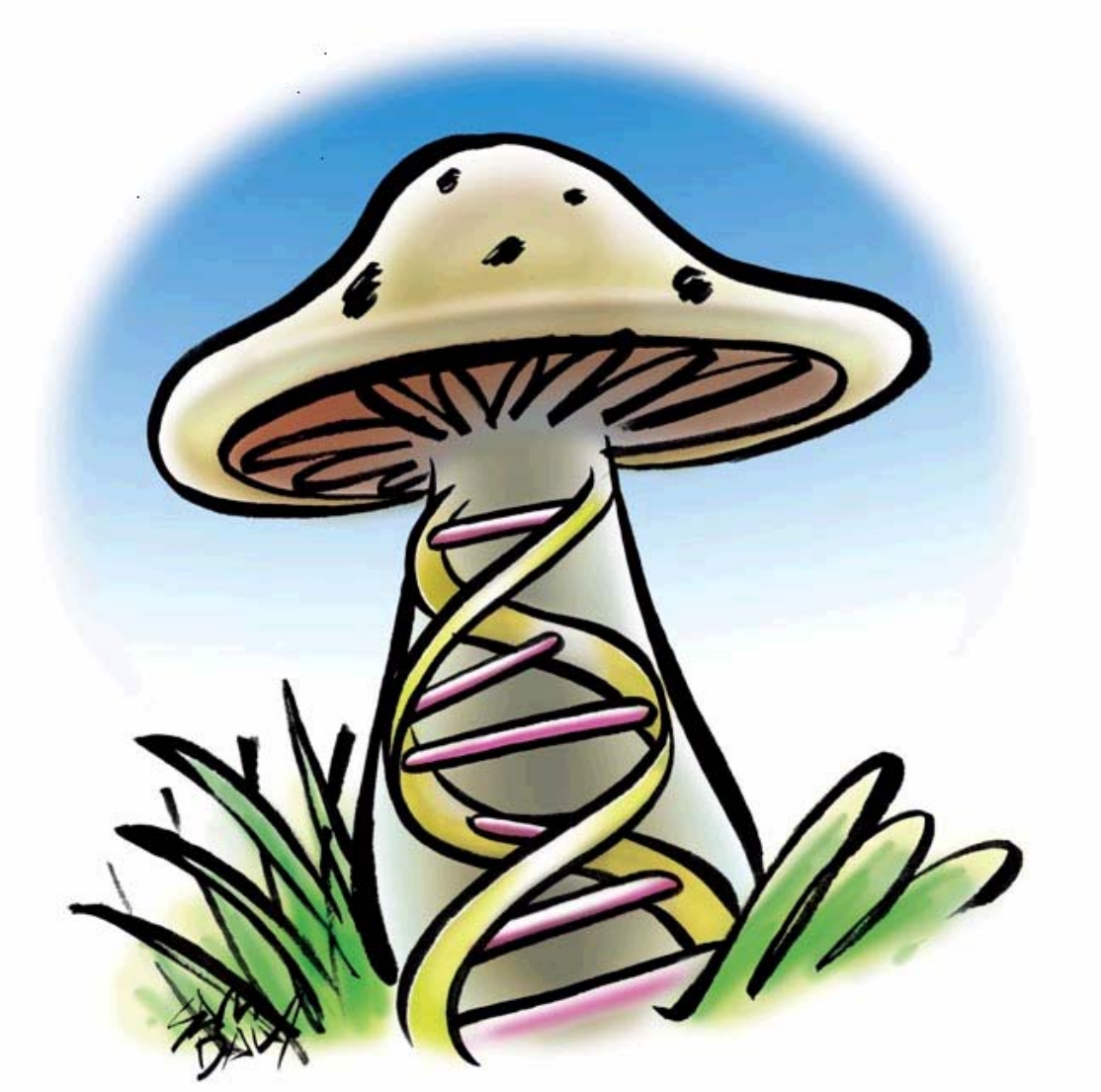
IXth International Fungal Biology Conference &
16th *New Phytologist* Symposium

Impact of genomics on fungal biology

Nancy, France
18–20 September 2006



Programme, abstracts & participants

New Phytologist

Programme, abstracts & participants

IXth International Fungal Biology Conference & 16th *New Phytologist* Symposium

Impact of genomics on fungal biology

Congress Hall of Nancy, France
18–20 September 2006

Organizing committee

Francis Martin (*INRA-Nancy, France*)

Nicholas J Talbot (*University of Exeter, UK*)

Holly Slater (New Phytologist, *Lancaster, UK*)

Steering Committee of the International Fungal Biology Conference

Jesus Aguirre
Solomón Bartnicki-García
Gordon Beakes
Louise Glass
Neil Gow
Harvey Hoch
Regina Kahmann
Kurt Mendgen
Robert Roberson
Jose Ruiz-Herrera

Acknowledgements

The IXth International Fungal Biology Conference &
16th *New Phytologist* Symposium is funded by the *New Phytologist* Trust and supported in part by INRA (Institut National de la Recherche Agronomique) and the Région Lorraine.

***New Phytologist* Trust**

The *New Phytologist* Trust is a non-profit-making organization dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.

Programme, abstracts and participant list compiled by Helen Pinfield-Wells. Mushroom illustration by Sam Day, www.samday.com

Table of contents

Monday 18 September

08:00–8:45 **Registration**

08:45–9:00 **Welcome & Introductions,** IJ Alexander, NJ Talbot & F Martin

Session 1: Fungal Structure & Development
Chairperson: JW Taylor, University of California, Berkeley, CA, USA

09:00–9:35 **1.1. Spatial control of mitosis in *Ashbya gossypii***
A Gladfelter, Dartmouth College, Hanover, NH, USA

09:35–10:10 **1.2. Through the looking-glass: dynamic imaging of living fungal cells**
PC Hickey, LUX Biotechnology Ltd., UK

10:10–10:40 Coffee break

10:40–11:15 **1.3. Electron tomography and its application to revealing fungal cytoplasmic order**
RW Roberson, Arizona State University, USA

11:15–11:50 **1.4. Woronin body genesis provides new insights into the molecular and cellular organization of the fungal colony**
G Jedd, National University of Singapore, Singapore

11:50–12:25 **1.5. Endocytosis is essential for pathogenic development in the corn smut fungus *Ustilago maydis***
U Fuchs, Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

12:30–14:00 Lunch

Session 2: Fungal genomics: from sequence to application I
Chairperson: NJ Talbot, University of Exeter, UK

14:00–14:35 **2.1. A comparative analysis of genome evolution in *Aspergillus***
A Rokas, Broad Institute of MIT & Harvard, Cambridge, MA, USA

14:35–15:10 **2.2. Examination of the role of gene content and gene expression in the virulence of *Cryptococcus* species**
J Kronstad, University of British Columbia**,** Vancouver, BC, Canada

15:10–15:45 **2.3. Structural and functional analysis of fungal pathogenesis: the rice blast fungus genome project**
RA Dean, North Carolina State University, Raleigh, NC, USA

15:45–16:20 **2.4. Post-genomics and functional analysis of *Neurospora crassa***
KA Borkovich, University of California Riverside, CA, USA

16:20–19:00 Coffee Break & **Posters**
(17:00–18:00) IFBC Business section for steering committee members
Chairperson: Jesus Aguirre

19:00–21:00 **Cocktail reception**

Tuesday 19 September

Session 3: Fungal genomics: from sequence to application II
Chairperson : SG Oliver, University of Manchester, UK

09:00–9:35	**3.1. Two b or not two b: regulatory cascades during pathogenic development of the smut fungus *Ustilago maydis*** J Kämper, Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany
09:35–10:10	**3.2. The visible touch: plant protein-protein interactions during host cell entry by powdery mildew fungi** R Panstruga, Max-Planck-Institute for Plant Breeding, Köln, Germany
10:10–10:40	Coffee break
10:40–11:15	**3.3. The genome of the white rot Basidiomycete *Phanerochaete chrysosporium*: implications relevant to wood decay mechanisms** DJ Cullen, USDA Forest Products Laboratory, Madison, WI, USA
11:15–11:50	**3.4. The genome of the symbiotic basidiomycete *Laccaria bicolor*: soil ecology, evolution and metabolism** F Martin, INRA-Nancy, France
11:50–12:30	**Posters**
12:30–14:00	Lunch

Session 4: Secretion & transport systems
Chairperson: K Mendgen, University of Konstanz, Germany

14:00–14:35	**4.1. Characterization of secreted proteins from *Magnaporthe oryzae*** D Ebbole, Texas A&M University, College Station, TX, USA
14:35–15:10	**4.2. Functional genomics of transporters in symbiotic fungi** M Chalot, University of Nancy, France
15:10–15:45	**4.3. Haustoria and their roles in biotrophy** RT Vögele, University of Konstanz, Germany
15:45–16:30	Coffee Break & **Posters**
16:30–17:30	**Discussion Session:** Post-genomics challenges (Leader: NJ Talbot)
19:00–22:00	**Apéritif & Conference Dinner (Hôtel de Ville – Stanislas Square)**

Wednesday 20 September

Session 5: Metabolomics
Chairperson: J Aguirre, National University of Mexico

09:00-9:35	**5.1. Metabolomics in yeasts and filamentous fungi** SG Oliver, University of Manchester, UK
09:35-10:10	**5.2. Functional genomics of pathogenicity in *Magnaporthe grisea*** NJ Talbot, University of Exeter, UK
10:10–10:40	Coffee break
10:40–11:15	**5.3. A secondary metabolite involved in recognition of the blast fungus *Magnaporthe grisea* by resistant rice cultivars** MH Lebrun, CNRS–Bayer crop science, Lyon, France
	**********
11:15–12:00	**5.4 Concluding Keynote Address: Evolutionary variation and comparative genomics** JW Taylor, University of California, Berkeley, CA, USA
	**********
12:00–12:15	**Concluding Remarks** *J Aguirre, National University of Mexico*
12:30–14:00	Lunch

***

Speaker Abstracts

Session 1: Fungal Structure & Development
Chairperson: JW Taylor, University of California, Berkeley, CA, USA

1.1. Spatial control of mitosis in *Ashbya gossypii*

AMY GLADFELTER
Dartmouth College, Hanover, NH, USA

Nuclei in the filamentous, multinucleated fungus *A. gossypii* divide asynchronously. We have investigated what internal and external signals spatially direct mitosis within these hyphal cells. Mitoses are most common near cortical septin rings found at growing tips and branch points. In septin mutants, mitoses are no longer concentrated at branch points suggesting that the septin rings function to locally promote mitosis near new branches. Similarly, cells lacking AgSwe1p kinase (a Wee1 homologue), AgHsl1p (a Nim1-related kinase) and AgMih1p phosphatase (the Cdc25 homologue that likely counteracts AgSwe1p activity) also have mitoses distributed randomly in the hyphae as opposed to at branch points. Surprisingly, however, no phosphorylation of the CDK tyrosine 18 residue, the conserved substrate of Swe1p kinases, was detected in normally growing cells. In contrast, abundant CDK tyrosine phosphorylation was apparent in starving cells resulting in diminished nuclear density. This starvation-induced CDK phosphorylation is AgSwe1p dependent and overexpressed AgSwe1p is sufficient to delay nuclei even in rich nutrient conditions. In starving cells lacking septins or AgSwe1p negative regulators, the nuclear density is further diminished compared to wild type. We have generated a model in which AgSwe1p may regulate mitosis in response to cell intrinsic morphogenesis cues and external nutrient availability in multinucleated cells.

1.2. Through the looking-glass: dynamic imaging of living fungal cells

PATRICK C. HICKEY
LUX Biotechnology Ltd, King's Buildings, Edinburgh, EH9 3JF, United Kingdom.

A combination of novel probes, high quality optics, a 'rainbow' of lasers and excitation sources coupled with sensitive detection technologies are pushing light microscopy to its limits. Fluorescent proteins (e.g. GFP) and fluorescent dyes are powerful tools for targeting organelles, tracking growth and analysing cellular dynamics of fungal hyphae.

Optimising settings e.g. laser power, scanning speeds and gain are crucial to obtain the highest quality images possible, whilst minimising the phototoxic damage to cells and photobleaching of fluorescent probes. We present some novel techniques for preparing samples for microscopy, with the aim of obtaining the best possible optics, and keeping cells alive for prolonged periods of time. An overview of useful software for image processing and time lapse animation is provided.

LUX Biotechnology Ltd is a research based company specialising in fluorescent and luminescent technologies. LUX have investigated a range of novel fluorescent and luminescent proteins, and expressed them in fungal cells. The importance of live cell analysis and the use of fungi in high throughput imaging applications will be discussed. We also introduce a unique range of calibration standards, designed to provide quality assurance for luminescent and fluorescent applications.

1.3. Electron tomography and its application to revealing fungal cytoplasmic order

ROBERT W. ROBERSON
School of Life Sciences, Molecular and Cellular Biology Faculty, Arizona State University, Tempe, AZ 85287-4501, USA

The development of the transmission electron microscope (TEM) has vastly increased our understanding of multiple biological systems. However, when attempting to visualize and understand the organizational and functional complexities that are typical of cells and tissues, the standard two-dimensional analyses that TEM affords often fall short. With the advances in specimen preparation, instrumentation design, and computer image analysis, the use of electron tomography is now providing a wealth of three-dimensional biological data with high spatial resolution (5–10 nm in all directions) and fidelity that is proving invaluable in refining our interpretations of cytoplasmic complexities, order, and function. This presentation will provide a brief introduction into the methods of cryo-preservation, a practical background of electron tomography and reconstruction, and a case study where electron tomography is helping reveal ultrastructural complexity in the hyphal apex of *Aspergillus nidulans.*

1.4. Woronin body genesis provides new insights into the molecular and cellular organization of the fungal colony

GREGORY JEDD
Temasek Life Sciences Laboratory and Department of Biological Sciences, National University of Singapore, Singapore 117604

Woronin bodies are peroxisome-derived organelles centered on a crystalline core of HEX-1 protein. This organelle functions to seal the septal pore in response to cellular damage and is found in all vegetative hyphal compartments. Time-lapse confocal microscopy shows that Woronin bodies form in the apical compartment where they are generally transported in a tip-directed manner. These vesicles undergo maturation entailing membrane fission and associate with the cell cortex roughly coinciding with septation. Cortical association retains Woronin bodies in newly formed sub-apical compartments and the continuous execution of this process ensures that all hyphal compartments inherit a certain number of Woronin bodies.

To investigate the genetic control of Woronin body formation, we examined the localization of YFP expressed from *hex-1* regulatory sequences and observed a tip-high fluorescent gradient, suggesting that *hex-1* gene expression is polarized. To directly assess the spatial distribution of various mRNAs, we developed a method to fractionate the fungal colony into apical and increasingly sub-apical compartments. Examining RNA from these regions we found that *hex-1* mRNA is highly enriched in apical hyphal compartments, whereas other transcripts accumulate in sub-apical compartments or show no spatial bias. When the *hex-1* structural gene was expressed from regulatory sequences of an abundant, sub-apically localized transcript, Woronin body formation was re-determined to this region of the colony. Together, these results define the genetic differentiation of apical hyphal compartments and show that polarized gene expression is a key determinant of apically localized Woronin body formation.

1.5. Endocytosis is essential for pathogenic development in the corn smut fungus *Ustilago maydis*

UTA FUCHS, GERD HAUSE[1], ISABEL SCHUCHARDT and GERO STEINBERG
MPI für terrestrische Mikrobiologie, Karl-von-Frisch-Straße, D-35043 Marburg
[1]Martin-Luther-Universität Halle-Wittenberg, Biozentrum, Weinbergweg 22, D-06099 Halle/Saale

It is well established that polarized exocytosis is essential for fungal virulence. In contrast, the contribution of endocytosis is unknown. We made use of a temperature-sensitive mutant in the endosomal t-SNARE Yup1 and demonstrate that endocytosis in *Ustilago maydis* is essential for the initial steps of pathogenic development, including pheromone perception and cell-cell fusion. Furthermore, spore formation and germination was drastically reduced, while colonization of the plant is only slightly inhibited. The function of endocytosis in recognition of mating pheromone through the G protein-coupled pheromone receptor Pra1 was analyzed in greater detail. Biologically active Pra1-GFP localizes to the plasma membrane and is constitutively endocytosed. Yup1^{ts} mutants that are blocked in fusion of endocytic transport vesicles with early endosomes are impaired in pheromone perception and conjugation hyphae formation. This is due to an accumulation of Pra1 carrying endocytic vesicles in the cytoplasm and the depletion of the receptor from the membrane. Consistently, strong Pra1 expression rescues the signaling defects in endocytosis mutants, but subsequent cell fusion is still impaired. Thus we conclude that endocytosis is essential for recognition of the partner at the beginning of the pathogenic program, but has additional roles in mating, as well as spore formation and germination.

Session 2: Fungal genomics: from sequence to application I

Chairperson: NJ Talbot, University of Exeter, UK

2.1. A comparative analysis of genome evolution in *Aspergillus*

ANTONIS ROKAS
Microbial Genome Analysis and Annotation, The Broad Institute of MIT & Harvard, Cambridge, MA 02139, USA

Eukaryotic genomes evolve through the modification, acquisition, deletion and/or rearrangement of genetic material. Identification of the forces that have shaped eukaryotic genomes and the types of changes that they cause is fundamental to our understanding of biology and evolution at the molecular level. The filamentous fungi of the genus *Aspergillus* provide a unique opportunity to study eukaryotic genome evolution as – despite their morphological similarity – they are remarkably diverse at the molecular level. Currently, genome sequences of 3 species are finished and those of 5 others are available in draft form, offering an excellent sample choice for reconstructing the major genomic events in the ~200 Myr long evolution of the genus. We have developed algorithms that enable us to delineate the regions of conserved synteny in the 8 *Aspergillus* genomes, while also retaining information about the structural rearrangements that have occurred in their evolutionary history. Thus, we have begun examining the types of structural rearrangements prevalent in *Aspergillus* evolution, the tempo and mode of structural evolution and whether it differs from that of sequence evolution, the evolution of genome size differences, the evolution of secondary metabolite gene clusters, and the evolution of the mating-type loci.

2.2. Examination of the role of gene content and gene expression in the virulence of *Cryptococcus* species

JAMES KRONSTAD, WON HEE JUNG & GUANGGAN HU
Michael Smith Laboratories, The University of British Columbia, 2185 East Mall, Vancouver, British Columbia, V6T 1Z4, Canada

Cryptococcus neoformans causes life-threatening disease in immunocompromised people and is a major problem among AIDS patients. Recently, a separate species, *C. gattii,* has emerged as a pathogen of immunocompetent people. Three genomes for *C. neoformans* and two for *C. gattii* have been sequenced. We used the *C. gattii* genomes to perform comparative genome hybridization (CGH) experiments and found extensive genomic variation (insertions/deletions) that may contribute to the differences in virulence observed between strains. We used the *C. neoformans* genomes in transcriptome studies with serial analysis of gene expression (SAGE) and microarrays to examine virulence gene expression. Iron levels control virulence factor expression and we therefore identified both the set of iron-responsive genes and a transcription factor that is a global regulator of virulence in *C. neoformans*. We also used SAGE to examine the transcriptomes of mutants defective in the catalytic and regulatory subunits of protein kinase A. This led to the identification of a target of the cAMP pathway that influences the size of the polysaccharide capsule that is the major virulence factor of the fungus.

2.3. Structural and functional analysis of fungal pathogenesis: the rice blast fungus genome project

RALPH A. DEAN
Center for Integrated Fungal Research, Dept. Plant Pathology, North Carolina State University, Raleigh, NC 27606, USA

Magnaporthe grisea is the causal agent of rice blast, the most devastating disease of rice world-wide and is a seminal model to elucidate the basis of pathogen–host interactions. The recent completion of the genome sequence for both *Magnaporthe* and rice as well as the genome sequences for several other pathogenic and non-pathogenic filamentous fungi has provided a wealth of new information regarding the raw components of the pathogen's offensive arsenal and host's defenses. In my presentation I will discuss some of the novel discoveries that have only come to light as a result of having access to the genome sequences, such as novel classes of secreted proteins, surface receptors and large suites of enzymes involved in secondary metabolism that may play a role in the disease process. I will also highlight recent results from functional analyses including transcription profiling and high throughput gene knockout experiments. I will close with some thoughts on other strategies, including proteomic and comparative evolutionary approaches we are undertaking, as well as other resources needed to fully appreciate the molecular basis of fungal pathogenesis.

2.4. Post-genomics and functional analysis of *Neurospora crassa*

KATHERINE A. BORKOVICH[1], HILDUR V. COLOT[2], GYUNGSOON PARK[1], LIUBOV LITVINKOVA[1], SUSAN CURILLA[2], PATRICK D. COLLOPY[2], LORENA ALTAMIRANO[1], CAROL RINGELBERG[2], GLORIA E. TURNER[3], RICHARD L. WEISS[3] AND JAY C. DUNLAP[2]
[1]*Department of Plant Pathology, University of California, Riverside, CA 91711, USA;*
[2]*Department of Genetics, Dartmouth Medical School, Hanover, NH 03755, USA;*
[3]*Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA*

The filamentous fungus *Neurospora crassa* has served as a model organism for studies of metabolism, development, environmental sensing, gene silencing and pathogenesis. We

have initiated a large scale functional genomics project for *Neurospora*, implementing gene knockouts, transcriptional profiling, EST sequencing, implementation of a community annotation database and construction of a single nucleotide polymorphism (SNP) map. Our laboratories are focused on the gene knockouts and we have successfully implemented a high-throughput protocol that utilizes yeast recombinational cloning, *Neurospora* recipient strains with a high rate of homologous recombination, robotics, custom software and a laboratory information management system (LIMS). To date we have mutated nearly 1000 genes and have deposited the strains in the Fungal Genetics Stock Center. The first large group of genes included more than 100 transcription factors. Analysis of the resulting mutants revealed new functions for previously-studied proteins as well as important roles for uncharacterized transcription factors. The current status of the project, including recent technical innovations and characterization of new classes of mutants will be presented.

Session 3: Fungal genomics: from sequence to application II
Chairperson : SG Oliver, University of Manchester, UK

3.1. Two *b* or not two *b*: regulatory cascades during pathogenic development of the smut fungus *Ustilago maydis*
MIROSLAV VRANEŠ, MARIO SCHERER, CHETSADA POTHIRATANA, KAI HEIMEL, RAMON WAHL & JÖRG KÄMPER
Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany

In the phytopathogenic fungus *Ustilago maydis*, the switch from saprophytic to biotrophic growth is controlled by the two homeodomain proteins bE and bW encoded by the *b*-mating type locus. By means of DNA microarrys, we have identified more than 350 *b*-dependently regulated genes. Amoung these, a systematical analysis of genes with potential regulatory functions has led to the identification of four novel pathogenicity factors: the zinc finger proteins Rbf1 and Biz1, the homeodomain transcription factor Hdp2, and Clp1*,* a protein with unknown function. Rbf1 plays a crucial role for the expression of nearly all *b*-regulated genes, and thus has a central role within the *b*-regulatory network. *biz* is required for the expression of genes induced on the plant surface, including *pst1* and *pst2* that encode *U. maydis*-specific secreted proteins essential for biotrophic growth. We hypothesize that *biz1*-dependent genes are involved in suppressing plant defense reactions and reprogramming of the host. *clp1* is thought to be required for the release of the cell cycle arrest in planta, since *clp1* mutant strains are able to infect, but are blocked in development before the first cell division. The emerging picture shows that the regulatory network of these regulatory proteins is used to control the expression of pathogenicity related proteins during infection, but also to synchronize cell cycle and cell division during in planta propagation.

3.2. The visible touch: plant protein-protein interactions during host cell entry by powdery mildew fungi
RIYAZ A. BHAT, CHIARA CONSONNI, PAUL SCHULZE-LEFERT & RALPH PANSTRUGA
Department of Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Köln, Germany

Host cell entry comprises an early and crucial step during pathogenesis of obligate biotrophic powdery mildew fungi. Presence of particular members of the family of serpentine MLO proteins is a prerequisite for successful host cell entry by powdery mildews on monocot as well as dicot plant species. We demonstrate physical interaction of MLO proteins with syntaxins, polypeptides belonging to the superfamily of SNARE domain proteins, using both a yeast-based interaction assay and *in planta* Fluorescence Resonance Energy Transfer (FRET) studies. These findings, in combination with genetic data, suggest that MLO and syntaxin isoforms form an ancient and likely co-evolved protein complex

which possibly regulates exocytotic processes at the cell periphery. We hypothesize that the MLO-syntaxin complex is targeted by powdery mildew fungi to mediate defence suppression for successful entry into plant host cells.

3.3. The genome of the white rot basidiomycete *Phanerochaete chrysosporium*: implications relevant to wood decay mechanisms

DAN CULLEN[1], AMBER VANDEN WYMELENBERG[2] AND PHIL KERSTEN[1]
[1]USDA Forest Service, Forest Products Laboratory, Madison, WI 53726; [2]Bacteriology Department, University of Wisconsin, Madison, 53706, USA

The U.S. Department of Energy has assembled a high quality draft genome of *Phanerochaete chrysosporium* (http://genome.jgi-psf.org/Phchr1/Phchr1.home.html). A general description of the most current assembly and annotations will be presented. Analysis of genes predicted to be involved in cellulose degradation reveals large and complex families of structurally related glycoside hydrolases. The ligninolytic system of *P. chrysosporium* also involves gene families including those encoding extracellular peroxidases, copper radical oxidases, and flavin-dependent oxidases. The expression of many of these genes has been confirmed by analysis of the corresponding cDNAs and/or by identification of specific peptides by LC-MS/MS. However, the role of gene multiplicity in *P. chrysosporium* remains uncertain. Possibly, the closely related sequences encode proteins with subtle differences in function, and such diversity is necessary to effectively break down wood polymers whose structure and accessibility vary with plant species and with the extent of decay. Consistent with this view, genes within families are often differentially regulated in response to nutrient conditions. Addressing the issue more directly, our current experiments focus on heterologous expression and detailed biochemical analysis of the copper radical oxidase genes. Recent progress will be presented.

3.4. The genome of the symbiotic basidiomycete *Laccaria bicolor*: soil ecology, evolution and metabolism

FRANCIS MARTIN[1], JAN WUYTS[2], IGOR GRIGORIEV[3], FREDERIC DUCHAUSSOY[1], P ROUZE[2], PAUL RICHARDSON[3], GOPI PODILA[4] & the DOE Joint Genome Institute Sequencing and Assembly Groups & the *Laccaria* Genome Consortium
[1]UMR IaM, INRA-Nancy, France; [2]Flanders Interuniversity Institute for Biotechnology, Ghent University, Belgium; [3]DOE Joint Genome Institute, CA, USA; [4]University of Alabama-Huntsville

Ectomycorrhizas have a beneficial impact on plant growth in natural and agroforestry ecosystems. Central to the success of these mutualistic symbioses is the exchange of nutrients between the partners. To elucidate the genetic basis of this ecologically important behavior, the US DOE Joint Genome Institute has sequenced the 65-megabase genome of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Agaricales, Tricholomataceae) to high draft using a whole genome shotgun method. This is the first symbiotic fungus genome to be sequenced. It contains about 20,000 intron-rich gene structures-more than twice as many as *Neurospora crassa* and *Phanerochaete chrysosporium*. Analysis of the gene set yields insights into unexpected aspects of *Laccaria* biology including the identification of genes potentially associated with wood and soil organic matter decay. This fungus also possesses an expanded family of G-protein-coupled receptors, small secreted cysteine-rich proteins, several virulence-associated genes and large suites of enzymes involved in transduction pathways, secondary metabolism and cell wall synthesis. The genome is rich in transposons belonging to various class I and II families. Comparison of the genomes of the different pathogenic and saprobic fungi with the *Laccaria* genome will be discussed. It will provide critical insights into the genetic makeup of plant-fungus interactions.

Session 4: Secretion & transport systems
Chairperson: K Mendgen, University of Konstanz, Germany

4.1. Characterization of secreted proteins from *Magnaporthe oryzae*

DANIEL J. EBBOLE, ERIC BHATTARAI, GUODONG LU, HANNO WOLF, CRISTINA FILIPPI, DAN LI, and YUE SHANG.
Department of Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Peterson Bldg. 120, Texas A&M University, College Station, TX 77843, USA.

Magnaporthe oryzae, the causal agent of rice blast disease, is an important model for plant-pathogen interactions. Analysis of the *M. oryzae* genome sequence reveals that approximately 750 genes encode secreted proteins based on SignalP and ProtComp analysis. We selected 300 genes for analysis and transformed histidine-tagged versions of them into the *M. oryzae* for overexpression studies. Purified proteins from *M. oryzae* culture filtrates were detected for 30% of the proteins tested, verifying the prediction of their secretion. Strains overexpressing secreted proteins were tested for effects on pathogenicity, and two strains were defective in causing infection on the susceptible cultivar M202. Larger quantities of proteins of particular interest were purified from a heterologous expression system to allow testing for elicitor activity. Testing these proteins for elicitor activity on rice plants is an on-going project.

4.2. Functional genomics of transporters in symbiotic fungi

MICHEL CHALOT[1], LUCIC EVA[1], BARBARA MONTANINI[1], DAMIEN BLAUDEZ[1], FRANCIS MARTIN[1], ANNICK BRUN[1], JGI Production Sequencing, Assembly & Annotation Staffs[2], and *Laccaria* Genome Annotation Consortium
[1]UMR INRA/UHP 1136 Interactions Arbres/Micro-organismes, Nancy-Université, 54506 Vandoeuvre-les-Nancy, France; [2]US DOE Joint Genome Institute, Walnut Creek, CA 94598, USA

Under natural conditions the majority of plants are believed to have mycorrhizal (MYC) associations (intimate symbiotic association between fungi and roots), either with arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi, which can have a significant role in the worldwide nitrogen cycle. This mycorrhizal status relies 1) on efficient uptake processes by the fungal partner 2) on the bidirectional transfer of nutrients between the two symbionts, which was first demonstrated by Melin and Nilsson >50 years ago. However, the identity of the N solutes (organic *versus* inorganic) taken up and translocated by MYC fungi and the question of whether or not fungal symbionts translocate a significant part of the newly absorbed or formed ammonia has remained unanswered until recently. The sequencing of the *Laccaria bicolor* genome and the use of appropriate functional tools offers an unprecedented opportunity to decipher between the different scenarios. Most particularly, the isolation and characterization of complete sets of genes encoding enzymes involved in N mobilization, for transporters for inorganic / organic N, and for assimilating enzymes will bring fundamental knowledge that will be used to tackle changes in capacities for N uptake and assimilation under natural conditions.

Acknowledgements. This project is a collaborative effort involving: DOE Joint Genome Institute (JGI, coordinator: P Richardson), INRA-Nancy (UMR IaM, F Martin *et al.*), University of Alabama-Huntsville (Department of Biological Sciences, G Podila *et al.*), Gent University (Bioinformatics & Evolutionary Genomics Division, P Rouzé *et al.*) and DOE Oak Ridge National Laboratory (Dr. J Tuskan & Dr. S DiFazio).

4.3. Haustoria and their roles in biotrophy

RALF T. VOEGELE
University of Konstanz, Department of Biology, Phytopathology, Universitätsstr. 10, 78457 Konstanz, Germany

On a global scale, some of the most devastating plant pathogens are obligate biotrophic parasites. This class of pathogens is characterized by the formation of specially differentiated hyphae. These so called haustoria form inside the infected plant cell and therefore provide an excellent location for the exchange of nutrients and information between the plant and the fungus. Our group is trying to elucidate the roles of these haustoria in establishing and maintaining the obligate biotrophic lifestyle using the legume rusts as a model system. We have so far been able to show that rust haustoria indeed function as nutrient uptake devises as envisaged ever since their discovery more than 150 years ago. We have also been able to show that hexose metabolism seems to be directly coupled to the suppression of host defense reactions, through the conversion of hexoses into sugar alcohols which in turn are capable of quenching ROS. Our latest finding involves the transfer of a haustorial secreted protein specifically into the infected plant cell. We are currently trying to elucidate the function and the mode of transfer of this potential biotrophic effector protein.

Session 5: Metabolomics

Chairperson: J Aguirre, National University of Mexico

5.1. Metabolomics in yeasts and filamentous fungi

STEPHEN G. OLIVER
Faculty of Life Sciences, The University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

The metabolome may be defined as the quantitative complement of low molecular weight metabolites present in the cell under a given set of physiological conditions. As such, the metabolome lends itself readily to functional analysis, since changes in a cell's physiology as a result of gene deletion or overexpression should be amplified through the hierarchy of the transcriptome and the proteome and so be more easily measurable via the metabolome. In addition, the default assumption underlying transcriptome and proteome analysis that an x-fold increase in transcript or protein necessarily results in x-fold increase in effective activity may very well not reflect reality: at any time the rate of an enzymatic reaction is a function of substrates, products and modifiers, as well as gene expression, and the formalism of metabolic control analysis tells us that while changes in the expression level of individual proteins may have little influence on fluxes, they can and do have major effects on the concentrations of intermediary metabolites. Consequently, metabolome analysis promises to be more sensitive than that of either the transcriptome or proteome as pathway activities will be reflected more clearly in the concentrations of pools of metabolites than in the concentrations of relevant enzymes (or indeed the concentrations of the mRNAs encoding those enzymes).

Metabolomics, however, should not be pursued in isolation from the other levels of functional genomic analysis. The availability of complete genome sequences has enabled the *in silico* reconstruction of the complete metabolic network of an organism. Such reconstructions permit an *in silico* approach to the identification of functional modules or correlated reaction sets (co-sets) within the metabolic network by using "flux coupling analysis" (FCA). The metabolome profiles of some make them outliers when compared to mutants of other genes in the same pathway. Outlying mutants encode enzymes or regulators that are uncoupled from the rest of the members of the pathway. Conversely, mutation of genes encoding enzyme co-sets produced similar metabolite profiles. This suggests that pattern recognition within metabolome profiles can be used as an empirical

tool to confirm the identity of modules or co-sets predicted by genome-scale metabolic models. More importantly, this approach can also show whether an enzyme that has been identified as being uncoupled in the *in silico* genome-scale model is functionally uncoupled under the particular cellular conditions examined. Thus, metabolome profiling of gene deletion mutants is a relatively simple empirical tool for the experimental validation and refinement of genome-scale metabolic models.

This, and other uses of both exo- and endometabolomic data from yeast, will be discussed in the broader context of the exploitation of metabolomics in fungal biology.

5.2. Functional genomics of pathogenicity in *Magnaporthe grisea*

NICOLAS J. TALBOT, THOMAS A. RICHARDS, DARREN M. SOANES, MARTIN J. GILBERT, RICHARD A. WILSON, GURPREET K. BHAMBRA, ZHENG-YI WANG, ZAIRA CARACUEL-RIOS.
School of Biosciences, University of Exeter, EX4 4QG, United Kingdom (n.j.talbot@ex.ac.uk)

The rice blast fungus *Magnaporthe grisea* causes one of the most serious diseases of cultivated rice. The availability of a full genome sequence for *M. grisea* has allowed the first opportunity to define the gene inventory associated with a fungal phytopathogen. Recently, we have shown that 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. We have also characterised a mutant of *M. grisea* that is impaired in polarised exocytosis. This mutant, *⊿Mgapt2*, is non-pathogenic and also fails to induce a hypersensitive reaction in an incompatible response, suggesting that it is unable to secrete fungal proteins during plant infection that may act as pathogenicity determinants or cultivar-specific elicitors of plant defence responses (avirulence gene products). MgApt2 is a Golgi-associated P-type ATPase that belongs to the aminophospholipid translocase family. We are using a multi-disciplinary approach, involving high throughput gene functional analysis, proteomics, cell biology and analytical biochemistry, to investigate the biology of plant infection by *M. grisea* and to exploit the genomic resources that are now available for its study. Comparative genomic analysis of *M. grisea* with phytopathogenic and free-living fungal species is central to this process and allows us to explore the evolutionary relatedness of the gene inventories of *M. grisea* with other pathogenic species.

Dean, R.A. *et al*. (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* **434**, 980–986
Gilbert, M.J., Thornton, C.R., Wakley, G.E., Talbot, N.J. (2006) A P-type ATPase required for rice blast disease and induction of host defence *Nature* **440**, 535–539
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

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

JÉRÔME COLLEMARE[1], SABRINA BOURAS[1], HEIDI U. BÖHNERT[1], ISABELLE FUDAL[1], ZHONGSHU SONG[2], RUSSELL J. COX[2], DIDIER THARREAU[3] AND MARC-HENRI LEBRUN[1]

[1]UMR2847 CNRS-Bayer Cropscience, Physiologie des plantes et des champignons lors de l'infection, Bayer Cropscience, 14-20 rue P Baizet, 69263 Lyon Cedex 09, France; [2]School of Chemistry, University of Bristol, Cantock's close, Clifton, Bristol BS8 1TS, UK; [3]UMR BGPI, CIRAD-INRA-AGROM, TA41/K, 34398 Montpellier Cedex 05, France

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.

Concluding Keynote Address

5.4. Evolutionary variation and comparative genomics

JOHN W. TAYLOR[1], THOMAS J. SHARPTON[1] and GARRY T. COLE[2]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA; [2]Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249, USA

Genome sequences are available for the human pathogenic Ascomycota fungal species *Coccidioides immitis* and *C. posadasii*, and their close non-pathogenic relative, *Uncinocarpus reesei*. Comparison of the genomes of these species will identify genes that are unique to the pathogens, genes that are shared with other ascomycete pathogens, and genes that are evolving unusually rapidly or under positive selection. These types of genes may include those important to pathogenicity. Use of *C. posadasii* genome sequence to design spotted, 70bp oligomer microarrays has enabled comparative transcription profiling of RNA isolated from pathogenic and non-pathogenic phases of *C. posadasii* from two individuals in the Arizona population. Transcription for the two individuals shows surprising variation including significant differences in the trend in transcription between saprobic and pathogenic life cycle phases. Comparison of genes with similar transcription in the two

individuals with those found to be interesting by the genome comparisons listed above further distills the pool of genes that may be important to pathogenicity. From this pool, genes most likely to be involved in causing disease are identified for experimental confirmation in mice.

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined

1. Comparative metabolic reconstruction of *Laccaria bicolor*
DAG G. AHRÉN
Microbial Ecology, Ecology Building, Lund University, 223 62, Lund, Sweden

Ectomycorrhizal fungi are dominating the soil in boreal forests much due to the access of large amounts of carbon from the photosynthesis of the symbiotic plant. With the genome sequence of *Laccaria bicolor* it is for the first time possible to reconstruct the metabolic map of an ectomycorrhizal fungus. By comparing the metabolic pathways with related organisms such as *Coprinopsis cinereus* and *Cryptococcus neoformans* it is possible to study the ectomycorrhizal symbiosis using a comparative genomics approach. The evolutionary rates of the different metabolic enzymes have been analyzed to identify enzymes and pathways that have an increased evolutionary rate in *L. bicolor* suggesting adaptation to the symbiotic nutritional strategy. Several pathways with importance to the mycorrhizal function have enzymes with increased relative evolutionary rate. These include sucrose, fructose and glucose related pathways, such as trehalose biosynthesis, sucrose and glucose degradation. A web resource of the entire metabolic reconstruction of *Laccaria bicolor* is available through the Laccaria Genome Consortium (http://mycor.nancy.inra.fr/IMGC/LaccariaGenome).

2. Identification of genes implicated in fruit body formation of *Ophiostoma novo-ulmi* and in the pathogenic interaction with its host *Ulmus americana*
MIRELLA AOUN, VOLKER JACOBI, JOSEE DUFOUR & LOUIS BERNIER.
Centre d'étude de la forêt (CEF), Université Laval, Québec (QC), G1K 7P4, Canada

The ascomycete *Ophiostoma novo-ulmi* is the most aggressive causal agent of Dutch elm disease. Here we present the first large-scale genomics study of two important aspects of the pathogen's life cycle: fruit body formation and host-pathogen interactions. Suppression subtractive hybridization cDNA libraries were constructed from perithecia and synnemata and from *Ulmus americana* calli inoculated with *O. novo-ulmi*. Approximately 500 clones of each library were screened for differential expression using subtracted and unsubtracted probes. The majority of clones of the host-pathogen interaction library were of plant origin, and many, implicated in defence mechanisms, were up regulated in the presence of *O. novo-ulmi*. Fungal genes up-regulated during fruit body formation included mannitol dehydrogenase, gtpase rho1, aquaporin, basic region leucine zipper and phosphoenolpyruvate carboxykinase. Differential expression of selected genes is being confirmed by quantitative PCR. Target genes will be disrupted to assess their importance for the physiological function under investigation.

3. Molecular genetics to identify genes involved in the pathogenicity of *Fusarium graminearum* on wheat ears
THOMAS K BALDWIN, MARTIN URBAN, JOHN ANTONIW AND KIM E. HAMMOND-KOSACK
Wheat Pathogenesis Programme, Plant Pathogen Interactions Division, Rothamsted Research, Harpenden, AL5 2JQ, UK

Fusarium Ear Blight (FEB) disease is caused by up to seventeen related *Fusarium* species and occurs on all Gramineae species, including the economically important crops, wheat, barley, rice and maize.

F. graminearum, an important global FEB pathogen, produces trichothecene compounds, including nivalenol (NIV) and deoxynivalenol (DON), that can cause health problems in animals and humans. *F. graminearum* has a haploid genome of 36MB. The genome of the strain PH-1 has been sequenced to 10x coverage and is predicted to contain 14 086 genes. EST collections (containing 40 000 sequences) are available from the COGEME (cogeme.ex.ac.uk) and the Broad Institute (www.broad.mit.edu) databases.

The aim of this project is to identify novel pathogenicity genes in *F. graminearum* by random insertional mutagenesis. A library of PH-1 mutants was created using a protoplast-PEG transformation method and tested for pathogenicity defects on wheat ears. Three mutants with reduced disease causing ability were identified for further molecular and phenotypic characterisation. Plasmid rescue was used to clone the disrupted gene in one of the mutants. A targeted deletion of this gene has verified its role in pathogenicity. Plasmid rescue of a second mutant revealed a multiple gene deletion event had occurred.

4. A gene from the oomycete plant pathogen *Phytophtora parasitica* potentially related to sulfate metabolism is highly expressed in invading hyphae during plant infection

GLORIA BERROCAL-TITO & FRANCK PANABIERES
INRA, Unité Interactions Plantes-Microorganismes et santé Végétale, 400 Route des Chappes BP 16706903 Sophia Antipolis Cedex, France.

The oomycete *Phytophthora parasitica* Dastur (*P nicotianae* Breda de Haan) is a broad host range soilborne pathogen, devastating numerous hosts, among which tobacco and tomato. A sequence encoding a hypothetical protein with discrete homology to bacterial sulfate transporters has been identified after mining an EST collection (Panabieres *et al*., 2005). The combination of *in silico* and experimental analyses indicated that this gene, designed as *PCysZ*, is induced during infection of tomato. .*PCysZ* encodes a 254 amino acid protein, with four transmembrane domains. The C-terminal moiety of the protein displays the DUF 540 domain, possibly involved in cysteine biosynthesis and proposed to be involved in sulfate transport. *PCysZ* appears to be differentially expressed during infection, with a more than 5-fold increase in expression during colonization of the host tissues compared to vegetative growth in synthetic medium. It is induced upon sulfate starvation and is highly expressed under water stress. Reverse genetics experiments will help to elucidate the role of *PCysZ* in *Phytophthora* growth, development and pathogenicity.

Panabieres, F.; Amselem, J.; Galiana, E. and Le Berre, J. (2005) Gene Identification in the oomycete pathogen *Phytophthora parasitica* during in vitro vegetative growth trough expressed sequence tags. *Fungal Genetics and Biology* 42: 611-23

5. Real-time PCR to study the distribution of two major rust species in Eastern North America.

BRIAN BOYLE[1], JOSEE GRONDIN[1], NICOLAS FEAU[1], LOUISE INNES[2], AND RICHARD C. HAMELIN[1].
[1]*Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 rue du P.E.P.S, Quebec, QC G1V4C7, Canada;* [2]*Direction de la protection des forêts, Ressources naturelles et faune Québec, 2700 rue Einstein, Québec, QC G1P 3W8, Canada.*

Foliar rust caused by *Melampsora* spp. is one of the most damaging diseases of hybrid poplars leading to premature defoliation and reduction in yield. The native *Melampsora medusae* f.sp. *deltoidae* and exotic *M. larici-populina* are encountered on wild poplars belonging to the botanical section Aigeiros and Tacamahaca and on a large range of hybrid clones deployed in poplar plantations. The objective of this work was to study the distribution

of these two species in several nurseries, plantations and natural stands of poplars from several locations in the province of Quebec. For this purpose we developed a real-time PCR assay that gave us the capacity to measure precisely the amount of pathogen present in a leaf sample. By relating the amount of pathogen DNA measured to the amount of poplar DNA we can identify and measure the extent of colonization of both rusts within a leaf. The distribution of the pathogens can be evaluated on different clones and tied up to the host or to the hybrid parentage type. Our analysis revealed that *Melampsora* species distribution varied according to the region and the host stand type.

6. The genome sequence of the symbiotic fungus *Laccaria bicolor:* genetic potential for nitrogen mobilisation and transport

ANNICK BRUN, LUCIC EVA[1], MARC BUEE, BARBARA MONTANINI, DAMIEN BLAUDEZ, FRANCIS MARTIN, MICHEL CHALOT, JGI Production Sequencing, Assembly & Annotation Staffs[2], and *Laccaria* Genome Annotation Consortium
[1]UMR INRA/UHP 1136 Interactions Arbres/Micro-organismes, Nancy-Université, 54506 Vandoeuvre-les-Nancy, France; [1]UMR INRA/UHP 1136 Interactions Arbres/Micro-organismes, INRA-Nancy, 54280 Champenoux, France; [2]US DOE Joint Genome Institute, Walnut Creek, CA 94598, USA

Tree species dominating forest ecosystems in boreal, temperate and montane regions develop symbiotic associations with soil fungi, so-called ectomycorrhizas. Central to the success of these mutualistic symbioses is the exchange of nutrients between the partners. To elucidate the genetic basis of this ecologically important behavior, the US Department of Energy Joint Genome Institute (JGI) has sequenced the 65-megabase genome of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Agaricales, Tricholomataceae) to high draft using a whole genome shotgun method. This is the first symbiotic fungus genome to be sequenced. It contains about 20,000 intron-rich gene structure. Analysis of the genes by the JGI and Genome Annotation Consortium yields insights into unexpected aspects of *Laccaria* biology. The isolation and characterization of complete sets of genes encoding transporters for inorganic and organic N, enzymes involved in N mobilisation and assimilation, will bring fundamental knowledge that will be used to tackle (i) changes in capacities for N mobilisation, uptake and assimilation, and (ii) the bidirectional transfer of nutrients between the two symbionts. Furthermore, comparisons with saprophytic or parasitic fungal genomes allow us to highlight symbiotic genetic markers.

7. *Coprinopsis cinerea* mutants forming etiolated stipes under normal fruiting conditions

WASSANA CHAISAENA, MONICA NAVARRO-GONZALEZ, PRAYOOK SRIVILAI & URSULA KÜES
Institute of Forest Botany, Section Molecular Wood Biotechnology, Georg-August-University of Göttingen, Göttingen, Germany

Fruiting body development in *Coprinopsis cinerea* is adapted to the light and dark phases of the normal day-night rhythm. If young developing structures (day 1 to day 4 of development) are transferred into constant darkness, normal cap and stipe differentiation arrests. Instead, the stipe base proliferates to give an elongated structure known as “etiolated stipe”. Recessive mutations in genes *dst3* and *dts4* cause such phenotype in the normal day-night regime. These genes are distinctive from *dst1* and *dst2* identified before in Japan. From morphological analysis of the cap tissues of the etiolated stipes, *dst3* and *dst4* appear to act later in development (at day 3 and day 4 of development, respectively) than *dst1* and *dst2* (defects at day 2 of development). Unlike *dst3* and *dst4*, *dst1* and *dst2* also act in light-control of asexual sporulation.

WC, PS and MNG acknowledge scholarships from the Rajamangala University of

Technology and the Mahasarakham University (Thailand) and CONACYT (Mexico), respectively. The Deutsche Bundesstiftung Umwelt financially supported the laboratory.

8. Two dimensional gel electrophoresis and mass spectrometry to study heavy metal tolerance mechanisms of an ericoid mycorrhizal fungus

MARCO CHIAPELLO, ELENA MARTINO & SILVIA PEROTTO
Dipartimento di Biologia Vegetale dell'Università di Torino, V.le Mattioli 25, 10125 Torino, Italy

Heavy metals represent major environmental hazards to natural ecosystems and to human health. A role for ericoid mycorrhizal fungi in protecting their host plant in contaminated sites has been established. We have focused on the ericoid strain *Oidiodendron maius* Zn, coming from a plot mostly contaminated with zinc and previously characterised for its ability to grow at high concentrations of metal ions. A proteomic approach has been used: 2DE followed by mass spectrometry allowed us to identified some proteins differentially expressed in the absence or in the presence of metal ions. Cadmium ions induced the over-expression of a heat shock protein 60. The over-expression of a DNA repair protein and of a protein belonging to the stress inducible protein family sti35 was observed in the presence of zinc ions. Activity assays of enzymes implied in the oxidative stress response have been performed. Superoxide dismutase, catalase, glutathione peroxidase and reductase activities showed to be all regulated in the presence of both zinc and cadmium ions.

9. Annotation, comparative and evolutionary analysis of fungal Carbohydrate Active enZymes (CAZymes).

ETIENNE G.J. DANCHIN, CORINNE RANCUREL, PEDRO M. COUTINHO AND BERNARD HENRISSAT.
Architecture et Fonction des Macromolécules Biologique (AFMB) UMR 6098 CNRS Universités Aix-Marseille I & II, Case 932, 163 Av. de Luminy, 13288 Marseille cedex 09. France.

Carbohydrate Active enZymes (CAZymes) play a central role in the biology of species having an intimate relation with sugars such as Fungi. Indeed, CAZymes are involved in important processes for this species such as biosynthesis and integrity of fungal cell wall, phytopathogenecity and ability to degrade carbohydrates from the biomass (starch, cellulose, pectin, etc). The wealth and biodiversity of fungal genomes already available allows deciphering the evolutionary history and adaptations of fungal CAZyme repertoires to the environment. We detected and annotated CAZymes from over 20 fungal genomes including both Basidiomycetes and Ascomycetes which allowed the definition of over 20 fungal CAZymes repertoires (or CAZomes). CAZymes represent on average 3% of a given fungal genome and this rate varies from 1% to 4% depending on lineages. Comparative analysis of CAZomes showed that these repertoires vary (in terms of size, distribution and diversity) both as a function of the phylogenetic distance and as a function of a species' lifestyle and ecology. We detected over- and under- represented families and attempted to correlate significant variations to adaptations to particular ecological niches and other functional aspects.

10. Improved Gene Targeting in *Penicillium chrysogenum*

PAULO DE BOER[1], HESSELIEN TOUW[2], JEROEN BASTIAANS[2], RICHARD KERKMAN[2], KAROLINA DUKIĆ[1], MARCO VAN DEN BERG[2], REMKO OFFRINGA[1]
[1]Add2X Biosciences BV, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands; [2]DSM Anti Infectives, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands

In most eukaryotes, including filamentous fungi, the predominant mode of DNA integration is via non-homologous recombination (NHR). For functional genomics studies and various other applications however, DNA integration via homologous recombination leading to gene targeting (GT) is the preferred pathway. The recent identification of key components of the NHR pathway, such as Ku70 and Ku80, has provided new tools for improving GT efficiencies. Knocking out the NHR pathway has resulted in efficient GT in a variety of eukaryotes, including several *Aspergillus* species. In the present study, we show that deletion of the *Ku70* or *Ku80* genes also results in significantly improved GT in the β-lactam producer *Penicillium chrysogenum*.

11. Regulation of fungal gene expression during the interaction between an ectomycorrhizal fungus and a mycorrhiza helper bacterium: a gene profiling approach.

A. DEVEAU, A. SARNIGUET, B. PALIN, J. GARBAYE, F. MARTIN, P. FREY-KLETT
INRA – Nancy, UMR INRA-UHP 1136 Interactions Arbres/Micro-organismes, IFR 110, 54280 Champenoux, FRANCE. deveau@nancy.inra.fr

Ectomycorrhizal fungi have a beneficial impact on tree nutrition and growth. In soils, they also interact with bacteria, some of which promote mycorrhiza formation. The mechanisms by which these fungi interact with their host plant have been extensively studied during the last decade. Recently, genomic tools such as expressed sequence tags (ESTs) and cDNA arrays have been developed to perform large-scale functional analyses of the ectomycorrhiza. However, the mechanisms controling the interactions between ectomycorrhizal fungi and mycorrhiza helper bacteria are poorly understood.

We have thus developed a gene profiling approach to identify the gene networks involved in the interactions between the ectomycorrhizal fungal strain *Laccaria bicolor* S238N and the mycorrhiza helper bacterial strain *Pseudomonas fluorescens* BBc6R8.The impact of the bacteria on the *L. bicolor* transcriptome was analysed at three key stages of the interaction: i.e, before, at the time of physical contact and after. Fungal RNAs were extracted, the cDNA synthesized and labelled with ^{33}P, then used to hybridize to microarrays containing 5000 cDNA from mycelium and sporocarp tissues.

The expression of about 2% of the genes that were analysed was up or down regulated during the interaction with a factor ranging between 2 and 8 fold. These regulated genes are involved various cellular processes. Transduction signal pathways were activated before contact, while genes involved in energy metabolism and protein synthesis were repressed after *Pseudomonas/Laccaria* contact. Based on the results of this large-scale functional analysis, we put forward a conceptual model of the cellular interactions between *L. bicolor* and *P. fluorescens*.

12. Annotation of signaling pathways genes families in the ectomycorrhizal basidiomycete *Laccaria bicolor* reveals an expansion of a G-proteins encoding genes family

SEBASTIEN DUPLESSIS[1], PRETI JAIN[2], AUDE GROSDEMANDE[1], FREDERIC DUCHAUSSOY[1], MIKE MURATET[2], GOPI K.PODILA[2], FRANCIS MARTIN[1] & DOE JOINT GENOME INSTITUTE[3]

[1]UMR 1136 INRA/UHP Nancy 1 Interactions Arbres/Micro-organismes, INRA Nancy, 54280 Champenoux, France; [2]Dept of Biological Sciences, University of Alabama, Huntsville, AL35899, USA, [3]DOE Joint Genome Institute, Walnut Creek, CA 94598, USA.

We have investigated the signaling pathways genes families in the genome sequence of the ectomycorrhizal basidiomycete *Laccaria bicolor*. We focused on the known components of the G-protein mediated signaling pathways (i.e., G-proteins, GPCRs, PKAs, Adenylate cyclases) that were recently described in other filamentous fungi. We also annotated several classes of Kinases (i.e., MAPKs, HKs and PKC); calcium-related signaling proteins, and different classes of phosphatases that had been previously described for their role in plant-microbes interaction signaling. Our results show a large expansion of the gene family coding for heterotrimeric G-proteiṇ α subunits in *L. bicolor* compared to other filamentous ascomycetes or basidiomycetes where only 3 to 4 copies had been described. Homologs of fungal G-α proteins known for their role in filamentous growth or mating were found in *L. bicolor* while no homolog of the G-α related to virulence in phytopathogenic fungi was detected. In addition, two new classes of G-ą were observed: one corresponding to shorter proteins and another one with similarities to G-α sequences described in *Ustilago maydis* (*UmGPA4*) and *Aspergillus oryzae* (*AoGaoC*). These new classes of G-α proteins may participate to fine-tune molecular crosstalk between the ectomycorrhizal fungus and tree roots during symbiosis development.

13. Intercontinental genetic structure of the poplar rust *Melampsora medusae* f.sp. *deltoidae*.

N. FEAU[1], M. BOURASSA[1], D.L. JOLY[1], A. ANDRIEUX[2], B. BARRÈS[2], R.C. HAMELIN[1], P. FREY[2].

[1]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 rue du P.E.P.S, Quebec, QC G1V4C7, Canada; [2]Centre INRA de Nancy, 54280 Champenoux, France.

Migrations of plant pathogens into regions or continents previously free of the disease are recent events relative to the co-evolutionary history of host and pathogen which occurred in the endemic area. Due to the stochastic nature of long-distance dispersal, founder populations are likely to represent a subset of the variation in the center of origin and are not expected to be in equilibrium. A genetically characterized set of SSCP (2 loci) and microsatellites (4 loci) developed for the poplar leaf rust fungus *Melampsora medusae* f. sp. *deltoidae* is used on an extensive worldwide collection in order to infer migration events and sources of introductions across three continents. Isolates from North America exhibited a very high genomic diversity with multiple alleles per locus (8, 14, and 13 to 17 alleles for each of two SSCP and four microsatellite loci, respectively). In contrast, a clonal structure was observed in the European and South-African populations. African and European alleles represent a subset of the allelic diversity present in North America. This is consistent with scenarios of long-distance and independent migration events.

14. Taxonomic EST sampling over four poplar leaf rusts (*Melampsora* spp.): applications for the identification of candidate loci implicated in host adaptation

NICOLAS FEAU, DAVID L. JOLY, RICHARD C. HAMELIN
Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 rue du P.E.P.S, Quebec, QC G1V4C7, Canada

Positive selection signatures can be an indicator of genomic regions that contain genes or gene families of functional importance in host-pathogen interactions. We scanned the transcriptome of poplar leaf rust pathogens for such candidate loci by generating four Expressed Sequence Tag (EST) databases. Two libraries were based on multi-genotype infections of poplar leaves with *Melampsora medusae* f.sp. *deltoidae* (5,500 clones sequenced) and *M. larici-populina* (3,000 clones sequenced), respectively. This approach allowed the detection of Single Nucleotide Polymorphisms (SNPs) directly from contig assemblies. Two additional EST libraries were constructed based on single genotype infections with *M. medusae* f.sp. *tremuloidae* (3,000 clones sequenced) and *M. occidentalis* (3,000 clones sequenced), allowing us to reconstruct ancestral polymorphisms. Clustering and assembly analyses performed within each library with the TGI Clustering tools (TGICL) resulted in 4847 unique sequences from 1,127 contigs and 3,720 singletons. A comparative analysis of the libraries using the blastn algorithm showed that 46 % of sequences in the *M. medusae* f.sp. *deltoidae* library had matches (e-value < 10^{-30}) with the data set containing all the other libraries. The intra- and interspecific SNPs detected are currently validated with a base quality method using the Phred/Phrap/PolyBayes softwares before being tested for positive selection.

15. Whole genome sequencing of the fungal plant pathogen *Botrytis cinerea*, and preliminary results of the comparison with *Sclerotinia sclerotiorum* genome.

SABINE FILLINGER[1], JOËLLE AMSELEM[1,2], FRANÇOIS ARTIGUENAVE[2], ALAIN BILLAULT[3], MATHIAS CHOQUER[1], ARNAUD COULOUX[2], CHRISTINA CUOMO[4], MARTIN DICKMAN[5], , ELISABETH FOURNIER[1], CORINNE GIRAUD[1], LINDA KOHN[6], FABRICE LEGEAI[2], CAROLINE LEVIS[1], CYRIL POMMIER[2], JEAN-MARC PRADIER[1], EMMANUEL QUEVILLON[2,8], JEFFREY ROLLINS, BÉATRICE SÉGURENS[3], ADELINE SIMON[1], MURIEL VIAUD[1], JEAN WEISSENBACH[3], PATRICK WINCKER[3] & MARC-HENRI LEBRUN[8]
[1]PMDV, INRA, Rte de St-Cyr, 78026 Versailles, France; [2]URGI, INRA, 523 place des Terrasses, 91000 Evry, France; [3]Génoscope - CNS, 2 rue Gaston Crémieux, 91000 Evry, France; [4]Broad Institute, 320 Charles St., Cambridge MA 02141, USA; [5]Inst for Plant Genomics and Biotech, 2123 TAMU, College Station, TX, USA; [6] University of Toronto, 3359 Mississauga Rd North, Mississauga ON L5L 1C6, Canada; [7] Department of Plant Pathology,University of Florida PO Box 110680, Gainesville, FL, USA; [8] Plant and Fungal Physiology, UMR 2847, CNRS-BayerCropScience, 69009 Lyon, France

Botrytis cinerea is a fungal plant pathogen (leotiomycete) that provokes grey mould on more than 200 dicotyledonous plant species including grapevine. In 2005, the French national sequencing center (Génoscope) commenced sequencing the genome of the grapevine, *Vitis vinifera* and two of its pathogens: Stolbur phytoplasm and *B. cinerea*.

The genome of *B. cinerea* T4 strain (40 Mb) was sequenced with a 10.5 x coverage (approx. 600 000 reads) and 50,000 ESTs are underway. Genomic sequences (3, 10 and 50 kb inserts) were assembled in 2281 contigs and 118 supercontigs. Structural annotation will be based on automatic gene prediction using *ab initio* (FgenesH, Eugene) and similarity (genome/cDNA and genome/known proteins comparisons) softwares. Automatic gene prediction will be validated by a manual annotation process involving an international consortium of 20 research groups.

In 2005, the Broad Institute released the assembly of a 4-5 x genomic sequence from *B. cinerea* strain B05-10 (TMRI/Syngenta) as well as the 7-8 x genomic sequence of the closely related necrotrophic fungus *Sclerotinia sclerotiorum* (Broad Institute/NFS). These data allowed us to compare both *B. cinerea* strains on one hand and *B. cinerea* B05.10 gene calls with those from *S. sclerotiorum* on the other hand. Both species present a high degree of sequence similarity/identity, but also synteny at the contig level was observed.

16. Impact of methionine synthase deletion on metabolism of the phytopathogenic fungus *Magnaporthe grisea*

OCEANE FRELIN[1], MARIE EMMANUELLE SAINT-MACARY[1], MARIE JOSEPH GAGEY[1], CHRISTELLE BARBISAN[1], ROLAND BEFFA[1], MARC-HENRI LEBRUN[1], GERALDINE MEY[2] & MICHEL DROUX[1]

[1]UMR2847 CNRS/Bayer CropScience, Physiologie des plantes et des champignons lors de l'infection, Bayer CropScience, 14-20, rue Pierre Baizet – 69009 LYON; [2]Université Claude Bernard Lyon I, UMR CNRS-INSA-UCB 5122, 10 rue Dubois, Bât Lwoff, 69622 VILLEURBANNE Cedex, France.

Sulphur plays an essential role in all living organisms. After sulphate assimilation, reduced sulphur is incorporated in organic molecules to generate the two sulphur amino acids, cysteine and methionine. In filamentous fungi, their biosynthetic pathways are complex and consist in features reported in both plant and bacteria as well as those described in the yeast *Saccharomyces cerevisiae*.

In this study, we focused on methionine metabolism in the plant pathogenic fungus *Magnaporthe grisea* as a model. With this aim, a mutant of the methionine synthase was obtained using gene replacement. Our goal is to further understand the impact of the methionine synthesis on general metabolism in *M. grisea*. For this view, we performed targeted metabolites analysis through reversed chromatography using HPLC. Results show drastic differences in amino acid profiles between wild type strain and the *ΔMS* mutants. We also highlight the role of methionine in the polyamine biosynthesis.

17. Annotation and expression of hydrophobins in the symbiotic fungus *Laccaria bicolor*.

JULIEN GIBON[1], RAJESH VELAGAPUDI[2], PATRIK J. HOEGGER[2], URSULA KÜES[2] & FRANCIS MARTIN[1].

[1]UMR INRA/Université Henry Poincaré 1136, Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France; [2]Molecular Wood Biotechnology, Institute of Forest Botany, Georg-August-University Goettingen, Buesgenweg 2, D-37077 Goettingen, Germany

Laccaria bicolor is a symbiotic fungus interacting with many tree species in temperate and boreal forests. The recent release of its complete draft genome by the DOE Joint Genome Institute allows large scale and rapid analysis of gene families. Here we present our work concerning annotation and expression of *L. bicolor* hydrophobins. Hydrophobins are small, self-assembling, cystein rich proteins involved in the production of aerial structure and probably in hyphae agregation. We have identified 14 gene models presenting homology with known hydrophobin sequences. They comprised 13 putative class I hydrophobins and one hydrophobin-like protein presenting a chitinase domain on its N-terminal part and a hydrophobic domain on its C-terminal part. Sequence alignments revealed two groups containing five and six genes, respectively. The phylogenetic analysis showed that *Laccaria* hydrophobins are closely related to subgroups of the 34 different hydrophobins in *Coprinopsis cinerea*. The 20 hydrophobins of *Phanerochaete chrysosporium* are more distantly related. Using RT-PCR, we have studied the expression of eight *Laccaria* hydrophobin genes in the vegetative mycelium, fruiting body tissues and ectomycorrhizal

tips; transcripts for six of these hydrophobin genes were found in the investigated tissues. Further studies will aim at understanding the role of these hydrophobins in the fungal development.

18. Through the looking-glass: dynamic imaging of living fungal cells

PATRICK C. HICKEY
LUX Biotechnology Ltd, King's Buildings, Edinburgh, EH9 3JF, United Kingdom.

A combination of novel probes, high quality optics, a "rainbow" of lasers and excitation sources coupled with sensitive detection technologies, are pushing light microscopy to its limits. Fluorescent proteins (e.g. GFP) and fluorescent dyes are powerful tools for targeting organelles, tracking growth and analysing cellular dynamics of fungal hyphae.

Optimising settings e.g. laser power, scanning speeds and gain are crucial to obtain the highest quality images possible, whilst minimising the phototoxic damage to cells and photobleaching of fluorescent probes. We present some novel techniques for preparing samples for microscopy, with the aim of obtaining the best possible optics, and keeping cells alive for prolonged periods of time. An overview of useful software for image processing and time lapse animation is provided.

LUX Biotechnology Ltd is a research based company specialising in fluorescent and luminescent technologies. LUX have investigated a range of novel fluorescent and luminescent proteins, and expressed them in fungal cells. The importance of live cell analysis and the use of fungi in high throughput imaging applications will be discussed. We also introduce a unique range of calibration standards, designed to provide quality assurance for luminescent and fluorescent applications.

19. Towards a database for the identification of fungi in wood

PATRIK J. HOEGGER, ITHZAZ MALIK, SUDHAKAR PEDDIREDDI, ANNETTE NAUMANN & URSULA KÜES
Institute of Forest Botany, Section Molecular Wood Biotechnology, Georg-August-University of Göttingen, Göttingen, Germany

Early detection and identification of fungi colonizing and degrading wood is of great economical importance in living trees as well as in timber in use. Visual inspection of wood allows detection only in severe cases of infestation and late stages of decay. Furthermore, species identification normally depends on the presence of fruiting bodies or asexual spore production. In contrast, molecular or spectroscopic methods (e.g. DNA sequencing of ITS region or FTIR spectroscopy) can lead to early fungal detection and also to species identification independent of morphological structures. Both approaches are applied in our lab to establish specific databases for use in the detection and identification of wood-inhabiting fungi.

This project is supported by the BMBF in the network project "Forst-Holz-Wertschöpfungskette Buche/Küstentanne" and the laboratory by the Deutsche Bundesstiftung Umwelt. AN holds a grant by the Deutsche Forschungsgemeinschaft DFG ("eigene Stelle", NA 749/1-1).

20. Identification of fungal biotrophy genes by insertional mutagenesis of the crucifer anthracnose pathogen, *Colletotrichum higginsianum*

AURELIE C. HUSER, HIROYUKI TAKAHARA, & RICHARD J. O'CONNELL
Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Carl von Linné Weg 10, 50829 Köln, Germany

The ascomycete fungus *Colletotrichum higginsianum* invades *Arabidopsis* plants by means of a two-stage, hemibiotrophic infection process. Following penetration by appressoria, specialised intracellular hyphae develop biotrophically inside living epidermal cells before the fungus switches to destructive necrotrophic growth. Our aim is to identify fungal genes required for the initial biotrophic phase. A library of over 7,800 random insertional mutants has been generated in *C. higginsianum* using *Agrobacterium*-mediated transformation. To date, 31 non-pathogenic or reduced pathogenicity mutants have been identified by screening on *Arabidopsis* seedlings. Seventeen of these mutants show defects in pathogenesis that are expressed after appressorium formation and penetration, e.g. failure to differentiate biotrophic hyphae, maintain host cell viability or make the transition to necrotrophy. Nucleotide sequences flanking the T-DNA insertion are recovered by inverse PCR or thermal asymmetric interlaced PCR and used to isolate the disrupted genes from a cosmid genomic library. The cytological and molecular characterisation of selected mutants will be presented.

21. TRACEability of Arbuscular Mycorrhizal fungi as plant-beneficial micro-organisms in agro-environments

MARLEEN IJDO[1], MARIA NAUMANN[2], MARLIS REICH[3], HERBERT STOCKINGER[4], PAOLA BONFANTE[2], STEPHANE DECLERCK[1], FRANCIS MARTIN[3] and ARTHUR SCHUESSLER[4]
[1]Université catholique de Louvain, Faculté d'ingéniérie biologique, agronomique et environnementale;Unité de microbiologie, Mycothèque de l'UCL (MUCL), Place croix du Sud, 3, 1348 Louvain-la-Neuve, Belgium; [2]Università degli Studi di Torino, Department of Plant Biology, Laboratory of Biology of Symbiosis, Viale Mattioli 25, 10125 Turin, Italy; [3]Institut National de la Recherche Agronomique, INRA /UHP UMR 1136, Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France;[4]University of Technology Darmstadt, Department of Biology (FB10), Institute of Botany, Schnittspahnstrasse 10, 64287 Darmstadt, Germany

Arbuscular mycorrhizal fungi (AMF) supply the majority of land plants with nutrients, thus increasing plant production and conferring resistance against stress factors. The exploitation of these symbionts in agro-environments is of high environmental relevance, economic value, and societal impact. However, it is not possible to produce these obligate symbiotic fungi axenically and therefore the ' classic' mass-production of AMF inoculum is based on *in vivo* material which is prone to contamination. This makes quality control problematic as actively growing AMF cannot be identified only by morphology, and real traceability in the field is yet impossible. Root Organ Culture (ROC) offers the possibilities to develop molecular detection systems for AMF under contaminant-free conditions. This also counts for their endobacteria that are difficult to study but are supposed to play an important role in this symbiosis. At present a rapidly growing sequence database of AMF gets available which now allows developing tools like DNA barcodes and DNA microarrays for the detection of the AMF community and the tracing of AMF species within the field. The objective of TRACEAM is i) to increase the quality of inoculum by developing an *in vitro* bioreactor cultivation system, ii) to develop methods for molecular identification (DNA barcodes), and iii) to design in-field applicable molecular tracing tools ('phylochips', taxonomic DNA microarrays).

22. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses.

RENAUD IOOS[1,2], AXELLE ANDRIEUX[1], BENOÎT MARÇAIS[1] & PASCAL FREY[1]
[1]INRA, UMR 1136 IAM, Equipe de Pathologie Forestière, 54280 Champenoux, France, [2]LNPV, Unité de Mycologie Agricole et Forestière, Domaine de Pixérécourt, 54220 Malzéville, France.

The different taxa of the *Phytophthora alni* complex, *P. alni* subsp. *alni* (*Paa*), *P. alni* subsp. *uniformis* (*Pau*) and *P. alni* subsp. *multiformis* (*Pam*) are recent and widespread pathogens of alder in Europe. They are believed to be a group of emergent heteroploid hybrids between two phylogenetically-close *Phytophthora* species. Nuclear and mitochondrial DNA analyses were performed, using a broad collection of *P. alni* and two closely related species, *P. cambivora* and *P. fragariae*. *Paa* possesses three different alleles for each of the nuclear genes we studied, two of which are present in *Pam* as well, whereas the third matches the single allele present in *Pau*. Moreover, *Paa* displays common mtDNA patterns with both *Pam* and *Pau*. A combination of the data suggests that *Paa* may have been generated on several occasions by hybridization between *Pam* and *Pau*, or their respective ancestors. *Pau* might have *P. cambivora* as a species ancestor, whereas *Pam* seems to have either been generated itself by an ancient reticulation or by autopolyploidization.

23. *Agrobacterium*-based gene transfer for post-genomic research in *Laccaria bicolor*

M. KEMPPAINEN[1], S. DUPLESSIS[2], F. MARTIN[2] & A.G. PARDO[1]
[1]Laboratorio de Interacciones Microorganismos-Plantas, Programa de Investigación en Interacciones Biológicas, Universidad Nacional de Quilmes, B1876BXD Bernal, Provincia de Buenos Aires, Argentina. E-mail: apardo@unq.edu.ar. [2]UMR INRA-UHP "Interactions Arbres/Micro-Organismes"IFR 110 "Genomique, Ecophysiologie et Ecologie Fonctionnelles" INRA-Nancy, 54280 Champenoux, France

Sequencing of the genome of the symbiotic basidiomycete *Laccaria bicolor* was accomplished by a whole-genome shotgun approach by the Joint Genome Institute (DoE, USA). The availability of this sequence opens a large array of possibilities for functional analysis of the ectomycorrhizal symbiosis and the biology of this fungus. However, an efficient use of this new information requires the availability of an efficient genetic transformation system of the fungus. *Agrobacterium*-mediated transformation offers an easy and manageable method for genetic manipulation of *L. bicolor* (Kemppainen et al 2005). We have optimized a mycelium-based transformation protocol for dikaryotic and compatible monocaryotic strains of *L. bicolor* and evaluated the use of several basidiomycete and ascomycete promoters in this fungus. The analysis of plasmid-rescue recovery of T-strand patterns revealed a random integration of transgenes in both coding and non-coding sequences, thus, proving that the method is suitable for validation of gene function in *Laccaria*. Besides, cytosolic and mitochondrial directed expression of sGFP proposes the use of this maker protein, with some limitations, in genetic studies of *L. bicolor*. Currently, we are evaluating the requirements for directed gene disruption and RNA interference by *Agrobacterium*-mediated transformation in *Laccaria bicolor*.

24. NACHT/NB-ARC domain proteins: a multigene family in *Laccaria bicolor*

ANNEGRET KOHLER & FRANCIS MARTIN
Unité Mixte de Recherche INRA/UHP 1136 « Interactions Arbres/Microorganismes », Institut National de la Recherche Agronomique, Centre de Recherches de Nancy, 54280 Champenoux, France

The U.S. Department of Energy Joint Genome Institute (DOE JGI) recently released the first complete draft genome sequence of an ectomycorrhizal fungus, *Laccaria bicolor*. In order to learn more about the signal transduction repertoire of this fungus and the pathways involved

in sensing, developmental signals, as well as stress factors, we have annotated the complete set of putative proteins containing NACHT/NB-ARC domains. The later proteins have been extensively studied in the context of apoptosis, pathogen response in animals and plants, and transcriptional regulation in bacteria. These P-loop NTPase domains have been detected in approximately 5-10% of the predicted gene products in the sequenced prokaryotic and eukaryotic genomes and emerged probably very early in evolution. In addition to the NACHT or NB-ARC domain, these proteins typically contain DNA- or protein-binding domains, such as WD40, tetratricopeptide repeat (TPR), leucin-rich repeat (LRR) and ankyrin repeat. In *Laccaria bicolor*, we identified putative proteins containing TPR, WD40 as well as ankyrin-repeats fused to NACHT or NB-ARC domains, but not LRR. The largest gene family with more than 70 members encodes putative NACHT/NB-ARC proteins with TPR repeats, a second multigene family of about 40 genes codes for NACHT-WD40 proteins. The size of these gene families indicates an important role for these gene families in *Laccaria bicolor*.

25. The secretome of *Coprinopsis cinerea* and other higher basidiomycetes

ANDRZEJ MAJCHERCZYK, DOROTHEA FRAGNER, RAVI CHANDRA DWIVEDI, MOJTABA ZOMORRODI & URSULA KÜES
Division of Molecular Wood Biotechnology, Institute of Forest Botany, Georg-August-University of Göttingen, D-37077 Göttingen, Germany

Basidiomycetes secrete many different proteins for degradation of complex substrates. Only few members of the complex fungal secretomes can be studied by direct detection of enzymatic activities and changes in substrate composition. To understand and to follow the degradation processes, we use mass spectrometry for the analysis of tryptic digests of proteins with a Mascot database containing known protein sequences, annotated genomic sequences and ESTs from *Coprinopsis cinerea*, *Trametes versicolor* and *Pleurotus ostreatus*, respectively. Analysis of the *C. cinerea* secretome showed an increasing complexity with rising age of cultures. Several different glucosidases, oxidative enzymes (e.g., laccases, manganese-dependent peroxidases, glyoxal and glucose oxidases), lectin-like proteins and multiple proteolytic enzymes have been identified in liquid culture. Other enzymes effective in substrate degradation localize to the fungal cell wall and methods are developed to identify their individual nature.

Part of this work is supported in frame of a Common Lower-Saxony-Israel Project (VW-Vorab). The Deutsche Bundesstiftung Umwelt financially supported our laboratory.

26. DIFFERENTIAL ANALYSIS OF FILAMENTOUS FUNGI *CHRYSONILIA SITOPHILA* PROTEOME INDUCED BY PENTACHLOROPHENOL – A CASE STUDY FOR ASCOMYCETE BIOREMEDIATION POTENTIAL

I. MARTINS[1]; M. CARVALHO[1]; R. AMEY[2]; P.T.N. SPENCER-PHILLIPS[2], C. SILVA PEREIRA [1,*] & M.V. SAN ROMÃO[1,3]
[1]ITQB-UNL/IBET, Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa / Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; [2]University of the West of England, Bristol, UK; [3]EVN, Estação Vitivinícola Nacional, Dois Portos, Portugal.
** Corresponding author, spereira@itqb.unl.pt*

Pentachlorophenol (PCP) was considered by the WHO (2004) as one of the most hazardous substances and classified as a probable human carcinogen and an endocrine disruptor. Although its use is EU-wide restricted, PCP and its degradation sub-products are still frequently detected in all environmental spheres. PCP is environmentally persistent (photostable) and its water solubility together with its moderate mobility makes soil/water interactions acute. This favours its use as a model molecule for assessing the behaviour of other POPs. The aromatic matrix of lignin/suberin resembles the basic molecular skeleton of

PCP, suggesting that the fungal species biodegradation potential is somehow correlated with its bioremediation potential. Some fungal species present amongst the cork colonising consortia of microorganisms were recently observed to be able to perforate the complete thickness of the highly recalcitrant cork cell wall (enriched in lignin - 20% and suberin - 40%), suggesting an active biodegradation ability (Silva Pereira et al., 2006). These filamentous ascomycete species were able to tolerate PCP to levels above 15 mg/L. *C. sitophila* could apparently growth solely in the presence of PCP and its growth rate in the presence of glucose declines as the concentration of the toxic increases. The proteome of *C. sitophila* during growth on different substrates has been determined by 2D-DIGE. This has enabled differences in the fungal protein expression profile to be evaluated in relation to substrate composition. Preliminary results indicated the appearance of new proteins in the extracellular proteome, but apparently not in the intracellular proteome. Mass spectrometry techniques are being used for protein identification. *C. sitophila* PCP main degradation pathways (and stress associated proteins) identification is now being attempted.

Silva Pereira, C, *et al.* M. Effect of Fungi Colonisation in Cork Mechanical Performance. Int. Biodeterior. Biodegrad. 2006; 57 (4): 195-250.

27. Tissue development and differentiation during fruiting in the basidiomycete *Coprinopsis cinerea*

MONICA NAVARRO-GONZALEZ, WASSANA CHAISAENA & URSULA KÜES
Institute of Forest Botany, Section Molecular Wood Biotechnology, Georg-August-University of Göttingen, Göttingen, Germany

For more than a century, *Coprinopsis cinerea* is a model fungus for studying fruiting body development of basidiomycetes. It takes seven days from the first hyphal aggregation over tissue formation and differentiation within primordia to obtain the mature fruiting bodies that within a few hours will autolyse to release the sexual basidiospores. Stages of fruiting body development have been described in the 1920ties by Buller in his book series “Researches of fungi”. Lateron, Rejinders presented a schematic diagram of tissue distributions within a fully established primordium and Moore and coworkers studied the morphology of the stipe during elongation and of the hymenium covering the gills. It is then surprising to note, that the complete process of fruiting body development is not documented in the literature in descriptions and/or photos. Here, we present a picture catalogue throughout the whole process of tissue development and maturation in the process of fruiting body development in *C. cinerea*.

MNG was supported by CONACYT (Mexico). The laboratory was supported by the Deutsche Bundesstiftung Umwelt.

28. Distribution of mating-types in a population of environmental *Aspergillus fumigatus* isolates from Ireland

CÉLINE M. O’GORMAN[1,2], HUBERT T. FULLER[1] & PAUL S. DYER[2]
[1]UCD School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Republic of Ireland; [2]School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.

Aspergillus fumigatus is the most common airborne opportunistic fungal pathogen of man. In immuno-compromised patients it causes life-threatening diseases, such as invasive aspergillosis. Its spores are also the causal agent of allergies in patients with atopic immune systems. In spite of its increasing medical importance, many aspects of the biology of the fungus remain unknown. In particular it is not clear whether the pathogen has a sexual reproductive phase, which might be of importance for variation and evolution of the species, and could lead to increased difficulties for disease control. 90 environmental isolates of *A*.

fumigatus collected from aerial samplings in Dublin were examined for the characteristics of a sexual population. The isolates were typed by a novel multiplex mating-type assay to determine whether they contained the *MAT1-1* or *MAT1-2* genotype. Results showed that the Irish population consisted of equal proportions of each mating type, with 50% *MAT1-1* and 50% *MAT1-2*. This 1:1 distribution ratio is consistent with sexuality. Work is underway to characterise the isolates by RAPD-PCR fingerprinting methods using four separate primers. The patterns generated will be used to assess the extent of genetic variation within samples and whether clustering of mating type is evident.

29. Genetic linkage between growth rate and intersterility genes in *Heterobasidion annosum* s. l.

ÅKE OLSON, MÅRTEN LIND and JAN STENLID
Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences. Box 7026, 75007 Uppsala, Sweden, ake.olson@mykopat.slu.se. http://www.mykopat.slu.se/

Quantitative trait loci (QTL) for mycelial growth rate were identified and positioned on a genetic linkage map of *Heterobasidion annosum sensu lato* (s.l.), a devastating root rot pathogen on conifers. The mycelial growth rate was analysed among 84 progeny isolates in two different temperature regimes, 12 and 24 °C, and segregated as a continuous character. The assay identified three QTL for growth rate at low temperature positioned on linkage groups 1, 17 and 19 with peak LOD values of 3.2, 2.9 and 4.8, respectively. At high temperature corresponding QTL on the same linkage groups, with peak LOD values of 1.3, 2.8 and 2.2, were identified. The QTL for the low temperature regime explained 20.9 %, 18.1 % and 24.0 % of the variation in mycelial growth rate, respectively. The broad-sense heritability was estimated to 0.97 and 0.95 for growth rate at low and high temperature, respectively. Two of the QTL for mycelial growth rate were shown to be tightly linked to the intersterility genes S and P, which control mating within and between closely related species and intersterility groups of *H. annosum* s.l. Isolates with a plus allele at the intersterility loci had a higher growth rate than isolates that harboured minus alleles.

30. Cell wall degrading enzymes produced by *Fusarium graminearum*

VINCENT PHALIP & JEAN-MARC JELTSCH
UMR 7175, Ecole Supérieure de Biotechnologie de Strasbourg, Université Louis Pasteur, Boulevard S. Brandt, BP 10413, 67412 Illkirch-Graffenstaden Cedex, France

Dialog between plants and pathogenic fungi, including parries and ripostes from the two partners, is complex and therefore difficult to study as a whole. However, some aspects could now be studied thanks to advances in genomics. We are interested in one of the assaults driven by filamentous fungi in the presence of plant material: cell wall degrading enzymes (CWDE) secretion. *Fusarium graminearum* / *Humulus lupulus* (hop) interaction was studied at transcriptomic, proteomic and enzymatic levels. The common features of these studies were the growth of the fungus onto a hop cell wall preparation and the necessity of genomics data for interpretation. The exoproteome of *F. graminearum* displayed 84 unique proteins, mainly putative CWDE falling into 24 different EC classes. *F. graminearum* genome encodes a lot of xylanases and our quantitative study revealed that no fewer than 30 were actually and differentially expressed on plant cell wall. Finally, enzymatic assays proved that these CWDE are highly active on many polymeric and oligomeric substrates. The results obtained by the combination of transcriptomic, proteomic and enzymatic approaches and interpreted via available genomic data are very coherent from one to another and contribute to a better understanding of plant / fungi interactions.

31. Quantification of morphology of *Trichoderma harzianum* during biosynthesis of chitinase

G. N. RAMESHAIAH and TAPOBRATA PANDA*
Biochemical Engineering Laboratory, Department of Chemical Engineering, Indian Institute of Technology Madras, Chennai – 600036, Tamilnadu, India. * Corresponding author:
E-mail: pandaiitm@yahoo.co in Phone no: +91-044-2257-6160

Understanding morphology of fungi is essential. Many parameters influence the morphological developments of filamentous fungi. Quantitation of fungal morphology will be useful in translating laboratory observations into commercial practice. Methods have been developed for quantitative estimation of process parameters during morphological developments in fungi in various bioprocesses. Chitinases are found in bacteria, plants, fungi, invertebrates, and other micro organisms. Chitinases produced from bacteria and plants are less stable than that produced from fungi during other metabolites production. The present investigation focuses on the morphology related production of chitinases from *Trichoderma harzianum.* Chitinase is well known to degrade chitin containing cell wall of many fungi. This property makes it most valuable in the field of pest control, pollution abatement, basic, and commercial biology. Using *Trichoderma harzianum,* biocontrol activity on phytopathogens is accomplished by different mechanisms. *Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. The development of a reliable transformation system is a prerequisite for improving the understanding of its genetics and molecular biology leading to enhancing its application.

Morphology of *Trichoderma harzianum* was estimated for quantitative characterization using microscopic technique. The morphological measurements showed that morphology of filamentous organisms is a function of initial glucose concentration. The morphological parameters increased with time and remained constant after the exponential phase. The chitinase fermentation process utilizing glucose as the sole carbon source was investigated in shake flask cultivation. Chitinase production varied rapidly at various initial concentration of glucose along with morphology. In microscopic analysis, free, dispersed mycelia, and pellets of filamentous fungi were observed. This work emphasizes on the estimation of the morphological parameters (mean hyphal length, mean hyphal growth unit, tip extension rate, and mean equivalent diameter) relating to chitinases production. An attempt to develop suitable kinetic mechanism for the synthesis of this is made.

32. The genome sequence of the symbiotic fungus *Laccaria bicolor:* Lipid metabolism

M. REICH[1,2], C. GOEBEL[3], F. MARTIN[2], I. FEUSSNER[3], A. POLLE[1]
[1]Institute of Forest botany, Georg-August-Universitaet Goettingen, Germany ; [2]INRA, Tree-Microbe Interactions Departement, Nancy, France ; [3]Institute Albrecht-von-Haller, Departement of Bio-chemistry of plants, Georg-August-Universitaet Goettingen, Germany

Tree species dominating forest ecosystems in boreal, temperate and montane regions develop symbiotic associations with soil fungi, so-called ectomycorrhizas. Central to the success of these mutualistic symbioses is the exchange of nutrients between the partners. To elucidate the genetic basis of this ecologically important behavior, the US Department of Energy Joint Genome Institute (JGI) has sequenced the 65-megabase genome of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Agaricales, Tricholomataceae) to high draft using a whole genome shotgun method. This is the first symbiotic fungus genome to be sequenced. It contains about 20,000 intron-rich gene structures. Analysis of the gene by the JGI and Genome Annotation Consortium yields insights into unexpected aspects of *Laccaria* biology.

We have annotated genes involved in lipid metabolism and assessed the changes in fatty acid profiles in *Laccaria* vegetative mycelium and ectomycorrhiza. We found striking

differences between *L. bicolor* and other fungi, including gene structure, clustering and compartmentation of gene coding for fatty acid synthesis and degradation of fatty acids via the β-oxidation cycle. The expression of these lipid metabolism genes in different *Laccaria* tissues will be discussed.

33. Optimization of laccase production by transformants of *Coprinopsis cinerea*

MARTIN RÜHL, SREEDHAR KILARU, KARIN LANGE, PATRIK J. HOEGGER, ANDRZEJ MAJCHERCZYK, & URSULA KÜES
Division of Molecular Wood Biotechnology, Institute of Forest Botany, Georg-August-University of Göttingen, D-37077 Göttingen, Germany

Laccases from basidiomycetes have various industrial applications, e.g. in the paper and pulp and in the wood industries. Overexpression of these enzymes in ascomycetes does not give satisfactory yields and, often, the enzyme show altered properties. Therefore, we use the basidiomycete *Coprinopsis cinerea* as a host for high level production of laccase from efficient promoter gene constructs. The *gpdII* promoter of *Agaricus bisporus* was found most efficient in driving laccase production. Various laccase genes have by now been expressed in *C. cinerea* under control of the *gpdII* promoter. However, yields of enzymes differed between the genes. Alteration of growth conditions (media, temperature, aeration) lead so far to a 20fold increase in total enzyme yields. With the most efficient gene, at our current best cultivation conditions, laccase activities of up to 30 U/ml are obtained.

Our laboratory is financially supported by the Deutsche Bundesstiftung Umwelt (DBU).
Present address of S. Kilaru: University of Bristol, School of Biological Sciences, Woodland Road, Bristol, BS8 1UG, UK

34. The endobacteria of *Gigaspora margarita* affect gene and protein expression during the spore germination

ALESSANDRA SALVIOLI, LUISA LANFRANCO, MARCO CHIAPELLO, PAOLA BONFANTE
Dipartimento di Biologia Vegetale dell' Università degli Studi, Viale Mattioli 25 10125 Torino, Italy

Symbiotic associations between endocellular bacteria and eukaryotic cells are widespread among animals and plants, but only a few examples have been described in the fungal kingdom. In *Gigaspora margarita* BEG 34 a homogenous population of bacteria is hosted inside the spore and is vertically transmitted through fungal generations. A protocol based on repeated passages through single spore inocula caused a dilution of *Candidatus* Glomeribacter gigasporarum, eventually leading to bacteria-cured spores. The cured spores represent a stable variant of the original genotype, showing differences in cytoplasm, cell wall organization and growth pattern during the presymbiotic phase.
To gain a better insight on the influence of the endobacteria on their fungal host, molecular approaches were applied, from gene expression profiles to assays of comparative proteomics. RNA was extracted from germinating spores with and without endosymbionts and used to perform macroarray experiments and to construct a suppression subtractive hybridization cDNA library. A number of differentially expressed genes were identified from both the experiments. For the proteomic analysis, proteins from both fungal lines were extracted and analyzed by two-dimensional gel electrophoresis. Ten spots representing differentially expressed proteins were detected. First results show that the two fungal lines differ in both gene and protein profiles; the expression of genes related to lipid metabolism seems to be affected in the cured spores.

35. Comprehensive analysis of the developmental processes associated with pathogenesis in the rice blast fungus.

ANE SESMA[1], SARA TUCKER[1], STEVE LENHERT[2,3], BRUNO MOERSCHBACHER[2] and ANNE OSBOURN[4]
[1]Dept. of Disease and Stress Biology, John Innes Centre NR4 7UH Norwich, UK; [2]Westfälische-Wilhelms-Universität Münster, Institut für Biochemie und Biotechnologie der Pflanzen, Hindenburgplatz 55, D-48143 Münster, Germany; [3]current address: Forschungszentrum Karlsruhe GmbH,Institut für NanoTechnologie 76344 Eggenstein-Leopoldshafen, Hermann-von-Helmholtz-Platz 1,Germany; 4Dept. of Metabolic Biology, John Innes Centre NR4 7UH Norwich, UK; e-mails: sesma@bbsrc.ac.uk; Steven.Lenhert@int.fzk.de; bruno.moerschbacher@uni-muenster.de; anne.osbourn@bbsrc.ac.uk

M. grisea is considered as a foliar pathogen and it penetrates leaves by means of a specialised structure called appressorium (Talbot, 2003). It can also infect cereal roots and new developmental processes associated with root infection have been identified such as penetration structures called hyphopodia (Sesma&Osbourn, 2004). These results highlight the extraordinary ability of *M. grisea* to recognise and develop diverse mechanisms associated with pathogenesis on different plant tissues. On the other hand, general determinants are also required by this fungus for both leaf and root infection such as the MAPK signalling pathway controlled by PMK1.

Apressoria can be induced in hydrophobic polystyrene (PS-PHOB) surfaces and several appressorium-deficient mutants have been isolated in this way. We have tested surfaces with different chemical and physical properties to identify *in vitro* hyphopodium-inducing conditions. We have found that bulbous swollen growth resembling the invasive growth of *M. grisea* within the plant cell can be induced by hydrophilic polysterene (PS-PHIL) surfaces. No morphological changes were observed during *M. grisea* growth on glass surfaces (also hydrophilic). Interestingly, this bulbous growth is PMK1-dependent. Chitin conversion to chitosan by de-N-acetylation has been identified on fungal structures when colonising their plant hosts (El Guedari, 2002). Remarkably, a decrease on chitin composition and presence of chitosan was also monitored on the hyphae developed on PS-PHIL membranes as well as on roots. To date 709 hygromycin resistant transformants have been identified for abnormal growth on PS-PHIL surfaces. Six of these transformants show an altered behaviour and are defective in infection. Taken together, we have developed an *in vitro* system which induces a developmental checkpoint regulated by the PMK1 MAP kinase pathway required by *M. grisea* for plant infection. Now, this new system offers an easy and fast method to screen for mutants defective in this specific pathogenesis-related developmental pathway.

36. Identification and expression analysis of hyphal growth genes involved in the infection process of *Botrytis cinerea.*

EVELYN SILVA, CAROLINA AGUAYO, CHRISTINA MUELLER, FELIPE SCHAERER & PABLO D.T. VALENZUELA
Fundación Ciencia para La Vida, Instituto MIFAB, Universidad Andrés Bello and Universidad Católica de Chile. Avenida Zanartu 1482, Nunoa, Santiago. Chile.

The disease ‘gray mold’ produced by the fungus *Botrytis cinerea* is responsible for great economic losses on many crops during growth but also during storage and transport of the agricultural products. The availability of genomic resources for this pathogenic fungus allowed bioinformatics analyses that provided us a new view of the gene repertoire involved pathogenic and cellular processes. The present work describes a preliminary analysis of a (9,980 ESTs) cDNA library from *B. cinerea* the bioinformatics and experimental identification of genes involved in the development, differentiation and polarity of hyphae. Similarity searches against this cDNA library detected putative orthologs of *SOD* and *SEP* genes from *Aspergillus nidulans* and *COT* and *VMA-1* from *Neurospora crassa.* Significant differences in

the expression levels of these genes were observed when we compared *B. cinerea* strains with different virulence phenotypes. These results provide new insights and opportunities for the investigation of the molecular basis of virulence in this pathogenic fungus.

37. Identification of differentially expressed proteins of *Verticillium longisporum* during infection of *Brassica napus* (Rapeseed)

SEEMA SINGH, SUSANNA A. BRAUS-STROMEYER, OLIVER VALERIUS, SVEN KRAPPMANN, GERHARD H. BRAUS
Institute of Microbiology and Genetics, Department of Molecular Microbiology & Genetics, George-August University, Grisebachstr. 8, 37077 Göttingen, Germany.

Verticillium longisporum is a devastating vascular pathogen on rapeseed crops causing wilt disease. It is host specific on the family Brassicaceae. *V. longisporum* is a soil-borne pathogen that infects through the roots and colonizes the vascular system of the plant. The complex communication between *V. longisporum* and rapeseed when it penetrates and colonizes the plant has not been investigated fully yet. It is likely that some proteins of *V. longisporum* may be differentially expressed on receiving possible signals from the host-plant. Such proteins could play an important role during fungal growth or pathogenesis. This project aims at the identification of such differentially expressed proteins. It involves quantitative comparison of proteomes of cell extracts of different culture conditions i.e. the fungus cultured with or without xylem sap from *Brassica napus* by 2D-PAGE. ESI-MS/MS identification of the digested 2-D spots is employed later. Identified proteins will serve as a basis for the cloning of corresponding coding genes. Reporter constructs of identified genes will be generated with GFP and integrated in *V. longisporum*. These constructs will be tested on induction by xylem sap and in planta. This will establish a suitable system for the identification of the possible fungus-plant interaction by changed gene expression.

38. Sclerotial mycoparasitism by *Coniothyrium minitans*: identification of molecular components conserved in functionally diverse fungi

S. SREENIVASAPRASAD, S. MUTHUMEENAKSHI, C. ROGERS, M.P. CHALLEN, & J.M. WHIPPS
Warwick HRI, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, UK

Coniothyrium minitans is a sclerotial mycoparasite of the wide host range pathogen *Sclerotinia sclerotiorum*. Currently, *C. minitans* is exploited as a commercial biocontrol agent, but the molecular basis of the sclerotial mycoparasitism process remains largely unexplored. We are applying a range of molecular approaches to unravel the mechanisms underlying this specialised host-parasite interaction. Suppression subtractive hybridisation (SSH) was used to generate a cDNA library enriched for genes up-regulated during sclerotial colonisation. Sequencing and bioinformatic analysis led to the identification of more than 250 unisequences and their assignment to various functional categories. Expression analysis of a selection of the sequences revealed different levels of gene up-regulation in *C. minitans* during sclerotial colonisation. Further, through the insertional mutagenesis, eight mycoparasitism-deficient mutants were identified from 4000 transformants. Molecular analysis of some of these mutants enabled the identification of putative novel mycoparasitism genes. Gene silencing and complementation technologies are being used for functional analysis of some of the key genes. Comparative analysis of the genes identified during the sclerotial mycoparasitism process by *C. minitans*, suggests a role for signalling, transport, stress response and other components conserved in functionally diverse fungi. This lays the platform for further comparative and functional genomic analysis of sclerotial mycoparasitism.

39. A small GTPase affects vegetative and sexual development in *Coprinopsis cinerea*
PRAYOOK SRIVILAI, MONICA NAVARRO-GONZALEZ, & URSULA KÜES
Institute of Forest Botany, Section Molecular Wood Biotechnology, Georg-August-University of Göttingen, Göttingen, Germany

A mutation in codon 19 of a cloned *ras* gene of *Coprinopsis cinerea* altering a glycine into a valine codon (*ras*^{val19}) gives rise to a constitutively activated GTPase. The *ras*^{val19} mutant allele has been transformed into different monokaryons of *C. cinerea* and found to effect the mycelium of monokaryons and, after mating, also of dikaryons. Growth rates were reduced, hyphae lost growth orientation, abnormal short side-branches were formed and mycelial growth was invasive. *ras*^{Val19} dikaryons had many abnormal unfused clamps at hyphal septa whose hyphal tips tended to further elongate, thereby passing the subapical pegs formed for clamp cell fusion. In fruiting body development, tissue formation within the primordia was altered. Gills appeared to be oversized compared to the upper stipe tissues and the plectenchyma in the pileus. Fully developed fruiting bodies were minute (1-1.5 cm) compared to normal 4-5 cm sized fruiting bodies. Basidiospore production was affected in quantity and quality.

PS and MNG acknowledge scholarships from the Mahasarakham University (Thailand) and CONACYT (Mexico), respectively. The Deutsche Bundesstiftung Umwelt financially supported the laboratory.

40. Sequencing the genome of the forest pathogen *Heterobasidion annosum*
JAN STENLID, HALVOR SOLHEIM, MATTEO GARBELOTTO, FRANCIS MARTIN, JAMES B ANDERSON, URSULA KÜES
Dept Forest Mycology and Pathology Swedish University of Agricultural Sciences, Box 7026, Uppsala Sweden; Norwegian Forest Research Institute, Ås, Norway; University of California, Berkeley, CA, USA; INRA, Nancy, France; University of Toronto, Canada; University of Göttingen, Germany.

Heterobasidion annosum causes a devastating root rot in conifer plantations and natural forests throughout the northern hemisphere. In a collaboration with the Joint Genome Institute, the genome of *H. annosum* will be the first plant pathogenic homobasidiomycete to be sequenced allowing for new insights into plant-microbe interactions. Comparisons with plant pathogens with a gradient of taxonomic relatedness to *H. annosum* will help understanding the evolution of pathogenicity factors. Response of the model tree *Populus* to various types of trophic interactions can be studied including rust pathogen fungi and mycorrhizal mutualists. Furthermore, comparisons with the model white rotter *Phanerochaete crysosporium,* will deepening our understanding of wood degradation including ligninolytic and polysaccharide degradation pathways and several bioremediation applications. Moreover, this project will also gain insights into fungal evolutionary history and biology including development, non-self recognition, mating, and secondary metabolism.

41. Effects of trichostatin A on appressorium formation of *Magnaporthe oryzae*
OSAMU TAKEKAWA, KEN-ICHIRO SAITOH, TSUTOMU ARIE, TOHRU TERAOKA, MARIE NISHIMURA, MINORU YOSHIDA AND TAKASHI KAMAKURA
Department of Applied Biological Science, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, JAPAN

Recently, epigenetical control of gene expression is considered to be important for regulation of cellular functions. Controlling acetylaton of histones is one of such epigenetical regulations. Acetylation of histones is governed by histone acetyltransferase(s) and histone deacetylase(s), and plays an important role in the remodeling of chromatin superstructure which causes alteration of gene expressions. In this study, we have tested the effects of

trichostatin A (TSA), a well-known histone deacetylase inhibitor, on *Magnaporthe oryzae*. *M. oryzae* is the causal agent of rice blast disease. After germination on rice leaves, the tip of the germ tube differentiates into a unicellular infection specific structure called an appressorium. Appressorium formation is one of the key processes in *M. oryzae* infection. Therefore, knowledge of the genes involved in these processes is important for the development of effective disease control strategies. We found that TSA was a quite effective inhibitor of appressorium differentiation. TSA also inhibited the lesion appearance and extension on rice leaves. Analyses of gene expressions affected by TSA treatment using cDNA macro-array are now in progress.

42. Mapping N-dynamics in fungal colonies

MONIKA TLALKA[1], SARAH WATKINSON[1], MARK FRICKER[1], PETER ARRAH[1], KERRY BURTON[2] & DANIEL EASTWOOD[2]
[1]Plant Sciences, Oxford University, South Parks Road, Oxford OX1 3RB, UK; [2]Warwick HRI, University of Warwick, Wellesbourne, CV35 9EF, UK

Saprophytic cord-forming Basidiomycetes play a central role in nutrient cycling in terrestrial ecosystems. In a carbon-rich, nitrogen-poor woodland environment, fungi develop an extensive network of corded mycelium that can efficiently scavenge, transport and store nitrogen.

Based on a urea cycle model for mycorrhiza, it is proposed that in saprothrophs nitrogen is acquired from source regions and channeled through glutamate to arginine *via* the anabolic arm of the urea cycle. The vacuolar system moves arginine to sink regions. Carrier-mediated efflux is linked to arginine breakdown *via* the catabolic arm of the urea cycle. The NH_4^+ released is re-assimilated into glutamate for biosynthesis at sites of active growth. The expression of key genes of interest in N-assimilation and the urea cycle in sub-sampled regions of *Phanerochaete velutina* mycelium is being investigated using RT-PCR. In parallel, a time-lapse scintillation imaging of a non-metabolised amino-acid marker, ^{14}C-AIB is performed to link a predicted asymmetric expression profiles between source, sink and transport regions with the amino-acid distribution map.

43. Genes encoding transcription factors in *Glomus intraradices* and their expression at the appressoria stage of arbuscular mycorrhiza interactions

MARIE TOLLOT, DIEDERICK VAN TUINEN, VIVIENNE GIANINAZZI-PEARSON & PASCALE SEDDAS
UMR INRA/CNRS/U. Bourgogne Plante-Microbe-Environnement, CMSE-INRA, BP 86510, 21065 Dijon Cedex, France

Molecular pathways governing the life cycle of arbuscular mycorrhizal (AM) fungi and their interactions with root tissues are not yet fully understood. Most studies of fungal responses to host plants have targeted developmental stages before root contact (germinating spores), or after root colonization (intraradical mycelium). We are focusing on the early cell events of appressoria contact with the root surface. Recent monitoring of *Glomus intraradices* gene expression at this stage has revealed differential fungal responses to roots of host and non-host (Myc- mutants) *M. truncatula* (Seddas et al. submitted), suggesting a fine regulation of fungal genes by the host plant. Transcription factors are central regulators of gene expression which control many physiological and developmental processes but little is known of these elements in AM fungi. We have identified seven sequences in *G. intraradices* with similarity to fungal genes encoding transcription factors, from the *Glomus* sequencing project data (DOE, joint genome institute, USA). Transcript profiling (real-time RT-PCR) of the corresponding genes in appressoria formed on roots of wild-type or Myc- mutants of *M. truncatula* points to their differential expression when symbiosis-related plant

genes are inactivated, suggesting that the encoded transcription factors could play a role in regulating early events leading to successful mycorrhizal interactions.

44. Cyanovirin homologues: a family of structurally variegated sugar binding proteins in filamentous ascomycetes

ARTURO R. VISCOMI[1], LEONARDUS KOHARUDIN[2], ANDREA BIANCHI[1], BARBARA MONTANINI[3], RICCARDO PERCUDANI[1], ANGELA GRONENBORN[2] & SIMONE OTTONELLO[1]

[1]Department of Biochemistry and Molecular Biology, University of Parma, 43100 Parma, Italy; [2]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh PA 15261, USA;[3]UMR INRA-UHP "Interactions Arbres/Micro-Organismes", IFR 110 "Genomique, Ecophysiologie et Ecologie *Fonctionnelles" INRA-Nancy, Champenoux (France)*

Cyanovirin (CVN) is a mannose binding lectin and a potent anti-HIV agent from the cyanobacterium *Nostoc ellipsosporum*. Till recently, CVN was considered an orphan protein structure. A putative homolog of CVN, named TbCVNH1, was identified as a nutritional status responsive mRNA during a gene expression profiling study in the mycorrhizal ascomycete *Tuber borchii*. A number of TbCVNH1 homologs, collectively designated as CVNHs, were subsequently identified in a variety of ascomycete, but not basidiomycete, filamentous fungi. CVNHs display a modular architecture: some of them are composed by a single domain made up by an internally repeated motif, while others are multidomain proteins in which the CVN-like domain is either reiterated or associated with other protein modules, such as the chitin-binding domain LysM. The structural similarity between CVN and two monodomain CVNHs from *T. borchii* and *Neurospora crassa* (NcCVNH1) was experimentally assessed by multidimensional NMR analysis. TbCVNH1 and the paralogous gene TbCVNH2 are both strongly downregulated following N or C deprivation and upon shift to a poor C source (e.g., sucrose), whereas a much lower (or no) influence of the nutritional status is observed for NcCVNH1. A similar expression profile was revealed by immunoblot analysis. Preliminary results as to the sugar-binding functionality (and specificity) of CVNHs have been obtained by a glycoarray analysis conducted on a library of natural and synthetic glycans. Data will also be presented on the chitin-binding, Lys M containing protein MgCVNH2 from *Magnaporthe grisea*.

This work was supported in part by the Interuniversity Consortium for Biotechnology and by the Ministry of Education, University and Research (PRIN programs). Glycoarray analysis resources were provided by the Consortium for Functional Glycomics (Grant n. GM62116).

45. Badisiomycete genome annotation using the Eugene gene finder.

JAN WUYTS[1,2], PIERRE ROUZE[1], YVES VAN DE PEER[1] & FRANCIS MARTIN[2]

[1]Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology (VIB), Ghent University, Technologiepark, B-9052 Ghent, Belgium; [2]Centre de Recherche de Nancy, Institut National de la Recherche Agronomique, Champenoux, France.

We used the Eugene gene prediction platform to perform the automatic annotation of the genome of the ectomycorrhizal basidiomycete *Laccaria bicolor*. Eugene is able to take into account both extrinsic and intrinsic information to come to an annotation of the genome. All sources of evidence are presented to the system and are scored using weights that are determined by training the system on a set of manually annotated genes.
Splice sites are detected using a support vector machine algorithm implemented in the program SpliceMachine. This program was slightly modified to take into account the abundance of GC splice donor sites in *L. bicolor*. Other information presented to Eugene include an interpolated Markov model to distinguish coding from non-coding regions,

BLASTx information of the genome against trusted protein databases and tBLASTx information comparing the *L. bicolor* genome to that of *Coprinus cinereus*.
Since Eugene worked very well on the *Laccaria* genome we also used the same system to produce an automatic annotation of two other basidiomycete genomes: *Coprinus cinereus* and *Phanerochaete chrysosporium*

Participants

** S=speaker abstract; P=poster abstract*

Participant	Email	Establishment	Abstract No. *	Research interests
Aguirre, Jesus	jaguirre@ifc.unam.mx	Instituto de Fisiologia Celular-UNAM, Mexico		Fungal development, reactive oxygen species, oxidative stress, stress signal transduction, secondary metabolism
Ahren, Dag	dag.ahren@mbioekol.lu.se	Lund University, Sweden		Genomics, mycorrhiza, evolution, adaptation, parasitism, bioinformatics, metabolic networks, phylogeny, metabolism, gene duplications
Alexander, Ian	i.alexander@abdn.ac.uk	University of Aberdeen, UK		
Amselem, Joelle	joelle.amselem@versailles.inra.fr	INRA, France	P15	Bioinformatics, bioanalysis, fungal, annotation, structural annotation, functional annotation, gene prediction
Aoun, Mirella	mirella.aoun@rsvs.ulaval.ca	Université Laval, Canada	P2	Genomics, genes expression analysis, host -fungus interaction, fungal histopathology
Baldwin, Thomas	thomas.baldwin@bbsrc.ac.uk	Rothamsted Research, UK	P3	Fungal pathogens, plant-microbe interactions, fungal genomics, molecular genetics, *Fusarium*
Berrocal-Tito, Gloria	berrocal@antibes.inra.fr	INRA, France	P4	Fungal growth, pathogenicity, oomycetes
Bianchi, Andrea	andrea.bianchi1@nemo.unipr.it	University of Parma, Italy	P44	Genomics, gene expression, filamentous ascomycetes, phytopathogenic fungi, nutrient starvation stress
Blaudez, Damien	dblaudez@scbiol.uhp-nancy.fr	Nancy-University, France	S2.4, P6	Ectomycorrhizal fungi, metal homeostasis and tolerance, plant physiology, nitrogen metabolism, mineral nutrition, transport mechanism, fungal genomics, fungal transformation
Bolchi, Angelo	angelo.bolchi@unipr.it	University of Parma, Italy		Gemomics, gene expression, filamentous ascomycetes, mycorrhizal fungi, nitrogen assimilation, nutrient starvation stress
Borkovich, Katherine	Katherine.Borkovich@ucr.edu	University of California-Riverside, USA	S2.4	Signal Transduction, heterotrimeric G proteins, functional genomics, sporulation, two component systems, chemotropism
Braus-Stromeyer, Susanna A	sbraus@gwdg.de	University of Goettingen, Germany		Plant / Pathogen interaction, *Verticillium*, proteomics, fungal genetics, life imaging
Brozek, Viviane	viviane.brozek@bayercropscience.com"	Bayer CropScience, France		
Brun, Annick	Annick.Brun@scbiol.uhp-nancy.fr	Nancy-University, France	S4.2, P6	Functional genomic, nitrogen metabolism, nitrogen transport, functional diversity, metabolomic, mycorrhizal symbiosis, fungi metabolism, regulatory processes, transcriptomic analysis
Buée, Marc	buee@nancy.inra.fr	INRA de Nancy, France	P6	Fungal diversity, ectomycorrhizal fungi, molecular ecology, saprotrophe, organic matter, nitrate, *Laccaria bicolor*, functionnal diversity, proteases, carbon
Chalot, M	michel.chalot@scbiol.uhp-nancy.fr	Université H. Poincaré, Nancy France	S4.2	Mycorrhiza, nitrogen nutrition, metal tolerance, molecular mechanism, transportome

Chiapello, Marco	marco.chiapello@unito.it	University of Turin, Italy	P8, P34	Proteomic, trascriptomic, fungus, mycorrizal, ROS, mRNA differential display
Coutinho, Pedro	pedro.coutinho@afmb.univ-mrs.fr	AFMB - CNRS / Univ. Aix-Marseille I and II		Bioinformatics, classification of carbohydrate-active enzymes, genome annotation, comparative genomics
Courty, Pierre Emmanuel	courty@nancy.inra.fr	INRA Nancy, France		Ectomycorrhizas, transcriptomics, functional genomic, genome annotation, exoenzymes, organic matter
Cullen, Daniel	dcullen@facstaff.wisc.edu	USDA, USA	S3.3	Phanerochaete chrysosporium, cellulose degradation, lignin degradation, genomics, proteomics, transposons
Danchin, Etienne	etienne.danchin@afmb.univ-mrs.fr	AFMB CNRS Universites Aix-Marseille I & II, France	P9	Comparative genomics, genome evolution, fungal genomics, adaptation to the environment
Daval, Stéphanie	daval.rioux@orange.fr	INRA, France		*Gaeumannomyces graminis*, pseudomonas, trehalose
De Boer, Paulo	deboer@add2x.com	Add2X Biosciences BV, The Netherlands	P10	Gene targeting, DNA repair, recombination, fungal biology, molecular genetics, DNA therapy, protein therapy
Dean, RA	ralph_dean@ncsu.edu	North Carolina State University, USA	S2.3	
Deveau, Aurélie	deveau@nancy.inra.fr	INRA-Nancy, France	P11	Ectomycorhiza, helper bacteria, interactions, molecular level, microarrays, mutagenesis
Droux, Michel	michel.droux@bayercropscience.com	CNRS, France	P16	Fungi, sulfur, metabolism, regulation, pathogenicity, transcriptome, metabolome, proteome
Dubois Calero, Nathalie	nathalie.calero@planteck.com	BioEvolution Inc., Canada		Mycelial culture, fructification, wild edible mushroom, mycelial culture forestry management
Duplessis, Sébastien	duplessi@nancy.inra.fr	INRA, France	P12, P23	Tree/microbes interactions, genomics, transcriptomics, poplar*, Melampsora*, rust, ectomycorrhiza, disease resistance
Ebbole, D	d-ebbole@tamu.edu	Texas A&M University, USA	S4.1	Genomics, extracellular proteins, conidiation, transcription factors
Feau, Nicolas	nfeau@cfl.forestry.ca	Natural Resources Canada	P5, P13, P14	Fungal genomics, molecular evolution, population genetics, poplar diseases, *Melampsora* rusts
Fillinger, Sabine	fillinge@versailles.inra.fr	INRA Versailles, France	P15	Grey mould, *Botrytis*, resistance, fungicides, signal transduction, *Sclerotinia*
Frelin, Oceane	oceane.frelin@bayercropscience.com	CNRS, France	P16	Fungi, pathogenicity, metabolism, transport, regulation, transcriptome, metabolome, sulfur
Frey, Pascal	frey@nancy.inra.fr	INRA, France	P11, P13, P22	
Fuchs, Uta	uta.fuchs@mpi-marburg.mpg.de	MPI for terrestrial Microbiology, Germany	S1.5	Endocytosis, molecular motors, G protein coupled receptors, RabGTPases, cytoskeleton
Gladfelter, Amy	amy.gladfelter@dartmouth.edu	Dartmouth College, USA	S1.1	Cell cycle, morphogenesis, asynchrony, mitosis, septins, multinucleated cells

Hickey, Patrick	ph@luxbiotech.com	LUX Biotechnology Ltd., UK	S1.2, P18	Mycology, luciferase, bioluminescence, GFP, fluorescence, *Neurospora*, cell Biology, imaging, microscopy, calcium
Huser, Aurelie	huser@mpiz-koeln.mpg.de	Max Planck Institute for Plant Breeding Research, Germany	P20	Plant pathogens, mutagenesis, haustoria, biotrophy, appressorium, hemibiotrophs
IJdo, Marleen	ijdo@mbla.ucl.ac.be or marleen41@hotmail.com	Universite Louvain la neuve, Belgium	P21	AMF diversity, in vitro culture AMF, AMF ecology
Jacobi, Volker	volker.jacobi@rsvs.ulaval.ca	Laval University, Canada	P2	Forest pathology, host-pathogen interactions, fungus biology, genomics, molecular biology, virology
Jedd, Gregory	gregory@tll.org.sg	Temasek Life Sciences Laboratory, Singapore	S1.4	Cell biology, genetics, evolution, organelles, fungi
Kamakura, Takashi	kamakura@rs.noda.tus.ac.jp	Tokyo University of Science, Japan	P41	Rice blast disease, appressorium, differentiation, epigenetics, phytopathology, gene expression
Kämper, Jörg	kaemper@staff.uni-marburg.de	Max-Planck-Institute for Terrestrial Microbiology, Germany	S3.1	
Kemppainen, Minna	mkemppainen@unq.edu.ar	Universidad Nacional de Quilmes, Argentina	P23	Molecular biology, cellular biology, physiology, molecular genetics, genetic engineering, genomics, fungal Biology, ectomycorrhiza
Kohler, Annegret	kohler@nancy.inra.fr	INRA, France	P24	Plant-Microbe interactions, Ectomycorrhiza, Laccaria bicolor, Transcriptomics, Symbiosis, Genomics
Kronstad, James	kronstad@interchange.ubc.ca	University of British Columbia, Canada	S2.2	Signal transduction, cAMP signaling, Pathogenesis, *Cryptococcus*, morphogenesis, *Ustilago*, genomics, gene expression, iron regulation, plant pathology
Kües, Ursula	ukuees@gwdg.de	Georg-August-University Göttingen, Germany	P7, P17, P19, P25, P27, P33, P39, P40	Development, biotechnology, basidiomycetes, genomics, enzymes, proteomics, sexual reproduction, fruiting bodies
Le Tacon, François	le_tacon@nancy.inra.fr	INRA, France		
Lebrun, MH	Marc-Henri.Lebrun@bayercropscience.com	CNRS-BayerCropScience, France	S5.3, P15, P16	
Lichius, Alexander	A.Lichius@sms.ed.ac.uk	University of Edinburgh, UK		*Neurospora*, *Magnaporthe*, cell fusion, appressorium formation, pathogenicity, MAPK signalling, GPCR endocytosis, knock-out mutants, live Cell Imaging, genomics
Lin, Cherry	cherry.lin@danisco.com	Genencor, a Danisco company, USA		*Trichoderma reesei*, *Aspergillus niger*, protein secretion, gene expression
Martin, Francis	fmartin@nancy.inra.fr	INRA-Nancy, France	S3.4, S4.2, P6, P11, P12, P17, P21, P23, P24, P32, P40, P45,	

Mendgen, Kurt	kurt.w.mendgen@uni-konstanz.de	University of Konstanz, Germany		
Montanini, Barbara	barbara.montanini@scbiol.uhp-nancy.fr	INRA-Nancy, France	S4.2, P6, P44	Metal stress, nitrogen metabolism, fungal nutrition, ectomycorrhization, transcriptomic analysis, tuber (truffle), metal transporter
Naumann, Maria	maria.naumann@unito.it	University of Torino, Italy	P21	BLO's, endobacteria, AMF, Diversity, Interaction between plant, fungus and BLO
O'Connell, Richard	oconnel@mpiz-koeln.mpg.de	Max Planck Institute for Plant Breeding Research, Germany	P20	Pathogenicity, cell biology, biotrophy, appressoria, *Colletotrichum*, secretome, effectors
O'Gorman, Celine	celine.ogorman@ucd.ie	University College Dublin, Republic of Ireland	P28	Aerobiology, *Aspergillus fumigatus*, mating types, RAPD-PCR
Oliver, Steve	steve.oliver@manchester.ac.uk	University of Manchester, UK	S5.1	
Olson, Åke	ake.olson@mykopat.slu.se	Swedish University of Agricultural Sciences, Sweden	P29	Forest pathology, interactions, virulence factors, speciation, mating, somatic compatibility, stress tolerance, wood degradation
Ottonello, Simone	s.ottonello@unipr.it	University of Parma, Italy	P44	Gene expression, genomics, filamentous ascomycetes, mycorrhizal fungi, nitrogen assimilation, nutrient starvation stress
Panstruga, R	panstrug@mpiz-koeln.mpg.de	Max-Planck Institute for Plant Breeding Research, Germany	S3.2	
Pardo, Alejandro	apardo@unq.edu.ar	Universidad Nacional de Quilmes, Argentina	P23	Molecular biology, cellular biology, physiology, molecular genetics, genetic engineering, genomics, fungal biology, ectomycorrhiza
Pereda, Veronica	pereda@nancy.inra.fr	INRA Nancy, France		Endomycorhiza, ectomycorhiza, phosphate transport, poplar, *Laccaria*
Phalip, Vincent	vincent.phalip@esbs.u-strasbg.fr	Esbs, France	P30	*Fusarium*, xylanases, cellulases, hop, proteomics
Polle, Andrea	apolle@gwdg.de	Georg-August-University of Göttingen, Germany	P32	
Quevillon, Emmanuel	emmanuel.quevillon@versailles.inra.fr	URGI/CNRS, France	P15	Annotation, fungi, bioinformatic, functional annotation, protein domains
Rameshaiah, GN	gnrameshaiah@rediffmail.com	Indian Institute of Technology Madras, India	P31	Filamentous fungi, fungal morphology, enzyme production, structural kinetics, biosynthesis, biochemical studies, chitinase, *Trichoderma harzianum*
Read, Nick	Nick.Read@ed.ac.uk	University of Edinburgh, UK		Cell fusion, calcium imaging, live-cell imaging, sexual morphogenesis, hyphal tip growth, optical tweezers
Reich, Marlis	reich@nancy.inra.fr	INRA Champenoux, France	P21, P32	Mycorrhiza, fungal ecology, fungal genetics, array technics, genome
Roberson, Robby	robert.roberson@asu.edu	Arizona StateUniversity, USA	S1.3	Cell biology, morphogenesis, polarized growth, bioimaging
Rokas, Antonis	arokas@MIT.EDU	Broad Institute of MIT & Havard, USA	S2.1	Computational biology, genomics, gene regulation, genome evolution, annotation, infectious disease

Rouzé, Pierre	chtir@psb.ugent.be	VIB, Belgium	S3.4, P45	Bioinformatics, evolutionary biology, systems biology
Salvioli, Alessandra	alessandra.salvioli@unito.it	Università degli Studi di Torino, Italy	P34	AM Fungi, symbiotic associations, *Gigaspora margarita*, endobacteria, gene expression profiles, comparative proteomics
Sesma, Ane	sesma@bbsrc.ac.uk	John Innes Centre, UK	P35	Fungal development, fungal pathogenicity, cell biology, functional genomics, RNA metabolism, tissue-specific factors, root infection, secreted proteins
Shasky, Jeffrey	jfys@novozymes.com	Novozymes Inc. USA		*Fusarium*, protein secretion, metabolism, genomics, gene expression
Silva Pereira, Cristina	spereira@itqb.unl.pt	ITQB, Portugal	P26	Fungal proteomics, biodegradation, bioremediation, fungal genomics, species adaptation, biocatalysis
Silva-Moreno, Evelyn	evelyn_silva@bionova.cl	Fundacion Ciencia para La Vida, Chile	P36	*Botrytis cinerea*, pathogenicity factors, cDNA library, hyphal growth genes, strawberry
Singh, Seema	ssingh1@gwdg.de	Georg-August-University Göttingen, Germany	P37	Plant-pathogen interaction, proteomics, fungal genomics, *Verticillium*, phytopathogenic fungi
Sirven, Catherine	catherine.sirven@bayercropscience.com	Bayer Cropscience, France		Fungus, bioinformatic, comparative genomic, gene expression profiling
Slater, Holly	h.slater@lancaster.ac.uk	New Phytologist, UK		
Sreenivasaprasad, Surapareddy (known as Prasad)	s.prasad@warwick.ac.uk	University of Warwick, UK	P38	Fungal-plant interactions, fungal-fungal interactions, fungal morphogenesis, population biology, gene diversity & function, GPCRs, transporters, ETP gene cluster, phylogenomics
Stenlid, Jan	jan.stenlid@mykopat.slu.se	Swedish Univ Agricultural Sciences, Sweden	P29, P40	Forest pathology, pathogenicity, genomics, population structure, interactions, evolution, mating, taxonomy
Stockinger, Herbert	happo@gmx.at	Technical University Darmstadt	P21	Mycorrhiza, phylogeny, ecology
Talbot, Nick	N.J.Talbot@exeter.ac.uk	University of Exeter, UK	S5.2	
Taylor, John	jtaylor@nature.berkeley.edu	University of California, Berkeley, USA	S5.4	Speciation, selection, phylogenetics, comparative genomics
Tlalka, Monika	monika.tlalka@plants.ox.ac.uk	Oxford University, UK	P42	Nitrogen transport, imaging, gene expression, fungal morphogenesis, amino acid transporters, fungal network
Tollot, Marie	marie.tollot@epoisses.inra.fr	INRA-CMSE, France	P43	Mycorrhiza, fungi, genes, recognition, transcription factors
van Tuinen, Diederik	tuinen@epoisses.inra.fr	INRA, France	P43	Root symbiosis, arbuscular mycorrhiza, diversity, genomics
Viscomi, Arturo Roberto	arturoroberto.viscomi@nemo.unipr.it	University of Parma, Italy	P44	Gene expression, genomics, filamentous ascomycetes, mycorrhizal fungi, nitrogen assimilation, nutrient starvation stress
Visser, Jaap	eurofung@rulbim.leidenuniv.nl	Eurofung		Molecular microbiology, Aspergilli, fungal genetics

Vögele, T	Ralf.Voegele@uni-konstanz.de	University of Konstanz, Germany	S4.3	Rust fungi, haustoria, obligate biotrophy, molecular biology, biochemistry, cytology, fire blight