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Botrytis/Sclerotinia resources: an integrated system for structural and functional genome annotation

Joelle J. Amselem, Nicolas Lapalu, Julien J. Brault, Laetitia L. Brigitte, Jonathan J. Kreplak, Françoise F. Alfama-Depauw, Aminah A. Keliet, Erik E. Kimmel, Isabelle I. Luyten, Sebastien S. Reboux, et al.

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Botrytis Sclerotinia Post-Genome Workshop

BSPGW 2011

Botrytis-Sclerotinia Post-Genome Workshop

15th - 17th September 2011, Lyon, France

«The Most Studied Necrotrophic Fungal Plant Pathogens»

ABSTRACT BOOK

Organizing Committee

Geneviève Billon-Grand
Christophe Bruel
Mathias Choquer (Coordinator)
Cindy Dieryckx
Nathalie Poussereau
Christine Rascle

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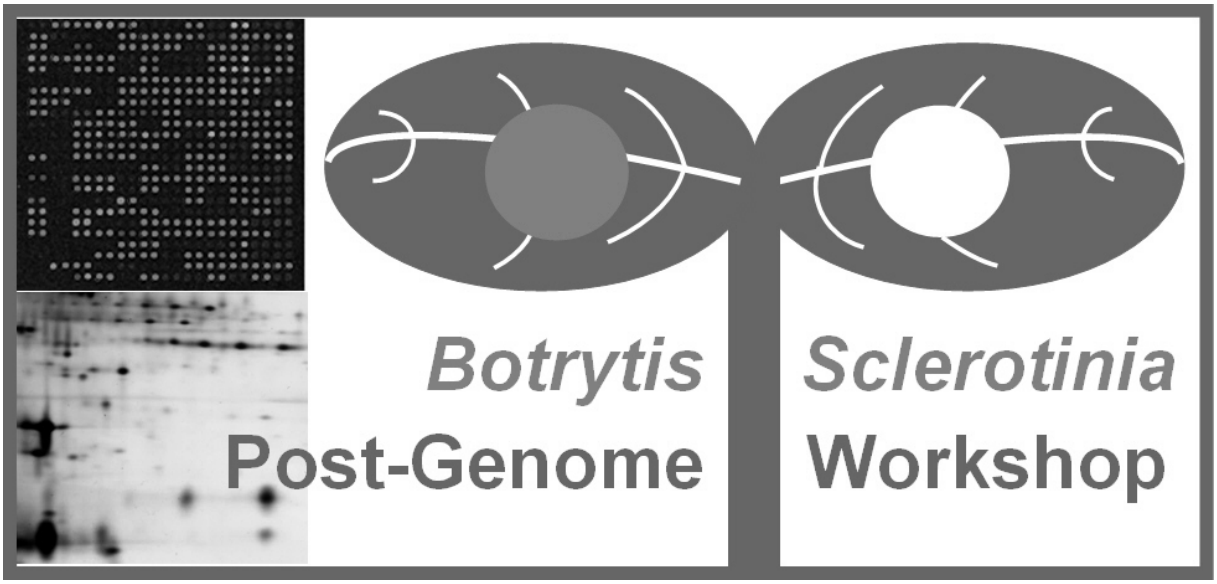

ABSTRACT BOOK

15th-17th September 2011
Lyon, France

Welcome message from the BSPGW2011 organizing committee

After the previous "Botrytis genome workshops" (BGW) in Kaiserslautern, Versailles and Tenerife, it's with great pleasure that we welcome you in Lyon for a "Botrytis/Sclerotinia Post-genome Workshop" (BSPGW).

With many preserved historical sites, Lyon is the archetype of a heritage city, and this has justified its recent classification by UNESCO. Lugdunum, the Roman name of the city, was officially founded in 43 BC and promoted Capital of Gauls in 27 BC. At the time of the Renaissance, the city was an important place for printing and for the production and weaving of silk by the "canuts" (silk workers). In more modern times, Lyon also happened to be the birthplace of cinema by Auguste and Louis Lumière and developed a reputation for itself as "France's capital of gastronomy". Lyon is also said to be the city of three rivers, the Rhône and the Saône rivers which converge there, and the Beaujolais wine ! Nowadays, Lyon is the third largest city in France and the centre of the second largest metropolitan area in the country. Economically, it is a major centre for chemical, pharmaceutical, and biotech industries and Lyon is the capital of both the Rhône-Alpes region and the Rhône "département".

We wished to rename the workshop as it is to mark the new post-genomic era into which Botrytis cinerea and Sclerotinia sclerotiorum have been taken via their annotated genome sequences made available and via the recent publication of their comparative genomic analysis. We hope this meeting will provide the opportunity to gather our scientific communities around new experimental approaches and strategies for capitalizing on genomic resources, to establish new contacts and to participate in scientific exchanges on all biological aspects of these two necrotrophic fungi.

The meeting brings together 4 invited lectures, 34 oral communications and 27 posters, with speakers being both leading and emerging scientists. Five sessions emerged from all the contributions, as seen by the scientific committee. These sessions are: 1."-Omics" technologies, 2. Adaptation to environment: fungicides and host plants, 3. post-genomic functional analyses, 4. host-pathogen interactions, and 5. bioinformatics and comparative genomics. In order for this workshop to be a moment of scientific interactions, the sessions have been organized with short talks, special open-discussion times and significant time for posters. Attention was also given to research on the plant hosts and to aspects related to the agronomic impact of the pathogens.

We wish to thank all the attendants for showing such interest in this workshop and for traveling from 17 countries around the world ! We wish to thank the scientific committee for its deep and efficient implication. We also wish to thank all the academic and industrial sponsors for their generous contributions. We hope everyone will enjoy the coming days and return to his/her laboratory invigorated by new ideas, strengthened links to fellow scientists and a taste of the traditional restaurants called "bouchons" (literally meaning "cork") ...

Christophe Bruel, Mathias Choquer and Nathalie Poussereau.

BSPGW2011 ORGANIZATION

Organizing Committee :

Laboratoire de Microbiologie, Adaptation et Pathogénie (MAP)

UMR5240: CNRS – Université Lyon 1 – INSA de Lyon – BAYER CropScience

http://map.univ-lyon1.fr

Equipe de Génomique Fonctionnelle des Champignons Pathogènes des Plantes

Geneviève Billon-Grand, Christophe Bruel, Mathias Choquer (coordinator), Cindy Dieryckx, Nathalie Poussereau, Christine Rascle.

Scientific Committee :

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Sabine Fillinger, INRA Versailles-Grignon, France.

Celedonio Gonzáles, Universidad de La Laguna, Spain.

Matthias Hahn, Technical University of Kaiserslautern, Germany.

Jeffrey Rollins, University of Florida, USA.

Paul Tudzynski, University of Muenster, Germany.

Jan van Kan, University of Wageningen, The Netherlands.

The workshop was sponsored by :

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Société Française de Phytopathologie (*Phytopathological Society of France*)

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LIBIOS

Université Lyon 1

Université de Lyon

CNRS

AVIESAN

INSERM

Région Rhônes-Alpes

Département du Rhône

INSA de Lyon

	THURSDAY 15th	FRIDAY 16th	SATURDAY 17th
8h	**8h00 Registration,**		
8h 15	**Poster installation &**		
8h 30	**Download Power Point slides**		
8h 45			
9h	**9h00 Opening -** Choquer M	**9h00 SESSION 3**	**9h00** Transfo *Bc* - Levy M
9h 15	**9h15 SESSION 1**	**Post-Genome Funct. Analysis**	**9h15 SESSION 5**
9h 30	**"-Omics" technologies**	I3 - Nowrousian M	**BioInfo. & Comp. Genomics**
9h 45	I1 - Martin F	O3.1 - Bruel C	O5.1 - Amselem J
10h	O1.1 - Rollins J	O3.2 - Cimerman A	O5.2 - Lapalu N
10h 15	O1.2 - Leroch M	O3.3 - Espino J	& Discussion, Chair: van Kan J
10h 30	**10h30 Coffee break**	**10h30 Coffee break**	**10h30 Coffee break**
10h 45	**&**	**&**	**&**
11h	**Poster viewing** (for all sessions)	**Poster viewing** (for all sessions)	**Poster viewing** (for all sessions)
11h 15			
11h 30	**11h30** O1.3 - Fillinger S	**11h30** O3.4 - Schumacher J	**11h30** O5.3 Gonzales C
11h 45	O1.4 - Fernandez-Acero FJ	O3.5 - Siegmund U	O5.4 van Kan J
12h	O1.5 - Heard S	O3.6 - Zhang L	
12h 15	O1.6 - GATC BIOTECH	O3.7 - Gonzales M	O5.5 St Quinton J
12h 30	Discussion	Discussion	Discussion & Conclusion
12h 45	Chair: Hahn M & Gonzales C	Chair: Rollins J & Poussereau N	Chair: van Kan J & Sharon A
13h	**13h00**	**13h00**	**13h00**
13h 15	***Lunch***	***Lunch***	***A Lunch box***
13h 30			**will be provided**
13h 45	**&**		
14h	**Poster viewing** (for all sessions)	**14h00 SESSION 4**	**End of workshop**
14h 15		**Plant-Pathogen Interaction**	*Thank you and have a nice trip !*
14h 30	**14h30 SESSION 2: Adapt. Envir.**	I4 - Schweizer P	
14h 45	I2 - Chevallier G	O4.1 - Simmonds D	
15h	O2.1 - Hahn M	O4.2 - Blanco-Ulate B	
15h 15	O2.2 - Bardin M	O4.3 - Seifi H	
15h 30	O2.3 - Laleve A	O4.4 - Colas S	
15h 45	**15h45 Picture of the meeting**	O4.5 - Levy M	
16h	**16h00 Coffee break**	**16h00 Coffee break**	
16h 15			
16h 30	**16h30** O2.4 - Clarkson J	**16h30** O4.6 - Kunz C	
16h 45	O2.5 - De Paula T	O4.7 - Sharon A	
17h	O2.6 - Sandor E	O4.8 - Israeli M	
17h 15	O2.7 - Johnston P	O4.9 - Frias M	
17h 30	Discussion	Discussion	
17h 45	Chair: Fillinger S & Bardin M	Chair: Powell A & Favaron F	
18h	**18h00** *30 min Metro*	**18h00** ***Conference Evening***	
18h 15	*LAENNEC to GRATTE-CIEL*	*20 min Metro*	
18h 30	*and 10 min walk in Art-Deco district*	*LAENNEC to BELLECOUR*	
18h 45	**18h45**	*and 10 min walk to the Pier*	
19h	***Welcome Reception***	***Guided River Boat Cruise***	
19h 15	***Cocktail*** *offered at the*	*on the Saône (1h)*	
19h 30	*VILLEURBANNE City Hall*		
19h 45	*Presence of the Mayor of the City*		
20h	*& President of University Lyon 1*		
20h 15	**20h30**		
20h 30		***Gala Dinner***	
20h 45		*at the "Auberge de Fond Rose"*	
21h	***Free evening***	*gastronomic restaurant*	
21h 15		*(starred in Guide Michelin)*	
21h 30		*with a Jazz Band*	
21h 45			
22h			
22h 15			
22h 30			
22h 45			
23h		**23h00** *Bus return, last metro 00:15*	

Botrytis Post-Genome Sclerotinia Workshop

Session 1

"-OMICS" TECHNOLOGIES

CHAIRPERSONS: Matthias Hahn and Celedonio Gonzalez

INVITED LECTURE

I1- BLURRED BOUNDARIES: LIFESTYLE LESSONS FROM ECTOMYCORRHIZAL FUNGAL GENOMES. Francis Martin, Jonathan Plett, Annegret Kohler, Emmanuelle Morin, Claude Murat, Elena Martino, Silvia Perotto, Pedro Coutinho, Bernard Henrissat, David Hibbett and Igor Grigoriev

ORAL COMMUNICATIONS

O1.1 - TRANSCRIPTOME PROFILING OF *SCLEROTINIA SCLEROTIORUM* COMPOUND APPRESSORIA. Xiaofei Liang, Ulla Benny, Li Lu, Mathias Choquer, George Casella, Jie Lang, Claudio Fuentes and Jeffrey Rollins

O1.2 - TRANSCRIPTOMICS OF *BOTRYTIS CINEREA* GERMINATION. Michaela Leroch, Evelyn Silva and Matthias Hahn

O1.3 - PHOSPHOPROTEOMICS TO TRACE SIGNAL TRANSDUCTION. Marlène Davanture, Benoît Valot, Michel Zivy and Sabine Fillinger

O1.4 - EXPLORING *BOTRYTIS CINEREA* PROTEOME: FROM CONIDIAL GERMINATION TO MEMBRANE PROTEINS. Francisco Javier Fernández-Acero, Thomas Colby, María Carbú, Anne Harzen, Carlos Garrido, Victoria E. González-Rodríguez, Eva Liñerio, Ursula Wieneke, Jürgen Schmidt and Jésus M. Cantoral

O1.5 - *SCLEROTINIA SCLEROTIORUM*: INVESTIGATING FUNGAL EXUDATES. Stephanie Heard, Kim E. Hammond-Kosack, Jon West, John Antoniw, Bruce Grieve and Sarah Perfect

O1.6 - GATC BIOTECH INTRODUCES THIRD GENERATION SEQUENCER PACBIO RS FOR NEW SEQUENCING APPLICATION. COMPLETE SOLUTIONS FOR DNA AND RNA SEQUENCING. Christophe Meynier and Zélie Dubreucq

POSTERS

P1.1 - LONGSAGE GENE-EXPRESSION PROFILING OF *B. CINEREA* GERMINATION SUPPRESSED BY RESVERATROL, THE MAJOR GRAPEVINE PHYTOALEXIN. Chuanlin Zheng, Mathias Choquer, Bing Zhang, Hui Ge, Songnian Hu, Huiqin Ma and Shangwu Chen

P1.2 - PROTEOMIC APPROACHES TO STUDY THE PHYTOPATHOGENIC FUNGUS *BOTRYTIS CINEREA.* Raquel González-Fernández, Inmaculada Redondo, Francisco J. Gómez-Gálvez and Jesús V.

P1.3 - ISOLATION OF NECROSIS-INDUCING PROTEINS FROM *BOTRYTIS CINEREA*. Yonatan Gur, Oliver Valerius, Gerhard Braus, Amir Sharon

P1.4 - THE SECRETOME OF *BOTRYTIS CINEREA*: ADAPTATION TO ENVIRONMENTAL PH. Cindy Dieryckx, Vincent Girard, Semcheddine Cherrad, Dominique Job, Claudette Job, Christine Rascle and Nathalie Poussereau

P1.5 - PROTEOMIC ANALYSIS OF PROTEINS SECRETED BY *BOTRYTIS CINEREA* IN RESPONSE TO METAL STRESS. Semcheddine Cherrad, Vincent Girard, Cindy Dieryckx, Isabelle R. Gonçalves, Jean-William Dupuy, Marc Bonneu, Claudette Job, Dominique Job, Sébastien Vacher and Nathalie Poussereau

I1- BLURRED BOUNDARIES: LIFESTYLE LESSONS FROM ECTOMYCORRHIZAL FUNGAL GENOMES

Francis Martin[1], Jonathan Plett[1], Annegret Kohler[1], Emmanuelle Morin[1], Claude Murat[1], Elena Martino[2], Silvia Perotto[2], Pedro Coutinho[3], Bernard Henrissat[3], David Hibbett[4] and Igor Grigoriev[5]

[1]UMR 1136, INRA-Nancy Université, Interactions Arbres/Microorganismes, INRA-Nancy, 54280 Champenoux, France; [2]Department of Plant Biology, University of Turin and IPP-CNR, Turin, Italy; [3]Architecture et Fonction des Macromolécules Biologiques, UMR 6098 CNRS-Universités Aix-Marseille I & II, 13288 Marseille Cedex 9, France; [4]Clark University, Worcester, MA, USA; [5]US DOE Joint Genome Institute, Walnut Creek, California 94598, USA.

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Soils contain a multitude of fungi with vastly divergent lifestyles ranging from saprotrophic to ectomycorrhizal symbionts (ECM) and parasites. It is unknown if all classes of symbiotic fungi share a common core set of genes required for the formation of symbiosis, or if the genetic mechanisms required for mutualism were reinvented each time it developed in evolutionary history. The study of symbiosis between ECM fungi and trees has seen some significant advances with the availability of the sequenced genomes of mutualistic fungi (*Laccaria bicolor*, *Tuber melanosporum, Paxillus involutus, Hebeloma cylindrosporum, Oidiodendron maius)*. The genomes of these fungi are proving especially useful in characterizing the genetic foundation of mutualistic symbiosis. New insights gleaned from these genomes, as compared to their saprotrophic and pathogenic cousins, have helped to redefine and shape our understanding of the nature of the symbiotic lifestyle. It would appear, based on the different molecular 'toolboxes' of these mycorrhizal genomes, that the evolution of the symbiotic lifestyle is quite divergent. Although commonalities exist, such as a reduction in plant cell wall-degrading enzymes, large differences are also seen, such as the secretion of effector-like small secreted proteins that are present only in Agarics. To understand if the differences between these species are due to their origins in the Ascomycota, versus the Basidiomycota, the genomes of 25 mycorrhizal fungi are currently being sequenced to serve as a comparison. The fact that mycorrhizal fungi appear to be independently derived from multiple saprobic lineages means that genomic data generated by current sequencing projects of saprotrophic agarics will provide independent assessments of the genetic underpinnings of mycorrhizal competence. I will also discuss how genomics and genome-wide transcriptomics allowed us to identify effector-like proteins of central importance in controlling symbiosis formation. One of these proteins, so-called MYCORRHIZAL iNDUCED SMALL SECRETED PROTEIN 7 (MiSSP7), the most highly symbiosis-upregulated gene from the ectomycorrhizal fungus *L. bicolor*, encodes an effector protein required for the establishment of mutualism. MiSSP7 is secreted by the fungus upon receipt of diffusible signals from plant roots, imported into the plant cell via phosphatidylinositol 3-phosphate-mediated endocytosis, and targeted to the plant nucleus where it alters the transcriptome of the plant cell. *L. bicolor* transformants with reduced expression of MiSSP7 do not enter into symbiosis with poplar roots. MiSSP7 resembles effectors of pathogenic fungi that are similarly targeted to the plant nucleus to promote colonization of the plant tissues and thus can be considered a mutualism effector. Implications of our findings and the potential role of effector-like proteins in mycorrhiza formation will be discussed.

This work was supported by the European Commission within Project EnergyPoplar (FP7-211917) and the NoE EVOLTREE (FP6-016322), and l'ANR (project FungEffector). This research was also sponsored by the Genomic Science Program of the US Department of Energy, Office of Science, Biological and Environmental Research under contract DE-AC05-00OR22725. Sequencing and assembly of the mycorrhizal and wood-decayer genomes were conducted by the U.S. DOE Joint Genome Institute and supported by the Office of Science of the U.S. DOE under Contract No. DE-AC02-05CH11231.

O1.1 - TRANSCRIPTOME PROFILING OF *SCLEROTINIA SCLEROTIORUM* COMPOUND APPRESSORIA

Xiaofei Liang[1], Ulla Benny[1], Li Lu[2], Mathias Choquer[3], George Casella[4], Jie Lang[4], Claudio Fuentes[4] and Jeffrey Rollins[1]

[1]Department of Plant Pathology, 1453 Fifield Hall, University of Florida, Gainesville, FL 32611-0680, USA; [2] Interdisciplinary Center for Biotechnology, 152 CGRC, University of Florida, Gainesville, FL, USA; [3]UMR5240, Joint Laboratory: University of Lyon 1 - CNRS - BAYER S.A.S., 14 impasse Pierre Baizet, BP 99163, 69263 Lyon Cedex 09, France; [4]Department of Statistics, 408 McCarty Hall C, University of Florida, Gainesville, FL 32611-0339, USA

Sclerotinia sclerotiorum produces three distinct multicellular developmental forms during its lifecycle: sclerotia, apothecia and compound appressoria. Each plays a unique and essential role in pathogenic success and persistence of the disease. The identification of genes associated with the function and regulation of each developmental stage may offer clues into strategies for blocking the disease cycle. In this study, we are focusing on transcriptome profiling during compound appressorium development utilizing microarray hybridization and analyses. These studies utilized an Agilent 4 by 44k long-oligo (60-mer) microarray platform designed from the predicted gene set of the *S. sclerotiorum* 1980 genome. Total RNAs extracted from (i) ascospores germinated 44h. in quarter strength potato dextrose broth versus (ii) ascospores germinated 44h. on quarter strength potato dextrose agar overlaid with a sheet of cellophane were reverse transcribed and labelled. Six biological replications of each condition were used for competitive hybridizations of (i) vs. (ii) cDNA. Signal intensities for predicted genes represented by two probes were analyzed by Anova and statistical significance assigned by F-test. Using a False Discovery Rate of 0.05, revealed 283 genes up-regulated greater than 2-fold and 351 genes down-regulated greater than 2-fold during compound appressorium development relative to undifferentiated hyphae. Among these is a cluster of ten contiguous genes and two unlinked two-member gene clusters containing orthologs of the recently identified *Botrytis cinerea* botcinic acid toxin biosynthetic cluster. These genes are co-ordinately up regulated 13 to 45-fold during compound appressorium development. This expression data has allowed us to generate hypotheses regarding the function of these and other genes in host penetration and early infection processes as well as compound appressorium regulation. These hypotheses currently are being tested through functional analyses of candidate genes.

O1.2 - TRANSCRIPTOMICS OF *BOTRYTIS CINEREA* GERMINATION

Michaela Leroch[1], Evelyn Silva[2] and Matthias Hahn[1]

[1]University of Kaiserslautern, Phytopatholoy Department, Kaiserslautern, Germany; [2]Fundación Ciencia para la vida and Instituto MIFAB, Santiago, Chile.

(Corresponding author: mleroch@rhrk.uni-kl.de)

Germination of spores is a fundamental event in fungal life, represented by the initiation of growth from a dormant state. In plant pathogens, germination immediately precedes host penetration, and therefore is of crucial importance for the success of infection.

We have performed transcriptome studies to follow gene expression changes during germination and differentiation of *Botrytis cinerea* conidia. The results showed that greatest changes of gene expression occur between 0 and 1 hour (before germ tube emergence), and between 4 and 15 hours. The genes that were specifically upregulated during germination (1-4 h.p.i.), were found to be enriched in genes encoding secreted proteins, indicating a strong secretory activity during the early stages of development. In a *bmp1* MAP kinase mutant, which is essential for germination on a hydrophobic surface and host penetration, upregulation of many of these genes was not observed. The microarray data were largely confirmed by RT-PCR data and promoter-GFP reporter strains for selected genes. One of the genes, encoding a putative chitin deacetylase (*cda1*), was found to be activated shortly preceding germ tube outgrowth, irrespective of germination conditions. In contrast, several cutinase genes were found to require host lipids for activation in germinating spores, and to be partly repressed by sugars.

Our further research focuses on the dissection of the regulatory mechanisms that control expression of germination-induced genes, and on the analysis of their function, in particular regarding their role in penetration and pathogenesis.

O1.3 - PHOSPHOPROTEOMICS TO TRACE SIGNAL TRANSDUCTION

Marlène Davanture[1], Benoît Valot[1], Michel Zivy[1] and Sabine Fillinger[2]

[1]PAPPSO, INRA-CNRS-University Paris XI-Agro ParisTech, Gif-sur-Yvette, France; [2]BIOGER CPP, INRA Versailles-Grignon, France.

(Corresponding author: sabine.fillinger@versailles.inra.fr)

Protein phosphorylation and dephosphorylation are cellular processes rapidly induced by external *stimuli* adapting regulatory circuits and enzymatic functions to changing environmental conditions. Signal transduction (ST) pathways necessary for signal perception and phosphorylation cascades are involved in many physiological processes such as development, stress adaptation, virulence etc. ST components may either constitute targets for agronomic fungicides or mediate resistance to these compounds.

In order to identify and to compare the proteins involved in transducing the signal perceived after phenylpyrrol treatment, we established a gel-free phosphoproteomic approach for systematic identification of phosphorylated peptides in *Botrytis cinerea* mycelia, treated or not with fludioxonil.

The gel-free phosphoproteomic approach combines two sequential steps after trypsin digestion : i) SCX chromatography (strong cation exchange); ii) IMAC (Immobilized metal affinity chromatography) for the enrichment of phosphopeptides prior to LC-MS/MS analysis. Differential dimethyl labelling of primary amines and of the N-termini of the peptides further allows to quantify the identified phosphopeptides between both conditions. Four independent biological replicates per condition were analysed.

We identified a total of 1743 unique phosphopeptides (peptide sequences covering a unique phosphorylation site) corresponding to 870 unique phosphoproteins. 18.5% of the phosphopeptides were only detected in the control samples and considered as dephosphorylated by the fludioxonil treatment; 21.5 % were specific of the treated samples and therefore considered as fludioxonil induced phosphorylated. A preliminary functional annotation of the qualitative phosphoproteome shows: i/ an enrichment of proteins involved in cell cycle (spindle pole body complex), control of translation and PKC1 among the dephosphorylated proteins; ii/ principally proteins involved in regulation (ST, transcriptional and translational control, protein fate) among the phosphorylated proteins. The statistical analysis of the quantitative data shall then complete and precise the functional categories of fludioxonil induced phosphorylations and dephosphorylations. Ultimately, the phosphoproteomic approach will not only help to unravel the mode-of-action of the antifungal compound fludioxonil but also to trace signal-transduction pathways.

O1.4 - EXPLORING *BOTRYTIS CINEREA* PROTEOME: FROM CONIDIAL GERMINATION TO MEMBRANE PROTEINS

Francisco Javier Fernández-Acero[1], Thomas Colby[2], María Carbú[1], Anne Harzen[2], Carlos Garrido[1], Victoria E. González-Rodríguez[1], Eva Liñerio[1], Ursula Wieneke[2], Jürgen Schmidt[2] and Jésus M. Cantoral[1]

[1]Laboratory of Microbiology, Marine and Environmental Sciences Faculty, University of Cádiz, Pol. Río San Pedro s/n, Puerto Real, Cádiz, Spain. [2]Max Planck Institute for Plant Breeding Research, MS group, Carl-von-Linné-Weg, Cologne, Germany.

In 2004, our group began to develop different proteomic approaches to investigate phytopathogenic fungi. Since these initials studies, our efforts have been directed towards elucidating the complex molecular mechanisms of the infection cycle and biology of these organisms. Specifically, our experimental approaches have focused on discovering new fungal pathogenicity/virulence factors which direct fungal attack. Following this main aim, our group has continued studying different subproteomes from *B. cinerea* in three different ways. By using 2DE and MALDI TOF/TOF approaches, we had studied the differences in the protein profile (i) of *B. cinerea* conidia during germination; (ii) of *B. cinerea* phosphorylated proteins during pathogenicity induction; and finally (iii) in membrane proteins of *B. cinerea* as signal translations key component. Most of the pathogenicity/virulence factors from *B. cinerea* listed in PHI-pathogen database belong to these categories.

During conidial germination the fungus produces proteins and toxins that may play an essential role in the establishment of a successful infection. Many of these proteins could be described as pathogenicity/virulence factors. A differential proteomic approach based on 2-DE and MALDI-TOF/TOF MS has been carried out to study the *B. cinerea* proteome during early stages of germination where 189 spots were identified, yielding 211 positive hits corresponding to 140 unique proteins. Most of the signalling cascades are based on the post-translational modifications of proteins. Some studies suggest that one-third of all proteins are modified by phosphorylation. We present the study of *Botrytis cinerea* phosphoproteome by 2-DE and MALDI/TOF-TOF. Our data support the hypothesis that each assayed carbon source produces different phosphoproteins 2-DE profiles. At present, we have identified 768 different protein spots. Membrane proteins play a key role in pathogenesis; we will report a subproteomic approach for the study of the membrane protein from *B. cinerea* under different pathogenic conditions.

O1.5 - *SCLEROTINIA SCLEROTIORUM*: INVESTIGATING FUNGAL EXUDATES

Stephanie Heard[1], Kim E. Hammond-Kosack[1], Jon West[1], John Antoniw[1], Bruce Grieve[2,3] and Sarah Perfect[2]

[1]Rothamsted Research, Plant Pathology and Microbiology, Hertfordshire, AL5 2JQ, UK; [2]Microscopy, Syngenta, Jealotts Hill, UK; [3]Univeristy of Manchester, Syngenta University Innovation Centre.

As the economic importance of *Sclerotinia sclerotiorum (Ss)* increases, a deeper understanding of how the production of fungal exudates is regulated and the identification of new pathogenicity factors is necessary for the design of novel control strategies. Many studies to date have looked only at the secretion of exudates after several days of growth. In this study our aim is to explore the secreted exudates during the early phases of spore germination. To do this a Metabolite Triple Fingerprinting analysis was used to determine the amino acid and sugar profiles of different samples collected after *Ss* spores had germinated and then grown in the presence of a range of host plant tissues. This technique uses Proton Nuclear Magnetic Resonance (^{1}H-NMR) in combination with ESI-MS (positive and negative modes) to investigate the metabolite profiles. The expression of genes linked to enzyme production, pathogenicity and carbon transporters from this study will be analysed using qRT-PCR to see how different plant tissue may change the expression of key genes.

Along with the metabolomics approach, a bioinformatics analysis has been used to predict the *Ss* secretome and this will be used to investigate the early secreted proteins. A putative secretome pipeline was developed based on specific sequence criteria. Molecular techniques such as PCR have been used to determine the presence/absence of these sequences in a range of natural European isolates. A qRT-PCR analysis will be used to determine whether the sub-set of genes identified in all *Ss* isolates are expressed during spore germination *in planta* at 48, 72 and 96hr after infection.

O1.6 - GATC BIOTECH INTRODUCES THIRD GENERATION SEQUENCER PACBIO RS FOR NEW SEQUENCING APPLICATION. COMPLETE SOLUTIONS FOR DNA AND RNA SEQUENCING.

Christophe Meynier[1], Zélie Dubreucq[2]

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GATC Biotech develops the potential of the third generation sequencer PacBio RS and its complementary to existing High Through Sequencer (NGS) for new sequencing application.
Our presentation will present the outlines of these available applications:

• Beautiful share on PacBio RS from Pacific BioScience
GATC Biotech acquired this third generation technology and will be the first commercial company in Europe to offer sequence reads longer than 1400bp in 2011.
The principal projection is real time sequencing on single molecule. Indeed, amplification step is excluded from the instrument protocol, making it possible to abstain from PCR's bias (emulsion or bridge).
The first applications available will focus on de novo genomes and improve the reliability of scaffolds of large genomes such as plants. The future potential of this technology show the possibility for further applications in transcriptome analysis.

Our strategy for unknown organism is optimized with a hybrid approach by combining three sequencing technologies: GS FLX plus of Roche, HiSeq2000 of illumina and PacBio RS of Pacific Bioscience. The new option, PAC Bio RS, with 1,4kb read length allows looking closely at repeat region.

• Transcriptome and regulome sequencing (RNA seq):
To discover its transcriptome we provide a wide choice of libraries such as normalized libraries that reduces the genes expression variations in order to create a reference transcriptome. Roche GS FLX plus is used to assemble a reference transcriptome with 700pb read length.
To search after expression level, GATC Biotech recommends a mapping of shorter fragments on a reference. We offer the Illumina Hiseq 2000 with new kits of 50pb or 100pb, sufficient size for a specific alignment.
The interest of the choice of Illumina technology lies in the depth of reading: up to 1.4 billion read pairs per flowcell.

P1.1 - LONGSAGE GENE-EXPRESSION PROFILING OF *B. CINEREA* GERMINATION SUPPRESSED BY RESVERATROL, THE MAJOR GRAPEVINE PHYTOALEXIN

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The Ascomycetes *Botrytis cinerea* is one of the most studied necrotrophic phytopathogens and one of the main fungal parasites of grapevine. As a defense mechanism, grapevine produces a phytoalexin compound, resveratrol, which inhibits germination of the fungal conidium before it can penetrate the plant barriers and lead to host cell necrotrophy. To elucidate the effect of resveratrol on transcriptional regulation in *B. cinerea* germlings, two LongSAGE (long serial analysis of gene expression) libraries were generated *in vitro* for gene-expression profiling: 41,428 tags and among them, 15,665 unitags were obtained from resveratrol-treated *B. cinerea* germlings, and 41,358 tags, among them, 16,362 unitags were obtained from non-treated *B. cinerea* germlings. *In-silico* analysis showed that about half of these unitags match known genes in the complete *B. cinerea* genome sequence. Comparison of unitag frequencies between libraries highlighted 110 genes that were transcriptionally regulated in the presence of resveratrol: 53 and 57 genes were significantly down- and up-regulated, respectively. Manual curation of their putative functional categories showed that primary metabolism of germinating conidia appears to be markedly affected under resveratrol treatment, along with changes in other putative metabolic pathways, such as resveratrol detoxification and virulence-effector secretion, in *B. cinerea* germlings. We propose a hypothetical model of cross talk between *B. cinerea* germinating conidia and resveratrol-producing grapevine at the very early steps of infection.

Zheng C[#], Choquer M[#], Zhang B[#], Ge H, Hu S, Ma H and Chen S (2011) LongSAGE gene-expression profiling of *B. cinerea* germination suppressed by resveratrol, the major grapevine phytoalexin. *Fungal Biology*, **115**: 815-832.

[#]These authors contributed equally to this work.

P1.2 - PROTEOMIC APPROACHES TO STUDY THE PHYTOPATHOGENIC FUNGUS *BOTRYTIS CINEREA*

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In the post-genomic era, proteomics —in combination with other techniques— has become a powerful tool to provide relevant information about fungal biology and their interaction with their hosts. The necrotrophic phytopathogenic fungus *Botrytis cinerea* produces a battery of weapons including cell wall-degrading enzymes, toxins (botrydial and botcinolides), and reactive oxygen species producing and scavenging enzymes, which subsequently promotes the invasion of the plant tissue. Proteomics can contribute to understand the plant-pathogen interactions, fungal infection strategies, and life cycle of this fungus, to identify virulence factors. As a consequence, proteomics is opening up new possibilities for crop disease diagnosis and to develop efficient and environment-friendly crop protection strategies. Within BOTBANK project, our research group is using a proteomic approach to characterize and validate a collection of mutants of *B. cinerea* whose infectious cycle is affected. This characterization involves both *in vitro* and *in planta* analyses of the mutants. In this work, we present different proteomic (gel-based and gel-free/label-free) approaches to study the biology and infection mechanisms of *B. cinerea* both *in vitro* and *in planta* analyses, starting with different wild-type strains with different virulence and host.

In gel-based analysis using mono-dimensional electrophoresis (1-DE), differences in the protein profiles were observed among strains both *in vitro* analyses of mycelia, secreted proteins and conidia, and *in planta* analyses made on grapes. Some of these differences were identified as proteins previously described as virulence factors. By gel-free/label-free analysis, several hundred proteins were identified and quantified, and the results obtained by 1-DE were confirmed.

In conclusion, 1-DE is a fast and reliable technique to identify virulence factors, and allows us to discriminate between phenotypes of different strains, especially in the case of comparative proteomics with a large number of samples. Gel-free/label-free analysis provides higher proteome coverage, and more important, different sets of proteins are identified, especially low abundant ones. In order to delve into the mechanisms of plant-pathogen interactions, proteomics can be a successful tool, through the use of different complementary techniques as gel-based (1-DE and 2-DE) or gel-free (label-free LC-MS/MS) approaches. These techniques are being applied successfully in the characterization of the mutants of *B. cinerea*.

This work is supported by MCINN (BOTBANK EUI2008-03686, FEDER co-financed), Andalusian Government and the University of Cordoba (AGR 164).

P1.3 - ISOLATION OF NECROSIS-INDUCING PROTEINS FROM *BOTRYTIS CINEREA*

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The grey mold fungus *Botrytis cinerea* is a necrotrophic plant pathogen infecting over 200 cultivated plant species and causing significant economic damage to crops worldwide. Early stages of Botrytis infection are characterized by appearance of small necroses from which infection spreads. Similar to other necrotrophic fungi, *B. cinerea* is unable to occupy living host cells and secrets enzymes and toxins, which induce cell death of host tissue. Recently, we showed that *B. cinerea* is exposed to massive, host-induced, cell death during the early infection phase. The fungus then quickly recovers and second infection phase (spreading lesions) is initiated from mycelium that resides within a necrotized area, in which the fungus is not exposed to plant defense compounds. We reasoned that production of the early necroses is assisted by fungal-secreted, necrotizing proteins (and possibly other types of compounds). Here we describe isolation and identification of a subset of proteins that are secreted by *B. cinerea* during early stages of infection.

In order to collect proteins from infected leaves, we developed a system that allows us to collect large amounts of secreted proteins from inoculated leaves during different stages of infection. An extract collected from leaves between 24-30 h post inoculation contained strong necrotizing activity when infiltrated into leaves. The necrosis-inducing activity was heat sensitive and could be precipitated in presence of ammonium sulfate, suggesting that it might contain proteins. The extract was partially purified, separated on SDS-PAGE and stained with silver. Bands were cut from the gel and subjected to MAS-Spectrometry analysis. Over 40 different proteins were identified and categorized according to function. The necrosis-inducing activity of specific candidate proteins is subject of current investigation.

P1.4 - THE SECRETOME OF *BOTRYTIS CINEREA*: ADAPTATION TO ENVIRONMENTAL PH

Cindy Dieryckx, Vincent Girard, Semcheddine Cherrad, Dominique Job, Claudette Job, Christine Rascle and Nathalie Poussereau

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Micro-organisms must adapt to environmental change to survive, and this is particularly true for fungal pathogens such as *Botrytis cinerea*. *B. cinerea* is found both in the environment and in diverse plant hosts. Their ambient pHs vary considerably, and therefore we have examined the response of *B. cinerea* to changes in ambient pH using a proteomic approach.

The expression of secreted proteins induced by controlled growth conditions, i.e. complete synthetic medium at pH 5.0, was identified by two-dimensional gel electrophoresis and image analysis software. All reproducible and statistically significant spots were identified by peptide mass fingerprinting, thereby extending our 2-DE map of the *Botrytis cinerea* to a total of 257 identified proteins. Proteins expressed in *B. cinerea* cells growing at pH 5.0 or 7.0 were compared by 2-DE. Qualitative and quantitative differences were observed related to the protein patterns whereas at both pH (5.0 and 7.0). Furthermore, characterization of a strain of *B. cinerea* deleted for the transcription factor PacC will clarify the role of the signaling pathway Pal/Pac on the establishment of the secretome of the fungus in both pH conditions.

Discussion will be done on the possible factors regulating secreted protein expression by pH in Botrytis. In addition, our data suggested that different fungal proteins could be involved in the infection process depending on the external pH.

P1.5 - PROTEOMIC ANALYSIS OF PROTEINS SECRETED BY *BOTRYTIS CINEREA* IN RESPONSE TO METAL STRESS

Semcheddine Cherrad[1,2], Vincent Girard[1], Cindy Dieryckx[1], Isabelle R. Gonçalves[1,3], Jean-William Dupuy[4], Marc Bonneu[4], Claudette Job[1], Dominique Job[1], Sébastien Vacher[2] and Nathalie Poussereau[1]

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Metal ions are essential elements in many cellular processes. However, metal excess becomes toxic and constitutes a global environmental hazard. Copper, Zinc and Nickel are essential for growth and survival of microorganisms, but become toxic at high concentrations. Cadmium has no known function and has a high toxicity even at low concentrations. A range of fungi from all major taxonomic groups may be found in metal-polluted habitats and the ability to survive and grow in the presence of potentially toxic concentrations is frequently encountered. To adapt to this stress, fungi have evolved several mechanisms at both intracellular and extracellular levels. In particular, fungi are well known for their ability to secrete a large panel of proteins. However, their role in the adaptation of fungi to metal toxicity has not yet been investigated. To address this question, here, the fungus *Botrytis cinerea* was challenged to copper, zinc, nickel or cadmium stress and secreted proteins were collected and separated by 2D-PAGE. One hundred and sixteen spots whose volume varied in at least one tested condition were observed on 2D gels. Densitometric analyses revealed that the secretome variation was correlated with physicochemical properties of tested metals. Thus Cd, which belongs to soft acids groups, not only affected the largest number of secreted proteins but also exerted a more potent effect on the accumulation level of metal-sensitive secreted proteins compared to Cu, Zn and Ni that are considered as essential metals and less toxic. Fifty-five of these 116 spots were associated with unique proteins and functional classification revealed that the production of oxidoreductases and cell-wall degrading enzymes was modified in response to metals. Moreover, this comparative analysis revealed that the accumulation levels of some secreted proteins increased specifically in response to one or several metals. In silico promoter analysis reveal that Rim101/PacC sites were statistically over-represented in the upstream sequences of the 31 genes corresponding to the varying unique spots suggesting a possible link between pH regulation and metal response in *B. cinerea*.

Session 2

ADAPTATION TO ENVIRONMENT: FUNGICIDES AND HOST PLANTS

CHAIRPERSONS: Sabine Fillinger and Marc Bardin

INVITED LECTURE

I2 - GLOBAL ECONOMIC IMPORTANCE OF *BOTRYTIS* AND *SCLEROTINIA* PROTECTION ON VEGETABLE, FRUIT AND INDUSTRIAL CROPS IN THE WORLD. Gilles Chevallier

ORAL COMMUNICATIONS

O2.1 - GREY MOULD ISOLATES FROM COMMERCIAL STRAWBERRY FIELDS SHARE GENETIC TRAITS OF *B. CINEREA* AND *B. FABAE.* Cecilia Plesken, Manuel Daumann, Michaela Leroch and Matthias Hahn

O2.2 - COMPARISON OF RESISTANCE MECHANISMS TO PYRROLNITRIN AND TO IPRODIONE IN *BOTRYTIS CINEREA.* Marc Bardin, Sakhr Ajouz, Anne-Sophie Walker, Philippe C. Nicot, Pierre Leroux and Sabine Fillinger

O2.3 - MUTAGENESIS OF *SDHB* AND *SDHD* GENES IN *BOTRYTIS CINEREA* FOR FUNCTIONAL ANALYSIS OF RESISTANCE TO SDHIS. Anaïs Lalève, Anne-Sophie Walker, Pierre Leroux, Valérie Toquin, Hélène Lachaise and Sabine Fillinger

O2.4 - POPULATION STRUCTURE OF *SCLEROTINIA SCLEROTIORUM* ON WILD AND AGRICULTURAL HOSTS IN THE UK. John Clarkson and Emma Coventry

O2.5 - VARIABILITY OF *SCLEROTINIA SCLEROTIORUM* IN MINAS GERAIS STATE, BRAZIL. Miller S. Lehner, Trazilbo J. Paula Jr., Braz T. Hora Jr., José Eustáquio S. Carneiro, Hudson Teixeira, Robson J. Nascimento and Eduardo S. Mizubuti

O2.6 - COMPARISON OF *BOTRYTIS CINEREA* POPULATIONS ISOLATED FROM TWO CULTIVATED HOST PLANTS. Mojtaba Asadollahi, Éva Fekete, Erzsébet Fekete, László Irinyi, Levente Karaffa, Mariann Árnyasi, Anikó Szojka, György J. Kövics and Erzsébet Sándor

O2.7 - CONTROLLING *BOTRYTIS* DISEASE IN VINEYARDS THROUGH MANIPULATING LOW-PATHOGENICITY POPULATIONS. Ross Beever, Peter Johnston and Karyn Hoksbergen

POSTERS

P2.1 - HOST PLANT SPECIES OF *SCLEROTINIA SCLEROTIORUM* AND *SCLEROTINIA MINOR* IN TURKEY. Cafer Eken

P2.2 - MOLECULAR BASIS AND POPULATION GENETICS OF MULTIPLE FUNGICIDE RESISTANCE IN *B. CINEREA.* Matthias Hahn, Michaela Leroch, Dennis Mernke and Cecilia Plesken

P2.3 - PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF RESISTANCE TO SDHI FUNGICIDES IN *BOTRYOTINIA FUCKELIANA* (*BOTRYTIS CINEREA*). Mario Masiello, Rita Milvia De Miccolis Angelini, Caterina Rotolo, Stefania Pollastro and Francesco Faretra

P2.4 - CHARACTERIZATION OF THE GENE CYP68,4 FOR THE FENHEXAMID RESISTANCE IN THE SPECIES *BOTRYTIS PSEUDOCINEREA*. Saad Azeddine, Alexis Billard, Pauline Solignac, Joclyne Bach, Danièle Debieu and Sabine Fillinger

P2.5 - ANTI*BOTRYTIS* SYNERGY BETWEEN INHIBITORS OF CHITIN SYNTHASES AND BETA-(1,3) GLUCAN SYNTHASE. Emmanuelle Galland, Pierre Genix, Stéphanie Gamet, Christine Rascle, Catherine Sirven, Marie-Pascale Latorse, Valérie Toquin, Roland Beffa and Mathias Choquer

P2.6 - BIOCONTROL EFFECT OF PLANT EXTRACTS AGAINST *BOTRYTIS CINEREA* AND *SCLEROTINIA SCLEROTIORUM* OF SEVERAL CROPS. Nazira Aitkhozhina

I2 - THE GLOBAL ECONOMIC IMPORTANCE OF *BOTRYTIS-SCLEROTINIA* CROP PROTECTION

Gilles Chevallier

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This presentation, in the form of a Quiz, will be focused on Horticulture Crops (+ OSR/Canola) where the biggest potential is. It will take into account the Sclerotiniacae markets, i.e. *Botrytis / Sclerotinia / Monilia :*

- How big is the *Botrytis / Sclerotinia / Monilia* market ?
- Among the 4 main Regions, where is the biggest market ?
- What are the main crop groups ?
- Crop Protection value (M€) by country ?
- Main products and Mode of Action ?

O2.1 - GREY MOULD ISOLATES FROM COMMERCIAL STRAWBERRY FIELDS SHARE GENETIC TRAITS OF *B. CINEREA* AND *B. FABAE*

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As a destructive pathogen of many fruit and vegetable crops, *B. cinerea* needs to be controlled by fungicide treatments. Grey mould isolates from German strawberry fields revealed a combination of specific and nonspecific (MDR-type) fungicide resistance mechanisms. A high proportion of these isolates showed high frequencies of resistance to all currently used fungicides. A new MDR phenotype ($MDR1^h$) was detected, with higher resistance levels than the previously described MDR1. The majority of German strawberry isolates, including all $MDR1^h$ isolates, were shown to belong to a novel genetic group. Based on the sequence comparisons of several genes, we found that these isolates (called group 3) were genetically intermediate between *B. cinerea* group II strains (*B. cinerea sensu stricto*) and *B. fabae*, a species that only infects legume hosts. However, sequences from different genes resulted in different phylogenetic groupings, indicating that group 3 isolates might represent hybrids. Morphologically and phenotypically, group 3 strains were almost indistinguishable from *B. cinerea*, except for poor sporulation on grapes under suboptimal conditions. Using PCR-based markers, group 3 isolates were found to be common also in strawberry fields in France, Spain and Greece. By using next generation sequencing technology, we want to clarify the genetic identity and phylogenetic origin of group 3 isolates, and to explain their different host preference compared to *B. cinerea*.

O2.2 - COMPARISON OF RESISTANCE MECHANISMS TO PYRROLNITRIN AND TO IPRODIONE IN *BOTRYTIS CINEREA*

Marc Bardin[1], Sakhr Ajouz[1], Anne-Sophie Walker[2], Philippe C. Nicot[1], Pierre Leroux[2] and Sabine Fillinger[2]

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In a recent study, we reported the development of resistance to pyrrolnitrin in laboratory-induced mutants of *Botrytis cinerea*, suggesting a possible loss of efficacy of pyrrolnitrin-producing biological control agents. Pyrrolnitrin is a structural analogue of phenylpyrrole fungicides and it was shown that highly-resistant mutants to phenylpyrroles generally acquire resistance to iprodione, a dicarboximide fungicide. In the present study we compared the mechanisms of resistance to pyrrolnitrin and to iprodione in *B. cinerea*. This knowledge may be helpful to predict the risk related to the loss of efficacy of pyrrolnitrin-producing biological control agents against *B. cinerea* in the field. Pyrrolnitrin-induced mutants and iprodione-induced mutants of *Botrytis cinerea* were produced *in vitro* on media containing pyrrolnitrin or iprodione. For the pyrrolnitrin-induced mutants, high level of resistance to pyrrolnitrin was associated with a high level of resistance to iprodione, for the four tested isolates. For the iprodione-induced mutants, the high level of resistance to iprodione detected generated variable levels of resistance to pyrrolnitrin, for the five tested isolates. All selected mutants showed hypersensitivity to high osmolarity, suggesting a defect in the osmosensing signal transduction pathway. The sequences of the osmosensing class III histidine kinase encoding gene *bos1* showed different mutations in both types of mutants, although different isolates derived from a same strain displayed sometimes identical mutations suggesting an influence of the genetic background on the selected mutation. Almost all mutations affect the signal transduction or HAMP-domains of the histidine-kinase including yet undetected replacements. Altogether our analyses show that each of the six HAMP domains is important for signal-transduction. The impact of the replacements on HAMP structure and function will be discussed.

O2.3 - MUTAGENESIS OF *SDHB* AND *SDHD* GENES IN *BOTRYTIS CINEREA* FOR FUNCTIONAL ANALYSIS OF RESISTANCE TO SDHIS

Anaïs Lalève[1,2], Anne-Sophie Walker[1], Pierre Leroux[1], Valérie Toquin[2], Hélène Lachaise[2] and Sabine Fillinger[1]

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Grey mould causes serious losses of the worldwide harvest. Chemical control remains the principal means to curb this disease. Respiratory inhibitors play an increasing role in the control of grey mould. **S**uccinate **d**e**h**ydrogenase **i**nhibitors (SDHIs, including carboxamides) inhibit the fungal respiration by blocking the ubiquinone-binding site of the mitochondrial complex II. Old SDHIs (i.e. carboxin), essentially active against Basidiomycetes were replaced in the 2000s by a new generation of SDHIs with a broader spectrum including Ascomycetes. Boscalid is the sole SDHI registered to deal with grey mould on grapevines even if several chemical subgroups of SDHIs have been synthesized. A few years after its market introduction, field mutants of *B. cinerea,* resistant to boscalid were isolated and characterized in France and Germany.

At least six different phenotypes, named CarR1 to CarR6 (**Car**boxamides **R**esistant 1 to 6), have been pinpointed by characterizing their resistance pattern towards 20 SDHIs. CarR1 to R4 phenotypes exhibit low to medium level of resistance, whereas CarR5 and R6 show high levels of resistance to different SDHIs, including boscalid. CarR1 and CarR2 strains are currently the most frequent strains detected in German and French vineyards.

The resistance mechanism was investigated for the different phenotypes by searching for putative alterations in the Sdh proteins, which could be responsible for the observed resistance. Our findings show that except for CarR2, point mutations occurring in the *sdhB* gene lead to a specific amino acid change in SdhB for each phenotype. For CarR2 strains, we distinguished at least 3 sub-groups: strains with i/ a point mutation in the *sdhB* gene, ii/ a point mutation in the *sdhD* gene and iii/ no mutation in any of the four *sdh* genes. Isogenic mutants have been generated through a gene replacement strategy to confirm the role of these mutations in the various SDHI resistance phenotypes. We have shown that each mutation in *sdhB* gene confers resistance to at least one SDHI. Isogenic mutants show similar resistance factor compared with fields mutants carrying the same mutation. Sdh enzyme activity and inhibition by different SDHIs were measured in these mutants. Our results provide evidence for differential Sdh inhibition profiles according to the tested mutations, correlating with the mutants' resistance pattern.

O2.4 - POPULATION STRUCTURE OF *SCLEROTINIA SCLEROTIORUM* ON WILD AND AGRICULTURAL HOSTS IN THE UK

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Sclerotinia disease caused by *Sclerotinia sclerotiorum* is a major problem on a variety of crops in the UK. The pathogen can also infect wild hosts which can potentially act as sources of inoculum for crops. Although the population structure of *S. sclerotiorum* has been examined in Canada, USA, and Australia, no study has been carried out for the UK. There is also little information on populations from wild hosts. UK isolates of *S. sclerotiorum* were therefore obtained from different crops and locations and also from wild *Ranunculus acris* (meadow buttercup). These isolates were characterised using microsatellite markers and by sequencing a section of the intergenic spacer (IGS) region of the ribosomal RNA (rRNA) gene.

The microsatellite analysis identified 228 haplotypes within 384 isolates from a total of 12 *S. sclerotiorum* populations. Each population comprised multiple haplotypes, but with one occurring at a much higher frequency than the rest, with the majority of other haplotypes found only once or a few times. A common microsatellite haplotype was distributed widely and at high frequency across different crop and buttercup hosts, locations and years. IGS sequencing identified a total of 14 haplotypes within the 12 *S. sclerotiorum* populations of which 13 were found in buttercup compared to only 8 for agricultural hosts. Although common haplotypes were found between *S. sclerotiorum* populations, there was some evidence of subdivision particularly for one geographically isolated buttercup population. Comparison of IGS sequences with those on Genbank showed that several of the most common UK IGS haplotypes were also found in *S. sclerotiorum* populations from USA, Canada and New Zealand. An IGS haplotype found only on buttercup in the UK was also found among isolates from *R. ficaria* (lesser celandine) in Norway.

In conclusion, *S. sclerotiorum* populations from buttercup and crop hosts in the UK have a similar multi-clonal population structures with limited evidence of differentiation. The high frequency of one microsatellite haplotype in the majority of populations in different years with distribution over different areas of the UK suggests spatial mixing at both local and wider scales. The implication of these results in relation to *S. sclerotiorum* evolution and disease management will be discussed.

O2.5 - VARIABILITY OF *SCLEROTINIA SCLEROTIORUM* IN MINAS GERAIS STATE, BRAZIL

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White mold caused by *Sclerotinia sclerotiorum* is the most destructive disease of common beans (*Phaseolus vulgaris*) in Minas Gerais State (MG), Brazil. We collected 127 isolates of *S. sclerotiorum* from bean fields located in four regions of MG to assess the amount and distribution of genetic variability in the state. Each single-sclerotium isolate was purified by transferring a single hyphal tip onto potato dextrose agar medium. The isolates were grown at 23℃ in a liquid synthetic medium. DNA extraction was accomplished using the CTAB protocol. A total of eight SSR loci were analyzed: 7-2:AF377902, 9-2:AF377903, 12-2:AF377906, 13-2:AF377907, 36-4: AF377914, 92-4: AF377919, 106-4:AF377921 and 114-4:AF377923. Each locus was labeled with one of the three fluorescent dyes (FAM, NED and HEX) in multiplex reactions. SSR patterns were automatically sized with the GeneMapper™ 3.5 software. The diversity indices of Stoddart and Taylor's (G), Shannon-Wiener's (H') and Hills N_1 as well as the evenness indices E_1 ($E_1 = H'/\ln g_{obs}$) and E_5 ($E_5 = G - 1/N_1 - 1$) were used for estimation of the genotypic diversity. We found 59 alleles varying from 3 (locus 36-4: AF377914) to 13 (locus 106-4: AF377921) per locus. Of the 65 genotypes detected, 49 were represented by a single isolate. A single genotype represented by 24 isolates was detected in all regions of MG and there was high genetic diversity ($G = 17.7$, $H' = 3.6$, $N_1 = 37.4$, $E_1 = 0.87$ and $E_5 = 0.46$). We concluded that the diversity within *S. sclerotiorum* populations in MG is high. Further analyses are now being conducted to better characterize the genetic structure of the population of *S. sclerotiorum* in MG.

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O2.6 - COMPARISON OF *BOTRYTIS CINEREA* POPULATIONS ISOLATED FROM TWO CULTIVATED HOST PLANTS

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Botrytis cinerea Pers.:Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel), the cause of gray mold, is a necrotrophic pathogen causing pre- and post harvest diseases in at least 230 plant species, causing severe damage on numerous crops including strawberries and raspberries. It is also the agent of noble rot on grapevine, used for the elaboration of sweet wines. *Botrytis cinerea* has long been thought to be a single but morphologically variable and generalist species. Several recent studies have shown that *B. cinerea* was likely to form a species complex, with restricted gene flow between different cryptic genetic groups. More recently, studies of DNA polymorphism of different nuclear genes in *B. cinerea* populations showed that *B. cinerea* isolates consistently clustered in two different clades in the different gene phylogenies, Group I and Group II, which were therefore proposed to be phylogenetic species. *B. cinerea*, considered for a long time as a generalist fungal pathogen of a multitude of plants, was nonetheless shown to exhibit significant population diversity in Chile, France and England in a host-specific manner. However, differences could not be detected between *B. cinerea* populations from different hosts in Tunisia and California.

We sampled populations of *B. cinerea* on sympatric strawberry and raspberry cultivars for three years. Altogether, 490 of Group II *B. cinerea* isolates were analyzed. Standard population genetic data were computed from three different data sets: (i) PCR-RFLP pattern of *ADP-ATP translocase* and *nitrate reductase* genes, (ii) MSB1 minisatellite sequence data, and (ii) fragment size of five microsatellite loci. The structure of the different populations was similar as indicated by Nei's gene diversity and Shanon's information index, and also by haplotype diversity. It should be noted, that a reduced sample size was accompanied by reduced population diversity. The computed index of differentiation (G_{st}), and gene flow indicated differentiation within the sympatric populations. However, the differentiation was also affected by the sample size and the year of isolation. Population genetic parameters were also influenced by the level of polymorphism of the data sets. These results support the possibility of sympatric divergence associated with host use in generalist parasites.

O2.7 - CONTROLLING *BOTRYTIS* DISEASE IN VINEYARDS THROUGH MANIPULATING LOW-PATHOGENICITY POPULATIONS

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In this project we are exploring the possibility of utilizing natural variation within *Botrytis cinerea* to manipulate vineyard populations of this pathogen to reduce their pathogenic potential. The overall aim of the project is to reduce the environmental impact of disease control through reduced fungicide use. Genetic diversity within New Zealand vineyards has been surveyed for species of *Botrytis* and for transposon occurrence within those species. The occurrence of transposons has been compared across seasons. Transposon-characterised isolates have been compared in relation to fungicide resistance, and to pathogenicity tested using detached fruit. Results will be discussed in relation to whether there might be potential to manage populations with naturally low levels of pathogenicity to help control *Botrytis* diseases in New Zealand vineyards.

P2.1 - HOST PLANT SPECIES OF *SCLEROTINIA SCLEROTIORUM* AND *SCLEROTINIA MINOR* IN TURKEY

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Sclerotinia sclerotiorum and *S. minor* are two closely related plant pathogens that cause significant losses worldwide in many economically important crops. *S. sclerotiorum* has a host range of more than 400 species and a worldwide distribution. *S. minor* has a narrow host range relative to *S. sclerotiorum. Sclerotinia* species are sexually reproducing ascomycetous fungi lacking an asexual conidial spore stage, but instead have an asexual sclerotial somatic stage for survival and dispersal. Both *S. sclerotiorum* and *S. minor* survive mainly as sclerotia in soil for many years. In Turkey, *S. sclerotiorum* and *S. minor* are among the most important pathogens causing disease in several crops and considerable losses occur in agriculture due to *Sclerotinia* diseases. In this review, the host plant species of *S. sclerotiorum* and *S. minor* in Turkey until now are summarized.

P2.2 - MOLECULAR BASIS AND POPULATION GENETICS OF MULTIPLE FUNGICIDE RESISTANCE IN *B. CINEREA*

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To protect fruit and vegetable crops against grey mould, fungicide treatments are effective but bear the risk of resistance development. Monitoring of *B. cinerea* strains in French and German vineyards revealed an increasing occurrence of strains with multidrug resistance (MDR) and target site resistance against the SDH inhibitor boscalid. The MDR2 phenotype, has been shown to be caused by two rare rearrangement mutations that probably originated in French vineyards, and strains carrying one of these mutations have spread eastward into German vinegrowing regions.

In contrast to 1-3 fungicide treatments per season against *Botrytis* in vineyards, treatments in commercial strawberry fields often occur weekly during flowering, resulting in repeated use of the same fungicides and high selection pressure. *B. cinerea* strains isolated from German strawberry fields revealed high occurrence of multiple fungicide resistant strains, due to a combination of specific and MDR resistance mechanisms. Many of the isolates showed resistance to all currently used fungicides. We have developed PCR-based markers for rapid identification of the most common resistance mutations against boscalid, QoIs, and for MDR phenotypes, and analysed the distribution of the major resistance types in different strawberry growing areas. The majority of these strains showed a novel, stronger MDR1-like phenotype (called $MDR1^h$), due to further increased constitutive overexpression of the AtrB efflux pump. In many but not all $MDR1^h$ strains, small deletions in the Mrr1 transcription factor encoding gene were detected. $MDR1^h$ strains are only rarely found in vineyard isolates, and belong to a subpopulation that is genetically clearly separated from common *B. cinerea* strains (see abstract by Plesken *et al*.).

P2.3 - PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF RESISTANCE TO SDHI FUNGICIDES IN *BOTRYOTINIA FUCKELIANA* (*BOTRYTIS CINEREA*)

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The great adaptability of the grey mould fungus *Botryotinia fuckeliana* (de Bary) Whetzel (an. *Botrytis cinerea* Pers.) and the repeated use of fungicides with a specific mode of action can explain the rapid development of resistance to fungicides that is frequently observed in field populations of the pathogen. Succinate dehydrogenase inhibitors (SDHI), inhibiting the complex II of the succinate-ubiquinone oxido-reductase in the mitochondrial electron transport chain, are classified by FRAC as fungicides at medium to high risk of resistance. Several nucleotide substitutions in the *SdhB* gene causing amino acid changes in highly conserved regions of the iron-sulphur protein of the succinate dehydrogenase complex are responsible for resistance of *B. fuckeliana* to SDHIs. Representative fungal isolates carrying allelic variants of the *SdhB* gene associated with different phenotypes of sensitivity/resistance to the pyridine-carboxamide boscalid were characterised for their response to the new fungicide, the pyridinyl-ethyl-benzamide fluopyram. Colony growth and conidial germination assays were carried out on acetate minimal medium amended with different concentrations of fungicides (0.001-300 mg l^{-1} fluopyram; 0.01-100 mg l^{-1} boscalid). Cross-resistance between the two SDHIs appeared to be limited to specific boscalid-resistant genotypes. Point mutations resulting in proline to leucine replacement at position 225 (P225L) of the target protein conferred a high level of resistance to both fungicides (RF>1000) whereas the replacement of the same amino acid with phenylalanine (P225F), detected in *B. fuckeliana* mutants highly resistant to boscalid, was associated with a moderate level of resistance to fluopyram (RF=10). The replacement of asparagine with isoleucine at position 230 (N230I) conferred moderate resistance to both SDHIs. Mutations at codon 272 resulting in histidine to tyrosine (H272Y) or arginine (H272R) replacements, responsible for a high level of resistance to boscalid, did not confer resistance to fluopyram. In detail, the H272R substitution did not confer any variation in the response to fluopyram whereas the H272Y change caused an increased sensitivity to the fungicide as compared to the wild-type strains. The strict association between the profiles of resistance to boscalid and fluopyram and allelic variants of the *SdhB* gene was then confirmed on about 90 *B. fuckeliana* isolates through AS-PCR analysis using allele-specific primers. Resistance to SDHIs in *B. fuckeliana* were recently detected in vineyards and strawberry commercial fields in Southern Italy where pathogen's populations had been exposed to sprays with boscalid in last years. Resistant mutants were mainly if not exclusively highly resistant to boscalid but sensitive to fluopyram and carried the H272Y or H272R substitutions. However, *B. fuckeliana* isolates resistant to both boscalid and fluopyram, carrying mutations at 225 or 230 codons of the *SdhB* gene, were rapidly selected in an experimental vineyard after only one season of repeated (up to 5) sprays with fluopyram.

P2.4 - CHARACTERIZATION OF THE GENE *CYP68,4* FOR THE FENHEXAMID RESISTANCE IN THE SPECIES *BOTRYTIS PSEUDOCINEREA.*

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The *Botrytis species* complex responsible for the grey mold disease on multiple crops harbors two species. The major one is *Botrytis cinerea,* the second one was called *Botrytis pseudocinera.* Despite their genetic polymorphism both species cannot be morphologically distinguished. However they differ for their sensitivity to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. Whereas *B. cinerea* is sensitive to this hydroxyanilide, but can acquire resistance through target site mutations, *B. pseudocinerea* is a naturally resistant species. We found a strong synergism between fenhexamid and sterol 14α-demethylation inhibitors (DMIs), especially on *B. pseudocinerea*. Since DMIs (Demethylation inhibitors) inhibit Cyp51 a cytochrome P450 protein, we suggested detoxification of fenhexamid by a cytochrome P450 similar to Cyp51 to be involved in *B. pseudocinerea's* resistance. The gene with the highest similarity to *cyp51,* named *cyp68.4*, was deleted in a *B. pseudocinerea* strain. *cyp68.4* knock out mutants exhibit increased fenhexamid sensitivity and decreased fenhexamid metabolisation, showing that *cyp68.4* encoding a cytochrompe P450 is responsible for *B. pseudocinerea's* (HydR1) natural resistance to fenhexamid. Although *cyp68.4* is also present in *B. cinerea* sensitive to fenhexamid, we observed several polymorphisms: i/ In *B. pseudocinerea* the *cyp68.4* promoter shows a deletion of 25 bp, ii/ the peptide sequence varies by 5 amino acid residues between the species. We are currently establishing the *cyp68.4* expression profiles in both species in order to analyze the impact of the promoter deletion on its expression. We will then study which part of the gene is/are responsible for fenhexamid resistance in *B. speudocinerea* prior to establish its physiological and enzymatic functions.

P2.5 - ANTI*BOTRYTIS* SYNERGY BETWEEN INHIBITORS OF CHITIN SYNTHASES AND BETA-(1,3) GLUCAN SYNTHASE

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Fungi are major agents causing diseases in crops with a strong yield reduction and an alteration of the quality as main consequences. Further knowledge of the infection process can bring new insights to develop novel antifungal strategies. The cell wall of fungi is quite specific and essential for their viability and their aggressiveness towards their hosts. How plant-pathogenic fungi cope with cell wall alterations was explored in *Botrytis cinerea* as a model of necrotrophic and phytopathogenic fungi. Inhibition of the cell wall biosynthesis pathways was assessed using two commercial inhibitors, Nikkomycin Z and Caspofungin, targeting respectively chitin synthases and β-(1,3) glucan synthase, responsible for the synthesis of the two major polysaccharides of the fungal cell wall. Each compound alone inhibits the *in vitro* growth of *B. cinerea*, and the combination of both inhibitors shows a significant synergistic effect. It was previously reported that inhibition of the synthesis of β-(1,3) glucans and chitin leads to a compensation phenomenon in several fungi. Our data suggest that, in the case of synergy, *B. cinerea* was not able to compensate all the cell wall deficiencies induced by the application of both inhibitors together. The regulation of the expression of the genes involved in the cell wall metabolism and the associated signalling pathways will be further studied in *B. cinerea* to better understand the role of these compensation phenomenons during the plant infection.

P2.6 - BIOCONTROL EFFECT OF PLANT EXTRACTS AGAINST *BOTRYTIS CINEREA* AND *SCLEROTINIA SCLEROTIORUM* OF SEVERAL CROPS

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Concerns associated with the use of chemical fungicides encourage the search of alternative strategies for economically feasible control for fungal diseases. Extracts of many higher plants are reported to exhibit antimicrobial activity for inhibition of causal agents *in vitro*, and suppression of crop disease in field tests. In this study, plant extracts of *Brassicaceae* family plants including their seed's crude extract and tuber's tissue atmosphere were tested for antifungal activity against *B.cinerea* and *S.sclerotiorum* isolated from kazakhstani tomato and soya beans leaves, stem, and fruits. Various concentrations, e.g. 0,25, 0,5 and 1% water extracts of powdered mustard (*Brassica nigra* L.) seeds and tuber tissue atmosphere of radish (*Raphanus sativus* L.) and horseradish (*Armoracea lapathifolia*), were tested for antifungal activity under laboratory conditions to determine the effect on mycelial growth, reproduction and sclerotia forming capability of pathogenic isolates of *B.cinerea* and S.sclerotiorum. Significant reduction of *B.cinerea* and *S.sclerotiorum* (70- and 50%, respectively) radial growth in plates amended with 1% water extract of powdered mustard seeds was observed. No sclerotia on fungal colonies were observed. The highest reduction (up to 90%) of both fungi growth was observed in plates during the exposure to atmosphere of ground radish and horseradish tuber tissue. The plant extracts and volatile substances produced by ground tissues appear to be active against disease caused by *B.cinerea* and *S.sclerotiorum* in semi-field tests. The same results were obtained when tomato and soya beans planting material (e.g. seeds or seedlings) were treated with mustard water extracts or exposed under the ground tuber tissue atmosphere just before the planting. Our 2-years field experiments conducted on tomato and soya beans plants has revealed the potent biocide action of *Brassica* plants on the health and crops yield. This strategy deserve further research.

Session 3

POST-GENOME FUNCTIONAL ANALYSIS

CHAIRPERSONS: Jeffrey Rollins and Nathalie Poussereau

INVITED LECTURE

I3 - GENOMICS AND TRANSCRIPTOMICS TO CHARACTERIZE FUNGAL DEVELOPMENTAL GENES. Minou Nowrousian, Ines Teichert, Gabriele Wolff and Ulrich Kück

ORAL COMMUNICATIONS

O3.1 - THE HOMEOBOX ARABESQUE GENE IN *BOTRYTIS CINEREA*; GROWTH AND SHAPES UNDER CONTROL. Zsuzsanna Antal, Christine Rascle, Agnès Cimerman, Muriel Viaud and Christophe Bruel

O3.2 - FUNCTIONAL ANALYSIS OF FUNGAL VIRULENCE FACTORS IN THE GRAPE / *BOTRYTIS* INTERACTION. Agnes Cimerman, Adeline Simon, Guillaume Morgant, Jani Kelloniemi, Marc Fermaud, Benoit Poinssot and Muriel Viaud

O3.3 - FUNCTIONAL CHARACTERIZATION OF *IN PLANTA* EXPRESSED GENES IN *BOTRYTIS CINEREA.* Jose J. Espino, Nora Temme, Anne Viefhues and Paul Tudzynski

O3.4 - T-DNA-MEDIATED INSERTIONAL MUTAGENESIS REVEALS LESS VIRULENT MUTANTS THAT ARE AFFECTED IN LIGHT-DEPENDENT DEVELOPMENT. Julia Schumacher, Sabine Giesbert and Paul Tudzynski

O3.5 - THE NADPH OXIDASE COMPLEXES IN *BOTRYTIS CINEREA*. Ulrike Siegmund, Sabine Giesbert and Paul Tudzynski

O3.6 - THE D-GALACTURONIC ACID CATABOLIC PATHWAY IN *BOTRYTIS CINEREA.* Lisha Zhang and Jan A. L. van Kan

O3.7 - *BOTRYTIS CINEREA* O-MANNOSYLTRANSFERASES PLAY A MAJOR ROLE IN GROWTH, MORPHOGENESIS AND VIRULENCE. Mario González, Nélida Brito, Marcos Frías and Celedonio González

POSTERS

P3.1 - *BOTRYTIS CINEREA* BCMCD1: CELLULAR LOCALIZATION AND POSSIBLE ROLE IN APOPTOSIS. Olga Bunis, Adi Doron and Amir Sharon

P3.2 - THE SESQUITERPENE FAMILY OF *BOTRYTIS CINEREA:* ROLE OF THESE SECONDARY METABOLITES IN THE INFECTION PROCESS. Bérengère Dalmais, Pascal Le Pêcheur, Guillaume Morgant, Jean-Marc Pradier, Agnès Cimerman, Javier Barua, Isidro Gonzalez Collado and Muriel Viaud

P3.3 - IMPLICATION OF *BOTRYTIS CINEREA* POLYKETIDE SYNTHASES IN THE INFECTION PROCESS: HUNTING CAN START… Pascal Bally, Agnès Cimerman, Bérengère Dalmais, Adeline Simon and Muriel Viaud

P3.4 - CONSTRUCTION OF A *B. CINEREA* ONE-HYBRID LIBRARY; SCREENING TO IDENTIFY TRANSCRIPTION FACTORS REGULATING THE SECONDARY METABOLISM. Adeline Simon, Guillaume Morgant , Bérangère Dalmais, Manon Rivière and Muriel Viaud

P3.5 - THE BOTBANK PROJECT : EVIDENCE OF A NEW GENE IMPLIED IN THE EARLY PHASE OF PATHOGENIC DEVELOPMENT OF *BOTRYTIS CINEREA.* Sophie Kaiser, Lucie Chandat, François Villalba, Marie-Pascale Latorse, Julia Schumacher, Paul Tudzynski, Géraldine Mey and Nathalie Poussereau

P3.6 - B05.10 vs. T4 – VELVET MAKES THE DIFFERENCE. Julia Schumacher, Stefanie Traeger, Javier Moraga, Isidro G. Collado, Muriel Viaud, Paul Tudzynski and Bettina Tudzynski

P3.7 - A YEAST RECOMBINATION-BASED CLONING SYSTEM FOR HIGH-THROUGHPUT GENE ANALYSES IN *BOTRYTIS CINEREA.* Julia Schumacher

P3.8 - EXPLOITING GFP AS TOOL FOR *BOTRYTIS CINEREA* . Michaela Leroch and Matthias Hahn

P3.9 - DO ALPHA-TUBULINS PLAY THE SAME ROLE IN PATHOGENIC AND SYMBIOTIC FUNGI ? Aurélie Perrin, Yohann Faivre, Jeanne Doré, Christine Rascle, Isabelle Gonçalves, Christophe Bruel and Gilles Gay

I3 - GENOMICS AND TRANSCRIPTOMICS TO CHARACTERIZE FUNGAL DEVELOPMENTAL GENES

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Next-generation sequencing (NGS) techniques have revolutionized the field of genomics/functional genomics. We have recently sequenced and assembled the genome of the filamentous ascomycete *Sordaria macrospora*, a model organism for fungal development, solely from NGS reads (PLoS Genet 6:e1000891). We are currently applying NGS in two approaches for the identification and characterization of developmental genes. (I) With laser capture microdissection, we can separate protoperithecia from the surrounding hyphae. RNA isolation and amplification from 150 protoperithecia yields enough material for RNA-seq analysis. The resulting data were compared to RNA-seq data from whole mycelial exctracts to characterize the genome-wide spatial distribution of gene expression during sexual development. Additionally, we used the RNA-seq information to improve the predicted *S. macrospora* gene models, and annotated UTRs for more than 50 % of the genes. (II) We sequenced the genomes from three mutants that were generated by conventional mutagenesis, and identified the three causative mutations through bioinformatics analysis. One mutant carries a mutation in the known developmental gene *pro41*. The second, a spore color mutant, has a point mutation in a gene that encodes an enzyme of the melanin biosynthesis pathway. In the third mutant, a point mutation in the stop codon of a conserved fungal transcription factor causes the sterility of the mutant. For all three mutants, transformation with a wild-type copy of the affected gene restored the wild-type phenotype. These data show that whole genome-sequencing of mutant strains is a rapid method for the identification of developmental genes.

O3.1 – THE HOMEOBOX ARABESQUE GENE IN *BOTRYTIS CINEREA*; GROWTH AND SHAPES UNDER CONTROL

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Homeobox genes encode DNA-binding homeodomain-containing proteins that essentially act as transcription factors. In insects, animals, plants and fungi, these proteins have been shown to play major roles in developmental processes such as differentiation and reproduction. In fungi, few homeobox genes have been described, but most of the ones that have been investigated are involved in hyphal growth, sexual development, appressorium formation or either conidia or microconidia production.

In *Botrytis cinerea* or *Sclerotinia sclerotiorum*, homeobox genes have not yet attracted much attention, and this work is a contribution to our knowledge of this gene family in these pathogens. Based on genomic data mining, a global view of these genes in the two fungi will be presented. Based on transcriptomics data of the early steps of *B. cinerea*'s development, and based on the work by Gioti *et al*.[(1)], one homeodomain gene was selected for functional analysis and the results will be detailed.

Deletion of the chosen homeobox gene in both the *B. cinerea* T4 and BO5-10 genetic backgrounds led to similar phenotypes, among which a curved, arabesque-like, hyphal growth on hydrophobic surfaces. In the two arabesque mutants, reduced growth rate and abnormal infection cushion formation were registered. Asexual reproduction was also shown to be affected with reduced conidiation and dramatic changes in conidial shape. In parallel, expression of the arabesque gene was shown to be high in the parental strains' conidia. Finally, an effect of the mutation on the fungal virulence towards different host plants was monitored.

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1. A Gioti, A Simon, P Le Pêcheur, C Giraud, J M Pradier, M Viaud, C Levis (2006) Expression profiling of *Botrytis cinerea* genes identifies three patterns of up-regulation in planta and an FKBP12 protein affecting pathogenicity. J Mol Biol vol. 358 (2) pp. 372-86

O3.2 - FUNCTIONAL ANALYSIS OF FUNGAL VIRULENCE FACTORS IN THE GRAPE / BOTRYTIS INTERACTION

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Botrytis cinerea is one of the major diseases of grapevine causing important damage on mature berries. The aim of the *SafeGrape* ANR project is to provide a first integrated view of the molecular mechanisms involved in grapevine infection. We hypothesize that a comprehensive kinetic description of the transcriptomes of both *B. cinerea* and of its plant host will answer important questions regarding fungal virulence factors and plant defense. Using Nimblegene chips we have identified the *B. cinerea* genes that are up-regulated during the early stages of infection. Several secondary metabolism gene clusters were shown to be mobilized such as the botrydial and botcinic acid clusters. One bZIP transcription factor was also characterized as highly up-regulated during the infection. Such candidate genes are inactivated in the B05-10 model strain to validate their role in virulence.

Transcriptomic data also suggested that plant and fungal oxylipins are mobilized during the interaction. Oxilipins are unsaturated-fatty-acids (PUFAs, C18 and C20) that are oxygenated by adding of molecular oxygen by non-enzymatic (ROS-lipid peroxidation) or enzymatic ways (dioxygenases (DOX), lipoxygenases (LOX), Psi-factor producing oxygenases (PPO), CytP450). They are considered as signal molecules in animals, plants, insects and fungi. Plant oxilipins play a role in the reproduction, in the development and in the mechanisms of resistance / defense to pathogens attack (Liavonchanka and Feussner 2006). Recent studies in *Fusarium* and *Aspergillus* spp. showed that fungal oxylipins are involved in the sexual / asexual development, mycotoxin production, lipid homeostasis and virulence (Horowitz Brown *et al.* 2008; Tsitsigiannis and Keller 2007). Oxylipins are mainly synthesized by LOX enzymes in plants and by PPO enzyme in fungi but their chemical structure is similar enough to make possible functional substitutions as shown in the peanut / *Aspergillus* interaction (Brodhagen *et al.* 2008, Burow *et al.* 2000, Tsitsigiannis and Keller 2006). The *B. cinerea* genome annotation allowed the identification of five key genes potentially involved in oxylipin synthesis: three *LOX* genes and two *PPO* genes. We are currently investigating the role of the *B. cinerea* oxylipins in phytotoxins production and virulence by a gene KO approach.

This work is funded by the SafeGrape ANR project and by the Comité National des Interprofessions des Vins d'appellation d'origine (CNIV).

O3.3 - FUNCTIONAL CHARACTERIZATION OF *IN PLANTA* EXPRESSED GENES IN *BOTRYTIS CINEREA*

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Many efforts have been made to manage the disease caused by *Botrytis cinerea*, the grey mould, in more than 200 plant species worldwide. But until now there are more and more doubts about its mechanisms of infection, especially at the early stages, when conidia germinate and penetrate the plant surface. In order to know more about the host-fungus interactions, a microarray was performed to identify the genes whose expression was induced *in planta* but not in conidia. Thanks to the genome annotation of both B05.10 and T4 strains, we could identify 179 genes in these conditions. More than a half of these genes were expressed since the 6 hours post-inoculation on bean leaves, postulating an important role of them in the establishment of the fungus on the plant surface. We are performing further studies to elucidate the function of these genes in the early stages, such as real time PCR and promoter-GFP fusions to study the gene expression, GFP fusion proteins for localization studies, and generating knock out strains used for pathogenicity assays on different hosts.

O3.4 - T-DNA-MEDIATED INSERTIONAL MUTAGENESIS REVEALS LESS VIRULENT MUTANTS THAT ARE AFFECTED IN LIGHT-DEPENDENT DEVELOPMENT

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By using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach in *B. cinerea* B05.10, we generated a library with 2,350 transformants carrying random integrations of a hygromycin resistance cassette flanked by LB and RB sequences. A first virulence screen of all transformants on detached tomato leaves resulted in the identification of 560 less virulent strains. 231 of these have been undergone a second screening on primary leaves of *Phaseolus vulgaris*, and the less virulent phenotype has been confirmed for 169 strains. Virulence phenotypes on *P. vulgaris* have been defined as follows: Type 1 mutants are are unable to produce lesions due to defects in germination or penetration, Type 2 mutants are producing primary lesions but are unable to further colonize the plant, and Type 3 mutants are hampered in their secondary spread and/ or conidiation *in planta*. The phenotypes of 30 less virulent ATMT mutants were further characterized by analysing the resistance/sensitivity to ROS, the formation of ROS and oxalic acid and the light-dependent differentiation. Interestingly, many of the less virulent mutants are affected in light-dependent development as they are either impaired in conidiation, sclerotia formation or both. One ATMT mutant of Type 2 exhibits the “VELVET phenotype“ (reduced virulence on *P. vulgaris*, loss of oxalic acid formation, conidiation in the dark) and carries the T-DNA insertion 1608 bp upstream of an ORF encoding a GATA transcription factor. Remarkably, two other less virulent ATMT mutants have been identified that are tagged in the same gene locus. Another ATMT mutant (Type 3) is severely reduced in growth, produces ROS in great quantities and shows a fluffy phenotype when incubated in the dark. TAIL-PCR analyses revealed that the T-DNA is inserted 1307 bp upstream of an ORF encoding a helix-loop-helix transcription factor. The detailed analysis of these transcription factors by analysing deletion and overexpression mutants is currently in progress. Furthermore, we have identified a protein likely involved in histone modification (demethylation of H3K36) as a factor important for virulence, stress response and light-dependent development. In the respective ATMT mutant the T-DNA is inserted in the ORF causing the disruption of the gene. Deletion mutants are impaired in plant colonization (Type 3), in growth on minimal medium, and are hypersensitive to hydrogen peroxide. Strikingly, the mutants are forming sclerotia even in the light! However, so far we do not have an explanation why these regulators of light-dependent development *in vitro* are important for virulence as the infection itself is not dependent on light. Experiments using color filters (blue, green, yellow, red) have been undertaken to further analyze the influence of the different light qualities on development and virulence. Hence, strain B05.10 produces conidia when incubated in blue light and predominantly sclerotia when incubated in yellow and red light, implying the involvement of blue and red light receptors in regulation of conidiation and sclerotia formation, respectively.

O3.5 - THE NADPH OXIDASE COMPLEXES IN *BOTRYTIS CINEREA*

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Reactive oxygen species (ROS) are generated in all aerobic environments and therefore play a major role for many organisms depending on oxygen. For example they act as messenger molecules for intercellular signaling or play a role during defense mechanisms against pathogens (Takemoto *et al.,* 2007). One good example is the oxidative burst; plants rapidly produce large amounts of ROS as the first defence reaction towards pathogen attacks. NADPH oxidases (Nox) are the most common enzymatic system to produce these ROS. Nox are enzymes, which transport electrons through biological membranes and therewith reduce oxygen to superoxide. In fungi they are shown to be involved in differentiation processes and pathogenicity (reviewed in Takemoto *et al.,* 2007) and are therewith in our focus to gain insights into plant - fungi interactions.

Here, the functions of the two identified catalytic subunits BcNoxA and BcNoxB as well as of the putative regulator BcNoxR will be introduced. Both catalytical subunits are involved in sclerotia formation and influence pathogenicity. But while BcNoxA is necessary for colonization of the plant tissue Δ*bcnoxB* mutants are retarded in primary lesion formation (Segmueller *et al*., 2008). Additionally, BcNoxA and BcNoxR are involved in hyphal germling fusions.

Nox are enzymes functioning as multi-enzyme complexes. In mammals the regulatory process and the participating proteins are well described, but not all the components have been identified in fungi, yet. For *B. cinerea* interaction studies with potential candidates have been intensively conducted and several interactions partners have been identified. It was shown that besides the regulatory subunit BcNoxR the small GTPase Rac, the GEF BcCdc24, the scaffold protein BcBem1 and the p-21 activated kinase BcCla4 are involved in the establishment of the BcNox complex.

- Segmueller N., Kokkelink L., Giesbert S., Odinius D., van Kahn J., and Tudzynski P. (2008) NADPH oxidases are involved in differentiation and pathogenicity in *Botrytis cinerea*. *Mol Plant Microbe Interact* **21:** 808-808-819.
- Takemoto D., Tanaka A., and Scott B. (2007) NADPH oxidases in fungi: Diverse roles of reactive oxygen species in fungal cellular differentiation. *Fungal Genet Biol* **44:** 1065-1076.

O3.6 - THE D-GALACTURONIC ACID CATABOLIC PATHWAY IN *BOTRYTIS CINEREA*

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D-galacturonic acid is the major component of pectin, which can be degraded by saprotrophic and pathogenic fungi; galacturonic acid potentially is an important carbon source for microorganisms living on decaying plant material. A catabolic pathway was proposed in filamentous fungi, comprising three enzymatic steps, involving D-galacturonate reductase, L-galactonate dehydratase, and 2-keto-3-deoxy-L-galactonate aldolase. The *Botrytis cinerea* genome contains two non-homologous galacturonate reductase genes (*Bcgar1* and *Bcgar2*), a galactonate dehydratase gene (*Bclgd1*), and a 2-keto-3-deoxy-L-galactonate aldolase gene (*Bclga1*). Their expression levels were highly induced in cultures containing galacturonic acid, pectate, or pectin as the sole carbon source. The four proteins were expressed in *Escherichia coli* and their enzymatic activity was characterized. Targeted gene replacement of all four genes in *B. cinerea*, either separately or in combinations, yielded mutants that were affected in growth on galacturonic acid, pectate, or pectin as the sole carbon source. The virulence of *B. cinerea* mutants in the D-galacturonic acid catabolic pathway was unaltered on tomato leaves, apple fruit and bell pepper. However, the mutants showed reduced virulence on leaves of *Nicotiana benthamiana* and *Arabidopsis thaliana*. Cell wall composition analysis revealed that the amount of uronic acid was 2-fold higher in *N. benthamiana* and *A. thaliana* leaves than in tomato leaves. Currently, we are investigating the relationship between the galacturonic acid content of different plant leaves and the virulence of *B. cinerea* galacturonic acid catabolic deficient mutants on these plants.

O3.7 - *BOTRYTIS CINEREA* O-MANNOSYLTRANSFERASES PLAY A MAJOR ROLE IN GROWTH, MORPHOGENESIS AND VIRULENCE

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O-mannosyltransferases (PMTs) are Endoplasmic Reticulum associated proteins that catalyze the first step in protein O-glycosilation, i.e. the addition of the first mannose residue to a Ser or Thr residue in target proteins. They are evolutionary conserved from yeast to humans but have not been found in plants. PMTs are classified into three subfamilies (PMT1, PMT2 and PMT4) on the basis of sequence similarity, which have been shown to have different substrate specificities. Bioinformatic analysis of *B. cinerea* B05.10 and T4 genomes showed that only one protein of each PMT subfamily is present (named BcPmt1, BcPmt2, and BcPmt4) and knock-out mutants were generated for the three of them. In contrast to other fungi, the three mutants were viable, although all of them showed a reduced growth rate in comparison with the wild type, especially the *ΔBcpmt2* mutant. This defect could be reversed by the addition of osmotic stabilizers in the case of *ΔBcpmt1 and ΔBcpmt2, but not in the case of ΔBcpmt4*. All three mutants were completely (*ΔBcpmt2*) or partially (*ΔBcpmt1* and *ΔBcpmt4*) unable to conidiate. *ΔBcpmt1* and *ΔBcpmt4* also showed a higher tendency to form sclerotia. Microscopic examination revealed altered hyphal morphology for the three mutants, which also could be reversed by the addition of osmotic stabilizers. *ΔBcpmt2* was completely avirulent when inoculated on intact plant leaves, while wounding the leaves with a needle at the site of inoculation allowed the formation of a limited number of slow-growing lesions. *ΔBcpmt1* and *ΔBcpmt4* also produced lesions in plant leaves that appeared at a lower frequency and had a reduced growth rate, in comparison to the wild type. Wounding the leaves also greatly increased the frequency of infection for *ΔBcpmt1* and *ΔBcpmt4*. In conclusion, *B. cinerea* PMTs play a major role in growth, morphogenesis, conidiation and virulence, and could be useful in the development of novel control strategies.

P3.1 - *BOTRYTIS CINEREA* BCMCD1: CELLULAR LOCALIZATION AND POSSIBLE ROLE IN APOPTOSIS

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MCD1 is a conserved protein, subunit of the cohesin complex that regulates sister chromatid cohesion and ordered chromosome segregation. Recent studies revealed that the *S. cerevisiae* Mcd1p and the human homologue hRad21, have another important role; under apoptosis-promoting conditions, these proteins are cleaved, and the resulting C-terminal fragment is translocated from the nucleus to mitochondria (in *S. cerevisiae)* or the cytosol (in human), and activates cell death. Studies in *S. ce*revisiae also showed that last 40 amino acids of the protein are responsible for the death promoting effect. Here, we report on the isolation and analysis of BcMCD1, the *Botrytis cinerea* homologue of *S. cerevisiae* MCD1.

A single homologue was found in *B. cinerea* (BC1G_02757.1). The gene was isolated and over expression and deletion (partial) strains were produced. Strains over-expressing *BcMCD1* showed increased sensitivity to high temperature (28ºC) and H_2O_2. Further analyses of the phenotypes of the over-expression as well as the deletion strains are underway. Tagging the BcMCD1 with GFP on its C terminus revealed that the protein localizes to the nucleus (as expected), but unlike the yeast or human counterparts, it was retained in the nucleus also under stress conditions. In order to look for conserved amino acids at the c terminal part (the last 40 amino acids that induce PCD in yeasts), we use 40 amino acids from the carboxyl end of Mcd1p to search the all available fungal databases. Multiple sequence alignment of the identified sequences (a total of 77 sequences) revealed a putative consensus, which was also found in the 42 amino acids near the carboxyl end of BcMcd1. This sequence will be analyzed for death promoting activity by expression in yeasts and *Botrytis.*

P3.2 - THE SESQUITERPENE FAMILY OF *BOTRYTIS CINEREA:* ROLE OF THESE SECONDARY METABOLITES IN THE INFECTION PROCESS

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Secondary metabolites are low molecular weight metabolites and are often highly bioactive molecules. They are not necessary for normal growth or development. Secondary metabolites are diversified in function like pigments (eg.bikaverin), antibiotics (eg.penicillin) as well as toxins (eg.aflatoxin). Some of this toxins are able to induce host's defence machinery and divert this machinery to the fungus (Rossi, Garriz et al. 2011) or are useful to facilitate plant colonization (Howlett 2006). Hence, secondary metabolites study is particularly informative to understand plant-fungi interaction during the infection process. Currently, only few active metabolites were identified in *B. cinerea* cultures, for example botrydial (a phytoxin), botcinic acid (a phytotoxin) and abscisic acid (a phytohormone). However, the whole genomic sequence annotation made it possible to predict the presence of about 40 putative gene clusters involved in the biosynthesis of secondary metabolites, including many polyketides, peptides and terpenes. Sesquiterpenes are synthesized by sesquiterpene cyclase enzymes (STC), which catalyze various farnesyl pyrophosphate (FPP) cyclisations. Within the genome, 6 genes coding for putative STCs have been identified. To study them, we have started different approaches: gene expression analysis, comparative genomic study, and gene inactivation. We have tested STC mutants by three ways: growth tests in three different media, chemical analyses to detect presence/absence of secondary metabolites and virulence on two host plant (Tomato cv “Moneymaker” and Bean cv “Caruso”). This work is in progress but some first results can already be set: (1) STC1 is the sesquiterpene cyclase responsible for the first stage in the botrydial biosynthesis; (2) STC1, STC2 and STC4 are involved in the pathogenicity of *Botrytis cinerea*, the null mutants for these genes displayed a notably reduced virulence. Some questions seem interesting to evoke: (a) By which mechanisms STC2 & STC4 act in the infection process; (b) Which metabolites are produced from FPP cyclisation by STC2 and STC4 ? (c) What role for the others sesquiterpenes cyclases in the biology of this fungus ? (d) How is regulated the expression of these sesquiterpenes cyclases ?

P3.3 - IMPLICATION OF *BOTRYTIS CINEREA* POLYKETIDE SYNTHASES IN THE INFECTION PROCESS: HUNTING CAN START…

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Polyketides are a large family of fungal secondary metabolites involved in defence, aggressiveness and communication. Genes involved in biosynthetic pathway of such compounds are organized in clusters within the genomes of filamentous fungi such as *Botrytis cinerea*. Each gene of a cluster leads to a specific step of the polyketide biosynthesis, transport or regulation. The key enzyme responsible of the polyketide biosynthesis is called Polyketide Synthase (PKS).

Botrytis cinerea possesses 21 PKS predicted genes (Kroken et al. PNAS 2003). Among them, two are up-regulated during tomato leaves infection (PKS6 and PKS9). PKS6 and PKS9 are both involved in the production of the phytotoxin botcinic acid which is involved in the infectious process (Dalmais *et al*. Mol Plant Pathol 2011).

In order to find other secondary metabolites clusters involved in the infection process, we initiated a transcriptomic analysis. Clusters that are up-regulated during specific developmental stages such as appressorium formation (PKS8) or grape berries infection (PKS11) were identified (SafeGrape Project).

An other approach, based on random mutagenesis, permitted to isolate a new PKS which seems to be involved in the last stages of the infection process (PKS7) (Botbank Project).

These genes are inactivated in the B05-10 model strain to validate their role in virulence.

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P3.4 - CONSTRUCTION OF A *B. CINEREA* ONE-HYBRID LIBRARY; SCREENING TO IDENTIFY TRANSCRIPTION FACTORS REGULATING THE SECONDARY METABOLISM

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Botrytis cinerea genome sequencing enabled comparative genomics studies, in particular with the closely related species *Sclerotinia sclerotiorum*. One of the major findings is the identification of *B. cinerea* specific genes regarding secondary metabolism, transcription factors (TFs) and transporters. These genes have assumed or proven roles in some life traits of the fungus including polyphagia, and the frequent occurrence of resistance to fungicides. To understand the regulation of genes specific to *B. cinerea* and / or involved in the infection process, we developed a post-genomic strategy innovative for filamentous fungi, based on the "one-hybrid" system, which allows the identification of physical interactions between TFs and promoters of interest.

The approach involved, first, the construction of a library containing the 400 *B. cinerea* TFs in a strain of *Saccharomyces cerevisiae* and on the other hand, the cloning of promoters of interest upstream of a reporter gene in a second yeast strain. By mating the yeast strains, TFs, which regulate the expression of genes of interest can thus be selected and identified.

The library screening with the BcBot1/BcBot2 promoter (Botrydial synthesis) revealed an interaction with a TF (C2H2 type) previously unexplored. This result was validated by an independent one-hybrid experiment involving the mating of the individual promoter BcBot1/BcBot2 with the identified TF. The TF was then inactivated in the *B.cinerea* B05-10 strain, in order to explore the mutant pathogenesis on different plants, and the secondary metabolism production. Currently, the BcBot1/BcBot2 genes expressions are quantified in the mutant and wild-type strains in different experimental conditions. In a second step, the overall transcriptomes willl be studied by hybridization to NimbleGen chips containing all *B. cinerea* predicted genes, to identify other genes regulated by this TF.

P3.5 - THE BOTBANK PROJECT : EVIDENCE OF A NEW GENE IMPLIED IN THE EARLY PHASE OF PATHOGENIC DEVELOPMENT OF *BOTRYTIS CINEREA*

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The goal of the BOTBANK project is to develop a collection of tagged mutants of *Botrytis cinerea* and to validate the creation of this library with the identification of the functions required for the infectious cycle and the spread of the parasite.

A random insertional mutagenesis strategy based on the *Agrobacterium tumefaciens*-mediated transformation (ATMT) is used to enlarge an existing mutant library (2367 lines, Tudzynski *et al*.). 2144 T-DNA integrated transformants have been generated. The insertion site(s) of the T-DNA are determined using TAIL-PCR and the genome sequences (Broad Institute, Genoscope) and capacity to infect plant is assayed.

An exploitation of the mutant library focuses on the characterization of mutants whose parasitic development *in planta* is hampered. We present here an example of the study of a new gene encoding a DnaJ domain protein. The T-DNA mutant exhibited a drastic alteration of the infectious process on bean leaves. Deletion of the studied gene confirmed this phenotype and revealed that colonization process was also altered on different host plants. A defect in penetration and an abnormal infection cushion formation were registered. A dramatic reduced conidiation and an abnormal hyphal morphology were also observed. Resistance/sensitivity to ROS, formation of ROS and oxalic secretion were investigated. Finally, *in vitro* assays for the secretion of lytic enzymes are currently developed to start the exploration of the secretome produced by this strain and attribute a function to this new gene.

This work is funded by the trilateral ANR project BotBank.

P3.6 - B05.10 vs. T4 – VELVET MAKES THE DIFFERENCE

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Like in other filamentous fungi, the differentiation program in *B. cinerea* leading to asexual or sexual reproduction is dependent on light. Hence, strain B05.10 produces conidia in the light and sclerotia (as prerequisite for sexual reproduction) in the dark. Strikingly, conidiation in strain T4 does not reply on photoinduction as the strain produces abundant conidia in continuous darkness. A release of sporulation from photocontrol was previously also reported for *Aspergillus nidulans veA1* mutants, and this finding has led later to the identification of VELVET (VeA) as a bridging factor in a protein complex which coordinates light signals with fungal development and secondary metabolism. In *A. nidulans,* the responsiveness to light is achieved by the white collar homologues LreA/LreB and the single phytochrome FphA, while the output relies on further VeA-interacting proteins i.e. LaeA, VelB and VosA (for review see Bayram *et al*. 2010). Four predicted proteins have been identified in *B. cinerea* that exhibit the characteristic “VELVET” protein domain: BcVEL1 corresponds to VeA, BcVEL2 to VelB, BcVEL3 to VosA, and BcVEL4 is likely the homologue of VelC. In order to investigate the function of the VELVET complex in differentiation, secondary metabolism and virulence, we generated *bcvel1* deletion mutants in B05.10. While growth rates are not affected by the mutation, striking differences have been found for light-dependent differentiation processes: the mutants fail to produce sclerotia; instead they produce high numbers of conidia. Furthermore, the mutants do not secrete oxalic acid and produce a dark pigment (likely melanin) in great quantities. Notable is the effect of the mutation on virulence: the mutants do infect primary leaves of *Phaseolus vulgaris* resulting in restricted lesions but they are impaired in growing invasively. Interestingly, the defect in virulence is not associated with the loss of phytotoxin production as the expression of botrydial (BOT)- and botcinic acid (BOA)-biosynthetic genes is still detectable in Δ*bcvel1*-infected plant tissue. Evidently, the VELVET phenotype resembles the phenotype of T4 that also includes the absence of oxalic acid secretion and reduced aggressiveness on *P. vulgaris*. When looking for *bcvel1* in the T4 genome sequence, we found a SNP that results in a stop codon and consequently in a truncated VELVET protein ($BcVEL1^{T4}$) of 184 aa instead of 575 aa ($BcVEL1^{B05.10}$). Both versions have been fused to *gfp* in order to monitor the localization within the cell. In accordance with the observed function of VELVET in the dark (repression of conidiation and induction of sclerotial development), we found that $BcVEL1^{B05.10}$-GFP is located in the nucleus when the fungus is incubated in permanent darkness, and in the cytosol when the mutant is incubated in the light. In contrast, the truncated protein $BcVEL1^{T4}$-GFP is exclusively found in the cytosol. As the introduction of $bcvel1^{B05.10}$ into T4 resulted in “restoration” of oxalic acid production and virulence, we conclude that VELVET indeed makes the difference!

P3.7 - A YEAST RECOMBINATION-BASED CLONING SYSTEM FOR HIGH-THROUGHPUT GENE ANALYSES IN *BOTRYTIS CINEREA*

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An important aspect of molecular genetics of *B. cinerea* is the high efficiency (70–100%) of targeted gene inactivation, which allows a rapid functional analysis of putative pathogenicity-related genes. However, molecular tools based on the expression of reporter gene constructs are hampered by weak expression levels and insufficient fluorescence of the reporters when conventional expression vectors are used. Hence, we have initiated an approach to establish an expression system for *B. cinerea* regarding the following aspects: (i) the targeted integration of the constructs at defined gene loci which are dispensable under standard conditions, (ii) the use of promoter and terminator sequences allowing optimal gene expression, (iii) the use of modified reporter genes, (iv) the use of different selection markers, and (v) the choice of a highly efficient cloning system. We chose the gene loci of the nitrate reductase (*niaD*) and the nitrite reductase (*niiA*) for the targeted integration of the reporter gene constructs after demonstrating that both gene loci are not essential for growth on complex media, for differentiation processes and virulence on French bean *Phaseolus vulgaris*. Promoter and terminator sequences derived from *A. nidulans* (P*oliC*, T*trpC*) and *B. cinerea* (P*actA*, T*gluc*) were used to control the reporters eGFP (enhanced green fluorescent protein) and mCherry (momoneric red fluorescent protein) whose nucleotide sequences have been adapted to the codon usage of *B. cinerea* (Leroch *et al*. 2011). The linkage of the constructs with the three available selection markers in *B. cinerea* (hygromycin, nourseothricin, phleomycin) furthermore allows the integration of several constructs in mutant backgrounds. In order to facilitate large-scale protein investigations, we employed the yeast recombination-based cloning technology, which has the advantage of fast and easy cloning of different genes into destination vectors without the use of restriction and ligation enzymes. We created a set of cloning vectors permitting protein fusions in all possible protein orientations and demonstrated the successful application of the expression system for labeling the cytosol, nuclei, membranes and F-actin by fusing reporter genes to selected *B. cinerea* genes. In addition, cloning vectors for bimolecular fluorescence complementation (BiFC) analyses for studying protein–protein interactions *in situ* have been generated. *B. cinerea* BiFC constructs are based on the complementation of two separately expressed N- (at *bcniaD* locus) and C-terminal fragments (at *bcniiA* locus) of the modified GFP, whose C or N terminus can be fused to putative intercting proteins. A first BiFC assay for validating the constructed vectors is in progress.

Leroch M, Mernke D, Koppenhoefer D, Schneider P, Mosbach A, Doehlemann G, Hahn M (2011) Living colors in the gray mold pathogen *Botrytis cinerea*: codon-optimized genes encoding green fluorescent protein and mCherry, which exhibit bright fluorescence. Appl Environ Microbiol. 77:2887-2897.

P3.8 - EXPLOITING GFP AS TOOL FOR *BOTRYTIS CINEREA*

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The green fluorescent protein (GFP) and its variants have been widely used in modern biology as reporters that allow a variety of live-cell imaging techniques. However, GFP has been rarely used in the gray mold fungus *Botrytis cinerea* because of low fluorescence intensity. The codon usage of *B. cinerea* genes strongly deviates from that of commonly used GFP encoding genes, and revealed a lower GC content compared to other fungi. We report the development and use of a codon-optimized version of the eGFP encoding gene (Bc-*gfp*) for improved expression in *B. cinerea*. Both the codon optimization and, to a smaller extent, the insertion of an intron resulted in higher mRNA levels and increased fluorescence. We found that Bc-*gfp* is a valuable tool for several live cell imaging applications:

1. As a reporter system for analysis of gene expression, namely:
- drug induced transporter gene expression (*artB*, *atrD*, *mfs19*)
- different cutinase genes that are induced by different host lipid signals and by different physical properties of the surface
- genes that are specifically induced during germination and host infection
2. As a reporter for subcellular localization, using translational GFP fusions:
- nuclear localization using the N-terminal 150 amino acids of the transcriptional activator mrr1
- labeling of actin using an actin binding protein fused to *gfp*
- translational *gfp* fusion of a cutinase gene
3. Following the infection process into the host tissue, using a *Botrytis* strain that constitutively expresses GFP.

We will report our experiences about the potential and the limitations of GFP for these applications. While our first experiments with GFP expression were made with *B. cinerea* strain B05-Hyg3 (Noda *et al.*, 2007, Mol. Plant Pathol. 8: 811-6), we found that this strain shows reduced infection on several host plants (tomato, Arabidopsis). Using expression cassettes developed by J. Schumacher, we have constructed new GFP reporter strains in the *B. cinerea* B05.10 background. Due to defined integration sites of these vector cassettes, genotypic analysis of transformants regarding multiple integration and ectopic integration events can be easier checked.

P3.9 - DO ALPHA-TUBULINS PLAY THE SAME ROLE IN PATHOGENIC AND SYMBIOTIC FUNGI ?

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In all terrestrial ecosystems, including agro-ecosystems, plants live in close interaction with numerous fungi, and the interaction has a negative or positive effect on the plant fitness when it involves a pathogenic or symbiotic fungus. Fungi hence have a key influence on the structure and dynamics of plants communities and, consequently, a dramatic impact on the growth and productivity of crops. In this context, pathogenic fungi are acknowledged a major concern in agriculture, but the beneficial effect of symbiotic fungi has been much less considered. The recent release of the genome sequence of two ectomycorrhizal fungi, namely *Laccaria bicolor* and *Tuber melanosporum*, did not reveal any major difference with pathogenic fungi, thus reinforcing the idea that the difference between pathogenic and mutualistic fungi is mostly to be searched at the regulatory network level. In this framework, we developed an original approach in an attempt to compare the way pathogenic and mycorrhizal fungi interact with their host plant. This approach aimed at identifying functions or regulatory cascades involved in both interactions. In both pathogenic and mycorrhizal interactions, early steps rely on a dramatic reorientation of hyphal growth polarity, leading to the differentiation of specialised structures allowing host colonisation, such as appressoria or fungal sheaths. Several data suggest that these changes in polarity involve cytoskeleton remodelling, and we reasoned that the highly conserved tubulins could be involved. We investigated this hypothesis using *Botrytis cinerea* and *Hebeloma cylindrosporum* as biological models. *B. cinerea* can attack a wide range of plants by differentiating either appressoria or infection cushions, hyphal structures that show some similarity with the fungal sheath formed by ectomycorrhizal fungi. *H. cylindrosporum* is an ectomycorrhizal fungus that can easily be grown and manipulated under laboratory conditions. It can be genetically transformed using *Agrobacterium tumefaciens* and two mutant libraries have been constructed. It forms typical fungal sheath and Hartig net when colonising its habitual host plant *Pinus pinaster.* The genome of both fungi contains two alpha-tubulins encoding genes, but curiously their evolutive history is very much different. The corresponding proteins show very high conservation, and this suggests potential redundancy within each fungus. However, the respective promoters differ significantly and the expressions of the two genes were shown to be differently regulated during the early developmental stages of *B. cinerea*. In both fungi, neither down-regulation nor inactivation of the «minor» alpha-tubulin encoding gene affected growth on solid media. It however affected the stability of microtubules as appreciated by the level of resistance to benomyl or to taxol. The question as to whether the two alpha-tubulins play similar roles in the pathogenic or symbiotic interaction with the plant is currently under investigation.

Session 4

PLANT-PATHOGEN INTERACTION

CHAIRPERSONS: Ann Powell and Francesco Favaron

INVITED LECTURE

I4 - QUANTITATIVE POWDERY MILDEW RESISTANCE IN BARLEY: ONE TRAIT - MANY GENES. Patrick Schweizer

ORAL COMMUNICATIONS

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O4.2 - CELL WALL-OMICS OF THE INTERACTION BETWEEN TOMATO FRUIT AND *BOTRYTIS CINEREA*. Barbara Blanco-Ulate, Dinesh Kumar Barupal, Dario Cantu, Sivakumar Pattathil, Virginia Brown, Heather McArthur, Sindhu Kandemkavil, John L. Labavitch, Alan B. Bennett, Michael G. Hahn, Oliver Fiehn and Ann L.T. Powell

O4.3 - MULTIFACETED DEFENSE MECHANISM IN *SITIENS*, AN ABSCISIC ACID DEFICIENT TOMATO MUTANT, AGAINST THE NECROTROPHIC FUNGUS *BOTRYTIS CINEREA*. Hamed Seifi and Monica Höfte

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O4.8 - MOLECULAR AND FUNCTIONAL ANALYSIS OF *BOTRYTIS CINEREA* INHIBITOR OF APOPTOSIS PROTEIN BCBIR1. Ma'ayan Israeli, Neta Shlezinger and Amir Sharon

O4.9 - THE *BOTRYTIS CINEREA* PROTEIN BCSPL1 BINDS TO PLANT CELL MEMBRANE AND ELICITS THE HYPERSENSITIVE RESPONSE. Marcos Frías, Celedonio González, Mario González and Nélida Brito.

POSTERS

P4.1 - RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN BRASSICA AND VARIATION IN PATHOGEN AGGRESSIVENESS. John Clarkson and Emma Coventry

P4.2 - CHARACTERIZATION OF PROTEASE INHIBITORS IN THE GRAPEVINE-*BOTRYTIS CINEREA* INTERACTION. Clémentine Gérard, Jérome Crouzet, Christophe Clément, Florence Mazeyrat-Gourbeyre, Laurence Monti-Dedieu and Fabienne Baillieul

P4.3 - CHARACTERIZATION OF *BOTRYTIS CINEREA* POLYGALACTURONASES AND LACCASES DURING GRAPE BERRIES INFECTION. Luca Sella, Silvana Odorizzi, Carla Castiglioni, Marco Lucchetta, Renato D'Ovidio and Francesco Favaron

P4.4 - THE *BOTRYTIS CINEREA* ELICITOR BCSPL1 IS A POTENT INDUCER OF SAR IN TOBACCO, EVEN THOUGH THE FUNGUS ITSELF IS NOT. Marcos Frías, Celedonio González, Mario González and Nélida Brito

I4 - QUANTITATIVE POWDERY MILDEW RESISTANCE IN BARLEY: ONE TRAIT - MANY GENES

Patrick Schweizer

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Quantitative pathogen resistance is of high importance to plant breeders due to its durability. However, it is usually controlled by multiple quantitative trait loci and therefore, difficult to handle in practice. Knowing the genes that underlie quantitative resistance would allow its exploitation in a more targeted manner. In order to identify genes that mediate quantitative resistance of barley to the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) we have combined functional-genomics approaches based on transcript profiling and transient-induced gene silencing (TIGS) with an association-genetic and a QTL-mapping approach. Starting with 1400 gene candidates that are either up-regulated by pathogen attack, that have been mapped to one selected QTL region on chromosome 5H, or that belong to potentially important multigene families for disease resistance, we have obtained a first shortlist of approximately 20 candidates with converging evidence for an important role in quantitative disease resistance of barley. These candidates are implicated in cell-death regulation and cell-wall modification and are being validated in barley by larger-scale re-sequencing, QTL fine mapping, stable silencing or complementation, and by TILLING. We think that the integration of functional-genomic with genetic approaches allow us to accelerate the discovery of genes underlying complex, quantitative traits in crop plants.

O4.1 - IDENTIFICATION OF CANDIDATE DEFENCE-RELATED GENES TO *SCLEROTINIA SCLEROTIORUM* IN SOYBEAN AND FUNCTIONAL ANALYSIS IN SOYBEAN AND *ARABIDOPSIS*

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Transgenic soybean lines expressing the wheat germin gene (*gf-2.8*), oxalate oxidase (OxO), show a high degree of resistance to *Sclerotinia sclerotiorum* infection. Microarray studies have been used to examine the changes in soybean gene expression in response to infection of the transgenic (resistant) and parental (susceptible) lines. In addition, the effect of oxalic acid (OA), a major pathogenicity determinant of *S. sclerotiorum*, was evaluated by leaf infiltration with OA. Thousands of genes were found to be significantly differentially expressed in each of the two studies. To identify genes related to defence, genes were classified functionally, based on the annotation of their best sequence match in public databases. Cluster analyses identified many defence-related genes that were induced across the studies, including genes annotated as GSTs, P450s, MMPs, PR proteins, WRKYs and genes of the phenylpropanoid pathway. Many of the genes that were down regulated across the studies were related to chloroplasts and photosynthesis. Functional characterization is ongoing to verify a defence-related role of the candidate genes. Functional analyses are being conducted on soybean and the model plant Arabidopsis by gene over-expression or silencing (RNAi in soybean and T-DNA knockouts in Arabidopsis).

O4.2 - CELL WALL-OMICS OF THE INTERACTION BETWEEN TOMATO FRUIT AND *BOTRYTIS CINEREA*

Barbara Blanco-Ulate[1], Dinesh Kumar Barupal[2], Dario Cantu[1], Sivakumar Pattathil[3], Virginia Brown[3], Heather McArthur[3], Sindhu Kandemkavil[3], John L. Labavitch[1], Alan B. Bennett[1], Michael G. Hahn[3], Oliver Fiehn[2] and Ann L.T. Powell[1]

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The plant cell wall (CW), a complex matrix of diverse polysaccharides and proteins, is the major physical barrier to pathogen infection. It also provides a scaffold for proteins and small molecules involved in sensing, signaling and responding to biotic stress. Pathogens have evolved strategies to break apart the host CW to utilize CW components as nutrients, access the contents of the plant cells and expand further into plant tissues. The necrotrophic plant pathogen, *Botrytis cinerea*, possesses versatile enzymatic machinery capable of degrading a variety of tissues from many different plant hosts. However, plant CW susceptibility to pathogen disassembly depends not only on the array of enzymes secreted by the pathogen during infection, but also on modifications that alter CW integrity as part of normal developmental events. This dependence on CW modifications imposed by endogenous CW modifying proteins is particularly evident during tomato fruit ripening. Unripe (mature green, MG) tomato fruit are largely resistant to *B. cinerea* infection, but as they become ripe (red ripe, RR) fruit are infected rapidly with extensive tissue maceration. During ripening, CWs undergo extensive remodeling and disassembly leading to fruit softening of the pericarp. This progressive loss of CW integrity has been linked to an increased susceptibility to *B. cinerea* (PNAS 105: 859-864). Because of the crucial role played by CW in tomato fruit susceptibility, we applied two targeted metabolomic approaches to examine the events occurring *in muro* after *B. cinerea's* infection of MG and RR tomato fruit: 1) CW glycomics using ~150 glycan-directed monoclonal antibodies (Plant Physiol 153: 514-525) to determine infection-induced changes in CW composition and structure, and to correlate the presence/absence and arrangement of polysaccharides with susceptibility or resistance; and 2) CW metabolomics using GC/MS, to characterize putative signaling molecules, secondary metabolites and other small molecules that accumulate during *B. cinerea* recognition, as well, to detect products of plant and pathogen metabolism during their interaction. Using glycome profiling, we identified specific classes of tomato fruit CW polysaccharides that appear to be targeted by *B. cinerea* during active infections. In particular, as confirmed by further chemical analyses, the arabinogalactan side-branches of the fruit CW rhamnogalacturonans appear to be hydrolyzed as a result of infection by *B. cinerea*. In the CW metabolomic analysis of infected and healthy tomato fruit, we detected ca. 500 small molecules of which 100 compounds exhibited significant changes in abundance depending on ripening stage and pathogen infection. Interestingly among these changes, we observed the accumulation of stress-related metabolites (proline, ornithine, salicylic acid, benzoic acid) in resistant MG fruit.

O4.3 - MULTIFACETED DEFENSE MECHANISM IN *SITIENS*, AN ABSCISIC ACID DEFICIENT TOMATO MUTANT, AGAINST THE NECROTROPHIC FUNGUS *B. CINEREA*

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The ABA deficient *sitiens* mutant of tomato is highly resistant to the necrotrophic fungus *Botrytis cinerea* via a timely and localized production of H_2O_2 followed by cell wall fortifications in the epidermis, which effectively arrest spreading of the pathogen. To further investigate how downstream defense responses are regulated in *sitiens*, the relative transcript level of several candidate genes was analyzed using qRT-PCR. Phenylalanine ammonia lyase was markedly upregulated in *sitiens* 8hpi, while the enzyme activity inhibition led to extreme susceptibility. Increased PAL expression level was paralleled with an early increase in expression of the cytosolic glutamine synthetase (GS1), which intimately functions with glutamate synthase via GS-GOGAT cycle. GS1 is commonly believed to be involved in ammonium re-assimilation during natural and stress-induced senescence. In spite of the early (8hpi) increase in GS1 expression level, no significant change was detected in total protein content of *sitiens* leaves even after 72hpi, indicating that upregulation in cytosolic GS could not be associated with the putative role of the enzyme. Interestingly, inhibition of GS activity by methionine sulfoximine (MSO) resulted in promoted cell death, and ultimately subdued the effective resistance in *sitiens,* while the early cell wall fortification in the epidermis of the mutant remained intact. Further qRT-PCR analyses in *sitiens*, showed increases in genes encoding for polyamines and GABA biosynthesis; suggesting a link between GS-GOGAT cycle and GABA-shunt. Furthermore, it was revealed that GABA application could significantly reduce susceptibility in wild type. According to these results we propose that a successful defensive response in *sitiens* consists of two crucially linked components: firstly, the rapid epidermal ROS accumulation and cell wall fortification and consequently arresting the pathogen; and secondly, a durable maintenance of the basic metabolism in the challenged tissue to retard the necrotroph-induced senescence.

O4.4 - SPATIO-TEMPORAL STUDY OF TWO MAJOR PR-PROTEINS OF GRAPE BERRIES DURING MATURATION, BIOTIC AND ABIOTIC STRESS

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Pathogenesis-related proteins are important elements of the plant defense machinery. In grapevine (*Vitis vinifera* L. cv. Pinot Noir) previous studies have shown that a chitinase (CHV5) and a thaumatin-like protein (TL) accumulate in berries during fruit maturation (Derckel *et al.* 1998; Davies and Robinson 2000) and have an antifungal effect against *Botrytis cinerea* (Derckel *et al.* 1998; Monteiro *et al*. 2003). However, *B. cinerea* is capable of growing on ripe berries. The aim of this work was to investigate firstly, the localization of these proteins in different tissues of berries during maturation and secondly, to study how their expression is affected by abiotic (UV-C) and biotic (*B. cinerea*) stresses. Localization of CHV5 and TL mRNAs/proteins was investigated by *in situ* hybridization and immunolocalization. Results show that during maturation without stress mRNAs/proteins of both were localized in the exocarp, and around all the vascular bundles of ripe berries. Following abiotic stress, after UV-C irradiation of berries at pre-veraison, *CHV5* and *TL* mRNAs accumulated in the exocarp and around all the vascular bundles. However, both proteins were localized in the exocarp and around the vascular bundles located in the mesocarp but not around those in the center of berries. In ripe berries from vineyards, CHV5 and TL mRNAs/proteins decreased during the infection by *B. cinerea*. Localization of both proteins in infected berries showed that proteins decreased around the site of infection, suggesting a degradation of both proteins by proteases secreted by the fungus (Have *et al*. 2004). Characterization of activities, properties, degradation of these two proteins produced by heterologous system is in progress. Finally, it is likely that these proteins, apart from being implicated in plant defense have also another function. To answer this question, transformation of grapevine plants, over- and underexpressing both genes are under progress.

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O4.5 - THE IMPACT OF GLUCOSINOLATES AND THEIR BREAKDOWN PRODUCTS ON NECROTROPHIC FUNGI

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In this study, we utilized different *Arabidopsis thaliana* mutants altered in their contents of glucosinolates (GSs) and glucosinolate-breakdown products (GSBs) to study the impact of these phytochemicals on phytopathogenic fungi. We compared the broad-spectrum fungus *Botrytis cinerea* to the Brassicaceae-specific fungus *Alternaria brassicicola*. *B. cinerea* isolates showed variable composition-dependent sensitivity to GSs and their hydrolysis products, whereas *A. brassicicola* was more resistant, especially to aliphatic GSs and to nitrile breakdown products. This correlates with the finding that *B. cinerea* stimulates GS accumulation to a greater extent than does *A. brassicicola*. We demonstrated that the impact of GSB type (isothiocyanates vs. nitriles) is greater than that of the GS group from which it was derived on *A. brassicicola*, as evidenced by the sensitivity of the Ler background and the sensitivity gained in Col-0 plants expressing epithiospecifier protein, both of which accumulate nitriles and not isothiocyanates. This also supports our hypothesis that the sensitivity to *A. brassicicola* in *cyp79B2/B3* double mutants in the Col-0 background is not only due to abolishment of camalexin accumulation but also to a reduction in GSs. Furthermore, we found that *in-vivo* hydrolysis products of indole GSs are involved in the defense response against *B. cinerea* but not against *A. brassicicola*. Finally, it appears that the Brassicaceae-specialist *A. brassicicola* has adapted to GSs and can cope with their high content and hydrolysis products, with a preference for aliphatic GSs and nitriles, whereas the generalist *B. cinerea* is more sensitive to these phytochemicals, in a manner similar to herbivores.

O4.6 - DISRUPTION OF CHITIN SYNTHASE GENE *BCCHS3A* IN *B. CINEREA* RESULTS IN OVER-STIMULATION OF HOST PLANT IMMUNITY

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Epidemics caused by the necrotrophic fungus *Botrytis cinerea* can be severe and economically damaging to many agricultural and horticultural crops. Chitin, an essential ultra-structural constituent of fungal cell walls, and chitin biosynthesis could be a suitable target for botryticides. The b-1,4 N-acetylglucosamine polymer is biosynthesized by a family of chitin synthases. Seven chitin synthase genes (*Bcchs*) have been identified in *B. cinerea* and several mutants have been constructed (1,2,3). We were especially interested in mutant *Bcchs3a* (class IIIa) as it shows a drastically reduced virulence on the major host plant grapevine and the model plant *Arabidopsis thaliana*. Mutant *Bcchs3a* grows normally in liquid culture media, has a 39% reduction of the chitin content in its cell wall and presents an unusual extra-cellular matrix (2). We hypothesised that a change in the cell wall constitution could lead to either (i) increased sensitivity to plant defence molecules or (ii) to over-induction of plant defence genes. We tested these hypotheses by inoculating *A. thaliana* mutants impaired in different defence mechanisms with *Bcchs3a* and wild type strain Bd90 mycelium. Hereby, we found restoration of *Bcchs3a* virulence on camalexin (phytoalexin) deficient *pad3-1 A. thaliana* mutant plants, but no increase of *Bcchs3a* virulence was found on any other *A. thaliana* mutant tested (4). We investigated the sensitivity of *Bcchs3a* and wild type strain Bd90 towards camalexin *in vitro* and *in planta* and found no difference in sensibility of the two strains towards this phytoalexin. Instead, we observed at the infection sites, where mutant *Bcchs3a* colonisation was arrested, higher amounts of camalexin accompanied by an increased expression of *PAD3*-1 (gene encoding a cytochrome P450 enzyme catalyzing the final step in camalexin biosynthesis). This correlation between phytoalexin accumulation at the infection site and arrest of fungal growth suggests a crucial role of camalexin in the resistance to the cell wall mutant (4). We hypothesise that this over-stimulation of host plant immunity is due to an efficient recognition by the host plant of an elicitor in the altered cell wall surface of mutant *Bcchs3a* that is either absent in the wild type strain Bd90 or present in a form not recognisable by the host. Interestingly, we did not find the same phenotype on *A. thaliana* leaves for mutant *bcchs3a* when we inoculated leaves with spore suspensions instead of mycelium. This indicates that BCCHS3a plays a different role during mycelium attack and that different mechanisms are involved in the *B. cinerea* conidia or mycelium infection process.

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2. Soulie *et al.* 2006. *Cell Microbiol*, 8 (8): 1310-21.
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4. Arbelet et al. 2010. MPMI 23 (10): 1324-34.

O4.7 - ANTI-APOPTOTIC MACHINERY PROTECTS BOTRYTIS FROM PCD-INDUCING PLANT METABOLITES AND IS ESSENTIAL FOR DISEASE ESTABLISHMENT

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Apoptosis, a form of programmed cell death, is a universal process. In animals, apoptosis is essential for development and homeostasis. In fungi, apoptotic-like programmed cell death (PCD) is important for proper development and is associated with aging and stress responses. Although PCD has been studied in a growing number of economically and medically important species, research of apoptotic cell death in fungi is still limited in scope. The study of PCD in pathogenic species, and possible role in pathogenicity is of special interest, since it could lead to better understanding of fungal pathogenesis and to the identification of novel targets for antifungal cures. Here we report on the characterization of PCD in *Botrytis cinerea* and on the role of the anti-apoptotic response in disease establishment.

To get a better picture of the available fungal components, we generated an automatic search protocol that is based on protein sequences together with a domain centered approach. We used this protocol to search all the available fungal databases for domains and homologues of human apoptotic proteins. Among all known apoptotic domains, only the BIR domain was found in fungi. A single protein with one or two BIR domains is present in most (but not all) fungal species, including in *Botrytis cinerea*. We isolated the BIR-containing protein from *B. cinerea* and determined its role in apoptosis and pathogenicity. Knockout or over expression strains of *BcBIR1* revealed that BcBir1 is anti-apoptotic and this activity was assigned to the N' terminal part of the protein. Using a strain expressing GFP-tagged nuclei and direct apoptosis assayed we found that the fungus undergoes massive programmed cell death during early stages of infection, but then fully recovers upon transition to second phase of infection. Further studies using the fungal mutants in combination with mutant *Arabidopsis* lines showed that fungal virulence was fully correlated with the ability of the fungus to cope with plant-induced PCD.

Together, our result show that BcBir1 is a major regulator of PCD in *B. cinerea* and that proper regulation of the host-induced PCD is essential for pathogenesis in *Botrytis*. Due to the general role of PCD in fungi and considering the common strategies of host invasion by pathogens, we propose that host-induced fungal PCD might be a general phenomenon, including in human pathogens.

O4.8 - MOLECULAR AND FUNCTIONAL ANALYSIS OF *BOTRYTIS CINEREA* INHIBITOR OF APOPTOSIS PROTEIN BCBIR1

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We have recently shown that during the early stage of infection, *Botrytis cinerea* undergoes massive apoptotic-like programmed cell death (PCD), resulting in almost complete elimination of the hyphae at 48h PI. However, upon transition to the second infection phase (>60h PI), the fungus quickly recovers and spreading lesions are initiated. Manipulation of *BcBIR1,* a homologue of the yeast anti-apoptotic gene *BIR1*, modified fungal sensitivity to PCD-inducing conditions, and as a result affected fungal pathogenicity. Thus, the anti-apoptotic response mediated by BcBir1 is important for establishment of infection. We have also found that the two BIR domains located at the N' terminal end of the protein were necessary and sufficient for the anti-apoptotic activity. To better understand the mode of action of BcBir1, we studied protein function and cellular localization using *Saccahromyces cerevisiae* as a supporting system.

Complementation assay of a *S. cerevisiae bir1* conditional mutant strain with the full length coding sequence of *BcBIR1* did not restore wild type phenotype in the mutant. This lack of functional complementation could have been attributed to differences at the C' terminal part, which is essential for the cell cycle regulating activity of the protein. However, expression of the N' part of BcBir1 restored the anti-apoptotic activity in the *S. cerevisiae bir1* mutant strain. GFP fusions with BcBir1 revealed that the protein is nuclear and remains in the nucleus under all conditions, including following treatment with hydrogen peroxide or acetic acid, which induce PCD in yeast cells. In contrast, GFP fusion with the N' part of BcBir1 was primarily cytoplasmic. Furthermore, expression of the N' part resulted in an anti-apoptotic activity in the yeast cells, whereas expression of the entire BcBir1 protein in yeast cells had no effect. These results suggest that the anti-apoptotic activity of BcBir1, and possibly of Bir1p as well, is associated with cytoplasmic localization. Further analyses to determine the parts in the protein that are essential for anti-apoptotic activity, and connection to cellular localization are underway.

O4.9 - THE *BOTRYTIS CINEREA* PROTEIN BCSPL1 BINDS TO PLANT CELL MEMBRANE AND ELICITS THE HYPERSENSITIVE RESPONSE.

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BcSpl1, a cerato-platanin family protein abundantly secreted by *B. cinerea*, was previously reported to contribute to *Botrytis cinerea* virulence and to cause necrosis when infiltrated in tomato, tobacco, and Arabidopsis leaves, with clear symptoms of the hypersensitive response (1). With the aid of BcSpl1-GFP fusions, we now have evidences that BcSpl1 also binds to the plasma membranes of tomato and tobacco protoplasts, and that the binding triggers morphological alteration such as loss of chlorophyll and cell shrinkage. Deletion studies of BcSpl1 shown that a 40-amino acids conserved region, in the central part of the protein, is sufficient and necessary for the necrosis inducing activity of the protein as well as for the ability to bind to tobacco and tomato plasma membranes. This region contains two discrete short amino acid sequence motifs well conserved in the cerato-platanin family, which group together to form a clear structural protuberance in the surface of the three three-dimensional structure of cerato-platanin. Chemically-synthesized peptides containing only one of these motifs show only partial necrosis inducing activity when infiltrated in plant leaves, but complement one another and display an activity similar to the whole BcSpl1 when infiltrated together. Moreover, the peptides with only one of the motifs act as inhibitors of the whole BcSpl1. Two experimental results suggest that the necrosis inducing activity of BcSpl1 occurs via recognition by the plant immune system as a MAMP, and the resulting activation of the hypersensitive cell death. In first place, the effect of BcSpl1 on Arabidopsis leaves is dependent on a functional BAK1 protein, a plasma membrane protein involved in responding to the archetypal MAMPs EF-Tu and flagellin. On the other hand, the Caspase-1 Inhibitor I is able to partially block BcSpl1 effect on plants, suggesting that the apoptotic-like programmed plant cell death is involved in the production of necrosis.

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P4.1 - RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN BRASSICA AND VARIATION IN PATHOGEN AGGRESSIVENESS

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Sclerotinia disease caused by *Sclerotinia sclerotiorum* causes major losses in oilseed rape (*Brassica napus*) worldwide and is increasing in the UK due both to the large area grown of this crop and the trend for shorter rotations. Currently, the disease is controlled by fungicide sprays but timing of applications is difficult and there are potential negative environmental and social impacts. Durable resistance to *S. sclerotiorum* is therefore very desirable but at present there are no resistant commercial oilseed rape cultivars in the UK. However, identifying sources of resistance is challenging as there is variation in aggressiveness between different *S. sclerotiorum* genotypes (as defined by markers such as microsatellites) causing inconsistencies in plant assays. Host genotypes with consistent resistance to the most prevalent *S. sclerotiorum* clones and an understanding of pathogen aggressiveness are therefore needed to develop successful screening and breeding programmes to combat this disease.

A stem inoculation procedure was developed for brassica plants (8 true leaves) using wheat grain colonised by *S. sclerotiorum*. Initially, 18 *S. sclerotiorum* isolates from different crop hosts and the wild host *Ranunculus acris* (meadow buttercup) were screened against *B. napus* (oilseed rape cv. Temple), *B. oleracea* (broccoli cv. Beaumont) and *B. rapa* (turnip cv. Manchester). There was wide variation in *S. sclerotiorum* aggressiveness for all three brassica types with isolates from *R. acris* generally being less aggressive than those from crop hosts, as determined by the number of leaves wilting and stem lesion development. Turnip was the most susceptible brassica followed by oilseed rape and broccoli. Based on these results, two *S. sclerotiorum* isolates (high / low aggressiveness) were used to screen a brassica diversity set developed through the UK Oilseed Rape Genetic Improvement Network (OREGIN; www.oregin.info). This consisted of genetically fixed lines representing a structured sampling of diversity across the *B. napus* gene pool. The diversity set exhibited a wide variation of responses to *S. sclerotiorum* inoculation from highly susceptible to strongly resistant. Further work is now confirming the response of potentially resistant lines including inoculation tests at the flowering stage.

P4.2 - CHARACTERIZATION OF PROTEASE INHIBITORS IN THE GRAPEVINE-*BOTRYTIS CINEREA* INTERACTION

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Pathogenesis-related (PR) proteins are important elements of the plant defence machinery against pathogens. Among these, PR-6 family includes proteases inhibitors (PIs) that are mostly studied for resistance to insects. Various studies suggest that the necrotrophic fungus *Botrytis cinerea,* the causal agent of gray mould, degrades defence proteins of grapevine by producing proteases (Manteau 2003; Marchal *et al,* 2006). Moreover, pepstatin, a chemical inhibitor of aspartic protease, was shown to reduce infection of carrot by *B. cinerea* (Movahedi & Heale, 1990). We hypothesize that the induction of PIs in grapevine may help the plant to counteract the infection by *B. cinerea*. The aim of this work is to identify PIs that could block fungal proteases and verify the impact of this inhibition on the ability of the fungus to degrade defence proteins of the plant. Two PIs, respectively part of the Potato Inhibitor I (VvPIN) and Kunitz Soybean Trypsin Inhibitor Family (VvKunitz16) were selected. Their expression pattern was studied by q-PCR according to various stresses including infection by *B. cinerea*. Preliminary results show that these two inhibitors are induced in berries by wounding, methyl jasmonate, an elicitor and during infection by the fungus but not by UV-C. Production of the two PIs is in progress and recombinants PI will be used to identify the fungal proteases interacting with them; to monitor their *in vitro* inhibitory activity against fungal proteases and their impact on the degradation of plant defence proteins. Finally, production of transgenic plants overexpressing PIs will allow checking their potential protector effect *in planta* against *B. cinerea*.

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P4.3 - CHARACTERIZATION OF *BOTRYTIS CINEREA* POLYGALACTURONASES AND LACCASES DURING GRAPE BERRIES INFECTION

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Botrytis cinerea, the causal agent of grey mould disease on grapevine, encounters an environment rich in polyphenols and proteins with potential anti-fungal activity when it infects the grape berries. In particular, the stilbenic phytoalexin *trans*-resveratrol and proteins structurally and functionally related to plant pathogenesis-related (PR) proteins, including mostly thaumatin-like proteins and chitinase. *B. cinerea* is thought to infect the host tissue by producing cell-wall degrading enzymes, such as polygalacturonases (PGs), and detoxification enzymes, for example laccase which is likely to be involved in *trans*-resveratrol detoxification.

The combination of *trans*-resveratrol and grape polyphenols or proteins induce *in vitro* a strong release of *B. cinerea* laccase activity which, in turn, neutralizes the toxicity of grape stilbenic phytoalexins and, by oxidizing grape polyphenols, causes the insolubilization of grape proteins. This mechanism could favour berry infection by the fungus and is in accordance with the observation that grape berries infected with *B. cinerea* show a strong reduction of the protein content in comparison to healthy ones.

Grape polyphenols have been also shown to inhibit *B. cinerea* PG activity *in vitro*. Nevertheless, the importance of *B. cinerea* PGs in grape berries infection cannot be ruled out since the fungus could have evolved an avoidance mechanism in order to escape the inhibitory effect of polyphenols.
To better understand the role played by *B. cinerea* PG and laccase activities during grape berries infection, we are analyzing the enzyme activities secreted by the fungus in the infected berry and the expression of the corresponding encoding genes.

P4.4 - THE *BOTRYTIS CINEREA* ELICITOR BCSPL1 IS A POTENT INDUCER OF SAR IN TOBACCO, EVEN THOUGH THE FUNGUS ITSELF IS NOT

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Systemic acquired resistance (SAR) is a potent plant defence system that, in response to a first contact with a plant pathogen, prepares the whole plant for subsequent attacks so that it becomes more resistant to the same and to other plant pathogens. *B. cinerea* has been shown to be a poor inducer of SAR (1). We have previously shown that BcSpl1, a cerato-platanin family protein abundantly secreted by *B. cinerea*, is required for full virulence and elicits the hypersensitive response in the host. Here we show that BcSpl1 also induces SAR in tobacco plants. Plants infiltrated with BcSpl1 were analyzed at different times after infiltration for their resistance to three plant pathogens, *B. cinerea*, *Fusarium oxysporum*, and *Pseudomonas syringae*, as well as for the induction of two markers of SAR, PR-1a and PR-5. The results show that the treated plants were clearly more resistant to the three pathogens than the control plants. Besides, infiltration with BcSpl1 produced a strong induction of the two SAR markers throughout the whole plant. The results were clearly different, however, when SAR was studied for infections with *B. cinerea*. The *B. cinerea*-infected tobacco plants showed only a marginal increase in resistance to the same three plant pathogens, as well as a small induction of the SAR markers, while control plants infected with the well-established SAR inducer *P. syringae* generated a response similar to the infiltration with BcSpl1.

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Session 5

BIO-INFORMATICS AND COMPARATIVE GENOMICS

CHAIRPERSONS: Jan van Kan and Amir Sharon

ORAL COMMUNICATIONS

O5.1 & O5.2 - *BOTRYTIS / SCLEROTINIA* RESOURCES: AN INTEGRATED SYSTEM FOR STRUCTURAL AND FUNCTIONAL GENOME ANNOTATION. Joelle Amselem, Nicolas Lapalu, Baptiste Brault, Laetitia Brigitte, Jonathan Kreplak, Françoise Alfama, Aminah Keliet, Erik Kimmel, Isabelle Luyten, Sébastien Reboux, Delphine Steinbach, Marc-Henri Lebrun and Hadi Quesneville

O5.3 - PREDICTION OF HYPER-*O*-GLYCOSYLATED REGIONS IN THE EXTRACELLULAR PROTEINS CODED BY EIGHT FUNGAL GENOMES. Mario González, Nélida Brito, Marcos Frías, Celedonio González

O5.4 - FROM GENOME SEQUENCE TOWARDS AN ENTIRE GENUS SEQUENCE: COMPARATIVE GENOMICS OF THE GENUS *BOTRYTIS.* Jan A.L. van Kan, Cizar Almalak, Martijn Staats

O5.5 - TOWARDS PROTECTING OCEANIC ISLAND BIODIVERSITY: A POTENTIAL ROLE FOR THE 'NOBLE ROT' AGAINST *RUBUS NIVEUS*. Jacqueline St.Quinton and Jane Faull

POSTERS

P5.1 - IMPROVING THE ASSEMBLY OF *BOTRYTIS CINEREA* GENOME USING A GENETIC MAP AND A BAC ENDS SEQUENCES LIBRARY. Jean-Marc Pradier, Isabelle Lebrun, Julien Pansiot, Pascal Bally, Angélique Gautier, Lilian Gout and Elisabeth Fournier

P5.2 - MICROTUBULE-ASSOCIATED PROTEINS IN *BOTRYTIS CINEREA*. Yohan Faivre and Christophe Bruel

P5.3 - COMPARISON OF COLONIZATION PROCESSES ON SUNFLOWER COTYLEDONS BETWEEN *BOTRYTIS CINEREA* AND *SCLEROTINIA SCLEROTIORUM*. Geneviève Billon-Grand, Christine Rascle, Jeffrey A. Rollins, Nathalie Poussereau

O5.1 & O5.2 - *BOTRYTIS / SCLEROTINIA* RESOURCES: AN INTEGRATED SYSTEM FOR STRUCTURAL AND FUNCTIONAL GENOME ANNOTATION

Joelle Amselem[1,2], Nicolas Lapalu[1,2], Baptiste Brault[1,2], Laetitia Brigitte[1,2], Jonathan Kreplak[1], Françoise Alfama[1], Aminah Keliet[1], Erik Kimmel[1], Isabelle Luyten[1], Sébastien Reboux[1], Delphine Steinbach[1], Marc-Henri Lebrun[2] and Hadi Quesneville[1]

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Nowadays with the recent development of new generation sequencing technologies, genomes are sequenced at a high pace, new sequences data are produced (RNAseq, ChIPseq,…), and large amounts of data have to be stored and queried. To face this challenge, the URGI (http://urgi.versailles.inra.fr) platform aims at providing tools to store and annotate entirely sequenced genomes comprising: pipelines, databases and user-friendly interfaces to browse and query the data.

We will focus here on systems developed at URGI to provide structural and functional annotations to a scientific community. We will present all these resources in the frame of the *Botrytis/Sclerotinia* genome project involving: *B. cinerea* T4, *B. cinerea* B0510 and *S. sclerotiorum*.

- A distributed annotation system allows the manual curation of gene structure. This system relies on the well known GMOD databases and interfaces (chado/GBrowse/Apollo). Curated data can be shared by the consortium as soon as they are committed in the database using the “pure JDBC” direct communication protocol between Apollo and Chado.

- A genome and a synteny browser allow exploring both genome annotations and the synteny between the genome sequences of *B. cinerea* T4, *B. cinerea* B0510 and *S. sclerotiorum.*

- A Genome Report System (GRS), gets structural and functional genomic data stored in a Chado database, to provide the users with a comprehensive list of gene related information including cross references, and Gene Ontology. An editing module (GRS edition) allows manual functional annotation.

- Quick and advanced search are proposed, respectively through GnpIS QuickSearch (http://urgi.versailles.inra.fr/gnpis) and Biomart (GMOD), to query genes by their functions.

O5.3 - PREDICTION OF HYPER-*O*-GLYCOSYLATED REGIONS IN THE EXTRACELLULAR PROTEINS CODED BY EIGHT FUNGAL GENOMES

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O-glycosylation of secreted protein has been found to be an important factor in fungal Biology and virulence. It consists in the addition of short glycosidic chains to Ser or Thr residues in the protein backbone via *O*-glycosidic bonds. Yeast usually have only linear sugar chains composed exclusively of mannose, but filamentous fungi may have branched chains containing also glucose or galactose. *O*-glycosylation has proven to be essential in fungi, and to have a role in enhancing the stability and solubility of secreted proteins, protecting them from proteases, as a sorting determinant, and in the development and differentiation of the fungal hyphae. Secreted proteins in fungi frequently display Ser/Thr rich regions that could be the site of extensive *O*-glycosylation. We have analyzed *in silico*, with the aid of NetOGlyc (www.cbs.dtu.dk), the whole sets of putatively secreted proteins coded by eight fungal genomes (*Botrytis cinerea, Magnaporthe grisea*, *Sclerotinia sclerotiorum, Ustilago maydis, Aspergillus nidulans, Neurospora crassa, Trichoderma reesei,* and Saccharomyces cerevisiae) in search of highly glycosylated regions. More than half secreted proteins in filamentous fungi were predicted to be *O*-glycosilated. The number of modified Ser/Thr residues in them averages 10 but varies widely, with some proteins displaying dozens or even hundreds of *O*-glycosilated residues. Besides, these residues have a tendency to be grouped together forming hyper-glycosilated regions of varying length, which can be found anywhere along the proteins but with a slight tendency to be at either one at the two ends. About one third of extracellular fungal proteins were predicted to have at least one hyper-glycosilated region, which consists of about 50 amino acids and displays, on average, a glycosylated Ser or Thr residue every four residues.

O5.4 - FROM GENOME SEQUENCE TOWARDS AN ENTIRE GENUS SEQUENCE: COMPARATIVE GENOMICS OF THE GENUS *BOTRYTIS*

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Comparative genomics is a powerful tool to infer the molecular basis of fungal pathogenicity and its evolution, by identifying differences in gene content and genomic organization between fungi with different hosts. The genus *Botrytis* comprises at least 21 specialist species that have a narrow host range, and the broad host-range species *B. cinerea*. Many *Botrytis* species infect agronomically important crops, including all major flower bulb and *Allium* crops. The *Botrytis* phylogeny has indicated that frequent host-jumps between distantly related host families has occurred, possibly through the acquisition of novel pathogenicity factors. The underling molecular mechanism conferring host-specificity are not known, but would be instrumental to designing rational strategies for disease control.

The recent determination of the whole genome sequences (WGS) of two *B. cinerea* strains has greatly accelerated genetic research and has improved our knowledge of the infection strategies of this necrotrophic pathogen. Preliminary results are presented of an improved version of the currently published *B. cinerea* B05.10 genome sequence. The new assembly contains larger scaffolds and a reduced number of sequencing errors. Second, preliminary genomic comparisons of WGS of specialist species with the generalist *B. cinerea* are presented, with which we anticipate to unravel some of the complexity associated with the ability to infect a wide range of hosts. Third, analyses will be presented on comparisons of WGS of phylogenetically close, yet distinct host-specific *Botrytis* species. Through comparative analysis, we will gain insights into the molecular diversification of *Botrytis* species. Moreover, we aim to identify pathogenicity-related genomic regions that might contain genes for host-specific virulence factors.

O5.5 - TOWARDS PROTECTING OCEANIC ISLAND BIODIVERSITY: A POTENTIAL ROLE FOR THE 'NOBLE ROT' AGAINST *RUBUS NIVEUS*

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In locations outside its native range – the Himalayan regions of Asia - the weed *Rubus niveus* (subgenus *Idaeobatus*) poses a significant threat to the biodiversity of the areas that it colonises. Notable areas under threat are the Galápagos and Hawaiian islands where this weed is being actively managed through manual cutting and application of manufactured herbicides. However, in Galápagos for instance, this manual intervention has been shown to be of short-term value and impractical to tackle the extensive coverage of this weed (some 60,000 ha). A biological control approach is being researched as an effective and sustainable alternative.

Die-back of *Rubus fructicosus* around the British countryside can be attributed to fungal attacks, e.g. *Septocyta ruborum* (Lib.) Petrak - or to drought conditions. However, the occurrence of wild bramble die-back noted in May 2011 in a riparian environment within a nature reserve - Hayesden Country Park, Kent, UK – and the absence of the diagnostic purple blotches associated with *Septocyta ruborum* - caused the drought hypothesis to be questioned. The isolation of a *Botrytis* species from moribund *R. fructicosus* leaves collected from this has resulted in its inclusion among the biotrophic and necrotrophic pathogens being evaluated within the framework of the biological control of *Rubus niveus*.

Work currently underway includes:

(a) The identification of this *Botrytis* species through traditional and molecular study.

(b) Pathogenicity evaluations carried out

(i) under laboratory conditions – using the detached-leaf technique

(ii) pot trials under quarantine conditions

Whilst issues of host-specificity may preclude the sole use of a *Botrytis* species as a biological agent against *Rubus niveus*, it could be of use in combination with other agents as part of an integrated weed management system.

P5.1 - IMPROVING THE ASSEMBLY OF *BOTRYTIS CINEREA* GENOME USING A GENETIC MAP AND A BAC ENDS SEQUENCES LIBRARY

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In the context of the sequencing and assembly of strain T4, a genetic map has been built. A crossing between strain T4 and strain 32 (kindly provided by Caroline Kunz) gave 68 usable progeny. About 134 polymorphic microsatellite markers were selected, mainly at the ends of the scaffolds built during the sequencing project (118 main scaffolds called SuperContigs or SuperSuperContigs), by using the GRAMENE and FONZIE software to find the microsatellites and the minisatellites sequences (1,2). The primers, bounding these markers, were designed with PRIMER3 (3) and the products separated by size directly on agarose gels or by using an adapted M13 fluorescent technology. From a collection of 140 SNPs devoted to *Botrytis* population studies, about 62 SNPs were found polymorphic in the T4X32 crossing and were also used in the constitution of this map. The genetic linkage groups were constructed by using MAPMAKER (4). The total length of the scaffolds appearing in the genetic map covered 31.8 Mbp, representing about 80% of the T4 sequence assembly (5). The use of an extra BAC end sequences library made in T4 permit to add information in the constitution of bigger scaffolds, often reinforced by linkage groups representing up to 87% of the total sequences produced with strain T4. This reduced the number of groups of sequences to 32 which leads closer to the 16 chromosomes described in this species (6). Only two scaffolds bt4_SupSuperContig_114_320_122_1 and bt4_SuperContig_29_1 seemed to be misassembled as pointed out by the BAC ends and the genetic data. This set of genetic markers has also shown to be useful in localizing fungicide resistance genes (7) or spontaneous mutations like the lack of sclerotia formation in strain T4.

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P5.2 - MICROTUBULE-ASSOCIATED PROTEINS IN *BOTRYTIS CINEREA*

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Successful Infection of a plant by conidia of *Botrytis cinerea* relies on their germination, growth of the germ tube, development of an unicellular appressorium or that of an infection cushion, penetration of the plant tissues and, lastly, necrotrophic invasion of the latter. Many, if not all, of these steps require the rearrangement of the fungal cytoskeleton because new material has to be brought to the cell apex, new cell growth orientation has to be given, new cell shape has to be created and dynamic cell-cell interactions has to be orchestrated.

Besides the very conserved tubulins that constitute the cell microtubules, microtubule-associated proteins (MAPs) are indispensable partners of the microtubule network. In plants, they have been shown to serve multiple functions among which the construction and maintenance of the microtubules themselves, the transport of specific cargo and the tethering of molecules involved in distinct cellular processes. In fungi, MAPs are likely of importance in the proper unfolding of their life or infection cycle, but they have not received much attention so far.

Based on the available genomic data of *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and in comparison to those available for *Aspergillus nidulans*, *Neurospora crassa* and the yeast *Saccharomyces cerevisiae*, we have collected all MAPs encoding genes putatively present in the two necrotrophic plant pathogens. Five families have been found that would contain 12 to 22 members each. All families are also present in the three other selected fungi, but gene distribution and conservation differ between species. Expression data analysis of *B. cinerea* early development steps (SafeGrape ANR project) showed differential expression of the MAPs genes and their grouping under six distinct expression profiles. Altogether, these data constitute the first review on fungal MAPs and would support the possible involvement of some of them at specific stages of the *B. cinerea* life cycle.

P5.3 - COMPARISON OF COLONIZATION PROCESSES ON SUNFLOWER COTYLEDONS BETWEEN *BOTRYTIS CINEREA* AND *SCLEROTINIA SCLEROTIORUM*

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During pathogenesis on sunflower cotyledons, *B. cinerea* (strain BO5.10) and *S. sclerotiorum* (strain S5) show a striking resemblance in symptom development. Based on the pH change profile of colonized tissues, the colonization process can be divided in two stages. The first stage is associated with a pH decrease, the second stage is correlated with a pH increase. For both fungi, citric and succinic acids are responsible for the observed pH decrease, oxalic acid being produced during the second stage of the colonization. The second stage is concomitant with a decrease in free amino acids and accumulation of ammonia in decaying tissues. Compared to *S. sclerotiorum*, *B. cinerea* produces 8-fold less oxalic acid and 2-fold more ammonia and these significant differences appear responsible for those observed for pH value fluctuations. At different stages of the colonization process, expression of genes encoding secreted proteases was investigated by RT-qPCR. This analysis shows that protease genes are predominantly activated during the second stage of the colonization process. Furthermore, the expression level of *B. cinerea* protease genes is from 2 to 4-fold higher than that of *S. sclerotiorum*. In this way, proteases potentially would be a more significant tool for the colonization process of *B. cinerea* relative to *S. sclerotiorum*. *In vitro* assays support these results. They also show that decreases in pH are linked to glucose consumption. For both fungi, these studies suggest that oxalic acid is not an essential factor for the colonization process on sunflower cotyledons since it is produced at a late stage of the colonization. They also suggest that *S. sclerotiorum* and *B. cinerea* significantly differ in glycolysis or in TCA cycle regulation. At last, they highlight that the pH dynamic, rather than the creation of a static acidic environment, seems to be important in determining colonization success.

ADDED ORAL COMMUNICATION

DELIVERY OF DNA TO *BOTRYTIS CINEREA* BY DIRECT HYPHAL BLASTING OR BY WOUND-MEDIATED TRANSFORMATION OF SCLEROTIA

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Botrytis cinerea, a necrotrophic ascomycete known as a major pathogen of fresh produce post-harvest, is responsible for 'grey mold' disease in over 200 plant species. Broad molecular research has been conducted on this pathogen in recent years, resulting in the sequencing of two strains, which has generated a wealth of information and helped in developing additional tools for molecular proteome and secretome investigations. Nevertheless, transformation protocols have remained a significant bottleneck for this pathogen, hindering functional analysis research in many labs, including ours. In this study, we used four different transformation methods: protoplasts, electroporation, air-pressure-mediated transformation and sclerotium-mediated transformation to determine the best one for high-throughput transformation with a higher yield of transformants. Our results suggest that the air-pressure- and sclerotium-mediated transformation methods are the easiest, cheapest and most efficient methods

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