia	Van Der Merwe MM, Clements MA, Nicotra AB	78
Granberg	Å	**0896**	PS8-479	Differences in conjugation patterns and sporidia production between populations of *Microbotryum violaceum* var *dioica*	Giles B E, Carlsson-Granér U	308
Griffith	G W	**0749**	PS5-572	Ecology and conservation of grassland macrofungi	Easton GL, Roderick K	380
Grobbelaar	J W	**0551**	PS8-475	Development of microsatellite markers to study the population biology of the wood-inhabiting fungus, *Ophiostoma quercus*	Wingfield MJ, Bloomer P, Solheim H, Wingfield BD	306
Groenewald	M	**0588**	PS4-431	Characterization and frequency of mating type genes in *Cercospora*	Groenewald JZ, Crous PW	290
Grube	M	**0554**	PS1-109	Molecular analysis of bacterial communities in lichen thall	Cardinale M, Puglia AM	56

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
de Gruyter	J	**0220**	PS1-43	Barcoding the genus *Phoma*: The next phase for *Phoma* taxonomic research in The Netherlands	Aveskamp MM, Crous PW	33
Gryzenhout	M	**0660**	PS1-121	Taxonomic revision of *Cryphonectria*	Wingfield BD, Wingfield MJ	60
Guo	L	**0840**	PS1-136	Smut fungi of Qilian mountains of China		65
Guo	S-Y	**0264**	PS8-457	Phylogeographical analysis of some species in the Umbilicariaceae based on the nrDNA ITS and partial LSU sequences	Wei Jiang-Chun	299
Gusmao	L	**0897**	PS1-146	New species and records of *Beltrania*-complex from brazilian semi-arid	Cruz A C R, Moraes Junior V O, Leão-Santos S M, Marques MFO, Barbosa FR	69
Guzman-Davalos	L	**0201**	PS1-36	A new bluing species of *Gymnopilus* (Cortinariaceae, Agaricales) from Mexico	Herrera M, Palomera V, Rodriguez A, Santerre A, Villalobos A	30
		0202	PS1-37	New records of *Gymnopilus* from America and Africa	ardona B, Herrera M, Mossebo DC, Saldarriaga Y	31
		0203	PS1-38	A taxonomic review of *Ganoderma resinaceum* (Ganodermatales, Ganodermataceae) complex	Torres-Torres MG	31
Hagen	F	**0281**	PS10-249	Where is the origin of the *Cryptococcus gattii* Vancouver Island outbreak?	Kuramae EE, Bovers M, Gerits DJC, Gerritzen C, Meyer W, Boekhout T	220
Hageskal	G	**0372**	PS6-221	Diversity of moulds species in Norwegian drinking water	Skaar I	129
Hallenberg	N	**0175**	PS1-27	Speciation and taxonomy in *Peniophora*		26
Hambardzumyan	L W	**0766**	PS10-364	Antifungal activity of the medicinal mushroom *Flammulina velutipes* (Curt: Fr) P Karst *in vitro*		226
Hambleton	S	**0651**	PS3-291	Using herbarium specimens to obtain DNA sequences and reveal genotypic diversity of two cereal rust pathogens	Liu M, Tropiano R, Robideau G	195
Handke	R	**0325**	PS5-525	Environmental Isolation of Entomophthorales from South Australia		362
Hara	K	**0518**	PS2-103	Cloning and Characterization of Secondary Metabolite Biosynthetic Genes from Lichens	Kominr M, Yamamoto Y	122
Hattori	T	**0345**	PS5-528	Diversity and distribution of polypores in Northern Thailand	To-anum C, Kakishima M	363
Hedayati	M	**0032**	PS10-335	Sera analysis of asthmatic patients for specific IgE to *Candida albicans* from Sari city-Iran	Myahi S, Aghili R, Goharimoghadam K, Mohammadpour R	214
Heller	G	**0364**	PS2-198	Transcriptomic analysis of biotic actions of the ectomycorrhizal fungus *Paxillus involutus*	LINDAHL Björn, TUNLID Anders, FINLAY Roger	120
Hemmes	D E	**0301**	PS5-522	Earthstars of the Hawaiian Islands: the five large *Geastrum* species	Desjardin D E	360
Himmelreich	U	**0624**	PS1-117	NMR spectroscopy: a tool for rapid yeast characterisation and screening	Dolenko B, Sorrell TC, Somorjay RL, Daniel H-M, Malik R	59

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Hirose	D	**0255**	PS1-46	*Oidiodendron* species isolated from the roots of *Rhododendron* spp growing the region near the tropic of Cancer in Taiwan	Tokumasu Seiji, Huang Jenn-Wen, Chang Ho-Shii, Kakishima Makoto	34
Ho	H M	**0318**	PS1-61	The genus *Coemansia* from Taiwan	Chien CY, Chuang SC	39
Ho	H H	**0224**	PS3-261	Patch canker disease of rubber trees on Hainan Island, China caused by *Pythium vexans*	Zeng HC, Zheng FC	182
Högnabba	F C H	**0335**	PS1-63	Evolution of cyanobacterial symbiosis in Ascomycota	Stenroos Soili, Thell Arne, Myllys Leena	40
Holighaus	G	**0680**	PS4-437	The enticing odour of fungi – A safety thread, tying insect-fungal associations?	Schütz S	293
		0911	PS4-446	Pathogenic Fungi on Horse Chestnut Trees– Volatile Organic Compounds as a Tool to Study Ecological Interactions	Johne A B, Weissbecker B, Schuetz S	295
		0912	PS4-447	The Enticing Odour Of Fungi – The role of Volatile Organic Compounds for a xylomycetophagous beetle and its fungal associates	Schuetz S	296
Hopkins	A J M	**0248**	PS5-515	Wood-Decay Fungi in CWD in Logged and Unlogged Wet Sclerophyll Forests in Southern Tasmania	Yuan Z-Q, Grove SJ, Wardlaw TJ, Yee M, Mohammed CL	358
		0250	PS5-516	Decayed Wood, Wood-Inhabiting Fungi and Saproxylic Beetles in Living Eucalyptus obliqua Trees in Southern Tasmania	Harrison KS, Grove SJ, Wardlaw TJ, Mohammed CL	358
Horn	B W	**0131**	PS6-219	Reduction of aflatoxins in wounded peanuts: role of competition by native Aspergillus species and applied nontoxigenic biocontrol strains	Dorner JW	128
Hosaka	K	**0431**	PS1-89	Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders	Bates ST, Beever RE, Castellano MA, Colgan W, Dominguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR, Simpson NB, Trappe JM	48
Hosoya	T	**0236**	PS5-513	A United Database of Fungal Specimens in Japan	Doi Yoshimichi, Kakishima Makoto, Hattori Tsutomu, Degawa Yousuke, Katumoto Ken, Maekawa Nitaro, Tsuda Mitsuya, Fukiharu Toshimitsu, Okada Gen, Kiyuna Tomohiko, Sugiyama Junta	357
Hou	Y-H	**0832**	PS10-371	Effects of drugs and chemical agents on cell-surface-hydrophobicity and adhesion of *Cryptococcus neoformans* to vero epithelial cells	Guo N-R, Wu S-X	229
Howlett	B J	**0887**	PS2-207	Phylogeny of gene clusters responsible for the biosynthesis of epipolythiodioxopiperazine (ETP) toxins	Cozijnsen AJ, Patron N, Waller RF	123
Hsieh	S Y	**0542**	PS4-425	Zoosporangium development and zoospore release of *Halophytophthora kandeliae*	Chan FL, Yuan GF	287

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Huang	X	**0952**	PS3-334	Pathogenic factors involved in infecting nematode by nematophagous fungi	Yang Jinkui, Dong Jinyan, Li Guohong, Luo Hong, Zhang Keqin	213
		0086	PS4-395	Purification and characterization of an extracellular serine protease serving as the potential pathogenic factor in *Clonostachys rosea*	Li Jun, Zhang Keqin	273
Hull	C	**0857**	PS10-374	Sex, virulence, and homeodomains in the fungal pathogen *Cryptococcus neoformans*	Stanton BC, Ekena JL, Staudt MW	231
Illman	B	**0508**	PS7-613	Putting fungi to work: mycoremediation of chemically treated waste wood		396
Inácio	C	**0970**	PS1-152	Novelties and conclusions from a monograph of the family Parmulariaceae	Paul F Cannon	71
Ivanov	D	**0380**	PS7-608	Prospects in use *Leccinum* sect Scabra as biomonitors of caesium-137 pollution in wood communities		394
Iwase	K	**0533**	PS9-176	Basidiomycetous fungi involved in orchid mycorrhiza	Yamato M, Yagame T, Suzuki A	81
Jacobs	K	**0375**	PS1-76	The genus *Leptographium*: a multigene approach to molecular characterization	Wingfield BD, Wingfield MJ	45
Jeamjitt	O A	**0405**	PS3-271	Coprophilous fungal diversity in Thailand: Studies on antagonistic activity against plant pathogenic fungi and secondary metabolites of *Ascodesmis macrospora* and *Sordaria fimicola*	Manoch Leka, Watling Roy, P Sharples George, Kijjoa Anake, Visarathanonth Niphon, Chamswarng Chiradej	186
Johnston	P R	**0207**	PS3-259	Detection of hyphae of endophytic fungi in leaf tissue	Sutherland PW, Joshee S	181
Jones	E B G	**0134**	PS1-20	Phylogeny of *Nemania eleiodoxae* based on 18S and 28S rDNA sequence analyses	Pinnoi A, Jeewon R, Hyde KD, Phongpaichit S	23
		0135	PS1-21	New fungal taxa described from peat swamps, mangroves, freshwater and on seeds, fruits and forest leaf litter in Thailand	Sakayroj J, Pinnoi A, Pinuran U, Somrithipol S, Pang KL	24
		0262	PS1-50	Basidiomycetes on plms and bamboo in Thailand, with special reference to the phylogeny of *Ganoderma colosuus* (*Tomophagus*) and *G. tsunodae* (*Trachyderma*)	Choeyklin R, Hattori T, Vikineswary S, Pang K-L	36
		0347	PS1-67	*Coelomycetes* and their teleomorphs, with special reference to the phylogeny of the cupulate genera *Infundibulomyces* and *Satchmopsis*	Plaingam N, Somrithipol S, Sakayaroj J	42
		0133	PS5-497	Endophytes of a peat swamp palm: *Licuala longecalycata*	Pinruan U, Pinnoi A, Hyde KD, Jeewon R	351
		0350	PS5-530	Fungal diversity of decomposing fruits and seeds in tropical forests in Thailand	Somrithipol S	364
Jones	E E	**0180**	PS3-255	Evaluation of *Trichoderma* bio-inoculant for control of Specific Apple Replant Disease (SARD)	Kandula DRW, Horner IJ, Tustin S, Stewart A	180

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Jones	E E	**0181**	PS3-256	Biological control of *Sclerotinia minor* in lettuce using *Trichoderma hamatum*	Rabeendran N, Moot DJ, Hunt JS, Stewart A	180
Jones	L	**0454**	PS3-278	Silencing of pathogenicity genes in the apple scab fungus, *Venturia inaequalis*	Plummer K M, Templeton M D, Cui W	189
Joshee	S	**0467**	PS5-538	Diversity and distribution of foliar fungal endophytes in New *Zealand podocarps*	Johnston PR, Paulus BC	367
Jung	H S	**0516**	PS5-545	Fungal diversity of the islands on the Yellow Sea of Korea	Kim CM	369
Juwadi	P R	**0498**	PS4-423	Identification of mating genes in the *Aspergillus oryzae* genome: studies towards understanding their role in its life cycle	Yamamoto Nanase, Maruyama Jun-ichi, Archer David B, Kitamoto Katsuhiko	286
Kageyama	K K	**0336**	PS5-526	Assessment of river environment using *Pythium* species	Hanai K, Tanahashi T, Senda M, Suga H	362
Kajimura	H	**0505**	PS1-102	Fungi isolated from an Attelabid beetle and its leaf roll	TAKABE Naoki, MASUYA Hayato	53
Kamgan Nkuekam	G	**0537**	PS1-104	*Ceratocystis* and *Ophiostoma* species associated with wounds on native South African trees	Roux J, Jacobs K, Wingfield M J	54
		0547	PS1-106	*Ophiostoma* spp associated with wounds on native broad-leaved trees in Norway	Roux J, Solheim H, Wingfield M J	55
Kaocharoen	S	**0704**	PS10-360	Molecular diversity of *Cryptococcus neoformans* isolates from cats	Banlunara Wijit, Younyouan Poomjit, Chindamporn Ariya	224
Karlshøj	K	**0852**	PS6-237	Potential of food safety prediction by detection of volatile biomarkers by electronic nose technologies	Nielsen PV, Larsen TO	136
Katiraee	F	**0136**	PS10-344	First Report of *Candida dubliniensis* in Iran Using Specific Primers	Khosravi Ali Reza, Soltani Minoo, Esmaeilzadeh Majid	218
Kaur	R	**0738**	PS3-304	Fluorescent *Pseudomonas* Isolates Suppressing Chickpea Wilt and Promoting Plant Growth in India	Astha, Singh Rama Shankar, Alabouvette Claude	201
Kauserud	H	**0275**	PS8-459	Phylogeography, cryptic speciation and hybridization in the cellar fungus *Coniophora puteana*	Bjorvand Svegården I, Hallenberg N, DeCock C	300
		0276	PS8-460	*Serpula lacrymans* as a model species to study micro-evolutionary processes	Högberg N, Svegården IB, Schmidt O, Stensrud Ø, Schumacher T	301
Kautto	L	**0991**	PS2-216	Characterisation of the *Trichoderma reesei* proteasome	Grinyer J, Bergquist PL, Te'o VSJ, Nevalainen KMH	126
Keirle	M	**0187**	PS8-454	Variability in the IGS1 region of *Rhodocollybia laulaha*	Avis Peter	298
Kemler	M	**0604**	PS1-113	Evolutionary patterns in *Microbotryum* - biological implications for basidiomycetous plant parasites	Begerow D, Göker M, Lutz M, Oberwinkler F	57
Khucharoenphaisan	K	**0673**	PS4-436	Highest thermostable xylanase produce from newly isolates *Thermomyces lanuginosus* strains	Kitpreechavanich Vichien	292
		0674	PS7-617	Selection beta-xylanase and beta-glucanase Produced *Aspergillus* and Factors Affecting Enzyme Production in Solid State Culture	Kitpreechavanich Vichien, Tongklib Cholnicha	398

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Kim	M S	**0642**	PS1-119	Molecular Characterization of *Fusarium commune* and *F. oxysporum* from a Conifer Nursery	Stewart Jane E, James Robert L, Dumroese R Kasten, Klopfenstein Ned B	60
Kim	S H	**0529**	PS2-204	SSH-based analysis of the genes expressed differentially in the primordia and basidioma of a medicinal mushroom *Hericium erinaceum*	Ko H K, Park H G	122
		0906	PS4-444	Analysis of cellobiohydrolase, pectinase, and xylanase in *Trichoderma* fungi using chromogenic media	Hyun MW, Yoon JH, Hong SB, Ko SJ	295
Kim	J G	**0982**	PS2-213	Proteome analysis of waito-c rice seedlings treated with culture fluid of gibberellin producing fungus, *Fusarium proliferatum* KGL0401	Rim SO, Lee JH, Hwang SK, Suh SJ, Lee IK	125
Kirisits	T	**0598**	PS5-560	Ophiostomatoid fungi associated with the spruce bark beetle *Pityogenes chalcographus* in Austria	Konrad H	375
Kitpreechavanich	V	**0791**	PS7-622	*Thermomyces lanuginosus* isolated from Thailand: high thermostable xylanase production and characterizations	Tokuyama Shinji, Khuchareanphaisan Khwanchai, Khonzue Parichart	400
Klenová	H	**0015**	PS3-241	Pathogenic variability of *Puccinia coronata* f sp *avenae* on oat in the Czech Republic	Šebesta Josef, Salava J	175
Klopfenstein	N B	**0640**	PS8-476	Phylogeographic patterns of *Armillaria ostoyae* in the western United States	Hanna JW, Kim M-S, McDonald GI, Moore JA	307
Kodsueb	R	**0050**	PS1-6	The family pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA	Jeewon Rajesh, McKenzie Eric H C, Lumyong Saisamorn, Aptroot André, Dhanasekaran Vijaykrishna, Hyde Kevin D	18
Kokaew	J	**0416**	PS3-274	Diseases of Weed in Vegetable Garden Plots and Their Potential Use for Biological Weed Control	Manoch Leka, Visarathanonth Niphon, Suwangul Duangporn	187
Kolarik	M	**0574**	PS5-556	*Scolytodes unipunctatus* (*Coleoptera*: Scolytinae): a new lineage of ambrosia beetles with a unique spectrum of nutritional fungi		373
		0578	PS5-557	*Geosmithia* fungi are highly diverse and consistent associates of bark beetles: a proof from their host preference in temperate Europe	Pazoutova S, Kubatova A	373
Kõljalg	U	**0621**	PS9-187	Frantic diversity of resupinate thelephoroid fungi on ectomycorrhizal tree roots	Tedersoo Leho, Kjøller Rasmus, Smith Matthew E	86
Komon-Zelazowska	M K-Z	**0286**	PS1-54	A recently emerging green mold disease in Eurasian oyster mushroom farms is caused by new species of *Trichoderma*	Zafari Dostmorad, Bissett John, Hatvani Lóránt, Zhang Chu-Long, Xu Tong, Woo Sheridan L, Manczinger László, Lorito Matteo, Kredics László, Kubicek Christian P, Druzhinina Irina S	37
Kondratyuk	S Ya	**0130**	PS1-19	To Revision of the Teloschistaceae (Ascomycota) of Australia	Karnefelt I	23

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Kopchinskiy	A	**0289**	PS1-56	TrichoMASTER: integrated multiloci database for *Hypocrea/Trichoderma* species identification powered by sequence diagnosis, oligonucleotide barcode and similarity search tools	Komon Monika, Kubicek Christian P, Druzhinina Irina S	38
Korolev	N S	**0111**	PS3-249	Evolution of *Botrytis cinerea* greenhouse population following introduction of marked selenate-resistant strains	Katan T, Elad Y	178
Koukol	O K	**0051**	PS4-390	Saprotrophic fungi transform organic phosphorus from spruce litter needles	Novak FN, Hrabal RH, Vosatka MV:	270
Kozlova	M	**0077**	PS4-394	Structural features in haloalkalitolerant ascomycete *Heleococcum alkalinum* Bilanenko et Ivanova responsible for its adaptation to extreme growth conditions		272
Krauss	G	**0391**	PS4-413	A strictly aquatic fungus can biotransform 1-naphthol	Schlosser D	282
		0392	PS5-531	Aquatic hyphomycetes – diversity and ecobiochemical response in polluted habitats		364
		0396	PS7-609	Bioconversion of trace contaminants by aquatic fungi	Martin C, Moeder M, Schlosser D	394
		0394	PS8-465	Use of 18S rDNA and TGGE to study aquatic hyphomycetes in polluted surface and groundwater	Solé M	303
Kshirsagar	A S	**0039**	PS1-4	The dilemma of Georgefischeriales systematics	Gandhe RV	18
		0052	PS4-391	Biochemical screening of some members of Xylariaceae	Gandhe RV, Wakharkar RD, Rhatwal SM	271
Kubono	T	**0211**	PS3-260	Disease Cycle of Japanese Cedar Sclerotia Dieback		181
Kullman	B	**0234**	PS4-401	Hybridization and heteroploidy as sources of biodiversity in filamentous fungi	Greve B, Severin G	276
		0239	PS4-403	Estimation of fungal genome size using DAPI- image cytometry	Teterin W	277
Kurihara	Y	**0190**	PS1-32	*Lecanicillium, Simplicillium, Pochonia*, and *Verticillium* species isolated from arthropod and soil samples collected in Indonesia	Sukarno Nampiah, Yuniarti Erny, Mangunwardoyo Wibowo, Park Ju-Young, Ando Katsuhiko, Widyastuti Yantyati	29
Kuvalekar	A A	**0063**	PS1-7	Phylogenetic positioning of *Ravenelia esculenta* using 18S rDNA sequence	Gandhe K R, Shauche Y C, Siddharth J	19
Kwan	H S	**0402**	PS2-199	Differential display, cDNA microarray, expressed sequence tags and serial analysis of gene expression (SAGE) reveal gene expression profiles of shiitake mushroom *Lentinula edodes* development	Chum WWY, Kwok ISW, Bian XL, Ng KTP, Shih RSM	120
Lättman	H	**0384**	PS8-464	Genetic variation in the SSU intron and the dispersal and migration history in Sweden of *Cliostomum corrugatum*	Mattsson J-E, Ekman S	302

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Lau	A	**0902**	PS10-378	Clinical utility of a panfungal PCR assay on tissue specimens for the rapid diagnosis of invasive fungal infection	Chen S, Sorrell TC, Marriott D, Halliday CL	233
Lawrie	A C	**0565**	PS9-179	Diversity And Germination Efficacy Of Mycorrhizal Fungi From *Caladenia formosa* GW Carr (Orchidaceae)	Huynh T, McLean CB	82
		0567	PS9-181	The Mycorrhizal Associations Of *Borya mirabilis* A Critically Endangered Australian Native Plant	Reiter NH, Walsh NG	83
		0580	PS9-183	Differences In Groups Of Orchid Mycorrhizal Fungi Infecting Groups Of *Caladenia* Species In Australia	Raleigh RE, Cross RG, Coates F	84
Lebel	T	**0343**	PS1-66	Weird sisters - affinities of some Australian truffle-like fungi		41
Lee	C-W	**0904**	PS4-442	Characterization of disruption mutant of phosphoinositide-specific phospholipase C gene plcA in the model filamentous fungus *Aspergillus nidulans*	Lee Eun Jin, Kim Jae Won	294
Lee	H-S	**0348**	PS3-268	Novel single-stranded RNA mycoviruses in edible mushrooms	Ro Hyun-Su, Kim Sung-Soon, Choi Jun-Oh, Kim Dong-Gyu, Won Hyo-Kyoung	185
Lee	H Y	**0330**	PS7-603	The Sexuality of Cordyceps militaris and Production of Cordycepin by Hybridization of *Monosprorous* strains	Su Ching-Hua	391
Lee	J S	**0244**	PS7-596	Isolation and characterization of a anticholesterolemic β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor from *Pholiota adiposa*	Lee D-H, No J-D, Lee D-H, Seo G-S, Cho S-M	389
		0245	PS7-597	Isolation and charaterization of a novel antithrombotic compound from mushrooms	Lee Dae-Hyoung, Lee Jae-Won, Cho Soo-Muk, Park Jeong-Sik	389
Lee	J W	**0225**	PS7-594	Evaluation on the utilization possibility of waste mushroom logs as biomass resource using enzyme analysis	Koo BW, Choi IG	388
		0848	PS7-629	Screening of wood rot fungi related to biodegradation of dimethyl phthalate and evaluation of its enzymes	Lee HJ, Park JY, Choi IG	403
Lee	T S	**0371**	PS10-351	Immunomodulatory and Antitumor Effects of Crude Polysaccharides Extracted from Korean Wild Medicinal Mushrooms	Shim Mi ja, Lee Min Woong	220
Lee	Y C	**0249**	PS7-598	Distribution Profiles of Ginsenosides in Korean Ginseng (Panax ginseng C A Meyer) Cultured with *Ganoderma lucidum* Mycelium	Rhee YK, Kwon MJ, Pyo YH	390
Leemon	D M	**0785**	PS10-366	Fungal biopesticides for tick and buffalo fly control		227
		0786	PS10-367	Fungal biocontrol of sheep lice (*Bovicola ovis*)	James P J, Sanson K R	227
Leslie	J F	**0880**	PS8-478	Diversity of *Gibberella zeae* populations isolated from wheat in Argentina	Ramirez M L, Reynoso M M, Farnochi M C, Torres A, Chulze S N	308

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Libkind	D	**0779**	PS1-130	Two novel ballistoconidia-producing yeasts species of the Sporidiobolales from aquatic environments in Patagonia, Argentina	van Broock M, Sampaio J P	63
Likhitekaraj	S	**0349**	PS5-529	Plant Diseases Herbarium in Thailand	Athipunyakom Pornpimon	363
Lima	N	**0877**	PS1-142	*Aspergillus ibericus*: a new species of section Nigri characterised by MALDI-TOF MS	Kallow W, Santos IM, Erhard M, Serra R, Venâncio A	67
		0406	PS6-222	Mycological examination and biofilm formation in drinking water	Paterson RRM, Gonçalves AB	129
Linde	C	**0444**	PS8-470	Origin And Expansion Of *Rhynchosporium secalis*, A Fungal Leaf Pathogen Of Barley	McDonald BA, Zaffarano PL	304
Liu	X Z	**0882**	PS1-143	Taxonomy and Molecular Phylogeny of Orbiliaceae from China	Liu B, Zhuang WY	67
Luangsa-ard	J J	**0265**	PS5-518	On the diversity of *Isaria* species from Thailand		359
Ludley	K E	**0176**	PS9-161	Mycelial response of ectomycorrhizal and saprotrophic fungi of coniferous forest soils to selected monoterpenes	Hartley-Whitaker J, Jickells S M, Chamberlain P M, Robinson C H	75
Lundén	K	**0223**	PS4-400	Gene expression during the switch from saprotrophic to pathogenic phases of growth in the root and butt rot fungi *Heterobasidion annosum*	Asiegbu F	276
Macreadie	I G	**0745**	PS10-363	Growth inhibition of *Candida* species and *Aspergillus fumigatus* by statins	Johnson G, Schlosser T, Macreadie P, Westermeyer C	225
Magan	N	**0087**	PS4-396	Water availability and metabolomic profiles of *Epicoccum nigrum* and *Sarophorum palmicola* grown in solid substrate fermentation systems	Aldred D, Penn J	273
		0089	PS6-217	Control of spoilage and ochratoxin a (ota) production in moist grain for animal feed using the biocontrol agent *Pichia anomala*	Mokiou S	127
		0807	PS6-236	Physiological relationship between food preservatives, environmental factors, ochratoxin and otapks gene expression by *Penicillium verrucosum*	Smidt-Heygdt M, Geisen R, Baxter E S	136
		0892	PS6-238	Identification of black aspergilli species responsible for ochratoxin A contamination of food using qualitative volatile fingerprints	Cabanes FJ, Sahgal N,	137
		0084	PS7-583	Differential breakdown of pesticide mixtures by *Trametes versicolor* and *Phanerochaete chrysosporium* by production of hydrolytic enzymes under different soil water potentials in soil microcosms	Fragoiero S	384

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Magan	N	**0090**	PS7-584	Medium optimization for the production of the secondary metabolite squalestatin S1 by a *Phoma* sp combining orthogonal design and response surface methodology	Parra R, Aldred D	384
		0097	PS7-585	Production of the biocontrol agent *Phlebiopsis gigantea* in controlled environmental and nutritional media for control of wood decay	Swanwick S, Aldred D	385
		0085	PS10-340	A population-based threshold model shows that manipulation of endogenous reserves increases virulence of insect pathogenic fungi	Chandler D, Anderson M	216
		0088	PS10-341	Use of volatile fingerprints (electronic nose) for early detection and discrimination between *Trichophyton* species (dermatophytes)	Sahgal N, Monk B, Wasil M	216
Manimohan	P	**0106**	PS4-397	Diversity of agarics on elephant dung	Thomas K A, Nisha V S	274
		0118	PS7-590	Isolation and *in vitro* cultivation of *Auriculoscypha anacardiicola*	Arun Kumar T K, Jisha E S	387
Mannanov	R N	**0094**	PS3-246	Diversity of fungal diseases in winter wheat fields under conditions of irrigated agriculture in Uzbekistan	Sattarova RK, Khakimova N	176
Mansouri	M	**0656**	PS6-231	Inhibitory Effects Of *Geranium Pelargonium* Oil On *Aspergillus flavus* Growth Rates	Khossravi Alireza, Chaichi Nosraty Arash, Modiri Leila, Faezi Ghasemi Mohammad	134
Mantle	P G	**0196**	PS7-593	An experimental strategy towards directing biosynthesis of communesin alkaloids by a *Penicillium* sp in submerged fermentation	Wigley LJ, Perry DA	388
Marin	M	**0932**	PS3-327	Genetic Characterization Of *Peronospora sparsa* On Rose In Colombia And Its Relationship With Sensitivity To The Qoi Fungicide, Fenamidone	Jaramillo S, Cotes JM, Argel LE, Ayala ML, Gúzman E	211
		0930	PS8-480	Phylogeny And Population Structure Of *Ceratocystis fimbriata* From Different Hosts In Colombia	Castro BL, Wingfield BD, Wingfield MJ	308
		0931	PS8-481	Genetic and Phenotypic Variability Of *Phytophthora infestans* From Colombia	Lagos LE, Jaramillo S, Raigosa N, Amaya MC	309
Maslen	M M	**0965**	PS10-385	Human and Animal Isolates of *Pseudallescheria boydii* and *Scedosporium* species Identified from 1977 to 1995		235
Masuya	H	**0183**	PS1-29	*Raffaelea* Species in the Gallery of Ambrosia Beetle, *Platypus quercivorus*	Ichihara Yu, Kubono Takanori	27
Matsuda	Y	**0103**	PS3-247	An Apparatus for Collecting Total Conidia of *Blumeria graminis* f sp *hordei* from Leaf Colonies using Electrostatic Attraction	Nonomura T, Toyoda H	177
Mattsson	J-E	**0905**	PS4-443	At what age becomes *Cliostomum corrugatum* adult?	Lättman H, Brand A, Hedlund J, Krikorev M, Olsson N, Rönnmark F	294

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Mattsson	J-E	**0531**	PS5-548	Macrolichen diversity in relation to diversity of woody plants	Vinter T, Lönn M	370
		0526	PS8-473	Genetic diversity and substrate preferences in *Hypogymnia physodes* in northern Europe	Hansson AC, Lindblom L	306
		0528	PS8-474	Patterns of genetic diversity of *Pseudevernia furfuracea* compared to chemistry, morphology and substrate ecology	Robeck A, Lindblom L	306
May	K J	**0910**	PS4-445	Expression patterns of a gene involved in secondary metabolism from the endophyte, *Epichloë festucae*	Bryant M, Zhang X, Ambrose B, Scott B	295
May	T W	**0709**	PS5-569	A global strategy for the conservation of fungi	Buchanan PK, Dahlberg A, Perini C	379
McLaughlin	D J	**0636**	PS5-563	Constructing a structural and biochemical database for the Fungi	Celio GJ, Padamsee M, Dentinger BTM	376
McLean	M S	**0200**	PS3-258	Prevalence and pathogenic variability of *Pyrenophora teres* f *maculata* from barley crops in Victoria	Platz GJ, Gupta S	181
McMullan-Fisher	S J M	**0515**	PS5-544	Will conservation based on vascular plants be sufficient for macrofungi and mosses - a Tasmanian study		369
McQualter	E	**0963**	PS9-192	SEM And PCR Study Of Mycorrhizal Fungi Isolated From The Australian Terrestrial Orchid: *Prasophyllum*	Cross R, McLean CB, Ladiges PY	88
Mejia	L C	**0913**	PS1-147	Redefinition of the genus *Cryptosporella* (Gnomoniaceae)	Castlebury L A, Rossman A Y, White J F Jr	69
		0482	PS5-540	Leaf litter and leaf age as factors affecting the assemblage of endophytes associated with particular hosts	Herre E A, Butler A, Rojas E I, Ramirez L, Van Bael S	367
Melo	I	**0222**	PS5-511	Fungi of Azores: *Corticiaceae* s l	Béltrán-Tejera E, Cardoso J, Dueñas M, Rodríguez-Armas J L, Salcedo I, Tellería M T	356
Minato	K	**0272**	PS10-247	Immunomodulating action of edible mushrooms, *Pleurotus cornucopiae* var *citrinopileatus* and *Pholiota nameko*	Kasahara S	219
Minchin	R F	**0450**	PS9-170	Effect of *Trichoderma* bio-inoculants on ectomycorrhizal colonisation of *Pinus radiata* seedlings	Hill RA, Condron LM, Ridgway H, Baldauf S, Jones EE	78
Minter	D W	**0412**	PS5-532	Conservation of non-lichen forming microfungi	Romero AI, Camino Vilaró M, Tykhonenko YuYa, Nanagulyan S, Sankaran KV, Rong I	364
Minter	D W	**0553**	PS5-554	Digital libraries for systematic mycology	Kirk PM	372
Mirabadi	S	**0679**	PS3-294	Natural occurrence of *Alternaria* sp the casual agent of stem spots on *Berberis thunbergii*	Golnaraghi Alireza, Rezaee Saeid	197
Mishra	J K	**0143**	PS4-398	Stachylina in India: occurrence and relationships with temperature and hydrogen-ion concentration of the waters		275

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Mnyazi	J J	**0927**	PS9-190	The composition and distribution of Arbuscular Mycorrhizal Fungi (AMF) under different ecological conditions	Vanlauwe B, Rashid M, Kahangi E, Odee D, Ruto L, van Asten P, Sinclair R, van Greuning V	87
Modiri	L	**0116**	PS4-397	A Qualitative Investigation On Tea Processing Houses Air Fungal Pollution In The North Of Iran , Gilan Province Estern Region	Chaichi-Nosraty Arash, Faezi Ghasemi Mohammad, Khosravi Ali Reza, Massiha Ali Reza, Roofigary Haghighat Shiva	274
Mohali	S	**0759**	PS3-306	Botryosphaeriaceae infecting *Eucalyptus, Acacia* and *Pinus* in Venezuela	Slippers B, Wingfield MJ	202
Mohammadi	A	**0839**	PS3-314	Detection of *Phytophthora nicotianae* from naturally infested soil, using soybean leaf disk baiting technique	Alizadeh Azizollah, Ranjbaran Masoumeh	205
		0866	PS3-319	A first report of *Phytophthora sojae* race1 from Moghan, Iran	Alizadeh Azizollah	208
Mohammed	C	**0686**	PS5-566	A decade of rotten research in the wet sclerophyll forest of Tasmania	Grove S, Yee M, Hopkins A, Harrison K, Stamm L, Zi Qing Y, Gates G, Wardlaw T	377
Mongkolsamrit	S	**0490**	PS1-98	Aschersonia badia from Thailand: one or more species?	Hywel-Jones NL, Spatafora J	52
		0499	PS1-101	A five-gene phylogeny for *Hypocrella* and its anamorph *Aschersonia*	Hywel-Jones NL, Sung Gi-Ho, Spatafora J	53
Montagna	M T	**0278**	PS10-248	Presence of fungi in the nose, throat and ear of Kurdish refugees in Apulia (Italy)	Napoli C, Iatta R, Tatò D, Da Molin G	220
Moosawi-Jorf	S A	**0758**	PS3-305	*Viable teliosporogenous* mycelia of *Neovossia indica* in infected grains of wheat		202
Mossebo	D C	**0112**	PS1-16	New records of *Termitomyces* (basidiomycetes) from Cameroon and Central Africa: taxonomy, ecology and phylogeny	Dominique Claude, Courtecuisse Régis, Kalman Vanky	22
Mostert	L	**0369**	PS1-73	Phylogenetic trends in the Calosphaeriales	Réblová M, Crous PW, Gams W	44
		0377	PS1-77	Further insights gained into *Togninia* (teleomorphs of *Phaeoacremonium*)	Gams W, Crous PW	45
		0664	PS3-293	Cylindrocarpon spp associated with black foot disease of grapevines in New Zealand	Fourie PH, Halleen F, Jaspers MV, Crous PW	196
Muid	S	**0561**	PS7-514	Poroid Mushrooms For Pulp Production	Mahidi Noreha	373
Muraguchi	H	**0476**	PS4-420	The *Coprinus cinereus eln6* gene involved in stipe elongation during fruit body maturation encodes a putative glycosyl transferase	Murayama T, Kamada T, Yanagi S O	285
Nakatani	A K	**0728**	PS1-128	Genetic diversity of *Rhizoctonia* species revealed by ITS sequencing	Souza NL, Boekhout T, Stalpers JA, Kuramae EE	63
Nasim	G N	**0535**	PS9-177	Title: "Role of mycorrhizae of grasses and ferns in controlling hill erosion in Mountainous areas of Pakistan"Authors: Ghazala Nasim & Rukhsana Bajwa	Bajwa Rukhsana	81

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Nasim	G N	**0538**	PS9-178	Response of wheat (*Triticum aestivum* cv Pak 81) grown in open top chamber system to various arbuscular mycorrhizal treatments	Bajwa Rukhsaana	82
Nauta	M M	**0895**	PS1-145	The flora agaricina neerlandica project: the importance of morphological studies		68
Nelson	B D	**0900**	PS2-208	Transformation of *Sclerotinia sclerotiorum* with the green fluorescent protein gene	de Silva A, Bolton M D	123
Neves	M A	**0080**	PS1-9	Phylogenetics of *Phylloporus* (*Boletales*) species based on the large subunit rDNA	Halling Roy E	19
Newbound	M G	**0479**	PS9-173	The influence of urbanisation and management practices on ectomycorrhizal fungal associates of two native species of *Eucalyptus*		79
Nonomura	T	**0105**	PS3-248a	Symptomatic Evidence for differential Root Invasion by *Fusarium Crown and root rot Pathogens between common tomato Lycopersicon esculentum* and its varieties	Y Matsuda, H Toyoda	177
Noordeloos	M E	**0694**	PS1-123	TAXONOMY and phylogeny of *Entoloma* (Agaricales, Basidiomycetes) in Tasmania	Gates G, Co DLV	61
Nordahl	M	**0530**	PS3-282	Effects of winter hardening and winter temperature shifts on *Pinus sylvestris - Gremmeniella abietina* plant-pathogen interactions	Stenlid J, Stenström E, Barklund P	191
Nordén	B	**0123**	PS5-496	Wood-fungi diversity, dead wood relations and habitat management in oak-dominated forest	Ryberg M	351
Novotny	D	**0356**	PS1-69	Ophiostomatoid fungi of the Czech Republic		42
		0359	PS1-70	Endophytic fungi of branches and leaves of the apple trees from the Czech Republic		43
		0360	PS1-71	Endophytes of branches and leaves of grapevine (*Vitis vinifera*) in the Czech Republic	Šilhánová M	43
Novotný	C	**0102**	PS7-586	Selection of ligninolytic fungi and application to bioremediation of contaminated water and soil	Svobodová K, Erbanová P, Schoeberl P, Pavko S, Rehorek A, Fuchs W	385
Nygren	K H M	**0981**	PS2-212	Evolutionary pressures on two pheromone receptor genes in heterothallic *Neurospora* species	Karlsson M, Johannesson H	125
Oertel	B	**0387**	PS1-82	Further Saccardo's omissions of non-lichenized fungi: the forgotten Sydow's lists for 1895-1918 and Petrak's real first "List" for 1919		46
Ogawa	Y O	**0346**	PS8-462	Geographical distribution of intraspecific groups of *Umbelopsis ramanniana* and the genetic variations of nLSU rDNA in their local populations	Yamazaki Y, Hirose D, Tokumasu S	302
Okada	G	**0464**	PS1-93	Synnematous fungi from Taiwan (1)	Tanaka K, Huang J-W, Chang H-S, Kakishima M	50

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Okada	G	**0474**	PS1-95	The anamorph of *Amphiporthe aculeans*, a pathogen of sumac and other Rhus species	Seifert KA	51
Okane	I	**0536**	PS5-549	Assemblages of endophytic fungi on *Salicornia europaea*	Nakagiri A	370
Okungbowa	F I	**0041**	PS3-242	Antifungal activity of leaf extracts of six asteraceous plants against *Fusarium oxysporium*	Edema NO	175
		0042	PS4-389	Mechanical disruption of *Candida* cells for protein estimation and enzymatic activity	Okungbowa MO	270
Olila	D	**0778**	PS10-365	Antibacterial activity of extracts of selected indigenous edible and medicinal tropical mushrooms	Opige M, Kateyo E, Kabasa JD	226
Olsson	J	**0560**	PS5-555	Prescribed Fire And Wood Inhabiting Fungi In A *Pinus sylvestris* Forest	Jonsson BG	372
Olstorpe	J M M	**0355**	PS6-220	Characterisation of the Fungal and Bacterial Diversity in Pig Feed Fermented with Whey, Wet Wheat Distillers´grain or Water at Different Temperatures	Lyberg K, Lindberg JE, Schnürer J, Passoth V	128
Orlovich	D A	**0447**	PS9-168	Molecular Diversity Of Ectomycorrhizal Fungi Associated With Southern Beech In New Zealand	Stephen MR, Draffin SJ, Roberts AE	77
Osmundson	T W	**0242**	PS5-514	Some noteworthy species of *Tylopilus* (Boletaceae) from northern Queensland, Australia	Halling RE	357
Osono	T	**0110**	PS5-493	Changes in microfungal communities during decomposition of leaf litter		350
Pampolina	N M	**0577**	PS9-182	Diversity of Mycorrhizal Fungi and Associated Plants Growing in Copper-rich Abandoned Mine Site	Aggangan Nelly S, Cadiz Nina M, Raymundo Asuncion K	84
Pang	K L	**0845**	PS7-628	Phthalate degradation by mangrove soil fungi	Wong MKM, Gu JD, Vrijmoed LLP	403
Park	J-Y	**0373**	PS1-74	Morphology and Molecular Phylogeny of a New Synnematous, Dimorphic *Bloxamia* Species from Indonesia	Inaba S, Mangunwardoyo W, Kanti A, Widyastuti Y, Ando K	44
Park	M S	**0251**	PS3-262	Development of the species-specific primer for the identification and detection of *Alternaria panax* causing leaf spot on ginseng	Lee Hye Min, Kim Won Ki, Yu Seung Hun	182
		0253	PS3-263	Molecular characterization of *Stemphylium* isolated from several host plants in Korea	Kong Dong Wook, Yu Seung Hun	182
Paul Gamboa-Trujillo	P G	**0685**	PS5-565	Etnomycology notes of eatable macromycetes in the Ecuador	Andi D, Grefa G	377
Paulus	B C	**0309**	PS5-523	Impact of logging on wood decay fungi in fine woody debris in a New Zealand forest	McDermott K, Johnston PR	361
		0315	PS5-524	Factors shaping microfungal communities in litter in Australian wet tropics rainforest	Gadek P, Hyde KD	361
Pavlik	M	**0421**	PS5-533	Macromycetes of the prosisko natural reserve	Pavlikova J	365

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Pavlik	M	**0597**	PS7-615	Evaluation of oyster mushroom growing on woody blocks		397
Pearce	C A	**0920**	PS5-578	Queensland DPI&F Biosecurity – enhancing North Queensland's protection against exotic plant diseases		382
Peintner	U	**0290**	PS9-163	Interactions of soil fungal communities with ectomycorrhizal host plants in an alpine environment	Oberkofler I, Mühlmann O, Kuhnert R, Bacher M	75
Perini	C	**0596**	PS5-559	A european red list for larger fungi in progress	Dahlberg Anders	374
		0590	PS9-184	Orchids and larger fungi: does a relationship exist above ground	Salerni Elena, Pecoraro Lorenzo, Leonardi Pamela, De Dominicis Vincenzo	85
Petrovic	T	**0520**	PS8-472	Genetic diversity of *Fusarium thapsinum* isolates from different hosts in Australia	Bentley A R, Leslie J F, Liew E C Y, Summerell B A, Burgess L W	305
Pfister	D H	**0287**	PS1-55	Chorioactidaceae: A new family of *Pezizales*	Hansen K, Slater C	37
Pieterse	Z	**0082**	PS3-245	The interaction between *Mycogone perniciosa*, the causal agent of wet bubble disease, and *Agaricus bisporus*	Aveling TAS, Labuschagne PM	176
Pilumwong	J	**0964**	PS6-240	Invasion pathway of peanut flower by green fluorescence protein *Aspergillus flavus*	Senthong Chuckree, Ingram Keith, Srichuwong Sombat, Meechoui Sawit	138
Piriyaprin	S	**0557**	PS3-284	Biological control of *Pythium* and *Sclerotium* root rot of sunn-hemp and mungbean by *Gliocladium virens*	Manoch Leka, Sunantapongsuk Vanlada, Somrang Ard	192
Plummer	K M	**0326**	PS2-196	Microarrays & gene silencing for functional genomics of *Sclerotinia sclerotiorum*	Sexton A, Drew M, Greenhill A, Edwards D, Gardiner D, Howlett BJ, Ridgeway H, Weld R	119
Promputtha	I	**0600**	PS4-432	Enzymatic activity of endophytic fungi on leaf decomposition	Hyde Kevin D, Peberdy John F, Lumyong Saisamorn	291
Rajagopal	K	**0022**	PS1-1	Detection of fungal endophyte DNA contamination using ITS primers in two grass species	Mahendran TS, Radhika Kumari R, Sathya TN, Shobana R	17
		0124	PS10-343	Characterization of two morphologically different *Phoma* species isolated from the same host using Somatic Incompatibility Test (SIT) and Isoenzyme analysis	MrTS Mahendran, MrVSuresh	217
Rakshit	A	**0016**	PS9-154	Significance of arbuscular mycorrhiza for P influx of maize and groundnut in an Oxisol	Bhadoria PBS, Ghosh S	72
Rameshaiah	G N	**0825**	PS7-627	Chitinases - Fungal Metabolites	Panda T	402
Ramos-Pamplona	M	**0886**	PS4-441	Fatty acid beta-oxidation pathways during *Magnaporthe pathogenesis*	Naqvi NI	294
Ranathunge	N P	**0901**	PS3-323	Infection of chilli pepper (*Capsicum* spp) plants by *Colletotrichum capsici* at early stages of growth	Ford R, Taylor P	209
Reddy	P	**0733**	PS3-301	The invasion and systemic transmission of *Aspergillus flavus* - a soybean seed pathogen	Berjak P	200

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Reddy	P	**0683**	PS6-232	The efficacy of a cathodic treatment in controlling inherent mycoflora levels in soybean seeds	Berjak P	134
		0805	PS6-235	Elimination of inherent seed-associated mycoflora of soybeans: efficacy of microwave irradiation versus cathodic protection	Berjak P	135
Redhead	S A	**0432**	PS1-90	Conservation of species names for economically significant mushrooms		49
Reese Næsborg	R	**0195**	PS1-35	A phylogenetic study of *Lecania* and closely related genera		30
Reiter	N	**0470**	PS9-172	Mycorrhizal associations of *Borya mirabilis* a critically endangered Australian native	Lawrie AC, Walsh N	79
Rerngsamran	P	**0864**	PS7-630	Biodegradation and adsorption of phenanthrene by fungi isolated from Thailand	Supanchanaburee Duangdoan, Visetkoop Sirirat	403
Reynaga-Peña	C G	**0928**	PS3-326	Understanding basic aspects of *Ustilago maydis* pathogenesis with the use of alternate hosts	Ruiz-Herrera J	211
Ribichich	K F	**0073**	PS2-193	A glance at *Blastocladiella emersonii* biology through a comparative EST analysis	Georg R C, Gomes S L	118
		0435	PS4-417	Characterization of Cdk-related kinases from *Blastocladiella emersonii* during its life cycle	Gomes S L	284
Ridkaw	R	**0314**	PS1-60	Molecular characterization of *Beauveria* species from Thai forests	Luangsa-ard JJ	39
Rittiboon	A	**0317**	PS7-599	Isolation, Selection, and Optimization for Xylanase Production from *Aspergillus niger* from Soil in Thailand	Prebou Rewadee, Somrithpol Sayanh	390
		0319	PS7-600	Isolation, Selection, and Optimization for Xylanase Production from *Aspergillus niger* Isolated from Soil in Thailand	Prebou Rewadee, Somrithpol Sayanh	390
		0712	PS7-618	Isolation, Selection and Optimization for Xylanase Production from *Aspergillus niger* Isolated from Soil in Eastern Thailand	Prebou Rewadee, Somrithpol Sayanh	398
Ro	H	**0742**	PS1-129	Molecular genetic classification of edible mushroom *Pleurotus eryngii* cultivated in Korea	Kim SS, Kim SW, Lee HS	63
Robinson	R M	**0213**	PS1-40	Stipitate hydnoid fungi of Southern Australia	Syme K, Lebel T, May TW	32
Rodtong	S	**0194**	PS1-34	Genetic Variation within ITS1 Region of *Hypoxylon* Species Found in Thailand	Suwannasai Nuttika, Thienhirun Surang, Whalley Anthony JS	30
		0219	PS1-42	Ribosomal DNA and ITS Sequence Heterogeneity of *Astrocystis* and *Rosellinia* from Thailand	Suwannasai Nuttika, Thienhirun Surang, Whalley Anthony JS	33
		0238	PS4-402	Lectin Accumulation in Edible Wild Mushrooms in Northeastern Thailand	Pikul-ngoen Yubon	277
Roets	F	**0260**	PS1-49	Molecular phylogeny of ophiostoma spp associated with *Protea* species	Dreyer L L, Wingfield M J, Crous P W, De Beer Z W	35

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Roets	F	**0261**	PS3-265	Fungus-arthropod mutualism and dispersal biology of ophiostoma spp inhabiting *Protea* flower-heads	Dreyer L L, Crous P W, Wingfield M J	183
Rosendahl	S	**0514**	PS5-543	Global and local genetic diversity of arbuscular mycorrhizal fungi		369
		0383	PS9-166	Low doses of fungicides influence competition and functioning of arbuscular mycorrhizal fungi	Gregersen R, Jakobsen I	77
Roux	J	**0338**	PS1-64	*Chrysoporthe* species on Myrtales in Southern Africa	Nakabonge G, Gryzenhout M, Wingfield MJ	40
		0339	PS1-65	*Sporendocladia bactrospora* associated with wounds of Norwegian broad-leaved trees: A potential pathogen	Solheim H, Kamgan Nkuekam G, Wingfield MJ	41
Rouxel	T	**0420**	PS2-200	The *Leptosphaeria maculans* genome initiative	BALESDENT M H, HOWLETT B J, WINCKER P	120
		0534	PS2-205	Genome environment is instrumental in evolution towards virulence at the *AvrLm1* avirulence locus of *Leptosphaeria maculans*	GOUT L, KUHN ML, BALESDENT M H	122
		0419	PS4-416	The H+-ATPase *LmPMA1* is involved in pathogenicity of *Leptosphaeria maculans* on oilseed rape	Remy E, Meyer M, Blaise F, Balesdent M H	283
Roy	B	**0790**	PS3-309	First report of *Alternaria* leaf blight caused by *Alternaria alternata* on *Hevea brasiliensis* in India	Zachariah C A, Jacob C K, Saha T	203
Rungjindamai	N	**0107**	PS1-14	The Internal Transcribed Spacer (ITS) Based Molecular Identification of Endophytic Fungi from *Garcinia* spp which Produce Antimicrobial Substances	Phongpaichit Souwalak, Rukachaisirikul Vatcharin, Sakayaroj Jariya	21
Ryberg	M	**0397**	PS1-85	Phylogenetic studies of the section Rimosae (Agariacales, *Inocybe*)	Larsson E, Jacobsson S	47
Ryley	M J	**0186**	PS1-30	A taxonomic revision of the Australian plant-infecting clavicipitalean fungi	Shivas RG	28
Sadravi	M	**0611**	PS5-561	Sixteen rust fungi from Northeast of Iran	Ono Yoshitaka, Pei Ming	375
		0614	PS9-186	Arbuscular mycorrhizal fungi of wheat in northeast of Iran		86
Sakalidis	M L	**0996**	PS1-100	*Botryosphaeria parva* and *B. ribis* - A question of species	Barber P A, Hardy Gest J, Wingfield B D, Burgess T I	
Sakamoto	Y H	**0243**	PS4-404	Identification and characterization of differentially expressed genes following harvest of the *Lentinula edodes* fruiting body	Nakade Keiko, Sato Toshitsugu	278
Salar	R K	**0033**	PS4-388	Extracellular enzymes from thermophilic fungi		269
Salcedo	I	**0285**	PS5-521	Mycocoenological characterization of evergreen oak forests in the Basque Country (North Spain) and its relation to biotic and abiotic factors	Sarrionandia E, Rodríguez N, Olariaga I	360
		0288	PS8-461	Intraespecific variability assessment in *Clavulina coralloides* complex, inferred from ITS region and morphological data	Olariaga I, Jugo B M	301

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Salcedo	I	**0603**	PS9-185	Inoculation with species of *Rhizopogon* and *Scleroderma* to improve the quality of forestal plants produced in nurseries	Rodriguez-Iturrizar N, Hormilla S, Sánchez-Zabala J, Sarrionandia E, Txarterina K, Duñabeitia MK	85
Sampaio	J P	**0947**	PS3-331	Postharvest biocontrol of *Penicillium* using yeasts of the genera *Pseudozyma* and *Rhodosporidium*	Alves L	212
Sanchez	C	**0894**	PS7-632	Isolation and identification of dioctyl phthalate-degrading fungi by enrichment cultures from soil	Kertesz M, Robson G	404
Sari	E	**0948**	PS3-332	Biological control of *Gaeumannomyces graminis* on wheat with *Bacillus* spp	Etebarian Hassan Reza, Aminian Heshmat allah, Rostaii Ali Mohammad	213
Sarma	V V	**0157**	PS5-503	Fungi on Nypa fruticans - a mangrove palm	Hyde KD	353
Sasikala	B	**0053**	PS10-336	Antifungal activity of Thuja occidentalis extracts on *Candida albicans*	Ramesh Velu, Senthil Kumaran Rangarajulu	214
Sato	H	**0510**	PS1-103	Four species of *Trichomycetes* collected in Thailand	To-anun C, Kakishima M, Tokumasu S	54
		0495	PS9-175	Recognition of cryptic species and host specificities in the ectomycorrhizal genus *Strobilomyces* using molecular markers	Murakami N	80
Sato	T	**0698**	PS1-124	A New species of the genus *Aprosporella parasitic* on a oriental orchid, *Cymbidium kanran*	Yamamoto I, Tomioka K	61
		0699	PS1-125	A new species of the genus *Pseudocercospora* isolated from *Dendrobium phalaenopsis*	Mori M, Tomioka K	62
		0697	PS3-297	Pathogenicity of *Ramularia pratensis* to *Rumex japonicus* and methods of its artificial reproduction and long term preservation	Tomioka K	198
		0700	PS5-567	Microorganisms section of the NIAS Genebank	Nagai T, Tomioka K, Aoki T, Sawada H	378
		0702	PS5-568	A Method of Long Term Preservation of *Phytophthora* and *Pythium* in Vapour Phase of Liquid Nitrogen	Takeuchi K, Tomioka K, Nagai T	378
Schatz	S	**0120**	PS10-342	Identification and estimation of fungi in the ocular tear film and the contact lens biofilm	Laubach H, Adams K	217
Schigel	D S	**0385**	PS1-80	Phylogeny of the *Postia* – *Oligoporus* complex of poroid Basidiomycetes	Niemelä T, Larsson KH, Larsson E	46
Schnürer	J	**0610**	PS6-224	Microbiological and nutritional properties of *Rhizopus oligosporus* fermented barley tempeh	Feng Xinmei, Passoth Volkmar, Eklund-Jonsson Charlotte, Alminger Marie	130
		0960	PS7-634	Domestication of Microorganisms (DOM) – A research programme on safety assessment, production and formulation of microorganisms for envirobiotech applications		405
Schrank	A	**0823**	PS7-626	Chitinases from the biocontrol agent *Metarhizium anisopliae*	Staats CC, Silva MS, Baratto CM, Boldo JT, Palma LP, Vainstein MH	402

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Scott	B	**0888**	PS3-320	Reactive oxygen species play a role in regulating a fungus-grass mutualistic interaction	Tanaka A, Takemoto D, Park P	208
		0889	PS3-321	Recruitment of a p67phox-like regulator controls hyphal branching in a fungal-plant mutualistic symbiosis	Takemoto D, Tanaka A	208
Sekimoto	S	**0279**	PS3-266	The ultrastructural morphology of *Eurychasma dicksonii*, an oomycete endoparasite of filamentous phaeophyte algae	Beakes GW, Küpper FC, Müller DG, Honda D	184
Senda	M S	**0341**	PS5-527	*Pythium* species in cool-temperate forest soil	Kageyama K	363
Senthil Kumaran	R	**0043**	PS1-5	Morphometric taxonomy in the diversity of some South Indian species of *Phyllosticta*	Muthumary John Paul	18
		0044	PS7-581	Studies on the production of the bioactive secondary metabolite, taxol - an anticancer drug by *Phyllosticta* spp	Muthumary John Paul	383
		0045	PS7-582	Effect of photoperiod on the growth and taxol production of *Colletotrichum capsici*	Muthumary John Paul, Sudhakaran Reshmi	383
Sergeeva	V	**0443**	PS3-275	Appendaged coelomycetes on grapevines in australia	Priest M, Nair N, Spooner-Hart R	188
Sergeeva	L E	**0828**	PS5-574	DIVERSITY OF MICROMYCETES IN LIBRARIAN ECOTOPES		380
Sexton	A	**0246**	PS2-195	Identification of *Sclerotinia sclerotiorum* genes expressed in early stages of *Brassica napus* infection	Cozijnsen A, Keniry A, Jewell E, Love C, Batley J, Edwards D, Howlett B	118
Shabbir	A	**0552**	PS3-283	High altitudinal plant ailments	Nasim Ghazala, Iqbal SH, Younas Maryam, Ali Muhammad	192
Shahi	S K	**0865**	PS3-318	Utilization of essential oil as herbal pesticide fight post harvest spoilage in fruits, *Malus pumilo*	Shahi MP	207
		0422	PS10-353	Botanical antifungal drug from lichen metabolites fights fungal infections	Shahi MP	221
Sharifnabi	B	**0095**	PS1-12	PCR-RFLP Patterns Of 58 S And ITS2 Genes In The Taxonomy Of Neotyphodium Endophytic Fungi	Dehghanpour Farashah, Saeede, Mirlohi Aghafakher	20
		0096	PS5-492	Endophytic Species Of *Neotyphodium* On Some *Gramineous* Species In Iran	Dehghanpour Farashah Saeede, Balali G Reza, Mirlohi Aghafakhr	350
Sheila Okoth	D A	**0989**	PS5-580	Land use systems and distribution of *Trichoderma* species in Embu Region, Kenya	Roimen R H, Mutsotso B, Owino J O, Okoth P	383
Shimizu	K	**0933**	PS2-209	Functional analysis of hybrid histidine kinase genes of *Cryptococcus neoformans*	Li Haoman, Kawamoto Susumu	124
Shimomura	N	**0257**	PS4-406	A homothallic mutant induced by UV irradiation in *Lentinula edodes*	Murakami Shigeyuki, Matsumoto Teruyuki, Maekawa Nitaro, Hasebe Kozaburo	279
Shirouzu	T	**0122**	PS5-495	Fungal succession on fallen leaves of an evergreen oak in Japan	Tokumasu S	350
Shivas	R G	**0113**	PS1-17	Some smut fungi (Ustilaginomycetes) from Thailand	Athipunyakom P, Vánky K	22

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Shnyreva	A V	**0075**	PS8-453	Population genetics of the basidiomycetous fungus *Pleurotus pulmonarius* based on haploid-dikaryotic life cycle		298
Shoji	J	**0461**	PS4-419	Pleiomorphic vacuoles in *Aspergillus oryzae* and their possible involvement in nutrient recycling	Arioka M, Kitamoto K	285
Shokohi	T	**0924**	PS10-380	Fungal keratitis in patients with corneal ulcer referred to a tertiary care hospital	Nowroozpoor-Dailami Kiumars, Moaddel-Haghighi Tahmineh, Hedayaty Mohamad-Taghi, Khalilian Ali-Reza	233
		0939	PS10-382	Propective typing of *Candida* species in oncology patients, A mulicenter study- preliminary results	Fatahi Meiababdi Masoomeh, Okhovatian Ali, Tamaddoni Ahmad, Ayaz Masoud, Moslemi Dariush, Karami Hossein, Hedayaty Mohammad Taghi	234
Simpson	J A	**0984**	PS5-579	The rusts of Myrtaceae	Thomas K, Grgurinovic C	382
Singh	R R	**0747**	PS5-571	Cytotoxic assessment of waters harbouring watermoulds		379
Singh	C J	**0732**	PS10-362	Hydrolytic enzymes as the virulence factors of dermatophytes		225
Sivichai	S	**0525**	PS5-547	Conservation and utilization of fungal biodiversity at BIOTEC Culture Collection (BCC) Thailand	S chunhametha, S Saidaengkham, W Potacharoen, K Kirtikara, M Tanticharoen	370
Skaar	I	**0389**	PS10-352	Possible impacts of fungi in sow-bedding for abortions and reproductive disorders	Christensen E, Framstad T, Jørgensen A, Lium B	221
Smith	Z	**0858**	PS9-189	Finding a mycorrhizal fungus for reintroductions of the threatened terrestrial orchid *Diuris fragrantissima*	McLean CB, James EA	87
Sogonov	M V	**0092**	PS1-10	Generic trends in the Gnomoniaceae, Diaporthales	Catslebury LC, Mejía LC, Rossman AY, White JF	20
Solarska	S	**0477**	PS7-612	Isolation of NOM degrading fungi	Roddick F, Lawrie A	396
Sonjak	S	**0083**	PS5-491	Filamentous fungi from coastal Arctic environment	Frisvad JC, Gunde-Cimerman N	349
Sotome	K	**0237**	PS1-45	Phylogenetic relationships of the *Polyporus* sensu lato and allied genera	Ota Y, Hattori T, Kakishima M	34
Srivastava	A	**0695**	PS3-294	Biocontrol of root rot of chili caused by *Rhizoctonia solani* with a formulation of *Trichoderma harzianum* secreting extracellular chitinase	Arora Dilip K	197
		0696	PS3-296	Anti-fungal properties of extracts of toxic *Microcystis aeruginosa*: potential to biocontrol the plant pathogens	Srivastava Madhumita, Tiwari Shree Prakash	197
Stadler	M	**0861**	PS5-576	Metabolic and molecular biodiversity – evidence from a survey of New Caldedonian Fungi and New Zealand Xylariaceae	Seng JM, Buchet S, Wetzstein HG	381
Stajic	M	**0114**	PS7-588	Ligninolytic Enzyme Production in *Pleurotus ostreatus* Depending on the Medium Composition and Cultivation Conditions	Vukojevic J, Wasser SP, Nevo E, Duletic-Lausevic S	386

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Stchigeli	A M	**0174**	PS1-26	Reappraisal of *Chaetomium ampullare* Chivers and *Coniochaeta emodensis* Udagawa & Y Hori from soil	Miller A N, Guarro J	26
Steffen	K T	**0563**	PS4-427	The litter- decomposing fungus Mycena epipterygia produces a novel hybrid enzyme of lignin and phenol-oxidizing peroxidases	Walter E, Ullrich R, Hintikka V, Hofrichter M	288
		0496	PS5-541	Oak (*Quercus petraea*) leaf litter degradation by litter-decomposing fungi	Cajthaml T, Snajdr J, Baldrian P	368
Stensrud	Ø	**0386**	PS1-81	Molecular phylogenies suggest polyphyly in morphotaxa of brown-spored agarics	Gulden G, Kauserud H, Høiland K, Schumacher T	46
		0388	PS1-83	Accelerated evolution and profound AT bias among lineages of the medicinal fungus *Cordyceps sinensis*	Schumacher T, Shalchian-Tabrizi K, Svegaarden IB, Kauserud H	47
Stepanova	A A	**0820**	PS10-370	THE ULTRASTRUCTURE OF *Aspergillus* SPECIES ISOLATED FROM PATIENTS AND THE SOIL	Sinitskaya I A	229
Stephenson	S L	**0145**	PS5-500	Distribution and ecology of dictyostelids in the Great Smoky Mountains National Park (eastern North America)	Landolt J C, Cavender J C	352
		0146	PS5-501	Dictyostelid cellular slime molds of Australia	Landolt J C, Cavender J C	352
Stukenbrock	E H	**0064**	PS8-451	GLOBAL migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*	Banke S, McDonald BA	297
		0065	PS8-452	ORIGIN and divergence of the fungal pathogen *Mycosphaerella graminicola*; from wild grasses to domesticated wheat	Banke S, McDonald BA	297
Subburaman	S	**0049**	PS9-155	The Peloton lysis (Pattern) in some endangered and endemic Orchids of Eastern Ghat's, India	John Britto Susai	72
Sugawara	K	**0353**	PS3-269	Detection of *Neotyphodium occultans* – new methods to deal with this hard to find grass endophyte using DIC and PCR	Inoue T, Yamashita M, Ohkubo H, Mikoshiba Y	185
		0363	PS3-270	*Puccinia striiformis* (sensu lato) in Japan – long term fluctuations of occurrence on different hosts	Abbasi M, Tajimi A, Tanabe Y, Kiyoshi T, Sanada Y, Ohkubo H, Mikoshiba Y	185
Suzuki	A	**0333**	PS1-62	Phylogenetic relationships among Asian and Australasian ectomycorrhizal ammonia fungi in *Hebeloma* subgenus *Porphyrospora*	Tanaka C, Buchanan PK, Bougher NL, Deng Z, Fukiharu T	40
Swett	C L	**0801**	PS3-310	Four Fusarium species causing diseases on orchids in Hawaii, including a potentially undescribed species in the *Gibberella fujikuroi* species complex	Uchida JY	204
Takahashi	K	**0555**	PS1-110	Taxonomic revision of the genus *Sticta* (Lobariaceae, Lichenized Ascomycota) in East Asia	Tsubota H, Deguchi H	56
Talbot	N J	**0854**	PS3-317	Investigating the early stages of plant infection by the rice blast fungus *Magnaporthe grisea*	Veneault-Fourrey C, Kershaw MJ, Egan MJ, Saunders DO, Wang ZY	207

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Tamaj	Y	**0358**	PS7-605	Cultivation properties of termite mushroom	Tamai Y, Cha J-Y, Wirawan IGP, Terazawa M	392
Tan	B S	**0342**	PS4-412	Respiration properties of *Pythium* species from cool-temperate forest soil	Senda M, Kageyama K	282
Tanaka	C	**0573**	PS4-429	The histidine kinase Dic1p regulates HOG1-MAPK involved in glycerol accumulation of *Cochliobolus heterostrophus*	Yoshimi A, Izumitsu K	289
Taniwaki	M	**0607**	PS6-223	The Influence of Fungal Metabolites on the Flavour of Coffee Beverages	Iamanaka BT, Copetti MV, Catharino RR, Marques LA, Eberlin MN, Teixeira AA, Teixeira ARR	130
		0612	PS6-225	Mycobiota of cocoa beans and toxigenic potential of *Aspergillus* species	Copetti MV, Iamanaka BT, Pereira JL, Pires JL	130
Tantiyaporn	M	**0208**	PS8-455	Genetic Variations of *Tricholoma crassum* in some Areas of Thailand by Isozyme Electrophoresis and PCR-RFLP Method	Veinggham Nattaporn	299
Tasanatai	K	**0491**	PS4-421	Isolation and in vitro cultivation of the fastidious insect pathogenic fungus *Cordyceps unilateralis*	Wongsa P, Kocharin K, Hywel-Jones NL	285
Tata	M H L	**0815**	PS1-133	Characterization of root colonizing fungi in two species of *Shorea* seedlings using mycelial isolation and direct sequencing	Summerbell RC	64
Taylor	P W J	**0776**	PS3-308	*Colletotrichum* species causing anthracnose of chilli (*Capsicum annuum*)	Than P P, Tshering K, Ranathunge Nalika, Hyde K, Jeewon R, Kanchana-Udomkan C, Ford R, Gurung A, Pongsupasamit S, Mongkolporn O	203
Tedersoo	L	**0169**	PS5-507	Does abandonment of a wooded meadow affect diversity and community structure of ectomycorrhizal fungi?	Suvi T, Larsson E, Kõljalg U	354
		0170	PS9-158	Molecular and morphological diversity of pezizalean ectomycorrhiza	Hansen K, Perry BA, Kjøller R	73
		0171	PS9-159	Partial mycoheterotrophy in some boreal ericoid subshrubs: isotopic evidence	Pellet P, Kõljalg U, Selosse M-A	74
		0172	PS9-160	Ectomycorrhizal fungi in Seychelles	Suvi T, Beaver K, Kõljalg U	74
Terekhova	V A	**0036**	PS5-486	Mycotest for ecological evaluation of aquatic and terrestrial ecosystems		347
Thi Hang	M	**0227**	PS5-512	Study on diversity of *Aspergillus* and *Penicillium* isolated From the mangrove forests of Vietnam and their potential application	Phan Thi Hoa, Nguyen Thi Hien	356
		0226	PS7-595	Study on using *A. niger* XP isolated from soy bean waste to produce acidic phytase for animal using cassava bagasse waste	Ngo Thanh Xuan	389
Thienhirun	S	**0079**	PS5-490	Xylariaceous Fungi in Doi Suthep-Pui National Park, Thailand	Rodtong Sureelak	349

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Thoen	E	**0599**	PS10-358	Comparison of two methods for quantification of viable *Saprolegnia* sp propagules in water samples	Bjøru Bjørn, Skaar Ida	223
Tibuhwa	D D	**0108**	PS1-15	Utility of the Macro-Micromorphological Characteristics Used in Classifying the Species of *Termitomyces*	Kivaisi AK, Buyck B, Magingo FSS	21
Tomsovsky	M	**0283**	PS1-53	Molecular phylogeny of European Trametes (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences	Kolarík M, Pažoutová S, Homolka L	37
		0282	PS5-520	Identification of *Armillaria* (Basidiomycetes, Agaricales) species in forest biotops of Central Europe from soil substrate	Lochman J, Májek T, Jankovský L	359
Tong	X Z	**0934**	PS2-210	Cloning and characterisation of a transcription factor GC-ZXT in *Glomerella cingulata*	Stowell K M, Farley P C	124
Torres-Torres	M G	**0299**	PS1-57	Secondary metabolite and their usefulness in the taxonomy of *Ganoderma*	López-Dellamary FA, Guzmán-Dávalos L, López-Iñiguez AI, Maya L	38
Toyoda	H	**0104**	PS3-248	Consecutive Monitoring of Lifelong Production of Conidia by Individual Conidiophores of *Blumeria graminis* f sp *hordei* on Barley Leaves by Digital Microscopic Techniques with Electrostatic Micromanipulation	Matsuda Y, Nonomura T	177
Tretiach	M	**0601**	PS4-433	How do endolithic lichens dissolve carbonates?	Crisafulli P, Favero-Longo S E, Mathieu M, Modenesi P, Piervittori R, Salvadori O	291
		0602	PS4-434	Endolithic lichens on carbonatic rocks: biodeterioration or bioprotection?	Crisafulli P, Modenesi P, Piervittori R, Furlani S, Cucchi F	292
Trilles	L T	**0874**	PS10-376	Molecular characterization of the *Cryptococcus neoformans* species complex from Brazil	Jover-Botella A, Ngamskulrungroj P, Maszewska K, Barbosa G G, Meyer W, Lazéra M S	232
Tschen	E F T	**0259**	PS1-48	Taxonomic studies on species of Russula in Taiwan	Tschen JSM	35
Tsui	K M	**0875**	PS1-140	Using RPB1 and RPB2 sequences to improve the phylogenetic inference among *Candida* species	Meyer Wieland	66
		0876	PS1-141	Aquaphila has phylogenetic affinity in Tubeufiaceae inferred from rDNA sequences	Sivichai Somsak, Rossman Amy R, Berbee Mary L	67
		0891	PS10-377	Establishment of a quality controlled internal transcribed spacer (ITS) sequence database as basis for routine clinical diagnostics of human fungal pathogens	Kong Fanrong, Sun Ying, Huynh Matthew, Halliday Catriona, Zeng Xianyu, Maszewska Krystyna, Porter Guy, Sorrell Tania, Meyer Wieland	232
Tunlid	A	**0794**	PS1-132	Molecular evolution of the hydrophobin gene family in the ectomycorrhizal fungus *Paxillus involutus*	Rajashekar B, Samson P, Johansson T	64

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Tzean	S-S	**0746**	PS4-439	Cloning and characterization of mating related genes in medicinal *Ganoderma lucidum*	Wu H Y	293
Unterseher	M	**0040**	PS5-487	Diversity and host specificity of leaf-inhabiting endophytic fungi in a temperate deciduous forest canopy	Finstermeier K, Reiher A, Otto P	347
		0878	PS5-577	Mycology in a temperate riparian forest canopy – the fungal side of the LAK-Project	Otto P, Morawetz W	382
Vainstein	M H	**0797**	PS10-368	Identification of genomic differences between *Cryptococcus gattii* and *Cryptococcus neoformans* var *grubii* by Representational Difference Analysis	Faganello J, Dutra V, Schrank A	228
Van Wyk	M	**0263**	PS1-51	A new *Ceratocystis* sp from *Phoracantha acanthocera* tunnels on Eucalyptus in Australia	Pegg G, Drenth A, Carnegie A, Lawson S, McDonald J, Wingfield MJ	36
Vanittanakom	N	**0941**	PS4-448	Comparison of physiological characteristics between environmental and clinical isolates of human pathogenic *Pythium insidiosum*	Supabandhu Jidapa, de Hoog Sybren	296
		0814	PS10-369	Isolation and characterization of cDNA encoding for heat shock protein 30 from *Penicillium marneffei*	Pongpom Patthama, Praparatanapan Jutarat, Sirisanthana Thira	228
		0940	PS10-383	Phagocytosis and killing of human pathogenic *Penicillium marneffei* and *Penicillium* sp by mouse macrophage J7741 cells	Thirach Sophit, Cooper Chester R	235
Vanittanakom	P	**0942**	PS10-384	Efficiency of immunoblot assay for rapid diagnosis of human pythiosis	Supabandhu Jidapa, Laohapensang Kamphol, Vanittanakom Nongnuch	235
Velzeboer	R	**0254**	PS3-264	Management of *Phytophthora cinnamomi* in native vegetation in South Australia		183
Venegas	R	**0916**	PS7-633	Prospective Cultivation Of The Wild Fungus "*Lentinula* sp" On Oak Sawdust In Mexico	Acosta-Urdapilleta L, Bautista N, Medrano F, Montiel E, Mora V	405
Verekar	S	**0735**	PS5-570	Keratinophilic fungi from Lonar meteorite crater soils (India)	Deshmukh Sunil	379
Verkley	G J M	**0692**	PS1-122	Characterization of Septoria using morphological, cultural and sequence data of the ITS region and partial calmodulin, actin and elongation factor-1 alpha genes	Starink-Willemse M	61
Vesentini	D	**0306**	PS4-408	An investigation into the production of fungal extracellular mucilaginous material (ECMM) with relation to stress conditions	Dickinson DJ, Murphy RJ	280
		0307	PS4-409	Mode(s) of action of chitosan: 1 The effect on fungal cell membranes	Singh T, Osman A, Singh AP	280
		0308	PS4-410	Mode(s) of action of chitosan: 2 The effect on fungal cell wall deposition	Steward D, Singh AP	281
Vettraino	A M	**0594**	PS5-558	*Phytophthora* species in forest ecosystems of western Nepal	Brown A, Brasier C, Patrizi E, Vannini A	374

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Visarathanon	N	**0575**	PS3-285a	Pathogenic fungi from banana, rambutan and rose apple	M Rattanachaot, L Manoch	193
Vohnik	M	**0566**	PS9-180	*Meliniomyces variabilis* (Variable White Taxon), isolated from a fruitbody of *Hydnotria tulasnei*, forms ericoid mycorrhiza and changes morphology of *Betula*, *Picea* and *Pinus* roots	Fendrych M, Gryndler M, Hršlerová H, Albrechtová J, Vosátka M	83
Vondrak	J V	**0074**	PS1-8	*Caloplaca soralifera*, a new sorediate species of *Caloplaca* (Lichenized Fungi, Teloschistaceae) lacking anthraquinones in its thallus	Hrouzek PH	19
Vukojevic	J	**0076**	PS4-393	Effect of the Medium pH and Cultivation Period on Mycelial Biomass, Polysaccharide and Ligninolytic Enzymes Production by *Ganoderma lucidum*	Stajic M, Duletic-Lausevic S	272
		0362	PS7-606	Using of the Spent *Pleurotus ostreatus* Substrat Based on Salt Cedar Sawdust, in the Ruminant Feeding	Milenkovic I, Adamovic M	393
Waipara	N W	**0646**	PS3-289	Safety record of plant pathogens introduced for weed biocontrol in New Zealand	Fowler S, Paynter Q, Winks C, Hona S, Smith L, Massey B, Wilkie P, Barton J	194
		0935	PS8-482	Morphological and genetic methods to differentiate strains of *Phoma clematidina* on *Clematis* in New Zealand	Kitchen H, Beever R, Harman H, Massey B, Parkes S, Wilkie P	309
		0669	PS10-359	Phyllosphere fungi of perennial pastures associated with ill thrift syndrome of livestock in New Zealand	Waipara N, Litherland A, Fraser T	224
Walbert	K W	**0316**	PS9-164	Ectomycorrhizal communities associated with a *Pinus radiata* plantation in New Zealand	Ramsfield T, Jones E, Ridgway H, Dick M	76
Walter	M	**0843**	PS3-315	Epiphytes on gorse and associated insects	Hee AKW, Yamoah E, Suckling DM, Jones EE, Stewart A, Waipara NW, Boyd-Wilson KSH, Weld RJ	206
Wang	Y	**0212**	PS1-39	A new thermophilic species of *Paecilomyces* from Xinjiang, China	Zhang Xiang-Min, Jiang Zheng-Qiang, Yang Shao-Qing	32
Wang	Y Z	**0205**	PS2-194	Secondary structures of ITS2 nuclear ribosomal DNA of some species of *Cercophora* and *Podospora* (Lasiosphaeriaceae)	Chan Jong-How, Kao Hsiao-Wei	118
Weckert	M A	**0445**	PS3-276	Grapevine wood fungal infection by soil/root transmission	Alonso M	188
Weeraratne	T	**0826**	PS3-313	Biology of leaf rust in plumeria spp caused by coleosporium sp	Adikaram N K B	205
Wei	J-G	**0029**	PS5-484	Diversity of endophytic *Pestalotiopsis* from Podocarpaceae, Theaceae and Taxaceae in southern China	Xu T, Guo LD	346
Weld	R J	**0449**	PS4-418	Genes involved in sclerotial differentiation in *Sclerotinia sclerotiorum*	Eady C C, Ridgway H J	284

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Wen	H	**0869**	PS7-631	Optimization of extraction and determination of polysaccharide in *Paecilomyces tenuipes* mycelia	Liu Gui-Jun	404
Whiffen	L K	**0782**	PS9-188	Soil organic carbon decline and the importance of arbuscular mycorrhizal fungi	McGee P A, Nehl D B	87
Wilson	A	**0711**	PS1-26	Molecular evolution and ecology of the basidiomycete genus *Calostoma* (Sclerodermatineae, Boletales)	M Binder, DS Hibbett	62
Wilson	B A	**0936**	PS9-191	Composition of arbuscular mycorrhizal fungal communities in saline soil in Wagga Wagga, New South Wales (NSW), Australia	Harper J, Nehl D, Jahromi F, Ash G	88
Wong	S F	**0844**	PS1-373	Histopathological and immunological changes in mice experimentally infected with *Candida albicans*	Mak Joon Wah, Zasmy Ngah, Peter Pook Chuen Keat, Ng Kee Peng	230
Wornik	S	**0414**	PS8-467	Population Genetic Studies Of Symbionts In Lichens With Different Propagation Modes	Grube M	303
Wright	M M	**0376**	PS9-165	The functional diversity of *Caladenia tentaculata* (Orchidaceae) mycorrhizal fungi from six distinct populations within Victoria, Australia	Cross R, Cousens R, McLean CB	76
Wu	S H	**0354**	PS1-68	*Brunneocorticium pyriforme*, A New Corticioid Fungal Genus And Species		42
Wu	M-L	**0417**	PS1-88	Some new species and new records of discomycetes from Taiwan	Su Yu-Chih, Liang Shih-Hsiung	48
Xu	T	**0147**	PS1-23	Phylogenetic relationship of *Pestalotiopsis* species based on parsimony analysis of rDNA ITS sequences	Wei Ji-Guang, Guo Liang-Dong, Liu Ai-Rong	25
		0258	PS1-47	Molecular and morphological description of *Pestalotiopsis hainanensis* sp nov, a new endophyte from tropical region in China	Liu Ai-Rong, Guo Liang-Dong	35
Yaguchi	T	**0395**	PS1-84	Classification of pathogenic *Aspergillus* section Fumigati	Horie Y, Tanaka R, Matsuzawa T, Ito J, Nishimura K	47
Yamada	T	**0164**	PS3-253	Influence of water stress and wetness of open wound on lesion expansion in the xylem of *Cryptomeria japonica* seedlings inoculated with a canker fungus *Guignardia cryptomeriae*		179
		0165	PS3-254	Occurrence of scab canker caused by *Scolecostigmina* sp on five-needle pines in Japan	Ikeda Hiroyuki	179
Yamaguchi	K Y	**0550**	PS1-108	Molecular phylogeny and morphology of aero-aquatic fungi, Diplocladiella and Candelabrum	Nakagiri A, Okane I, Ito Tad	56
Yamamoto	Y	**0462**	PS10-354	Screening for growth inhibition of animal-diseased bacteria in natural thalli of lichens	Sato Y, Hara K, Komine M, Inamoto T	222
Yamaoka	N Y	**0247**	PS4-405	The role of primary germ tube for the morphogenesis of *Blumeria graminis*	Matsumoto I	278

Poster Author List (List is according to presenting author)

Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Yamoah	E Y	**0442**	PS1-91	Fungal species associated with three phytophagous insects of gorse	Jones EEJ, Weld RJW, Waipara NW, Suckling DMS, BOourdöt GB, Stewart T AS	49
Yokoyama	R	**0962**	PS1-150	The phylogeny and taxonomy of the thraustochytrid strains isolated from Malaysia (Labyrinthulomycetes, Stramenopiles)	Salleh Baharudin, Honda Daiske	70
Young	C	**0460**	PS3-279	Genetic variation for *Phymatotrichopsis omnivora* tolerance in Alfalfa (*Medicago sativa* L)	Lee H-K, Marek S, Sledge M	190
Yurkov	A M	**0714**	PS7-619	Pigmented basidiomycetous yeasts are the perspective source of carotenoids, Pigmented basidiomycetous yeasts are the perspective source of carotenoids	Vustin MM, Sineoky SP	399
Zainuddin	N	**0532**	PS4-424	Isolation and screening of biologically active metabolites of selected marine fungi from Malaysia	Lee Choon Weng, Alias Siti Aisyah	287
		0544	PS5-553	Biodiversity of marine fungi from mangrove areas in Malaysia	Jones E B Gareth, Alias Siti Aisyah	372
Zare	R	**0488**	PS1-96	*Coniochaeta* species from Iran	Asgari B	52
		0723	PS1-127	A new species of *Rhizopus* from Iran	Zangeneh S	62
Zarrinfar	H	**0055**	PS10-337	Evaluation of the Effects of Incubation Temperature and pH on the *in vitro* susceptibility Test for ketoconazole Against *Candida albicans* Isolates from Women with Recurrent Vaginities	Yadegari mohamad hossein, Riazipoor Majid, Farahnejad Zohre, Katiraee Farzad	214
		0056	PS10-338	Comparison of the effect of D-glucose different concentrations on germ tube formation in *Candida albicans*	Jebali Ali	215
Zhang	G	**0802**	PS3-311	Development of Real-time PCR for the Rapid Detection of *Phytophthora sojae* Using TaqMan MGB Probe Assays	Cheng Yinghui, Wu Jiyun, Peng Jihuo, Yan Jin, Wang Ying, Yi Jianping, Yang Weidong, Jin Xianzhong	204
Zhao	R-L	**0098**	PS1-13	Ribosomal DNA phylogenies of *Cyathus*: Is the current infrageneric classification appropriate?	Jeewon Rajesh, Desjardin Dennis, Soytong Kasem, Hyde Kevin	20
		0957	PS1-149	A preliminary analysis of *Lactarius* Subgenus *Piperites* from Northern Thailand based on morphology and nrITS sequence data	Le Huyen Thanh, Nuytinck Jorinde, Verbeken Annemieke, Lumyong Saisamorn, Desjardin Dennis	70
Zhao	Z	**0166**	PS9-157	Dynamics of arbuscular mycorrhizal fungal diversity and community in a hot and arid ecosystem during conversion from natural to arable and subsequently to restoration lands	Li Lingfei	73
Zhou	T	**0661**	PS3-292	The multimechanisms of *Bacillus amyloliquefaciens* C06 in the biocontrol of peach brown rot caused by *Monilinia fructicola*	Liu WZ, Li XZ	196

Poster Author List (List is according to presenting author)

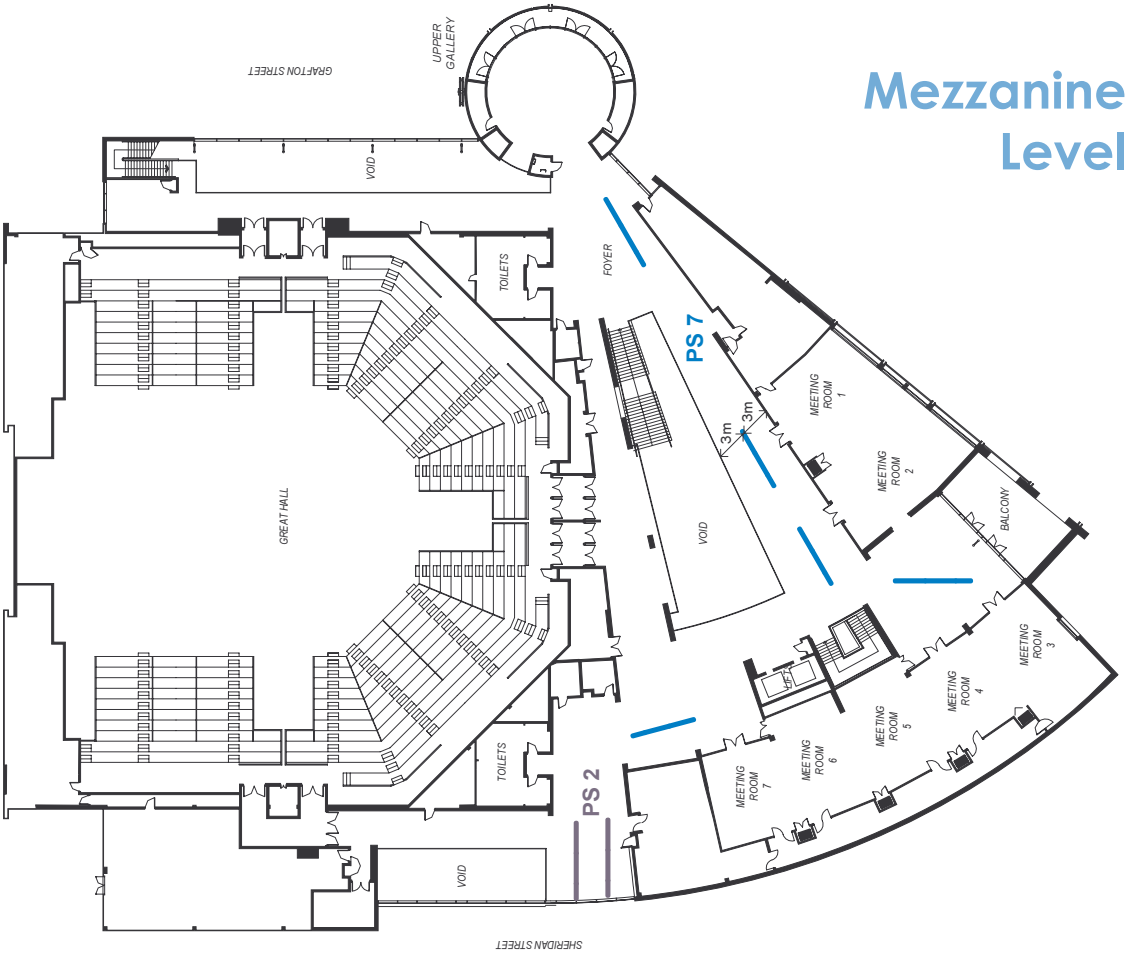
Last name	Initials	Abstract No	Session Board No	Title	All authors	Page No
Zhou	X	**0269**	PS1-52	A new *Leptographium* species associated with the pine root collar weevil *Hylobitelus chenkupdorjii* on *Pinus wallichiana* in Bhutan	Jacobs K, Kirisits T, Konrad H, Chhettri DB, Wingfield M J	36
		0267	PS8-458	Population biology of the sapstain fungus, *Ophiostoma* ips, reflects global movement of its bark beetle vectors	Burgess T, de beer ZW, Lieutier F, Yart A, Klepzig K, Carnegie A, Portales JM, Wingfield BD, Wingfield MJ	300
Zhuang	J Y	**0841**	PS5-575	Comparisons between the rust flora of Tibetan East Himalaya and the rust floras of India, Nepal and Pakistan		381

Map of Cairns

1. Civic Theatre & Ticketlink
2. Cinemas
3. Library
4. Museum
5. Art Gallery
6. Post Office
7. Casino
8. Police
9. Main Post Office
10. Cairns Convention Centre
11. National Bus Terminal
12. Ergon Energy Soundshell, Fogarty Park

- Information
- Bank
- Car park
- Hospital
- Toilets
- Railway Station

Mezzanine Level



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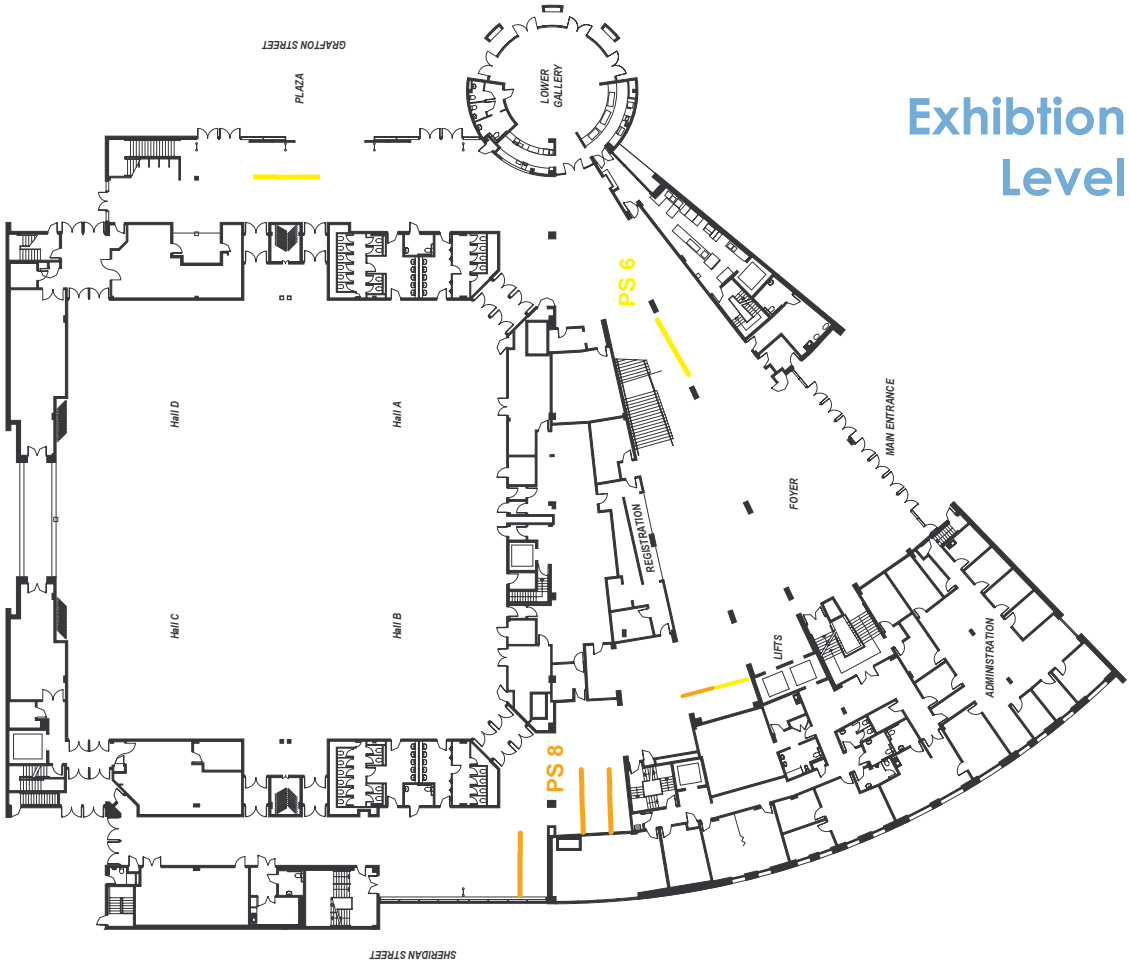
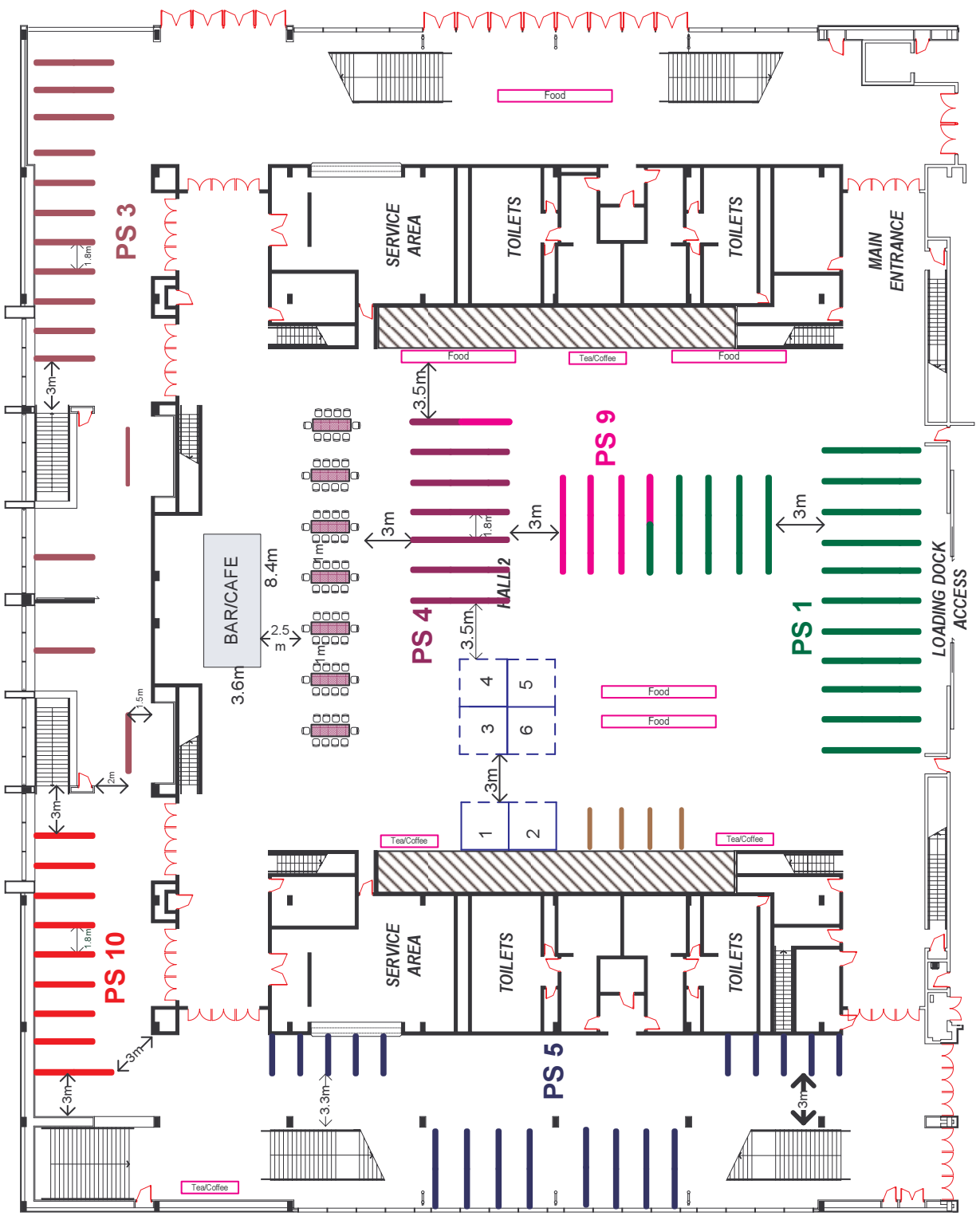


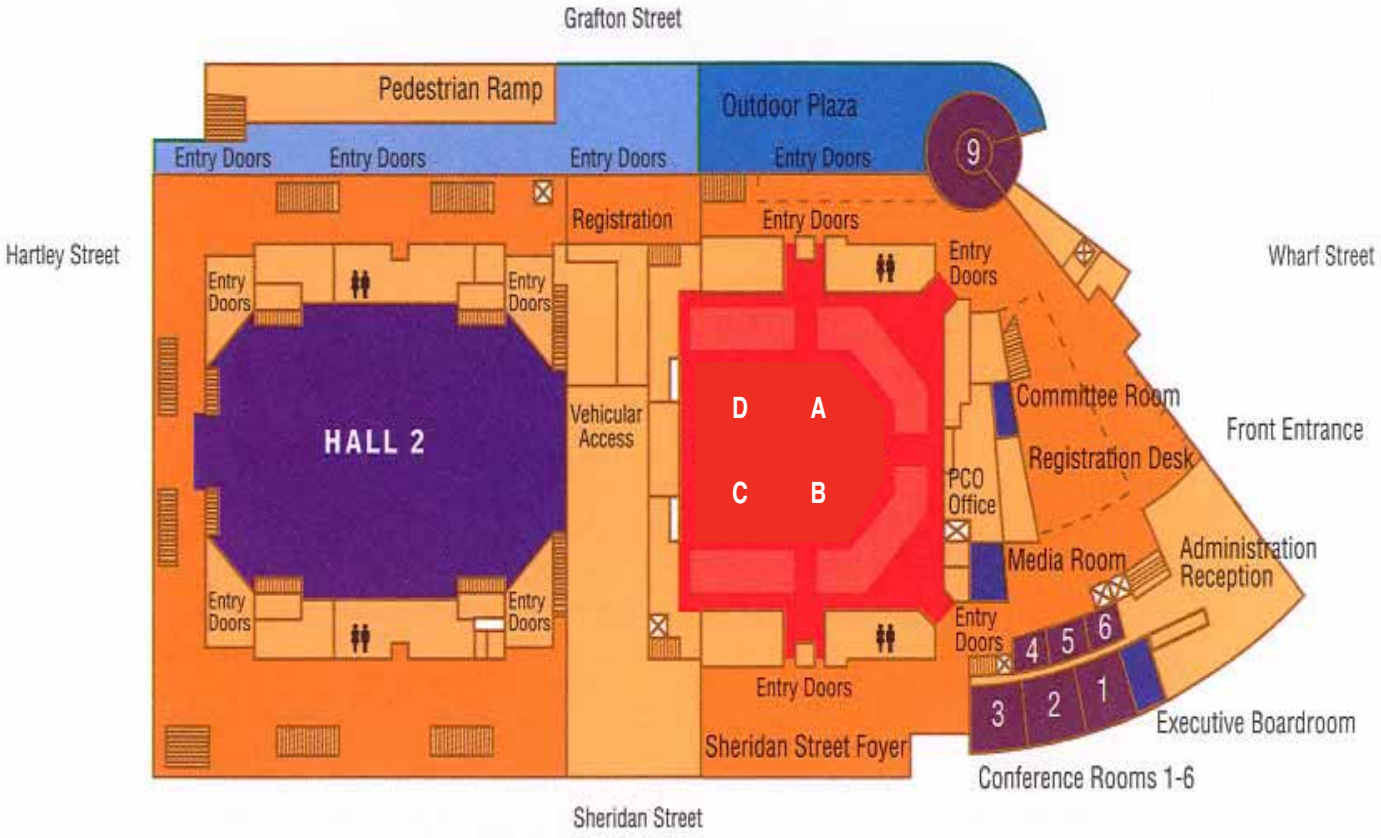
Hall 2



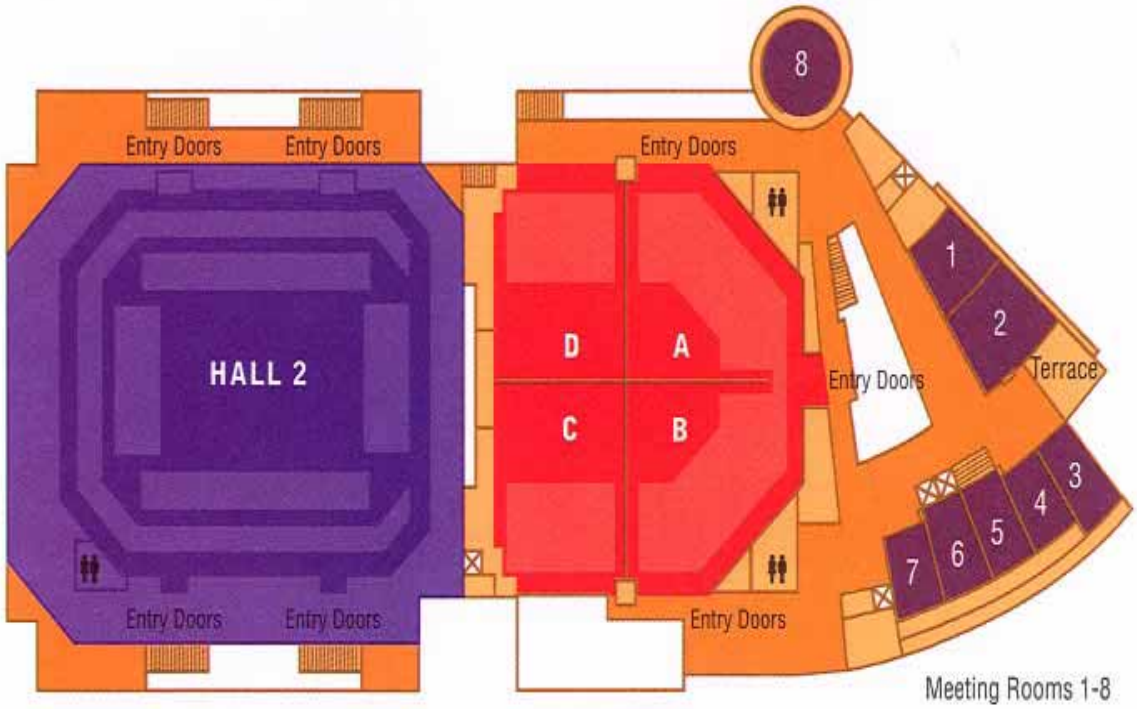
Exhibitors

4	Cambridge University Press
3	CBS
2	Fungal Diversity Press
6&5	Schering-Plough Pty Limited
1	Taylor and Francis

EXHIBITION LEVEL



MEZZANINE LEVEL

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