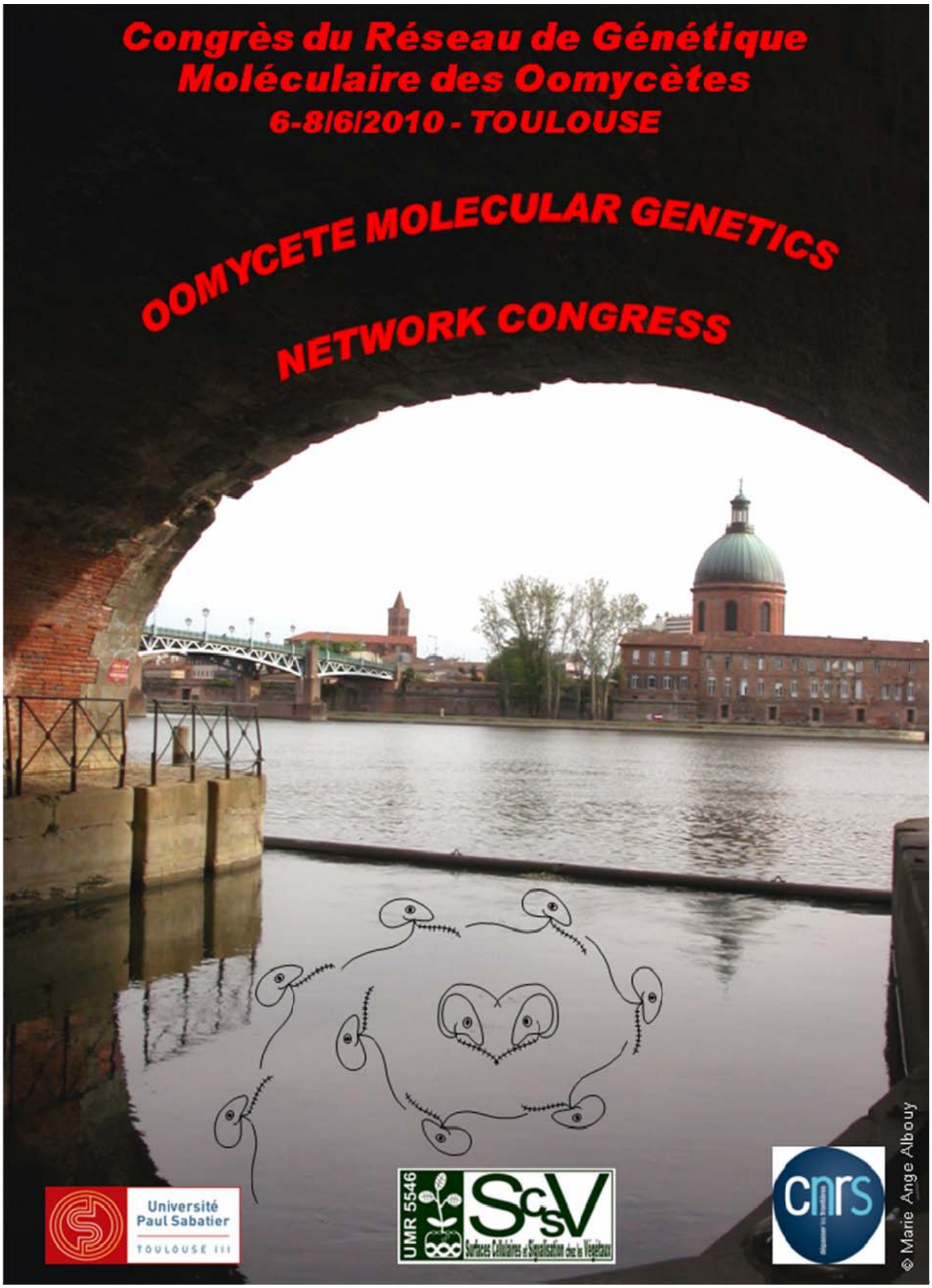
.

Congrès du Réseau de Génétique Moléculaire des Oomycètes

6-8/6/2010 - TOULOUSE

OOMYCETE MOLECULAR GENETICS NETWORK CONGRESS



Université Paul Sabatier TOULOUSE III

UMR 5546 SCSV Surfaces Cellulaires et Signalisation chez les Végétaux

cnrs

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Congress
of the Oomycete Molecular Genetics Network

Congrès
du Réseau de Génétique Moléculaire des Oomycètes

6-8/6/2010
Salons Marengo
Médiathèque José Cabanis,
Toulouse, France

Presentation - Présentation

Oomycetes are a diverse class of microorganisms comprising more than 600 species that have major impacts on plant and animal health. In the time between 1975, when Noel T Keen introduced the concept of race-cultivar specific elicitors, and present times, the deciphering of the molecular interactions between the oomycetes and their hosts has entered the genomic era. Scientists are now able to study a variety of molecules, such as PAMPs and hundreds of putative cytoplasmic effectors, all potentially interacting with components of their hosts' immune systems. A research community of the highest scientific quality has grown, focused on studying the molecular biology of oomycetes, and which the US National Science Foundation has funded to organize dedicated meetings since 2002. This international community now alternates its annual meeting between the USA and Europe. After Wageningen (The Netherlands) in 2006 and Birnam (Scotland) in 2008, the French "Ville Rose" welcomes you to discuss the latest exciting developments in oomycete molecular genetics and genomics, and also to discover some of the many still obscure aspects of their cell biology, evolution and ecological impact.

Les oomycètes sont les microorganismes pathogènes les plus dévastateurs des cultures dans le monde, et comptent des parasites majeurs d'animaux. Un effort de recherche important est nécessaire au niveau international pour mieux connaître les mécanismes de leur pouvoir pathogène et mieux contrôler ces parasites. Un réseau collaboratif de recherche, intitulé « Oomycete Molecular Genetics Network » (OMGN), est financé par la « National Science Foundation » aux Etats-Unis depuis plusieurs années. Il se consacre et promeut l'étude de la génétique moléculaire des oomycètes, qui connaît un développement important du fait du séquençage complet de plusieurs génomes et de l'excellence des groupes de recherche sur le sujet. Un colloque est organisé chaque année sous l'égide de l'OMGN, qui réunit des chercheurs et des étudiants en stage post-doctoral ou en thèse. Depuis quelques années les correspondants européens de l'OMGN organisent le colloque sur leur sol en alternance avec les réunions aux Etats-Unis. Après Wageningen (Hollande, 2006) et Birnam (Ecosse, 2008), la réunion de 2010 a lieu à Toulouse. Elle est organisée par l'UMR5546 CNRS-Université Paul Sabatier, au sein de laquelle l'équipe Interactions Plantes-Microorganismes (IPM) participe à l'OMGN depuis plusieurs années. A la suite de la France, la Chine prendra le relai pour organiser la réunion de 2012.

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van den Ackerveken, Guido (Plant-Microbe Interactions, Department of Biology Faculty, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands)

Bottin, Arnaud (UMR 5546 UPS/CNRS, Toulouse, France)

Diéguez Uribeondo, Javier (Molecular Systematics Laboratory, Department of Mycology, Royal Botanic Garden, CSIC, Plaza Murillo 2, 28014 Madrid, Spain)

Dumas, Bernard (UMR 5546 UPS/CNRS, Toulouse, France)

Gaulin, Elodie (UMR 5546 UPS/CNRS, Toulouse, France)

Panabières, Franck (UMR INRA 1064 / CNRS 6192, Sophia-Antipolis, France)

van West, Pieter (College of Life Sciences and Medicine, Institute of Medical Sciences, University of Aberdeen, Oresterhill, Aberdeen, AB25 2ZD, Scotland, UK)

Whisson, Steve (SCRI Invergowrie, Dundee, DD2 5DA, Scotland, UK)

Local organizing committee - Comité local d'organisation:

Albouy, Marie-Ange (UMR 5546 UPS/CNRS, Toulouse)

Tel : 05 34 32 38 24, Mel : albouy@scsv.ups-tlse.fr

Bottin, Arnaud

Dumas, Bernard

Gaulin, Elodie

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The organizers of the 2010 Oomycete Molecular Genetics Network Meeting (OMGN 2010) thank the institutional and private partners that have contributed to this international scientific congress.

Les organisateurs du Congrès 2010 du Réseau de Génétique Moléculaire des Oomycètes (OMGN 2010) remercient leurs partenaires institutionnels et privés qui ont contribué à la réalisation de cette manifestation scientifique internationale.

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UMR 5546 UPS/CNRS
Surfaces Cellulaires et Signalisation
chez les Végétaux

IFR 40
« Agrobiosciences, Interactions
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Programme of the meeting

Sunday, June 6

12h30-15h30	Registration	
15h30-17h00	Communications	I. Microbial Effectors (1)
17h30-18h30	Conference (M. Rep, NL)	"The molecular basis of pathogenicity in fungi"

Monday, June 7

8h30-10h00	Communications	I. Microbial Effectors (2)
10h00-10h30	Break	
10h30-12h00	Communications	II. Plant Immunity (1)
12h00-13h30	Lunch	
13h30-15h00	Communications	II. Plant Immunity (2)
15h00-17h00	Poster Session	
17h00-19h30	Guided Tour of Toulouse	
20h00-23h30	Conference Dinner	

Tuesday, June 8

9h10-10h40	Communications	III. Reproduction, Evolution and Epidemiology (1)
10h40-11h10	Break	
11h10-12h30	Communications	III. Reproduction, Evolution and Epidemiology (2)
12h30-14h00	Lunch	
14h00-15h30	Communications	IV. Cell Biology and Genomics (1)
15h30-16h00	Break	
16h00-17h30	Communications	IV. Cell Biology and Genomics (2)
17h45-18h30	Conclusion of the meeting	

Programme of the talks

Sunday, June 6

I. Microbial Effectors

15:30	Stassen J.H.M.	Effector proteins of the lettuce downy mildew pathogen *Bremia lactucae*
15:40	De Bruijn I.	Searching the genome of the animal pathogenic *Saprolegnia parasitica* for effector proteins and their molecular characterisation
15:50	McLellan H.	Identification of RxLR effectors essential for virulence in *Phytophthora infestans*
16:00	Liu T.	A subgroup of *Phytophthora sojae* effectors is required for pathogenesis by manipulating plant cell death
16:20	Huitema E.	Studies on targeting, trafficking and activities of the *Crinkler* protein family in *Phytophthora capsici*
16:30	Minor K. L.	Localisation studies of a putative RxLR effector from *Saprolegnia parasitica* to haustorium-like structures.
16:40	Kale S. D.	Mechanism of RXLR-mediated entry of oomycete and fungal effectors into host cells

Conference

17:30	Rep M.	The molecular basis of pathogenicity in fungi

Monday, June 7

I. Microbial Effectors

8:30	Armstrong M.	Identifying candidate host 'targets' of *Phytophthora infestans* translocated RXLR proteins
8:50	van Damme M.	CRN8 a *Phytophthora infestans* host-translocated effector with kinase activity
9:00	Chaparro-Garcia A.	*Phytophthora infestans* AVR3a effector protein is a suppressor of immune responses in plants
9:10	Breen S.	Avr2, an RXLR effector from *Phytophthora infestans*
9:20	Engelhardt S.	R3a-mediated recognition of AVR3a: Cell biological and biochemical characterization
9:30	Qutob D.	Gain-of-virulence by naturally occurring paramutation in *Phytophthora sojae*
9:50	Küfner I.	The NLP effector superfamily comprises cytolytic and non-cytolytic members

Monday, June 7 (continued)

II. Plant Immunity

10:30	Larroque M.	Plant cell wall integrity surveillance: a novel perception system for cellulose-binding oomycete effectors?
10:50	Nars A.	Are chitin synthases targets for antimicrobial compounds and sources of MAMPs in oomycetes?
11:00	Bouwmeester K.	The lectin receptor LecRK-I.9 is a novel *Phytophthora* resistance component and a host target for the RXLR effector IPI-O
11:10	Bozkurt T. O.	*Phytophthora infestans* RXLR effector AvrBlb2 supports host colonization by interfering with secretion of papain like cysteine protease C14
11:30	Schornack S.	Membrane-associated RXLR effectors of *Phytophthora infestans* alter localisation of plant receptor-like kinases and affect plant immunity
11:40	Fraiture M.	Identification of *Hyaloperonospora arabidopsidis* RxLR-(EER) effectors suppressing PAMP-triggered immunity in *Arabidopsis*
11:50	Borhan M.H.	Identifying effectors from *Albugo candida* by high throughput screening
12:00		***Lunch***
13:30	Fabro G.	*Hyaloperonospora arabidopsidis (Hpa)* candidate effectors suppress PTI enhancing plant susceptibility
13:50	Gilroy E.M.	Using knowledge of RXLR effectors to seek durable resistance
14:10	Lapin D.	Genetic mapping of broad resistance to downy mildew in *Arabidopsis thaliana* C24
14:20	As-Sadi F.	Towards the characterization of a quantitative resistance to Downy Mildew in cultivated Sunflower, *Helianthus annuus*
14:30	Gachon C.M.M.	Why brown algae may not have resistance genes, and proteomics of the interaction between *Ectocarpus siliculosus* and the oomycete pathogen *Eurychasma dickosnii*
14:40	Rey T.	Screening of *Medicago truncatula* symbiotic mutants with *Aphanomyces euteiches* revealed molecular crosstalks between symbiotic interactions and resistance to pathogens
14:50	Krajaejun T.	A 74-kDa Immunodominant antigen of the pathogenic Oomycete *Pythium insidiosum* is a Putative Exo-1,3-ß Glucanase

Tuesday, June 8

III. Reproduction, Evolution and Epidemiology

9:10	Diéguez-Uribeondo J.	A new phylogenetic lineage in the genus *Aphanomyces* (Oomycetes) comprising thermophilic strains isolated from the Ecuatorian Amazonia
9:20	Delmotte F.	Invasion history of grapevine downy mildew (*Plasmopara viticola*): a population genetic perspective
9:40	Harbaoui K.	*Phytophthora infestans* Tunisian isolates show a high genotypic diversity as revealed by a SSR multiplex PCR technique using 12 SSR markers
9:50	Magalon H.	Study of invasive French populations (2006-2008) of *Phytophthora infestans*, the oomycete causing potato late blight
10:10	Cooke D.	Ongoing change in populations of Phytophthora infestans in the GB potato crop
10:20	Valade R.	Genes flow in populations of *Bremia lactucae,* the causal agent of downy mildew of lettuce, in France
10:30	Blair J.E.	The utility of Genome Survey Sequences in developing anonymous loci for population-level studies in *Phytophthora* and *Pythium*
10:40		***Break***
11:10	Robideau G.P.	Evolution of oomycete flagella
11:30	Morris P. F.	Conservation of regulatory networks in the *Phytophthora sojae* genome
11:50	Tyler B.M.	Comparative and functional genomics of oomycete infection
12:10	Cano L. M.	Comparative genome analysis of a strain from the UK blue 13 clonal lineage of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes
12:20	Thines M.	Evolutionary and functional analysis of ATR1 and ART13 from sister species of *Hyaloperonospora arabidopsidis*
12:30		***Lunch***

IV. Cell Biology and Genomics

14:00	Jiang R.H.Y.	Comparative genomic analysis of *Saprolegnia parasitica* and *Phytophthora* species suggests host-specific evolution of effectors in oomycete animal and plant pathogens
14:10	Feng B.Z.	Isolation of nine *Phytophthora capsici* pectin methylesterase genes which are differentially expressed in various plant species
14:20	Raffaele S.	Comparative genome sequencing connects host adaptation and genome organization in the *Phytophthora infestans* lineage
14:30	Wawra S.	Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins
14:50	Vetukuri R.R.	Exploring the small RNA world in *Phytophthora infestans*
15:00	Phuntumart V.	Epigenetic gene regulation in *Phytophthora sojae*
15:10	Wang Y.	Development of high-throughput gene silencing tools in *Phytophthora sojae*
15:30		***Break***
16:00	Judelson H. S.	Oomycete Kinomes: structure, diversification, and cellular function
16:20	Hua C.	Characterization of GPCR-PIPKs, a novel class of proteins in *Phytophthora*
16:30	Grenville-Briggs L. J.	Identification of appressorial and mycelial cell wall and membrane-associated proteins from *Phytophthora infestans*
16:40	Guerriero G.	Chitin synthase: a potential target of anti-Oomycetes drugs
16:50	Garavito M. F.	Dihydroorotate dehydrogenase from the phytopathogenic Oomycete *Phytophthora infestans* as a novel target for crop control
17:00	García Bayona L.	Orotate phosphoribosyl transferase and orotidine-5-monophosphate decarboxylase as targets for control of *Phytophthora infestans*
17:10	Panabières F.	Functional analysis of the *P. parasitica* hexose kinase genes as a first step towards exploring the nutritional basis of pathogenicity of *Phytophthora*
17:30		***Break***
17:45		***Conclusion of the meeting***

TALKS

COMMUNICATIONS ORALES

I. Microbial Effectors

Effector proteins of the lettuce downy mildew pathogen *Bremia lactucae*

STASSEN J.H.M., VERGEER P., ANDEL A. AND G. VAN DEN ACKERVEKEN

Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Bremia lactucae is an obligate biotrophic oomycete pathogen of lettuce (*Lactuca sativa*) and constitutes a major problem in lettuce cultivation. The dominant resistance genes that have been used to control *Bremia* so far are rapidly overcome by new isolates. By gaining more knowledge about the molecular players in the interaction between pathogen and host a step towards more durable resistance can be taken. In order to identify pathogen proteins involved in this interaction we sequenced cDNA of heavily *Bremia*-infected lettuce by 454 sequencing. Additionally we sequenced spore-derived genomic DNA of *Bremia* by SOLiD sequencing. From a total dataset of over 2.5 million 454 reads of an average size of >200 bp and over 170 million 50 bp SOLiD reads we generated a *de novo* pool of effector candidates for further investigation. The effector predictions based on short read sequencing and bioinformatics are now being cloned for functional studies. Currently, the focus is on RXLR effector candidates and crinkler-like candidates. The activity of the cloned effectors is being investigated by *Agrobacterium*-mediated transient expression in a series of bioassays designed to test for disease enhancement or suppression of defence. Together with data about the level of sequence conservation of such effectors within the oomycetes as well as polymorphism data from allele sequencing of different *Bremia* isolates, this will reveal which candidates can be selected for future effector-assisted breeding programmes

Identifying effectors from *Albugo candida* by high throughput screening

BORHAN M.H., UTERMARK J., BEYNON E., LINKS M., SHARPE A. AND D. HEGEDUS*

*Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK., Canada. * NRC-Plant Biotechnology Institute, Saskatoon, SK., Canada.*

Albugo candida is the causal agent of white rust disease of Brassica species. Several *A. candida* races including *A. candida* race 2 (Ac2) and race 7 (Ac7) which are economically important pathogen of Brassica crops also infect *Arabidopsis thaliana*. We have cloned *WRR4*, a TIR-NB-LRR type resistance gene from *Arabidopsis* accession Col. *WRR4* confers resistance in *Arabidopsis* and *Brassica* to Ac2V and Ac7 as well as several other *Albugo* isolates. We have sequenced the genome of Ac2V and identified potential RXLR type effectors among the predicted secreted proteins. High throughput transient assay in tobacco has proved function for several Ac2V effectors. Results of functional analysis of one of these effectors and its potential host targets identified by Y2H will be presented

Searching the genome of the animal pathogenic *Saprolegnia parasitica* for effector proteins and their molecular characterisation

DE BRUIJN I.[1], MINOR K.L.[1], PHILLIPS A.J.[1], ROBERTSEN E.J.[1], ANDERSON V.L.[1], BAIN J.[1], WAWRA S. [1], RUSS C.[2], NUSBAUM C.[2], TYLER B.M.[3], SECOMBES C.J.[1] AND P. VAN WESTP.

[1]*University of Aberdeen, Scotland, UK.*
[2]*Broad Institute, MIT, USA*
[3]*Virginia Bioinformatics Institute, Virginia Tech, USA*

Water molds (oomycetes) are destructive pathogens of aquatic animals and terrestrial plants. *Saprolegnia* species cause Saprolegniosis, a disease that is characterized by visible white or grey patches of filamentous mycelium on the body or fins of freshwater fish. *Saprolegnia parasitica* is economically one of the most important fish pathogens, especially on catfish, salmon and trout species, causing millions of dollar losses to the aquaculture business worldwide. Several *Saprolegnia* species have also been linked to declining wild fish stocks and amphibian populations around the world. Recently, the 53 Mb genome sequence of *S. parasitica* (isolate CBS223.65) has been released comprising 1442 supercontigs. Further assembly and annotation will be supported by paired-end EST sequences derived from several life stages of *Saprolegnia*, *Saprolegnia*-infected fish cell-line, and *Saprolegnia* infected salmon-eggs. Analysis of the preliminary genome sequence and EST libraries resulted in the identification of a putative effector protein family in the *Saprolegnia* genome. Electron microscope analysis showed that *Saprolegnia* interacts with fish cells by forming haustoria-like structures. Detailed expression studies of several genes encoding for the members of the putative effector protein family were performed during the biotrophic and necrotrophic infection stages of *S. parasitica*. Also localization and uptake studies were performed to show a role of the effector proteins in the interaction of *S. parasitica* with a rainbow trout cell-line.

Identification of RxLR effectors essential for virulence in *Phytophthora infestans*

MC LELLAN H.[1], TIAN Z.[2], AVROVA A.O.[2], GILROY E.M.[2], PRITCHARD L.[2], WHISSON SC.[2] AND P.R.J. BIRCH [1]

[1]Division of Plant Science, College of Life Sciences, University of Dundee (at SCRI), Invergowrie, Dundee DD2 5DA, UK
[2]Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

The sequencing of the *Phytophthora infestans* genome, along with the discovery of the RxLR motif and subsequent computational analysis, has allowed the identification of upwards of 450 candidate RxLR effector genes. With a multitude of putative virulence factors now predicted, several criteria were used to prioritize a subset of effectors for further functional characterisation. These included expression analyses, conservation with other oomycete pathogens and the presence of known protein domains. Initial experiments employing transient RNAi hairpins to knockdown effector expression were used to identify those RxLRs which may contribute to pathogen virulence. These were followed by making stably transformed *P. infestans* lines containing inverted repeat constructs to knockdown gene expression. From this we have identified candidate RxLRs which may be essential for *Phytophthora* virulence on potato as in the absence of expression of these effectors the pathogen loses its ability to grow on its host. Further experiments where we have transiently expressed the silenced effector in the host has allowed the complementation of the missing RxLR and restored the ability of *Phytophthora* silenced lines to grow *in planta*.

A subgroup of *Phytophthora sojae* effectors is required for pathogenesis by manipulating plant cell death

LIU L., YE W., RU Y., LU S., YANG W., TAO K., DONG S., ZHENG X., WANG Y., AND D; DOU

Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China

Phytophthora sojae encodes hundreds of effectors with conserved FLAK motif following signal peptides, which was termed as Crinkler or crinkling and necrosis induced proteins (CRN).Its functions and mechanisms in pathogenesis are still largely unknown. Here, a *P. sojae* specific subgroup CRN effectors was chosen for functional analysis because of its high expression level and containing of the putative nuclear localization signals. Seven very similar genes are included in this subgroup, in which 5 of them are putatively pseudogenes. Functional analysis shows that those 2 genes have contradictory roles in *Nicotiana benthamiana*, PsCRN63 inducing cell death while PsCRN207 suppressing the cell death elicited by NIP or PsCRN63. The fragment of PsCRN63, abolishing signal peptides and FLAK motifs, is efficient to trigger cell death while the nuclear localization signals are required for this process, suggesting those genes are trafficked into host nuclear in the pathogenesis. Silencing of this subgroup of effectors in *P. sojae* stable transformants lead to the significant reduction of virulence on its host soybean. Furthermore, the cell death is observed only in the silenced transformants but not in the wild type, indicating that host cell death is alternated. Taking the results together, we inferred that this subgroup of CRN effectors contribute to *P. soaje* pathogenesis by manipulating cell death and the process is host nuclear localization.

Studies on targeting, trafficking and activities of the *Crinkler* protein family in *Phytophthora capsici*

JUPE J., VAN VUGT D. AND E. HUITEMA

Division of Plant Sciences, University of Dundee, Invergowrie, Dundee DD2 5DA

With the advent of Oomycete genomics and detailed functional studies of secreted pathogen proteins (Effectors), effectors are now emerging as the principal determinants of epidemics. Several classes of effectors have been identified in *Phytophthora*, some of which are targeted to the host apoplast (extra-cellular or apoplastic effectors) whereas others are delivered inside host cells (intracellular effectors). The Crinkler (CRN) gene family encodes a diverse class of intracellular effectors that target the host nucleus and are present in all plant pathogenic oomycetes studied to date. Our aim is to answer emerging questions regarding targeting, delivery and activity of CRN proteins during infection. How are pathogen proteins directed to and transported across the host membrane? How do they act inside host cells? What are the functional host targets? To address these questions and to illuminate the processes that underpin *Phytophthora* virulence, we have adopted *Phytophthora capsici* as a model organism. Here, we will report on the progress made towards achieving our aims.

Localisation studies of a putative RxLR effector from *Saprolegnia parasitica* to haustorium-like structures

MINOR K. L.[1], DE BRUIJN I.[1], WAWRA S.[,1], PHILLIPS A.[1], ROBERTSON E.J.[1], ANDERSON V. L.[1], BAIN J.[1], SECOMBES C. J.[2], AND P. VAN WEST[1]

[1]*Aberdeen Oomycete Laboratory, University of Aberdeen, Aberdeen, Scotland, UK.*
[2] *Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, Scotland, UK.*

Saprolegnia parasitica is a fish pathogenic oomycete capable of causing disease in freshwater fish. Since the ban of malachite green, previously used to control *Saprolegnia* infections, the disease has re-emerged as a major problem for the aquaculture, where it is estimated that 10% of all hatched salmon die as a result of Saprolegniosis. As a result of this rise in *Saprolegnia* infections there is an increased need to study and understand the interaction between *S. parasitica* and its host in order to develop new control strategies.

To enable us to study the fish-*Saprolegnia* interaction we have developed an *in-vitro* infection model, where a cultured-monolayer of a primary fish cell-line (RTG-2) is infected with cysts of *S. parasitica*. We have used this cell-line to investigate the interaction between *S. parasitica* and fish with a range of molecular, microscopic and biochemical techniques. Of particular interest, we have identified the localisation of a putative RxLR protein, SpHtp1, to haustorium-like structures. In addition we have demonstrated that recombinant SpHtp1 can be translocated into fish cells. Here we present our latest findings.

Mechanism of RXLR-mediated entry of oomycete and fungal effectors into host cells

KALE S.D.[1], GU B.[1,2], CAPELLUTO D.G.S.[3], DOU D. [1†], CRONIN A. [1], ARREDONDO F. D. [1], FELDMAN[1] E., FUDAL I. [4], ROUXEL T. [4], LAWRENCE C.B. [1], SHAN W. [2] AND B.M. TYLER [1*]

[1] Virginia Bioinformatics Institute, Virginia Polytechnic Institute Blacksburg, USA ; [2] College of Plant Protection and Shaanxi Key Laboratory of Molecular Biology for Agriculture, University, Yangling, Shaanxi China ; [3] Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, USA ; [4] INRA-Bioger, Campus AgroParisTech, Thiverval-Grignon, France ; [†] Current address: Department of Plant Pathology, Nanjing Agricultural University, Nanjing China

Each year oomycetes cause millions of dollars of damage to crops and forests. Many oomycetes utilize effectors to facilitate infection during their biotrophic phase of infection. Several of these effectors have been shown to suppress inducers of cell death. These effectors enter host cells through a highly conserved N-terminus RXLR-dEER motif. Characterization of the RXLR motif by bombardment assay has shown that it tolerates amino acid substitutions at position 2 and 4. Several amino acids have been identified that can replace the conserved arginine residue at position 1 and the leucine at position 3. Using these substitutions we have identified and experimentally validated putative RXLR motifs in many well-studied and putative effectors from oomycete and fungal pathogens of both plants and animals. We show that these effectors have the ability to enter both soybean root cells and airway epithelial cells and the entry mechanism is reliant on the RXLR motif. We also show that these effectors rely on phosphoinositide binding to enter host cells. We have biochemically characterized oomycete effectors Avr1b, Avr1k and Avh5. We have recently designed a highly efficient *E. coli* expression system with which we could achieve high-level expression of soluble full length Avr1b protein, as well as Avr1k and Avh5. When Avr1b or Avr1k proteins were infiltrated on soybean leaves with the *Rps*1b or *Rps*1k genotype respectively, strong programmed cell death (PCD) was triggered. Triggering of PCD by exogenous protein was dependant on the RXLR-dEER motif in both Avr1b and Avr1k. Several types of inhibitors that can inhibit effector cell entry suppressed the cell death induced by the effector-R gene interactions.

Identifying candidate host 'targets' of *Phytophthora infestans* translocated RXLR proteins

ARMSTRONG M.[1], BOEVINK P.[2], BUKHAROVA T.[1], MC LELLAN H.[1], RAMOS M.[1,2], GILROY E.[2], PRITCHARD L.[2], WHISSON S.[2], BEYNON J.[3] AND P.R.J. BIRCH [1,2]

[1]Division of Plant Science, College of Life Sciences, University of Dundee (at SCRI), Invergowrie, Dundee DD2 5DA, UK.
[2]Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

As part of a large collaborative effort to identify key host targets of translocated RXLR effector proteins, especially those targeted by distantly related oomycete pathogens, we have screened an infected potato Y2H library with 25 *P. infestans* RXLRs. The RXLRs were prioritized on the basis of their up-regulation during the biotrophic phase, presence of orthologues in other *Phytophthora* species, and bioinformatic prediction of known protein domains. We have also transiently expressed many of these RXLRs as fluorescently tagged fusions and established their sub-cellular localization within host cells by confocal microscopy. These approaches are being extended to further investigate RXLR-target protein interactions *in planta* using bimolecular fluorescence complementation. Data on the potential host targets of RXLR effectors, and on their localization inside host cells, will be summarized and compared to provide an overview of the cellular processes RXLRs appear to have evolved to modulate.

CRN8 a *Phytophthora infestans* host-translocated effector with kinase activity

VAN DAMME M.[1], SCHORNACK S.[1], BOSKURT T.[1], CAKIR C.[2], JONES A.[1] AND S. KAMOUN[1]

[1.] The Sainsbury Laboratory, Colney Lane, NR4 7UH Norwich, UK,
[2]USDA-ARS, Purdue University, West Lafayette, IN 47907, USA

Phytophthora spp. cause some of the most destructive plant diseases in the world. The most notable species is *Phytophthora infestans*, the causal agent of late blight on potato and tomato. It it is now well established that oomycetes, such as *P. infestans*, secrete an arsenal of effector proteins that modulate plant innate immunity to enable infection. Cytoplasmic effectors from *P. infestans* and other oomycetes with a conserved RXLR motif are being studied intensively, but the Crinkler (CRN) family containing the LFLAK motif is relatively unknown. CRNs were identified by an *in planta* functional expression screen of candidate *P. infestans* secreted proteins. Similar to RXLR effectors the N-terminal region of the CRNs is dispensable for cell death induction *in planta*, indicating the presence of modular domains that are involved in distinct processes. For one of the CRNs, CRN8, the C-terminal domain has similarity to a Serine/Threonine RD kinase. CRN8 has a striking similarity to plant kinase-like proteins. This intriguing finding raises the possibility that CRN8 mimics a specific class of plant enzymes, a feature that was noted for other pathogen effectors. The CRN8 effector is translocated inside the plant cell where it targets the plant cell nucleus. We show that CRN8 has kinase activity, depending on an intact RD catalytic site, when it is expressed within the host cell. Secreted kinases have not been reported from microbial plant pathogens so far. Results obtained by a combination of genetic and biochemical methods of the CRN8 kinase effector protein will be presented. These new findings will enable us to decipher how pathogens successfully colonize and and reproduce on their host plants.

Phytophthora infestans AVR3a effector protein is a suppressor of immune responses in plants

CHAPARRO-GARCIA A., SCHORNACK S., SCHWIZER S., BOS J., AND S. KAMOUN

The Sainsbury Laboratory, Colney Lane, Norwich, NR4 7UH, UK

Phytophthora infestans, the causal agent of potato late blight, secretes effector proteins to overcome plant immunity. One of these is the cytoplasmic RxLR effector AVR3a, which suppresses the cell death induced by *P. infestans* INF1 elicitin and induces R3a-mediated resistance. Mutants of AVR3a that fail to suppress cell death such as AVR3a^{Y147}, but still activate R3a, suggest that distinct amino acids condition the effector activities. INF1 is a conserved secreted protein of oomycetes with features of pathogen-associated molecular patterns (PAMPs). Heese *et al.*, (2007) previously reported that BAK1, a co-receptor of several receptor-like kinases (RLKs), is required for INF1 triggered cell death; hence a BAK1-dependent RLK might recognize INF1. We investigated whether suppression of INF1 cell death by AVR3a is achieved via interference with BAK1-dependent RLKs. We found that transient and stable expression of AVR3a in *N. benthamiana* suppresses the production of oxidative species (ROS) mediated by BAK1-dependent pattern recognition receptors (PRRs) pathways, whereas BAK1-independent PAMP recognition pathways are not affected. Moreover, different variants of AVR3a distinctly interfere with BAK1-dependent PAMP perception of the bacterial PAMP EF-Tu-derived peptide elf18. These data suggest that AVR3a perturbs BAK1-dependent PAMP triggered plant defense responses by manipulating specific receptor pathways. Further work is under way to dissect the underlying mechanisms

Avr2, an RXLR effector from *Phytophthora infestans*

BREEN S[1,2], GILROY E.M.[1], ARMSTRONG M.R.[2], MORALES J.G.[1], HEIN I. [1], DOUGLAS E.[1], BOEVINK P.C[1]., MC LELLAN H.[2], RANDALL E.[1], ZHENDONG T.[1], AVROVA A.O.[1], PRITCHARD L.[1], LOKOSSOU A.[3], GOVERS F. [3], VAN DER VOSSEN E. [3], VLEESHOUWERS V.[3], BRUNNER F.[4], WHISSON S.C. [1], AND P.R.J. BIRCH[1,2]

1. Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK;
2. Division of Plant Sc., College of Life Sciences, Univ.y of Dundee at SCRI, Invergowrie, Dundee DD2 5DA, UK;
3. Wageningen UR - Plant Breeding, P.O. Box 386, 6700 AJ Wageningen, the Netherlands;
4. ZMBP Eberhard-Karls-Univ., Tübingen D-72076, Germany;

An important research goal in the fight against potato late blight, caused by the Oomycete *Phytophthora infestans*, is to identify pathogen effector proteins likely to be secreted during infection. These effectors are translocated into host cells to manipulate host metabolism and defence responses. Avr3a, the first effector characterized from *P. infestans*, was found to contain an N-terminal RxLR and dEER motif required for transport across the host plasma membrane. Developing genomic resources have allowed large-scale computational screening for this conserved motif to reveal approximately 500 candidate rapidly diverging *P. infestans* effectors. We have identified the RxLR-EER effector *Avr2* from the sequenced isolate t30-4, which is recognised by *R2-like* genes. Cloning *Avr2* from virulent isolates collected around the world has revealed additional alleles. These alleles evade recognition by the *R2-like* genes. The recognition of the C- and N-termini of Avr2 has also been investigated. All *Avr2* alleles and the C- and N-termini will be used to identify interacting plant host proteins using the Yeast 2-Hybrid system. The library to be screened was generated from pathogen challenged resistant and susceptible potato cultivars. Once identified, we will be using virus induced gene silencing (VIGS) to examine the function of the identified Avr2 interactors.

R3a-mediated recognition of AVR3a: cell biological and biochemical characterization

ENGELHARDT S.[1,2], ARMSTRONG M.R.[1], BOEVINK P.C.[2], RAMOS M.[1,2], GILROY E.M.[2], AND P.R.J. BIRCH[1,2]

[1]Division of Plant Science, College of Life Sciences, University of Dundee (at SCRI), Invergowrie, Dundee DD2 5DA, UK. ;
[2]Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Plants utilize resistance (R) proteins which activate effector-triggered immunity (ETI) by detecting the presence of microbial effectors either directly or indirectly. However, to date the mode-of-action of this recognition process in plant-oomycete interactions is largely obscure. The potato cytoplasmic CC-NBS-LRR resistance protein R3a detects one of two forms of the effector AVR3a from potato late blight pathogen *Phytophthora infestans*. Here we show that transient co-expression of fluorescent protein fusions in *N.benthamiana* leads to a relocalization of R3a in the presence of the recognized AVR3a form, but not in the presence of the unrecognized form. Interestingly, a direct interaction of AVR3a and R3a could not be observed using Yeast-2-Hybrid (Y2H). However, a number of AVR3a-interacting host proteins have been identified using Y2H and we are currently investigating whether any of these mediate the AVR3a-dependent relocalisation of R3a. We intend to conduct *in planta* pull-down experiments and a Yeast-2-Hybrid screen to gain more insight into putative R3a interactors. Eventually we hope to unravel the R3a-Avr3a recognition process, especially to determine whether ETI is activated after a direct R3a-Avr3a interaction.

Gain-of-virulence by naturally occurring paramutation in *Phytophthora sojae*

QUTOB D., MUTIU I., KUFLU K., AND M. GIJZEN

Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, Canada, N5V 4T3

Several avirulence (*Avr*) genes of *P. sojae* have recently been identified, including *Avr1a*, *Avr1b*, *Avr3a*, *Avr3c*, and *Avr4/6*. Each of these *Avr* genes encodes a distinct RXLR effector that triggers immunity on host plants with the complementary resistance (*Rps*) gene. Allelic analysis has shown that naturally occurring gain-of-virulence mutations of these *Avr* loci fall into to different categories, which include amino acid changes, gene deletions, 5' UTR mutations, and loss-of-transcript (gene-silenced) polymorphisms. Additionally, *Avr1a*, *Avr3a*, and *Avr3c* exist in the genome as multiple copies in tandem arrays, with *Avr1a* and *Avr3a* displaying copy number variation among *P. sojae* strains. We have been studying gene-silenced alleles of the *Avr3a* and *Avr1a* loci by performing crosses between *P. sojae* strains and following segregation in F_1 and F_2 progeny. For *Avr3a*, gene-silenced alleles display normal (Mendelian recessive) or unusual (paramutagenic) segregation patterns, depending on the cross. Gene-silenced alleles that segregate normally carry deletion mutations in the promoter region of the RXLR effector; thus, the mutation likely interferes with transcription of the gene. However, gene-silenced alleles that are paramutagenic are identical in sequence to alleles from avirulent strains expressing the gene. We also observed paramutagenic segregation patterns for gene-silenced alleles of *Avr1a*, which are likewise identical in sequence to avirulent stains expressing the gene. Thus, paramutation and epigenetic gene silencing of RXLR effector genes in *P. sojae* represents a novel and naturally occurring gain-of-virulence mechanism that aids pathogen adaptation towards its host.

The NLP effector superfamily comprises cytolytic and non-cytolytic members

KÜFNER I.[1], OECKING C.[1], BRUNNER F.[1], CABRAL A.[2], VAN DEN ACKERVEKEN G.[2], OOME S.[2], SANTHANAM P.[3], THOMMA B.[3] AND T. NÜRNBERGER[1]

[1]Center of Plant Molecular Biology, University of Tübingen, Germany.
[2]Department of Molecular and Cellular Biology, University of Utrecht, The Netherlands;
[3]Laboratory of Phytopathology, Wageningen University, The Netherlands.

Necrosis and ethylene-inducing peptide 1-like proteins (NLP) constitute a superfamily of proteins that are produced by various phytopathogenic micro-organisms, comprising both prokaryotic and eukaryotic organisms (> 250 data base entries). We have previously shown that some NLPs are conserved virulence factors that contribute to host infection of dicot, but not monocot plants by plasma membrane destruction and cytolysis. Current research addresses the molecular mode of action of cytolytic NLPs. We have shown that NLPs likely bind to lipid components of plant plasma membranes prior to membrane pore formation and subsequent cytolysis. Recent progress in identifying the host docking site for microbial NLPs will be presented.

Several lines of evidence strongly suggest that NLPs have adopted physiological functions and biochemical activities that differ from those of cytolytic toxins. These include (i) the presence of multiple NLP sequences in genomes of obligate biotrophic phytopathogens (*Hyaloperonospora arabidopsidis*), (iii) the lack of cytotoxic activity of *H. arabidopsidis* NLPs (iii) massive evolutionarily recent NLP sequence duplication and sequence diversification events in biotrophic and hemibiotrophic plant pathogens, (iv) the identification of non-cytolytic NLPs in the necrotrophic fungal vascular wilt pathogens *Verticillium dahliae* and *V. albo-atrum*, and (v) the production of cytolytic NLPs in *Mycosphaerella graminicola*, a pathogen that grows only on NLP-insensitive monocot plants. We will provide recent progress in the functional analysis of non-cytolytic NLPs and discuss their potential role in host cell adhesion.

II. Plant Immunity

Plant cell wall integrity surveillance: a novel perception system for cellulose-binding oomycete effectors?

LARROQUE M., BORDERIES G., LAFITTE C., DUMAS B. AND E. GAULIN

UMR 5546- CNRS-Université Paul Sabatier Toulouse III, Pôle de Biotechnologie Végétale, BP 42617 Auzeville, 31326, Castanet-Tolosan

The plant cell wall is targeted by microbial degrading enzymes. Wall degradation results in the release of cell wall fragments that can be detected as DAMPs (Danger Associated Molecular Patterns) inducing plant immunity. Modification of the plant cell wall could also occur during the interaction of cellulose with non-enzymatic microbial proteins harboring CBM1s (Carbohydrate-Binding Modules family 1). CBM1s were originally characterized in fungal cellulases and in non-enzymatic CBEL proteins of *Phytophthora* which play an essential role in adhesion and recognition of cellulosic substrates. CBEL-like proteins were detected in all oomycete genomes analyzed so far, including the legume pathogen *Aphanomyces euteiches*. In this species, CBEL-like proteins containing up to six associated CBM1s have been identified. CBM1s from *P. parasitica* acts as PAMPs (Pathogen Associated Molecular Pattern) by inducing plant immune responses in several plant species including *Arabidopsis* and *tobacco* and a model involving a cell-wall integrity surveillance system linked to CBM1 perception was proposed. To validate this model, a reverse-genetic approach was developed. We first evaluated activity in *A. thaliana* of a CBM1 synthetic peptide designed and a recombinant protein obtained in yeast, corresponding to a repetition of three CBM1s. Both approaches led to molecules inducing defense responses in *Arabidopsis thaliana* and in sufficient yield to engage a screening strategy. Results concerning responses obtained with RLK and cell wall mutants will be presented.

Are chitin synthases targets for antimicrobial compounds and sources of MAMPs in oomycetes?

NARS A.[1], LAFITTE C.[1], HEUX L.[2], BADREDDINE I.[1], BONO J.J.[1], DUMAS B.[1], FLIEGMANN J.[1], AND A. BOTTIN[1]

[1] UMR5546 CNRS-UPS, 24 chemin de Borde Rouge, Auzeville, F-31326 Castanet-Tolosan, France
[2] CERMAV–CNRS and Université Joseph Fourier, BP53, F-38041 Grenoble Cedex 9, France

Chitin is a crystalline *N*-Acetyl-Glucosamine (GlcNAc) polymer that is essential to cell wall function in *Fungi*, and chitooligosaccharides derived from it are Microbe-Associated Molecular Patterns recognized by LysM-containing receptors. Whereas GlcNAc is usually a minor component in the cell wall of most oomycetes, large scale sequencing shows the presence of chitin synthase (CHS) genes in their genomes. We are interested in determining the biological role of oomycete, and their involvement in the generation of signals perceived by the plant cell. Our microbial models are *Aphanomyces euteiches*, a legume root parasite, and a *Phytophthora parasitica* strain pathogenic to tobacco. Whereas chitin has never been detected in *P. parasitica*, our data suggest that its CHS gene(s) play(s) an essential role. In *A. euteiches*, we recently showed that amorphous GlcNAc polymers (chitosaccharides) are involved in cell wall function (Badreddine et al 2008). We are now engaged in characterizing these chitosaccharides in order to determine their links to the other cell wall polymers, and to understand how the host plant *Medicago truncatula* distinguishes chitosaccharide-derived fragments from other microbial signals such as symbiotic Nod factors. Our approaches include ^{13}C-NMR studies of the cell wall polysaccharides, purification of the chitosaccharides after sequential chemical and enzymatic hydrolyses, elicitation bioassays involving the measurement of reactive oxygen species and expression of defense-related genes, and genetic studies targeting candidate genes of the LysM-containing putative receptors family. Last data obtained on both the microbe and plant sides will be presented.

The lectin receptor LecRK-I.9 is a novel *Phytophthora* resistance component and a host target for the RXLR effector IPI-O

BOUWMEESTER K.[1], DE SAIN M.[1], WEIDE R.[1], CANUT H.[2], AND F. GOVERS[1]

[1]Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.
[2]Université Paul Sabatier/CNRS-, UMR5546, Castanet Tolosan, France.

Phytophthora infestans secretes many RXLR effectors, one of which is IPI-O. The RXLR motif of IPI-O is in partial overlap with an RGD cell adhesion motif (RSLRGD), which has been shown to bind LecRK-I.9, a lectin receptor kinase of Arabidopsis. LecRK-I.9 was shown to mediate the adhesions between the cell wall (CW) and plasma membrane (PM), and it has been observed that IPI-O can disrupt these adhesions through its RGD motif. Transgenic Arabidopsis lines expressing *ipiO* and Arabidopsis *LecRK-I.9* knock-out lines (*lecrk-I.9*) were analyzed in their response to pathogen infection, in particular to *Phytophthora*. Both, the *ipiO* expressing lines and *lecrk-I.9* are impaired in their resistance to oomycete pathogens. To unravel the mechanisms underlying this phenomenon we analysed callose deposition upon MAMP treatment and investigated the strength of cell wall-plasma membrane adhesions by inducing plasmolysis. The obtained results indicate that LecRK-I.9 is not only important for the maintenance of the CW-PM continuum, but also in MAMP-triggered immunity. Moreover, Arabidopsis lines with constitutive *LecRK-I.9* expression display an enhanced *Phytophthora* resistance. Our observations strongly suggest that LecRK-I.9 plays a crucial role in disease resistance and point toward involvement of the RXLR effector IPI-O in the infection process.

Phytophthora infestans RXLR effector AvrBlb2 supports host colonization by interfering with secretion of papain like cysteine protease C14

BOZKURT T.O.[1], SCHORNACK S.[1], WIN J.[1], SHINDO T.[2], JONES A.[1], VAN DER HOORN R.[2], AND S. KAMOUN

1 The Sainsbury Laboratory,Norwich Research Park, Colney, Norwich NR4 7UH, UK.
2 Max Planck Institute for Plant Breeding Research Carl-von-Linne-Weg 10 50829 Cologne, Germany

Oomycetes deliver a variety of effector proteins into plant host cells to suppress immunity and enable colonization. However, the host targets of these effectors and their role in immunity remain largely unknown. *Phytophthora infestans* RXLR-type effector AVRblb2 is an important translocated effector because it is conserved across *P. infestans* isolates and recognized by the broad-spectrum resistance protein Rpi-blb2. Therefore, we decided to investigate how AVRblb2 contributes to host colonization and modifies defense responses. First, we challenged transgenic *Nicotiana benthamiana* plants stably expressing AVRblb2 with *P. infestans*. We found that *P. infestans* establishes better colonization on plants that express AVRblb2 when compared to control plants. Next, we searched for host targets of AVRblb2. We used an *in planta* co-immunoprecipitation (co-IP) procedure based on transient overexpression of epitope-tagged AVRblb2 in *N. benthamiana*. By using LC-MS/MS, we identified the papain-like cysteine protease C14 as a candidate target of AVRblb2. C14 was previously reported to localize into vesicles, vacuole and apoplast of plants. We discovered that expression of *Avrblb2* in *N. benthamiana* plants alters the subcellular localization of C14 by blocking its secretion into the apoplast. Furthermore, RNAi silencing of C14 in *N. benthamiana* resulted in enhanced susceptibility to *P. infestans* whereas transient C14 overexpression reduced colonization. Currently, we focus on identifying the contribution of C14 to disease resistance and plant innate immunity and how AVRblb2 affects this.

Membrane-associated RXLR effectors of *Phytophthora infestans* alter localisation of plant receptor-like kinases and affect plant immunity

SCHORNACK S., CHAPARRO-GARCIA A., CANO L., SCHWIZER S., AND S. KAMOUN

Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH, UK

The potato late blight pathogen *Phytophthora infestans* secretes RXLR-type effectors that are translocated inside host cells where they modulate plant immunity. Basal plant immunity is mediated by membrane-integral receptor-like kinases (RLK) that perceive non-self pathogen-associated molecular patterns (PAMPs) to initiate a defense response.
This work aims at identification of RXLR effectors that target host cell membrane structures and to characterise their impact on alteration of basal immunity. We applied transient and stable *Agrobacterium tumefaciens*-mediated expression in *Nicotiana benthamiana* to analyse the localisation of RXLR effectors and their effect on *P. infestans* susceptibility and PAMP-triggered immunity.
We found that overexpression of selected RXLR effectors confers enhanced susceptibility towards *P. infestans* infection. Some of these effectors associated with endomembrane compartments and/or showed focal accumulation at haustorial sites suggesting interference with the host machinery for secretion, maturation and quality control of secreted and membrane-integral proteins. Interference with the function of membrane-integral PAMP receptors is further supported by attenuated production of reactive oxygen species (ROS) upon expression of specific RXLR effectors. Importantly, we found that some *P. infestans* effectors are able to alter the cellular localisation of specific RLKs involved in PAMP-triggered immunity. Further research aims at defining the target specificity and to elucidate the molecular basis of altered RLK localisation.

Identification of *Hyaloperonospora arabidopsidis* RxLR-(EER) effectors suppressing PAMP-triggered immunity in *Arabidopsis*

FRAITURE M.[1], JÄNTSCH C.[1], CABRAL A.[2], YATUSEVICH R.[3], VAN DEN ACKERVEKEN G.[2], PARKER J. E.[3] AND F. BRUNNER[1]

[1] Department of Plant Biochemistry, Center for Plant Molecular Biology, University of Tübingen, D-72076 Tübingen, Germany
[2] Plant-Microbe Interactions, Department of Biology, Utrecht University, 3584 CH Utrecht, The Netherlands
[3] Department of Plant Microbe Interactions, Max-Planck Institute for Plant Breeding Research, D-50829 Köln, Germany

The oomycete *Hyaloperonospora arabidopsidis* causes downy mildew in *Arabidopsis*. Its genome encodes a large number of host-targeting RxLR-(EER) proteins that are thought to manipulate host cellular activities to the benefit of the pathogen. One possible virulence function would be the interference with plant immunity initiated by the recognition of invariant microbial structures, the so-called Pathogen-Associated Molecular Patterns (PAMPs). To identify the repertoire of *H. arabidopsidis* RxLR-(EER) effectors that suppress PAMP-triggered immunity, we use an *Arabidopsis* protoplast-based system to monitor the early gene expression response induced upon PAMP recognition. Protoplasts that transiently express candidate RxLR-(EER) effectors are tested for their responsiveness to flagellin (flg22) treatment. The enzymatic activity of luciferase, whose expression is under the control of the PAMP-inducible promoter *pFRK1,* serves as a read-out. This reporter assay allows fast pathogen-independent effector screening in a simplified *in vivo* background. The effector function and molecular target of RxLR23, revealed in this screen, is currently being investigated.

Identifying effectors from Albugo candida by high throughput screening

BORHAN H., UTERMARK J., BEYNON E., LINKS M., SHARPE A.*, HEGEDUS D.

*Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK., Canada;. * NRC-Plant Biotechnology Institute, Saskatoon, SK., Canada.*

Albugo candida is the causal agent of white rust disease of Brassica species. Several *A. candida* races including *A. candida* race 2 (Ac2) and race 7 (Ac7) which are economically important pathogen of Brassica crops also infect *Arabidopsis thaliana*. We have cloned *WRR4*, a TIR-NB-LRR type resistnce gene, from Arabidopsis accession Col. *WRR4* confers resistance in Arabidopsis and Brassica to Ac2V and Ac7 as well as several other Albugo isolates. We have sequenced the genome of Ac2V and identified potential RXLR type effectors among the predicted secreted proteins. High throughput transient assay in tobacco has proved function for several Ac2V effectors. Results of functional analysis of one of these effectors and its potential host targets identified by Y2H will be presented

Hyaloperonospora arabidopsidis (Hpa) candidate effectors suppress PTI enhancing plant susceptibility

FABRO G.*, J.D.G. JONES, THE SAINSBURY LABORATORY AND COLLABORATORS FROM THE ERA-PG EFFECTOROMICS CONSORTIUM

England: Mary Coates, Laura Baxter, Jens Steinbrenner, Jim Beynon, Warwick University
Germany: Shinpei Katou, Jaqueline Bautor, Jane Parker, Max-Planck Institute of Plant Breeding Research
Netherlands: Adriana Cabral, Guido van den Ackerveken, Utrecht University

We are focusing our studies in a set of candidate effectors from the oomycete *Hyaloperonospora arabidopsidis* (*Hpa*). This pathogen is an obligate biotroph of *A. thaliana* that is proposed uses a wide range of effectors to gain access to resources available in its host. At the same time is able to avoid and/or suppress host defence responses, keeping the tissues alive until it completes its asexual and sexual reproductions. We have found that pre-induction of strong PTI responses impairs the growth and reproduction of different *Hpa* races on compatible *A. thaliana* Ecotypes. In contrast, *Hpa* is able to actively suppress PTI. We observed that plant tissues pre-infected with different *Hpa* races are less responsive, regarding levels of ROS burst and callose deposition, to bacterial and fungal PAMPs. In a collaborative effort with the ERA-PG Effectoromics consortium we have indentified and cloned several candidate effectors (small secreted proteins containing a signal peptide and RxLR motif). Taking advantage of a Heterologous (EDV) System that allow us to deliver one effector at the time via the *Pseudomonas syringae* (Pst) TTSS (Sohn et al., Plant Cell 2007) we have assessed individually if a given *Hpa* effector could suppress PAMP triggered immunity (PTI). Our results indicate that around 50% of the effectors can suppress callose accumulation when delivered through the Pst ΔCEL mutant. Similarly, when we generated transgenic plants expressing constitutively 10 different Hpa effectors, we found in 7 out of 10 cases that these plants show reduced ROS burst and callose deposition in response to flg22. These transgenics are also more susceptible to compatible *Hpa* races and to different *P. syringae* strains infections.

Using knowledge of RXLR effectors to seek durable resistance

GILROY E.M[1]., HEIN I[1]., MC LELLAN H[1,2]., BREEN S[1]., ENGELHARDT S[1]., TIAN Z[1]., SEMAN, ZA[1]., PRITCHARD L[1]., ARMSTRONG, M[1,2]., RANDALL, E[1]., AVROVA A.O[1]., COOKE, D[1]., LEES, A[1]., VLEESHOUWERS VGAA[3]., VAN WEST P[4]., WHISSON S.C[1]., AND P.R.J. BIRCH[1,2]

[1]Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, uk,
[2]Division of Plant Sciences, College of Life Sciences, Univ. of Dundee at SCRI, Invergowrie, Dundee DD2 5DA, UK,
[3]Wageningen UR - Plant Breeding, P.O. Box 386, 6700 AJ Wageningen, The Netherlands
[4]Institute of Medical Sciences, Forresterhill, Univ. of Aberdeen, Aberdeen, AB25-2ZD, UK

165 years since the Irish Potato famine, *Phytophthora infestans* genotypes are still rapidly evolving to overcome resistances we currently deploy in potato fields. An influencing factor in these cultivar's disappointing performances is a failure to take account of the role and variability of the targeted effector/avirulence protein in populations of the pathogen. An important research goal in the fight against potato blight is to identify pathogen effector proteins likely to be secreted during infection and translocated into host cells to manipulate host metabolism and defence responses. The first effectors cloned from Oomycetes were found to contain an N-terminal RxLR and dEER motif, since demonstrated to be required for transport across the host plasma membrane. The recently published *P. infestans* genome sequence has allowed large-scale prediction of RXLR effector genes revealing around 500 candidate rapidly diverging effectors, the majority of which are located in expansive regions of the genome, rich in mobile genetic elements. This may give *P. infestans* significant power to rearrange its genome, accelerating its ability to evolve effectors that evade detection. The strategy of our project is to use knowledge of RXLR effectors to predict durable resistance. We identify those RXLR effectors expressed during infection and examine how conserved they are in *P. infestans* populations. We have identified RXLRs that are essential for virulence through silencing, and which are thus less likely to be lost if targeted by a resistance mechanism in the host. Essential, conserved RXLRs are then screened in cultivated and wild potato species to identify recognition by novel resistances.

Genetic mapping of broad resistance to downy mildew in *Arabidopsis thaliana* C24

LAPIN D.[1], MEYER R.C.[2] AND G. VAN DEN ACKERVEKEN[1]

[1] Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8 3584 CH Utrecht The Netherlands; e-mail address: D.Lapin@uu.nl
[2]Heterosis, Department of Molecular Genetics, Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466 Gatersleben, Germany

The downy mildew oomycete *Hyaloperonospora arabidopsidis* is an obligate biotrophic pathogen of *Arabidopsis*. Broad resistance to all tested isolates of this pathogen was identified in *Arabidopsis* accession C24. Downy mildew growth is clearly reduced in C24 at the level of haustorium formation and intercellular hyphal development resulting in the absence of sporulation. Different isolates of *H. arabidopsidis* cause diverse immune responses in C24 from strong HR-like reactions to the suppression of conidiophore formation. The attenuation of hyphal growth appears not to be correlated with HR development. Disease resistance in C24 is genetically complex. To identify the genetic basis of the resistance we take advantage of QTL mapping using recombinant inbred lines and near-isogenic lines derived from reciprocal crosses between C24 and Col-0. Quantitative assessment of pathogen growth and development is performed by three methods: (i) quantification of formed haustoria at 2 days post inoculation (dpi), (ii) DNA-based quantification with qPCR using TaqMan chemistry and (iii) appearance of conidiophores at 5 dpi. In parallel we have initiated a back-cross approach in which we introgress susceptibility loci from Col-0 into the C24 background, and are genetically analyzing other *Arabidopsis* accessions with a similar type of resistance. These different approaches to identify the molecular mechanisms underlying C24 resistance may reveal novel aspects of plant immunity or host genes involved in susceptibility to downy mildew.

Towards the characterization of a Quantitative Resistance to Downy Mildew in cultivated Sunflower, *Helianthus annuus*

AS-SADI F., POUILLY N., BONIFACE M-C., BORDAT A., BELLEC A.[1], HELMSTETTER N.[1], VAUTRIN S.[1], BERGES H.[1], TOURVIEILLE de LABROUHE D.[2], VEAR F.[2], VINCOURT P. AND L. GODIARD

Laboratoire des Interactions Plantes Micro-organismes (LIPM), INRA-CNRS, Castanet -Tolosan, France
[1]Centre National de Ressources Génomiques Végétales (CNRGV), INRA, Castanet - Tolosan, France
[2]UMR 1095 INRA-Université Blaise Pascal, Domaine de Crouelle, Clermont-Ferrand, France

Quantitative resistance to sunflower Downy Mildew caused by the oomycete *Plasmopara halstedii* was studied on a population of recombinant inbred lines (RIL) and on a F4 population, not carrying efficient major resistance gene, in fields naturally infested by one race of the pathogen (703 or 710) and in growth chamber (710). The major quantitative trait locus (QTL) localized on linkage group 10 explains almost 40% of variation, and is not linked to any of the known race-specific resistance genes called *Pl* genes. This QTL support interval is currently 1.5 cM long. We constructed and screened a BAC library of the RIL parent (XRQ) having the QTL with the closest genetic markers in order to build a BAC contig in the QTL region, a first step towards the positional cloning strategy. The polymorphic BAC ends are currently being used as new genetic markers on the RIL and F4 population. The evaluation of the resistant phenotypes of such recombinant plants may help restricting the QTL support interval. In order to characterize the expressed genes during the interaction from both partners, plant and oomycete, we performed a cDNA sequencing approach of infected sunflower plantlets using the 454® sequencing method. A database was created and used to identify 60 new *P. halstedii* sequences. Sequence polymorphism between races of *P.halstedii* was searched to identify new markers. Putative effectors having RXLR or Crinkler domains were also characterized.

Why brown algae may not have resistance genes, and proteomics of the interaction between *Ectocarpus siliculosus* and the oomycete pathogen *Eurychasma dickosnii*

GACHON C.M.M.[1], STRITTMATTER M.[1], ZAMBOUNIS A.[2], ELIAS M.[3], VAN WEST P.[4] AND F. C KÜPPER [1]

[1] Scottish Association for Marine S., Dunstaffnage Marine Lab., Oban United Kingdom;
[2] Univ. of Thessaly, School of Agricultural Sc., Department of Ichthyology and Aquatic Environment, Volos, Greece;
[3] Charles Univ. in Prague, Faculty of Science, Department of Botany and Department of Parasitology, Benatska 2 Prague, Czech Republic;
[4] Scottish Association for Marine Science, Dunstaffnage Marine Lab., Oban United Kingdom.

The intracellular, obligate-biotrophic pathogen *Eurychasma dicksonii* is the most widespread eukaryotic pathogen of marine brown algae, and also the most basal member of the oomycete lineage. This algal parasite has the broadest host range described so far for marine pathogens and occurs worldwide in cold and temperate waters. Currently, nothing is known about the molecular biology of the interaction between this generalist pathogen and its brown algal host. We will present the first results of a proteomic investigation of the susceptible interaction between *Ectocarpus siliculosus* CCAP 1310/4 and *Eurychasma* CCAP 4018/1 via two-dimensional electrophoresis.

Moreover, we will report on the annotation of candidate defense genes in the genome of *Ectocarpus*. We identified two families of candidate pathogen receptors, namely some LRR-GTPases of the ROCO family, and 24 NB-ARC-TPR proteins. They exhibit high birth and death rates, whilst diversifying selection is acting on their LRR (respectively TPR) domain, probably affecting ligand-binding specificities. Remarkably, each repeat is encoded by an exon, and intense exon shuffling underpins the variability of LRR and TPR domains. We conclude that the *Ectocarpus* LRR-ROCO and NB-ARC-TPR families are excellent candidates for being involved in recognition / transduction events linked to immunity. We further hypothesize that brown algae may not strictly rely on resistance genes for pathogen recognition, but rather might generate their immune repertoire *via* controlled somatic recombination comparable to the cassette mechanisms underpinning vertebrate adaptive immunity.

Screening of *Medicago truncatula* symbiotic mutants with *Aphanomyces euteiches* revealed molecular crosstalks between symbiotic interaction and resistance to pathogens

REY T.[1], GOUGH C.[2], CARBONNE F.[1], DUMAS B.[1], AND C. JACQUET[1]

[1] UMR5546, Pôle de biotechnologie Végétale, 24 chemin de Borde Rouge 31326 Castanet-Tolosan, France ;
[2] LIPM, UMR 441-2594 -INRA-CNRS BP 52627 chemin de Borde Rouge - Auzeville 31326 Castanet Tolosan France

Interactions between plants and soil-born microorganisms have a crucial impact on ecosystems and crop productivity, as they may lead to parasitism or symbiosis depending on the nature of the interacting microorganism. During the last few years, several results obtained in different plant species underlined structural and biochemical similarities between microbial signals and their plant receptors that are required either for symbiosis establishment or for plant defence activation. This is the case for the Nod factors produced by soil-bacteria called Rhizobia which establish symbiotic interactions with Legumes. Nod factors display a chitosaccharidic moiety and their perception involved LysM receptors, such as the *Medicago truncatula* (*M.t.*) protein NFP. LysM receptors have been also identified as key components of the perception of chitin fragments which are considered as Pathogen Associated Molecular Patterns (PAMPs). This raises the question of how plants are able to distinguish friends and foes and whether molecular crosstalk is involved in mechanisms leading to rejection or acceptance of the microbe. To answer these issues, the model legume *Medicago truncatula (M.t.)* was particularly well suited as i) a collection of mutants impaired in symbiotic interactions was available in the genetic background of the A17 *M t.* reference line and ii) A17 was shown to be resistant to the root oomycete *Aphanomyces euteiches*. The screening of eleven *M. t* symbiosis mutants upon *A. euteiches* infection showed that some of them displayed a significantly altered level of resistance to this oomycete compared to the A17 WT. We will focus the presentation on the cytological, transcriptomic and genetic analyses that were performed on the *nfp* and *hap2a* mutants that are respectively more susceptible and more resistant than WT line. The novel functions for these two genes as components of plant resistance will be discussed.

A 74 kDa immunodominant antigen of the pathogenic Oomycete *Pythium insidiosum* is a putative exo-1,3-ß glucanase

KRAJAEJUN T.[1,4], KEERATIJARUT A.[1,4], SRIWANICHRAK K.[1], LOWHNOO T.[2], RUJIRAWAT T.[2], PETCHTHONG T.[2], YINGYONG W.[2], KALAMBAHETI T.[3], SMITHIPAT N.[4], JUTHAYOTHIN T.[4], AND T. D. SULLIVAN

[1]Department of Pathology and [2]Research Center, Faculty of Medicine, Ramathibodi Hospital, [3]Department of Microbiology and Immunology, Faculty of Tropical medicine, and [4]Molecular Medicine Program, Faculty of Science, Mahidol University, Bangkok; [4]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, Thailand; [5]Department of Pediatrics, School of Medicine and Public Health University of Wisconsin, Madison, Wisconsin, USA

The oomycetous, fungus-like, aquatic organism *Pythium insidiosum* is the causative agent of pythiosis, a life-threatening infectious disease of humans and animals, living in tropical and subtropical areas of the world. Common sites of infection are the arteries, eyes, cutaneous/subcutaneous tissue and the gastrointestinal tract. Diagnosis of pythiosis is time-consuming and difficult. Radical excision of infected organs is the main treatment for pythiosis because conventional antifungal drugs are ineffective. An immunotherapeutic vaccine prepared from *P. insidiosum* crude extract showed limited efficacy in the treatment of pythiosis patients. Many pythiosis patients suffer life-long disabilities or die from an advanced infection. Recently, we identified a 74-kDa major immunodominant antigen of *P. insidiosum*, which could be a target for development of a more effective serodiagnostic test and vaccines. Mass spectrometric analysis, identified two peptides (s74-1 and s74-2) of the 74-kDa antigen, which perfectly matched a putative exo-1,3-ß glucanase (EXO1) of *Phytophthora infestans*. Using degenerate primers, derived from these peptides, a 1.1-kb product was produced by PCR and its sequence found to be homologous to the *P. infestans* exo-1,3-ß glucanase gene, *EXO1*. ELISAs targeting the s74-1 and s74-2 synthetic peptides demonstrated that the 74-kDa antigen was highly immunoreactive to pythiosis sera, but not to control sera. Phylogenetic analysis using part of the 74-kDa protein coding sequence divided 22 Thai isolates of *P insidiosum* into two clades. Further characterization of the putative *P. insidiosum* glucanase could lead to new diagnostic tests, antimicrobial agents and vaccines for the prevention and management of the serious and life-threatening disease of pythiosis.

III. Reproduction, Evolution and Epidemiology

A new phylogenetic lineage in the genus *Aphanomyces* (Oomycetes) comprising thermophilic strains isolated from the ecuatorian Amazonia

BETANCOURT CARGUA L.[1]; MARTÍN M.P. [2] AND J. DIÉGUEZ-URIBEONDO[2]

1. Universidad Internaciona, Menéndez Pelayo, Madrid, Spain.
2. Departamento de Micología, Real Jardín Botánico CSIC, Plaza Murillo 2, 28014, Madrid, Spain.

The phylogenetic position and temperature-developmental biology relationships of two strains obtained from river Napo and ponds of the Ecuadorian Amazonic region of Jatun Sacha (Ecuador) were studied. Their phylogenetic position was studied based on ITS sequences of rDNA of 12 species of the genus *Aphanomyces* de Bary (Oomycetes) available at the GenBank, and the analysis was based on a recent alignment for sequences of this genus available at TreeBase. The two strains isolated were asexual and had termophilic properties since it could grow, sporulate and their zoospores were motile at 45ºC. These strains were physiologically adapted to warm environments and their optimal temperature for growth, sporulation, and zoospore swimming was characteristic of warm environments. Phylogenetic analysis showed that these isolates constitute a new lineage in *Aphanomyces* that differs from the animal parasitic, plant parasitic lineages, and saprobiotic lineages previously described (Diéguez-Uribeondo et al., 2009). The analysis also showed that these strains clustered with one sequence isolated from catfish in North America. This study represents the first description of a different evolutionary lineage within *Aphanomyces*, the first isolation of a tropical *Aphanomyces* and an example of physiological adaptation to warm habitats within this genus.

Invasion history of grapevine downy mildew (*Plasmopara viticola*): a population genetics perspective

DELMOTTE F.[1], LOUVET G.[1], RICHARD-CERVERA S.[1], MESTRE P.[2], SCHILDER A.[3], AUSTERLITZ F.[4], AND M.C. FONTAINE[4]

[1] INRA, UMR 1065 SV, Institut des Sc. de la Vigne et du Vin, Centre de Recherches Bordeaux-Aquitaine, Bordeaux, France;
[2] INRA, UMR 1131 SVQV, Colmar, France;
[3] Michigan State University, Plant Pathology Department,East Lansing, USA ;
[4] ESE - Ecologie, Evolution et Systématique, CNRS/Université Paris-Sud XI, 91405 Orsay, France

Grapevine (*Vitis vinifera*, L.) is cultivated worldwide mainly for the production of wine, juice and fresh fruits. However, viticulture is threatened by numerous pathogens, most of them being introduced by human activities. The Oomycete *Plasmopara viticola* (Berkl. and Curt.) Berl. and de Toni., causal agent of grapevine downy mildew, is an obligate biotrophic pathogen that attacks numerous species of the *Vitaceae*. *Plasmopara viticola* is a native species from North America that was introduced accidently into Europe in the late 1870s. This introduction was an indirect consequence of the previous invasion by the devastating root-feeding aphid phylloxera, which imposed the grafting of European varieties on to phylloxera-resistant rootstocks imported from North America that probably contained downy mildew inoculums. The disease spread rapidly through most of continental Europe to become the most important vineyard diseases. Since then, grapevine downy mildew has expanded all over wine producing regions worldwide: it was officially reported for the first time in 1907 in the Eastern Cape Province of South Africa and in South America, in 1917 in Australia, and in 1926 in New Zealand.

We assessed the genealogical history of *P. viticola* using seven microsatellite markers, partial sequences from two nuclear genes (β-tubulin, 28S) and a mitochondrial gene (cyt*b*) from more than 600 isolates collected in North American, European (France, Spain, Italy, Germany, Switzerland, Austria, Hungary, Czech Republic, Romania, Greece) as well as in Australian and South African vineyards. Using population genetics and phylogenetics tools, we addressed the following questions related to the invasion history of *P. viticola* worldwide: what is the level of genetic diversity of *P. viticola* in its native range, i.e North America? From which populations in this native range originate the inoculums of *P. viticola* that were introduced in France in 1878? How many independent introductions occurred in Europe and is there a colonization gradient into the European vineyards? Did the subsequent introductions into new world wine producing areas (in particular in Australia and South Africa) result from a primary introduction from North America or a secondary introduction from European *P. viticola* populations?

Phytophthora infestans Tunisian isolates show a high genotypic diversity as revealed by a SSR multiplex PCR technique using 12 SSR markers

HARBAOUI K.[1], VAN GENT-PELZER M.[2], LI Y.[4], VLEESHOUWERS V.[3], HAMADA W.[1], AND VAN DER LEE T.[2]

[1] Genetics and Plant Breeding, National Institute of Agronomy, Tunis, Tunisia ; [2] Plant Research International B.V., UR Biointeraction &Plant Health, Wageningen, The Netherlands ; [3] UR Plant Breeding, Wageningen, The Netherlands ; [4] Institute of Vegetables and Flowers (IVF), CAAS, Beijing, China

In Tunisia late blight caused by *Phytophthora infestans* a serious threat to potato or tomato every day of the year. Weather conditions can be favourable in all seasons and the host crops tomato and potato are grown year round. Potato is planted and harvested in three overlapping intervals from August to June and tomato is grown both in open field and in greenhouses. Little is known about the consequences on the epidemiology and genetic variation of this pathogen in Tunisia and in North Africa. We collected 159 *Phytophthora infestans* isolates, from Northern West, North, Northern East and coastal zone of Tunisia between 2006 and 2008, and determined the genotypic diversity in comparison to reference isolates. Twelve SSR markers are used in this study in a single multiplex PCR. These SSR markers were developed based on *P. infestans* genome sequencing and integrated with previously available SSR markers (Li et al. 2010; Li et al unpublished). In addition we determined the mating type, the mitochondrial haplotype and (a) virulence on a differential set of potato plants that carry the resistance genes originating from *Solanum demissum*. Within Tunisia a large genotypic variation was found and isolates could be grouped in four major clusters. We also identified a clonal line that is dominant in several regions. This study is the first step to investigate in the genetic characteristics including the importance of the sexual cycle and the spread of clones by infected tubers in Tunisia.

Study of invasive French populations (2006-2008) of *Phytophthora infestans*, the oomycete causing potato late blight

MAGALON H., BOULARD F., GLAIS I., ANDRIVON D., AND R. CORBIERE

UMR BiO3P 1099, INRA/Agrocampus/Université Rennes 1, Domaine de la Motte, BP 35327, 35653 Le Rheu Cedex, France

The pathogen causing potato late blight, *Phytophthora infestans*, is a heterothallic oomycete able to reproduce sexually when its two mating types (named A1 and A2) meet. Since 2003, France is the site of a rapid expansion of A2 isolates in detriment of A1. In order to better understand the population dynamics of this pathogen and to assess the impact of sexual reproduction on the evolution of its populations, we explored the phenotypic (virulence and resistance to fungicide) and genotypic (12 microsatellite loci) variations of 480 isolates collected over three years (2006-2008) from three different potato production areas (Brittany, North, Center)) in France.

The A2 isolates exhibited virulence profiles more complex than those of A1. Moreover, the frequency of isolates resistant to phenylamide fungicides was greater for A2 isolates than for A1. These traits, possibly adaptive, might explain in part the expansion of A2 in France. Populations showed some genetic differentiation between mating types. Assignment tests will help us to detect recombining genotypes, which can play a key role in the development of epidemics and in the sustainable management of plant resistance.

Ongoing change in populations of *Phytophthora infestans* in the GB potato crop

COOKE D., LEES A., AND P. BIRCH

SCRI, Invergowrie, Dundee, DD2 5DA, Scotland, UK

Several thousand isolates of *P. infestans* have been collected from GB potato crops over the past 5 seasons as part of a Potato Council funded survey. Genetic fingerprinting using SSRs has revealed a dramatic shift in the population with a more aggressive A2 mating type clonal lineage (genotype 13_A2) now dominating. This lineage was first recovered in the UK in 2005 and has spread to all potato growing regions in the UK. Hyper-variable SSR loci have enabled the discrimination of sub-groups within this clonal lineage. Regional differences in the distribution of these sub-groups are apparent and reveal patterns of local and long-distance inoculum spread. The increase in the A2 mating type has, in theory, increased the risk of sexual recombination within the GB *P. infestans* population. However, in contrast to some other potato growing regions in Europe, SSR fingerprint data suggest that sexual recombination is uncommon in the UK. To date, the rare recombinants have remained at a low frequency suggesting that they are less fit and aggressive than existing clonal lineages. Understanding the factors that determine the evolutionary trajectory of these *P. infestans* populations is important in planning future management strategies. Studies on effector diversity and ploidy will be presented in this light.

Genes flow in populations of *Bremia lactucae,* the causal agent of downy mildew of lettuce, in France

VALADE R.[1], MAISONNEUVE C.[2], AND C. NEEMA[1]

[1]AgroParisTech, UMR BIOGER-CPP, Thiverval Grignon, 78850, France
[2] INRA, GAFL Domaine St Maurice, 84143 Montfavet cedex, France

Bremia lactucae, the causal agent of downy mildew on *Lactuca sativa*, is an important economic problem for lettuce crops. Breeders used genetic control to limit damages of this pathogen and more particularly specific resistances. However, under selection pressure, *B. lactucae* populations showed a rapid adaption to host resistances. A better knowledge is necessary to understand this rapid virulence evolution. A study was initiated to evaluate genetic diversity of this biotrophic, heterothallic and diploid pathogen, and to determine the impact of recombination and migration on the structure of pathogen populations. More than four hundred isolates were recovered in France from different regions and different varieties (carrying different resistance genes) from *L. sativa* and *L.* serriola which is a wild host species. More than one hundred isolates were characterized for their virulence. Neutral markers, microsatellites, were developed to assess population structure and gene flow in french populations of *Bremia*. Moreover, polymorphism in RxLR candidate genes was evaluated in relation with genotypic and phenotypic diversity. Results showed an important polymorphism for virulence in *B. lactucae* french populations and the occurrence of gene flow between populations and between the wild and crop pathosystems. These results will be discussed regarding durable resistance management.

The utility of Genome Survey Sequences in developing anonymous loci for population-level studies in *Phytophthora* and *Pythium*

BLAIR J.E. AND A.G. STAFFARONI

Department of Biology, Franklin & Marshall College, Lancaster, PA, USA

Genome Survey Sequences (GSS) are random clone libraries that represent a "snapshot" of an organism's genome. GSS libraries have several advantages over traditional cDNA libraries; sheared genomic DNA is used as the source material (as opposed to mRNA), so no special conditions are needed to obtain high quality nucleic acids. GSS data also contain both coding and non-coding sequences, which allows for the identification of simple sequence repeats (i.e., microsatellites) and other anonymous loci that are important for species- and population-level studies. In this project, we have generated GSS libraries for three species of floral pathogens in the genus *Phytophthora*; *P. cactorum*, *P. nicotianae*, and *P. cryptogea*. Genomic DNA was extracted from one isolate per species, sheared, cloned into a standard vector, and sequenced. Approximately 1150 sequences were generated (~1.4 million basepairs) for each of the three isolates; putative microsatellites were identified in each dataset, and primers were designed for PCR amplification from a collection of isolates representing both geographic and host diversity for each species. We are currently verifying the applicability of approximately 15 loci per species using both agarose gel electrophoresis and capillary electrophoresis on an Agilent 2100 BioAnalyzer. Additional GSS libraries are being planned for other Oomycetes, including species of *Pythium*.

Evolution of oomycete flagella

ROBIDEAU G.P.[1,2], RINTOUL T.L.[2], AND C.A. LÉVESQUE[2,1]

[1] Department of Biology, Nesbitt Building, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6
[2] Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada, K.W. Neatby bldg., 960 Carling Ave. / Edifice K.W. Neatby, 960 avenue Carling Ottawa, ON, Canada K1A 0C6

The kingdom Straminipila (Stramenopiles) is defined by the possession of an ornamented "straminipilous" or "tinsel" flagellum. With flagella being central to the classification of these organisms, it seems quite natural then, that the genes controlling flagellar structure and motility should be able to provide some very interesting insight into the evolution of different species and genera of oomycetes. Ultrastructural studies have already revealed the connection between flagellar apparatus morphology and phylogeny of zoosporic fungi. This concept has now been explored at the molecular level, with DNA sequencing of the axoneme central apparatus gene (ACA) and a tinsel flagellum tubular mastigoneme gene (OCM1) from a variety of Peronosporales (eg. *Pythium, Phytophthora*) and Saprolegniales (eg. *Saprolegnia, Aphanomyces*) species using degenerate primers. Recent sequencing and annotation of the entire *Pythium ultimum* var. *ultimum* genome has also provided new insight into a small family of putative proteins with similarity to the well studied tinsel flagellum mastigoneme proteins of *Ochromonas danica.* The evolution of this protein family in *Pythium* and *Phytophthora* is discussed.

Conservation of regulatory networks in the *Phytophthora sojae* genome

MORRIS P. F., TORTO-ALIBO T., TRIPATHY S., ARREDONDO F., VANDUZEN N., SCHMUCKER A., AND B.M. TYLER

Biological Sciences, Bowling Green State University, Bowling Green OH, 43403 (PFM, NV, & AS) ; Virginia Biofinformatics, Virginia Tech, Blacksburg, VA 24061(TTA, ST, FA, BMT)

Computational predictions of protein-protein interactions have been used to predict associations that are conserved across the major eukaryotic lineages of plants, animals and fungi. These predictions rely on identifying orthologous pairs of proteins that have been experimentally verified to predict protein-protein interactions in other organisms. We first used the ortholog search strategy of reciprocal smallest distance to identify conserved orthologs in *Phytophthora sojae* with respect to the *A. thaliana*, human and S. *cerevisiae* genomes. Conserved orthologs were then used to identify ortholog pairs in the predicted *A. thaliana* and *S. cerevisae* interactomes. This strategy captured 14,235 of 72,266 interactions in the *A. thaliana* interactome and 13,298 of 53,301 interactions from the *S. cerevisiae* interactome. Expression data from 9039 genes in 42 microarray experiments of germinating zoospores and mycelia has also been used to compare the co-expression of ortholog pairs from the predicted interactome. Co-expression analysis of P sojae genes with orthologs to only the *A. thaliana* genome, revealed more than 9300 predicted interactions with a Pearson correlation coefficient greater than R=0.7. However only 46 ortholog pairs were also part of the set of 14,235 interactions captured from the A*rabidopsis* interactome. While genes can be components of regulatory networks and not have high levels of co-expression, the low number of co-expressed genes that are also part of the predicted interactome suggest that no unique regulatory networks from the endosymbiont ancestral génome have been conserved in oomycete genomes.

Comparative and functional genomics of oomycete infection

TYLER, B.M.[1], TRIPATHY S.[1], KALE S.D.[1], ZHOU L.[1], FERREIRA A.[1], DOU D.[1,2], ARREDONDO F.D.[1], WANG Q.[2], HAN C.[2], GU B.[1,4], ANDERSON R.[1], HANLON R.[1], WANG X.[2], YU X.[2], LIU T.[2], YAO Y.[2], WANG X.[2], DONG S.[2], ZHANG Z.[2], ZHENG X.[2], SHAN W.[4], WANG Y.[2] AND J. MCDOWELL[1]

[1] *Virginia Polytechnic Institute and State University, USA;*
[2] *Nanjing Agricultural University, China;*
[3] *Ohio State University, USA;*
[4] *Northwest Agricultural and Forestry University, China*

Oomycete genome sequences have been developed so far for *Phytophthora sojae, P. ramorum, P. infestans, P. capsici, Hyaloperonospora arabidopsidis, Pythium ultimum and Saprolegnia parasitica,* and next generation (454 and Illumina) survey sequences of *P. phaseoli, P. mirabilis, P. andina, Peronospora tabacina, Pseudoperonospora cubensis, Bremia lactucae,* and *Albugo candida* have been produced. We have also compared the genome sequences of the four major genotypes of *P. sojae* using 454 pyrosequencing. Comparisons among these genome sequences have identified large numbers of rapidly evolving genes, including toxin and effector genes that are likely involved in the interaction with host plants. Closer examination of the sequences has revealed highly conserved members of effector families that may play key roles in infection. We have also used transcriptional profiling, including both Affymetrix GeneChips and ABI Solid™ sequence tags, to identify *P. sojae* genes that may play an active role in promoting infection,. Transcriptional profiling revealed that very large numbers of pathogen mRNAs change in level during infection. Combining information about the transcriptional program of *P. sojae* effector gene expression with high throughput functional screens for plant defense suppression has revealed a coordinated interplay among the effectors to maximize defense suppression.

Comparative genome analysis of a strain from the UK blue 13 clonal lineage of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes

CANO L. M. [1], **RAFFAELE S.** [1], **WIN J.** [1], **SCHORNACK S.** [1], **COOKE D.** [1], **BIRCH P.** [2], **AND S. KAMOUN** [1]

[1] *The Sainsbury Laboratory, JIC Norwich Research Park. NR47UH, Norwich, UK.*
[2] *SCRI, Invergowrie, Dundee DD25DA, Scotland, UK.*

Phytophthora infestans is an oomycete pathogen that causes the devastating late blight disease in potatoes. In 2005, a clonal lineage of the A2 mating type, termed genotype blue 13, was identified in the UK and now this strain has become the most prevalent in the country. *P. infestans* blue 13 strains are characterized by an increased aggressiveness and virulence on several resistant potato varieties. We hypothesize that the enhanced virulence activity in potato of *P. infestans* blue 13 is a result of sequence and expression variation of some effector genes. Genome analysis of the Illumina sequenced *P. infestans* blue 13 (063928-A2 strain) revealed contained RXLR effector regions with structural (copy number) variation designated by an increased sequence depth. De novo assembly of unmapped Illumina reads predicted some contained RXLR contigs that summed ~ 4 Mbp lengths in *P. infestans* blue 13 that are absent in *P. infestans* T30-4 genome. In addition, using a whole-genome microarray screen we identified a secreted RXLR effector family that showed a 2-fold increase in induction on potato with no induction in the less virulent *P. infestans* T30-4 strain. Together, our findings suggest that *P. infestans* blue13 exhibit significant sequence, structural and expression variation in effector genes. Further studies of the genetic variation of *P. infestans* blue 13 will provide insights for the understanding of the evolution of virulence in emerging plant diseases.

Evolutionary and functional analysis of ATR1 and ART13 from sister species of *Hyaloperonospora arabidopsidis*

THINES M[1,2], SCHMUKER A[2], CANO L[1], AND S. KAMOUN[1]

[1] *The Sainsbury Laboratory, Colney Lane, Norwich NR4 7UH, United Kingdom.*
[2] *University of Hohenheim, Institute of Botany 210, 70593 Stuttgart, Germany.*

So far, two cytoplasmic effectors with known avirulence phenotype and a cognate resistance gene have been reported for the downy mildews. These are the RxLR effectors ATR1 and ATR13, which show a high degree of variation and signatures of positive selection in different strains of *Hyaloperonospora arabidopsidis* (*Ha*). This is matched by highly polymorphic corresponding R-genes in *Arabidopsis thaliana*, indicative of an evolutionary arms-race between host and parasite.To date, none of these effector genes could be obtained from any other *Hyaloperonospora* species, including *H. brassicae* and *H. parasitica*. Here we report the cloning and sequencing of homologues of both ATR1 and ATR13 from species closely related to *Ha*.These showed only moderate similarity on the amino acid level and high degrees of positive selection when compared to *Ha*, indicative for avoidance of recognition or the adaptation to altered host targets. Substitution pattern analysis provided evidence also for the latter scenario. In addition, for ATR1, variability among species related to *Ha* was significantly lower and unlike in *Ha* no traces of positive selection within natural pathogen populations were observed. This could indicate that no R-genes recognising ATR1 or ATR13 homologues are present in the hosts of sister species of *Ha*.Transient expression analyses were carried out for ATR1 homologues in conjunction with RPP1 WsB and revealed differential recognition of ATR1 alleles from *Ha* and its sister species.

IV. Cell Biology and Genomics

Comparative genomic analysis of *Saprolegnia parasitica* and *Phytophthora* species suggests host-specific evolution of effectors in oomycete animal and plant pathogens

JIANG R.H.Y.[1], SYKES S. [1], YOUNG S.[1], DE BRUIJN I.[2], VAN WEST P.[2], NUSBAUM C.[1], TYLER B.[3], AND C. RUSS[1]

[1] *Broad Institute, Cambridge, MA 02142, USA,*
[2] *University of Aberdeen, Aberdeen AB25 2ZD, UK,*
[3] *Virginia Bioinformatics Institute, Blacksburg, VA 24061, USA*

Saprolegnia parasitica is an oomycete pathogen that causes severe disease in a wide variety of fish, amphibians and crustaceans, resulting in significant losses to aquaculture and damage to aquatic ecosystems. In contrast, its better-studied relatives in the genus *Phytophthora* are plant pathogens. We have generated a high quality assembly of the *Saprolegnia parasitica* genome, 53Mb in length with contig and scaffold N50 sizes of 34.5Kb and 280.9kb respectively. With the first animal pathogen oomycete now sequenced, significant opportunities arise for comparative genome analysis between oomycete plant and animal pathogens. Here we report on the genome annotation and present results from comparative genomic analysis of *Saprolegnia* and three *Phytophthora* species, *P. infestans, P. sojae* and *P. ramorum*. Preliminary BLAST search results suggest that ~60% of *Phytophthora* core proteome genes share homology with *Saprolegnia*. In contrast, minimal conservation is observed among genes encoding known secreted proteins. The largest plant pathogen effector families from *Phytophthora*, such as RXLR, crinkler and Necrosis Inducing Proteins (NIP), appear to be absent in *Saprolegnia*. However, several protein families implicated in *Saprolegnia* pathogenicity such as peptidases, elicitin-like proteins and secreted Cys-rich proteins are present and appear to be expanded. Intriguingly, canonical host-targeting domains from plant pathogen oomycetes were not found in *Saprolegnia*. Nevertheless, homology independent sequence motif searches reveal several effector candidates with variant-RXLR motifs, which may have evolved independently to achieve specific targeting of animal host cells.

Isolation of nine *Phytophthora capsici* pectin methylesterase genes which are differentially expressed in various plant species

FENG B.Z., AND X.G.ZHANG

Department of Plant Pathology, Shandong Agricultural University, Tai'an, 271018, China

Phytophthora capsici, the cause of blight, crown rots as well as stem, leaf, and fruit lesions on many plant species, secretes various pectin methylesterases during all stages of infection. The expression pattern of the encoding genes (*Pcpme* 1-9) was studied on three hosts: pepper, tomato, and cucumber using quantitative real-time PCR. All gene members were found to be differentially expressed, depending on the host species and stage of infection. The expression levels of the respective gene was gradually increased from 1 to 7 days post-infection (dpi) in pepper fruits, and expression patterns of most genes in tomato fruits were opposed to that of in pepper fruits from 1 to 5 dpi, but eventually reached definite peaks at 7 dpi in solanaceous plant fruits. In cucumber fruits, each gene showed minor levels of expression from 1 to 3 dpi, exhibited definite peaks at 5 dpi, and decreased from 5 to 7 dpi. Thus, evidence on *Pcpme* gene expression diversity during interaction with three hosts, suggested that the late stages of infection may be critical for the ability of *P. capsici* to successfully secrete PME and cause necrotic lesions. In addition, *Pcpme* 6 and *Pcpme* 1 showed significant expression levels in pepper, tomato, and cucumber fruits. *Pcpme* 6 may also play an important role in pathogenicity expressed during infection of two solanaceous plants, and *Pcpme* 1 performed a corresponding role during cucumber infection.

Comparative genome sequencing connects host adaptation and genome organization in the *Phytophthora infestans* lineage

RAFFAELE S.[1], FARRER R.[1], CANO L. M. [1], PIKE J.[1], DONOFRIO N.[2], EVANS T.[2], MEYERS B.C. [2], HAAS B.[3], JIANG R.H.Y. [3], ZODY M.[3], NUSBAUM C.[3], MACLEAN D.[1], STUDHOLME D.[1], AND S. KAMOUN[1]

[1] *The Sainsbury Laboratory, John Innes Centre, Norwich, U;*
[2] *Department of Plant & Soil Sciences, University of Delaware, USA ;*
[3] *Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA*

The Irish potato famine microorganism *Phytophthora infestans* is an economically important specialized pathogen that causes a destructive disease on *Solanum* plants. In Central Mexico, the *P. infestans* lineage specifically infects hosts that belong to three different botanical families, exhibiting an intriguing flexibility in adaptation to unrelated host plants. To determine the patterns and selective forces that shape sequence variation in this lineage, we performed Illumina sequencing in six genomes representing 3 species, including the re-sequencing of the reference *P. infestans* T30-4 strain. We identified presence/absence and single nucleotide polymorphisms (SNPs), gene copy number variation (CNV) and non synonymous vs synonymous changes (dN/dS) in the re-sequenced genomes. The genomes in *P. infestans* lineage experienced a repeat-driven expansion relative to other *Phytophthora* spp. In *P. infestans* T30-4 strain we observed an unusual distribution of gene density where effector genes localize to repeat-rich and gene-sparse regions (GSRs) of the genome (Haas *et al.*, 2009). Our results show that the frequency of presence/absence polymorphisms, CNVs, dN/dS average and variance are higher in GSRs. In addition, *in planta* gene expression pattern differs between GSRs and gene dense regions (GDRs). GSRs are enriched in effector families, secreted carbohydrate binding proteins and histone methylases. These findings indicate that genes in GSRs have experienced faster evolution than those in GDRs, and display a different expression regulation *in planta*. We conclude that *P. infestans* GSRs are genome niches that allow rapid host adaptation, providing support to the epi-transposon model for fitness landscape switch (Zeh *et al.* 2009).

Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins

WAWRA S.[1], GROUFFAUD S.[1,2], BAIN J.[1], WHISSON S.C.[2], MATENA A.[3], PORTER A.J.[1], BAYER P.[3], WHISSON S.[2], BIRCH P.[2], SECOMBES C.J.[1], AND P. VAN WEST[1]

[1] *University of Aberdeen, Aberdeen, Scotland, UK.*
[2] *Scottish Crop Research Institute, Invergowrie, Scotland, UK.*
[3] *ZMB/Faculty of Biology and Geography, Universität Duisburg-Essen, Germany*

Several Prokaryotic and Eukaryotic microbial pathogens have evolved intriguing mechanisms to translocate proteins into their host cells. The translocated proteins are called effectors as they can modulate molecular processes in their hosts in order to establish an infection and/or suppress their immune response. For example, certain types of bacteria possess a needle like injection system, the type III secretion system (T3SS), which allows a direct translocation of effector proteins into the cells under attack. Whilst the bacterial translocation machineries are well described, little is known about how effectors from Eukaryotic pathogens are delivered into their host cells. The early stage of infection caused by the eukaryotic oomycete pathogen *Phytophthora infestans* involves a biotrophic phase. In this early interaction stage the secretion of oomycete RxLR effectors takes place via haustoria, which are structures formed by the pathogen that are in intimate contact with the extra haustorial membrane produced by the plant. The mechanism by which oomycetes direct their RxLR effectors into host cells is as yet unknown and is the main focus of our research. It has been postulated that endocytosis processes or protein transporters are responsible. Here we present our latest results, which give insight into the mechanism of the oomycete RxLR-EER protein translocation system.

Exploring the small RNA world in *Phytophthora infestans*

VETUKURI R.R.[1], TELLGREN-ROTH C.[2], AVROVA A.O.[3], SAVENKOV E.I.[1], WHISSON S.C.[3], AND C. DIXELIUS[1]

[1]Dept. Plant Biology & Forest Genetics, Uppsala BioCenter, SLU, 750 07 Uppsala, Sweden;
[2]Dept. Genetics & Pathology, Rudbeck Lab. Uppsala University, 751 85 Uppsala, Sweden;
[3]Plant-Pathology Program, Scottish Crop Research Institute, Invergowrie, Dundee, United Kingdom.

Cyst life cycle stage. This pathogen has large numbers of effector genes that are expressed during infection. The question that we would like to answer is whether P. infestans isolates employ gene silencing to restrict the expression of specific effectors. The other interesting aspect that we are trying to understand is avirluence gene silencing.There is accumulating evidence that some oomycete avirulence genes may be transcriptionally inactivated, possibly via silencing, to avoid recognition by host plants.
To explore all the above mentioned possibilities deep sequencing (SOLiD) of small non-coding RNAs of two isolates belonging to A1 and A2 mating types and four life cycle stages was carried out. From the sequencing data obtained we aim to identify sRNAs targeting transposons, avirulence genes, effector genes, pathogenicity genes, mating type-specific genes, and other lifecycle stage-specific genes. So far from the data we have analyzed, the size distribution of sRNAs range from 18 to 40 nucleotides. The sRNAs targeting transposons, effectors, from different life cycle stages in two different mating *Phytophthora infestans* is an oomycete that causes late blight disease in potato. Our own studies have shown that components of known gene silencing machinery from other eukaryotes are well conserved in *P. infestans* and particularly active in the germinating types show high variation in terms of number and size. The progress of this work would be presented.

Epigenetic Gene Regulation in *Phytophthora sojae*

PHUNTUMART V.

Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403, USA

Unlike most eukaryotes where DNA methylation is an important feature of epigenetic, this was not the case for Phytophthora. The absence of DNA methylation for both adenine and cytosine nucleotides in *P. sojae* makes it an ideal eukaryote to study epigenetic inheritance. However, similar to other eukaryotes, *P. sojae* uses histone modifications as an epigenetic signal. Data presented will include results from immunoprecipitation coupled with high throughput DNA sequencing both chromatin immunoprecipitation sequencing (ChIP-seq) and methylated DNA immunoprecipitation sequencing (MeDIP-seq).

Development of high-throughput gene silencing tools in *Phytophthora sojae*

ZHAO W.[1], DONG S.[1], DAI T.[1], YANG X.[1], SHENG Y.[1], TYLER B.[2], GIJZEN M.[3], AND Y. WANG[1]

[1] *Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China*
[2] *Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*
[3] *Agriculture and Agri-Food Canada, London, Ontario, Canada*

We are developing high-throughput gene silencing tools for elucidating gene function in *Phytophthora sojae*. dsRNA mediated gene silencing, a post-transcriptional and highly conserved process in eukaryotes, could result in specific gene silencing through degradation of the target mRNA. Recently, we set up a dsRNA mediated transient gene silencing protocol combined with *in vitro* dsRNA synthesis and a PEG mediated transformation system. Single-gene silencing is accomplished by introducing unique dsRNA sequences into protoplasts, and multiple-gene silencing is performed with chimeric overlapping dsRNA fragments.The cell cycle regulated gene *PsCdc14* (a protein phosphatase that regulates the mitosis and the cell cycle) and the avirulence gene *PsAvr3a* (an RxLR effector that is recognized by the soybean *Rps3a* resistance gene) are transiently silenced by introducing *in vitro* synthesized dsRNA into *P. sojae* protoplasts. The results show that there is no reduction in *PsCdc14* or *PsAvr3a* mRNA in the transformants until 8 days after the specific gene dsRNA was transferred into protoplasts. From 9 to 15 days, the transformants exhibited significant gene silencing, with mRNA levels reduced by 50% to 95%. All the silencing transformants showed recovery of gene expression by 17 days. Thus, the gene silencing protocols could significantly reduce the mRNA level of *PsCdc14* and *PsAvr3a*, yielding detectable phenotypes. The successful setup of transient gene silencing could make *P. sojae* high-throughput gene function elucidation work feasible, facilitating our understanding of the development and pathogenicity mechanisms of this organism.

Oomycete Kinomes: structure, diversification, and cellular function

JUDELSON H. S.[1], AH FONG A.M.V.[1], SUNDQVIST G.[2], MADISHETTY K.[1], AND V. BULONE[2]

[1]Department of Plant Pathology and Microbiology, University of California, Riverside, USA
[2]School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden

Phosphorylation by protein kinases represents the most widespread signaling mechanism in eukaryotes. In *Phytophthora infestans*, inhibitor studies show that kinases play critical roles in zoosporogenesis and other life-stages. Dramatic changes in kinase activity occur during spore development based on in-gel phosphorylation assays. To help identify the proteins involved in these phenomena, a superfamily of 356 protein kinase genes was detected within the genome of *P. infestans,* and similar numbers were found in *P. ramorum* and *P. sojae.* Most belong to evolutionarily conserved families within the AGC, CAMK, CK1, CMGC, STE, and TKL groups, but RGC and TK kinases were not detected. Novel combinations of regulatory domains were detected in several kinase groups, some apparently the product of gene fusions. The TKL group, and especially an oomycete-specific TKL family, may include many transmembrane receptor kinase-like proteins. The total fraction of kinases with regulatory domains was small, suggesting that transcription plays the greatest role in their control. This was supported by microarray and qRT-PCR data which demonstrated that a large fraction were sporangia or zoospore-specific. Possibly reflecting their role in zoospores, about one-third fewer kinases are predicted to be encoded by *Hyaloperonospora arabidopsidis* which lacks the zoospore stage. To further address the role of kinases in *P. infestans,* stage-specific patterns of protein phosphorylation were identified using proteomics techniques. A family of fluorescent marker expression plasmids was also created to help study the subcellular localization of kinases, phosphatases, and other proteins.

Characterization of GPCR-PIPKs, a novel class of proteins in *Phytophthora*

HUA C.[1], MEIJER H.[1], DE KEIJZER J.[1], WEI Z[2], WANG Y.[2], AND F. GOVERS[1]

[1]*Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*
[2]*Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China.*

Mining and comparing of whole genome sequences of *Phytophthora* species uncovered a number of enzymes with aberrant combinations of catalytic and regulatory domains. One outstanding class of novel proteins comprises GPCR-PIPKs that contain an N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) combined with a phosphatidylinositol phosphate kinase (PIPK) domain at the C-terminus[1]. Each of the *Phytophthora* species sequenced so far, has 12 orthologous genes encoding GPCR-PIPKs. Gene expression analyses of these 12 genes in *P. infestans* and *P. sojae*, revealed mostly differential expression during asexual development and very low or no expression during infection. In order to elucidate the biological function of GPCR-PIPKs a silencing strategy was applied. Silencing of the GPCR-PIPK-D4 and GPCR-PIPK-D5 gene in *P. sojae* resulted not only in aberrant development but also in loss of virulence. To determine the subcellular localization of GPCR-PIPKs, we generated *Phytophthora* transformants carrying constructs encoding full-length or truncated GPCR-PIPKs fused to the monomeric red fluorescent protein (mRFP). These are currently analysed using fluorescence and confocal microscopy. An update of the ongoing analysis on GPCR-PIPKs in *Phytophthora* will be presented.

Identification of appressorial and mycelial cell wall and membrane-associated proteins from *Phytophthora infestans*

GRENVILLE-BRIGGS L.J.[1], AVROVA A.O.[2], HAY R.J.[1], WHISSON S.C.[2], AND P. VAN WEST[1]

[1]Aberdeen Oomycete Laboratory, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, UK
[2]Plant-Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.

Proteins embedded in the cell wall and plasma membrane of filamentous oomycetes and fungi provide a means by which these organisms can interact with their local environment. However, such proteins have often proved difficult to isolate using conventional proteomic techniques. Here we have used liquid chromatography tandem mass spectroscopy (LC-MS/MS) to facilitate rapid and sensitive quantification of the cell wall proteome. We report the use of LC-MS/MS to identify differentially regulated proteins from the cell walls of three different lifecycle stages of the oomycete plant pathogen *Phytophthora infestans*: non-sporulating vegetative mycelium, sporulating mycelium, and germinating cysts with appressoria. We have also used quantitative real-time RT-PCR to confirm that the transcripts corresponding to some of these proteins, namely those identified in cell walls of germinating cysts with appressoria, accumulate differentially throughout the lifecycle and may therefore be important for pre-infective development and early pathogenicity. Up to 31 covalently and non-covalently bound cell wall-associated proteins were identified. All of the proteins identified in germinating cysts with appressoria, and several of those from mycelial fractions, were classified as putative effector or pathogen-associated molecular pattern (PAMP) molecules, including members of the CBEL family, the elicitin family, the crinkler (CRN) family and two transglutaminases. Thus, the cell wall of *P. infestans* may represent an important reservoir for surface-presented, apoplastic effectors. Cell surface proteins included IPI-B like proteins, mucins, cell wall-associated enzymes and annexins.

Chitin synthase: a potential target of anti-Oomycetes drugs

GUERRIERO G., AND V. BULONE

KTH Biotechnology, Division of Glycoscience, AlbaNova University Centre, SE-106 91 Stockholm, Sweden

Oomycetes constitute a phylum of great economical importance, which comprises numerous devastating pathogens of plants and animals.
The cell walls of Oomycetes are mainly composed of (1→3)-β-glucans, (1→6)-β-glucans and cellulose and, unlike fungal cell walls, they contain no or little chitin. Chitin synthase (CHS) genes are however widespread in Oomycetes. Here we report the isolation and functional characterization of two CHS genes from *Saprolegnia monoica*, *CHS1* and *CHS2*, both characterized by the occurrence of a Microtubule Interacting and Trafficking domain (MIT) at their N-terminus. The potential role of these domains in the delivery of CHS to the plasma membrane and/or in the endosomal trafficking and recycling of CHS will be discussed in relation with the recent discovery that Oomycete CHS are located in plasma membrane microdomains similar to lipid rafts. Treatment of mycelium with the CHS competitive inhibitor Nikkomycin Z (NZ) causes an increase in *CHS1* and *CHS2* gene expression levels, together with swelling and bursting of hyphal tips. The drug also causes a decrease in biomass, but interestingly, the N-acetylglucosamine content does not change, suggesting the existence of a compensatory mechanism controlling chitin biosynthesis in the presence of the inhibitor. Our data show that CHSs represent promising targets for the development of environmentally friendly anti-Oomycete drugs.

Dihydroorotate dehydrogenase from the phytopathogenic Oomycete *Phytophthora infestans* as a novel target for crop control

GARAVITO M. F. (†‡), GARCÍA L. (‡†), LOZANO G. (†), BERNAL A. (‡), ZIMMERMANN B. H. (†), AND S. RESTREPO(‡)

(†) Grupo de investigaciones en Bioquímica y Biología Molecular de Parásitos, Bogotá D.C., Colombia
(‡) Laboratorio de Micología y Fitopatología Universidad de los andes LAMFU, Universidad de los Andes, Bogotá D.C., Colombia

The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of the tomato and potato late blight, causes tremendous crop and economic losses worldwide. In Colombia this pathogen is currently a devastating risk for the highlands dedicated to production of potato and tomato, hosts of this oomycete. Yet, current control strategies are far from being adequate and new ones are urgently needed. An interesting and unexplored alternative to control human parasites based on the inhibition of the *de novo* pyrimidine biosynthetic pathway might work as well in *P. infestans.* In this study we investigated the pathogen's dihydroorotase dehydrogenase DHODase, which catalyzes the fourth and only redox step of the pathway as a target to develop control strategies. We propose that this enzyme is member of the DHODase family 2, which compromises a mitochondrial bound enzyme with quinones as direct electron acceptors. *In silico,* preliminary molecular docking assays using homology modeled structures reveal that key structural aspects of the enzyme such as its apparent binding site flexibility could be exploited to develop species-selective inhibitors. A full length and an N-terminally truncated DHODase were expressed as recombinant proteins and complemented a DHODase-deficient bacterial host.

Orotate phosphoribosyl transferase and orotidine-5-monophosphate decarboxylase as targets for control of *Phytophthora infestans*

GARCÍA BAYONA L.[1,2], GARAVITO M.F.[1,2], BERNAL A.[1], ZIMMERMANN B.H.[2], AND S. RESTREPO[2]

[1]Laboratorio de Micología y Fitopatología Universidad de los andes LAMFU, Universidad de los Andes, Carrera 1 # 18A – 10 edificio J-205 ,Bogotá D.C., Colombia
[2]Grupo de investigaciones en Bioquímica y Biología Molecular de Parásitos, Bogotá D.C., Colombia

Phytophthora infestans, the causal agent of the tomato and potato late blight, generates high economic losses worldwide. Current control strategies are far from being adequate and an interesting and unexplored alternative for control could be based on the inhibition of the *de novo* pyrimidine biosynthetic pathway. Taking into account that striking similarities have been observed between the pathogenicity mechanisms of oomycetes and human apicomplexan parasites such as *Plasmodium falciparum*, common control targets could be proposed that have proven very promisory in the Apicomplexa. Indeed, inhibitors of some of the enzymes of pyrimidine synthesis have been proposed as therapeutic agents for a wide range of human parasites and some plant pathogens. The last two enzymes of the pathway, orotate phosphoribosyltransferase and orotidine-5-monophosphate decarboxylase, were selected as the most promising targets for inhibition, based on their low similarity to host enzymes, different predicted subcellular localization, architecture, predicted 3D structure and phylogenetic relations. In order to start their characterization, these two enzymes, which are fused and duplicated in *P. infestans*, were cloned and are in the process of being expressed and purified in their recombinant form Key aspects of their metabolic inhibition were also determined for future virtual screening of a compound library using molecular docking. To our knowledge, this is the first study of the pyrimidine biosynthesis in oomycetes.

Functional analysis of the *P. parasitica* hexose kinase genes as a first step towards exploring the nutritional basis of pathogenicity of *Phytophthora*

PANABIERES F[1]., LE BERRE J-Y[1]., AND M. LEMAIRE[2]

[1] Plant-Oomycete Interactions Group ,UMR-Interactions Biotiques et Santé Végétale, INRA1301-CNRS6243-Université Nice-Sophia Antipolis, 06903, Sophia Antipolis, France.
[2] UMR 5240 Microbiologie, pathogénie et adaptation, Université Claude Bernard Lyon1, 69622 Villeurbanne, France

To propagate and cause disease, oomycete plant pathogens must feed on their hosts, and deciphering mechanisms regulating *in planta* nutrition may help in the design of new anti-oomycete drugs. We intended to study metabolic changes occurring during *P. parasitica* infection of its host tomato through *in silico* comparative analyses. Mining *P. parasitica* EST libraries from various physiological stages, we identified three genes encoding glucokinases (GK) and a gene encoding a potential fructokinase (FK). These genes are related to sequences present in cyanobacteria and some amitochondrial protists. Biochemical analyses, using yeast complementation assays and determination of the enzymatic activity of recombinant proteins indicate that *P. parasitica* GKs and FK display substrate specificity for glucose and fructose, respectively. From an applied point of view, these hexose kinases (HK) might be attractive targets for potent anti-oomycete molecules. GK and FK genes displayed complex expression patterns during *P. parasitica* life cycle and respond differentially to various stresses, based on RT-PCR. A GK gene and FK are induced during plant infection, concomitantly to the induction of the host cell wall bound invertase, which is known to provide plant tissues with free hexoses, potent substrates for *P. parasitica* HKs. As induction of invertase is considered as a component of plant defense mechanisms, these data lead to draw hypotheses about potential links between manipulation of host plant defence responses, *in planta* nutrition and pathogenicity of *Phytophthora*.

POSTERS

I. Microbial Effectors

I.1 - Suppression of defense responses in distantly related plants by homologous RXLR effectors from *Hyaloperonospora arabidopsidis* and *Pytophthora sojae*

ANDERSON R. G. [1], FEE R. A.[1], DEB D.[1], KALE S. D. [2], TYLER B. M. [2], AND J.M. MCDOWELL[1]

[1]Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA
[2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA

We are using the interaction between the model plant *Arabidopsis* and its downy mildew pathogen *Hyaloperonospora arabidopsidis (Ha*) to investigate how plant cells are manipulated by oömycete effectors. The *Ha* genome has been sequenced and bioinformatic analyses have revealed at least 130 candidate effector genes. We are focusing on a pair of homologous effectors: *Ha98* and *Ps163* from *H. arabidopsidis* and *Phytophthora sojae*, respectively. Both effectors are expressed during the host-pathogen interaction. Furthermore, Ha98 and Ps163 carry a functional host targeting (HT) sequence that is required for translocation into the host cell. Transient assays indicate that they suppress diverse elicitors of programmed cell death in soybean including mammalian Bax and the *P. sojae* elicitor Avr4/6. In addition, transgenic *Arabidopsis* plants expressing either *Ha98* or *Ps163* partially suppress *RPP4*-mediated resistance to *Ha* EMOY2 and are more susceptible to *Ha* EMCO5. *Ha98* and *Ps163* expressing plants suppress the callose response to avirulent bacteria suggesting that both effectors are capable of suppressing PAMP-triggered immunity. Transient expression of *Ps163* alleles in *Nicotiana benthamiana* triggers cell death response that is allele-specific and requires RAR1 and Hsp90-1. These experiments suggest that these homologous effectors from distantly related oömycetes can suppress and induce defense mechanisms in diverse plant species.

I.2 - Rapid identification of *Hyaloperonospora arabidopsidis* RXLR effectors with virulence function in *Arabidopsis* using mixed bacterial infections

BADEL J.L., GREENSHIELDS D., FABRO G., PIQUEREZ S., ISHAQUE N., AND J.D.G. JONES

The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK.

The interaction between *Hyaloperonospora arabidopsidis* (*Hpa*) and *Arabidopsis* is a well established model system to study oomycete pathogenesis and plant defence response against obligate biotrophic parasites. *Hpa* has been divided into several races depending on the outcome of its interaction with different *Arabidopsis* genotypes. *Hpa* Emoy2, for instance, causes disease in Wei-0 and Oy-0, but not in Col-0, Ler-0, Nd-0, and Ws-0, due to the specific recognition conferred by RPP (recognition to *Peronospora parasitica*, former *Hpa* name) resistance genes. It is estimated that during infection Emoy2 delivers into plant cells more than a hundred effector proteins that work in concert to cause disease. A group of these effectors is characterized by the presence of an RXLR motif, which has been shown to be required for translocation of *Phytophthora* effectors into the plant cytoplasm (Duo *et al.*, 2008; Whisson, S.C., *et al*. 2007). Using the Effector Detector system (Sohn *et al*. 2007) for heterologous expression and mixed *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) infections we have been able to identify RXLR effector candidates that have virulence function during the interaction of Emoy2 with different *Arabidopsis* genotypes based on the competitive advantage of the bacterial strain carrying the plasmid-borne effector candidate. This work is funded by the Gatsby Charitable Foundation; JLB and DG are funded by the Human Frontier of Science Program (HFSP).

I.3 - Identification of *Phytophthora cactorum* genes expressed during infection of strawberry

CHEN X., KLEMSDAL S.S., AND M.B. BRURBERG

Bioforsk, Plant Health and Plant Protection Division, Department of Genetics and Biotechnology, Høgskoleveien 7, 1432 Ås, Norway

The oomycete *Phytophthora cactorum* causes crown rot in strawberry, resulting in big economic losses. To unravel the molecular mechanisms that are involved in the pathogenicity of *P. cactorum* on strawberry, two strategies were followed, SSH cDNA library and effector specific differential display (ESDD). Two cDNA libraries were made, enriched for *P. cactorum* genes upregulated during infection of strawberry or in *in vitro* germinating cysts (a developmental stage essential for infection). Recent characterization of oomycete AVR/effector genes revealed that they encode proteins with conserved RxLR-dEER motifs required for translocating these effectors into host cells. The presence of such a conserved "tag" has provided a tool for discovering the otherwise structurally diverse effector genes. To select RxLR effector genes from *P. cactorum*, ESDD was performed on seven cDNA populations including four developmental stages (mycelium, sporangia, zoospores, germinating cysts) as well as three time points during infection (3, 5, 7 days post-inoculation), using RxLR and EER primers. Using these strategies more than 230 gene fragments were isolated. Nearly 30 % of the genes could not be assigned a coding function, using sequence similarity-based function prediction, because either no similar sequences were detected or similarities were found only to putative proteins with unknown function (mostly oomycete). However, several genes potentially relevant for pathogenicity, including several putative RxLR effector and elicitor genes were discovered. The expression of 27 of these genes was studied in detail using real-time RT- PCR, and their upregulated expression during infection relative to dispersal propagules (sporangia) were confirmed for all but one.

I.4 - Characterization of the Conserved Effector Protein Ha23 from the Oömycete pathogen *Hyaloperonospora arabidopsidis*

DEB D.[1], ANDERSON R.G.[1], KALE S.D.[2], TYLER B.M.[2], AND J. M. MCDOWELL [1]

[1]Department of Plant Pathology, Physiology and Weed Science,
[2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA

Numerous plant pathogens are known to produce effector proteins that are exported to the interior of host cells. Effectors have been well characterized in bacteria but their roles in oömycete and fungal pathogens are poorly understood. Bioinformatic analysis of recently obtained genome sequences from oömycete pathogens *Phytophthora sojae*, *P. ramorum* and *Hyaloperonospora arabidopsidis (Ha)* have led to the identification of numerous candidate effector genes. These effector genes are defined by characteristic motifs (RxLR and dEER) that target the effectors into plant cells. Although these effector genes are very diverse, certain genes are conserved between *P. sojae* and *H. arabidopsidis*, suggesting that they fulfill key roles in pathogenicity. The goal of my project is to characterize a selected set of conserved effector candidates in regard to pathogenicity in *H. arabidopsidis* and *P. sojae*. We hypothesize that these effectors have important conserved roles in infection. My primary objective centers on identifying effector functions and *in planta* targets using both transient assays and stably transformed plants. From the preliminary experiments conducted to date, Ha23 seems to be a promising candidate effector. Firstly, Ha23 is expressed during the course of *Ha* infection of *Arabidopsis*. Secondly, Ha23 can suppress both immunity triggered by pathogen associated molecular patterns (PTI) and effector triggered immunity (ETI) *in planta.* Finally, an Ha23-GFP fusion localizes to the chloroplast. This is interesting because none of the oömycete effectors are yet known to localize to that organelle. Future experiments will determine the functional relevance of chloroplast localization of Ha23, and test potential virulence functions of the effector.

I.5 - Identification of *Phytophthora infestans* RxLR-(EER) effectors suppressing PAMP-triggered immunity

JÄNTSCH C.[1], CHEN Y.[1], GILROY E.[2], BIRCH P.[2] AND F. BRUNNER[1]

[1]*ZMBP, Plantbiochemistry, Tübingen 72076, Germany;*
[2] *Plant Pathology Programme, Scottish Crop Research Institute, Dundee DD2 5DA, UK*

Phytophthora infestans is an oomycete that causes late blight in potato and tomato. The genome of *P. infestans* encodes more than 100 RxLR-(EER) effectors that are assumed to manipulate diverse host cellular activities. To identify the repertoire of RxLR-(EER) effectors that suppress plant immunity triggered by the recognition of invariant microbial structures, the so-called Pathogen-Associated Molecular Patterns (PAMPs), we use an *Arabidopsis* protoplast-based system. The premise for testing *P. infestans* RxLR-(EER) effectors in a non-host plant species is that we hypothesise that some oomycete effectors have evolved to target universal plant factors. Our high-throughput assay takes advantage of the huge genetic resources existing in *Arabidopsis* and will help further to guide the work with natural hosts of *P. infestans*. Protoplasts expressing transiently candidate *P. infestans* RxLR-(EER) effectors are tested for their responsiveness to flg22. As a read-out, we measure the activity of luciferase, whose expression is under the control of a PAMP-inducible promoter (p*FRK1*). Two RxLR-(EER) effectors, 09680 and 13628, were identified as suppressors of PAMP-triggered immunity in this screen. A third RxLR-(EER) effector, Avr2, does not cause suppression of *pFRK1:: Luc* activity, but exhibits an interesting dot pattern when cell localization studies are performed with N-or C-terminal GFP fusion protein. With confocal microscopy we could show that Avr2 localises to small, fast-moving vesicles, in all points similar to what is observed when Avr2 is expressed in Solanaceae leaves.

I.6 - In silico analysis of nematode genes induced during parasitism

JAOUANNET M., DELEURY E., MAGLIANO M., ABAD P., AND M.N. ROSSO

INRA, UMR 1064, Université de Nice Sophia Antipolis, CNRS, UMR 6243, Interactions Biotiques et Santé Végétale, 400, route des Chappes, BP 167, F-06903 Sophia Antipolis, France.

Root-knot nematodes are biotrophe root parasites that maintain an intimate relationship with host plants and induce the redifferentiation of root cells into specialized feeding cells called giant cells. In order to identify nematode effectors, we searched for nematode genes induced during parasitism. We focused on *M. incognita,* for which the genome has been recently sequenced and for which local and public EST databases include ESTs from pre-parasitic (eggs, infective J2) and parasitic stages (parasitic J2, J3, J4, females). *In silico* clustering of ESTs identified 294 clusters found in parasitic juveniles and absent from pre-parasitic stages. We identified in the genome of *M. incognita* the gene models of 146 parasitic-specific EST clusters. For 24 EST clusters no gene model was found in *M. incognita* but we could identify homologs sharing more than 95% identity in *M. hapla*. Among the predicted proteins encoded by these EST clusters, 46 have a predicted signal peptide for secretion and no transmembrane domain, suggesting that these proteins could potentially be secreted by the nematode in the plant tissue. Predicted functions were deduced from blast searches and from InterProScan. In addition, we used ORTHOMCL to search for orthologs in the genome of free-living and parasitic nematodes. Gene family expansions or the specific presence of genes in *Meloidogyne sp.* were indications of evolutionary adaptation to parasitism. Genes expressed in secretory organs of the nematode will provide new candidate effectors.

I.7 - Oomycete effector traffic: How are Albugo effectors delivered to the host cell?

KEMEN A.C., KEMEN E., SMOKER M. AND J.D. JONES

The Sainsbury Laboratory, Colney Lane, Norwich, NR4 7UH, UK

Biotrophic plant pathogenic oomycetes produce effector proteins that are delivered to the host cell to manipulate host metabolism and defence. However, beside extensive effector analysis, the actual mechanisms for the effector delivery in oomycetes and fungi are not yet understood. An interesting system to study the transfer of effector proteins is the obligate biotrophic oomycete *Albugo laibachii*, causing white blister rust on *Arabidopsis thaliana*.
We are using three different approaches to discover export motifs and mechanisms involved in effector delivery.
1) We are testing a host-induced gene silencing system for the *Albugo-Arabidopsis* pathosystem to examine not only effector candidates but also components of the delivery mechanism.
2) To identify known and new classes of transfer motifs in *Albugo* we use the *P. capsici – N. benthamiana* translocation assay. This test system is based on Avr3a-mediated avirulence in plants carrying R3a. The Avr3a translocation domain is replaced by the N-termini of different classes of Albugo effector candidates. This test system will reveal the importance for delivery of the CHXC motif recently identified in *Albugo*.
3) By developing an *Albugo* transformation system we have established the first transformation system for an obligate biotrophic oomycete creating the unique opportunity to study the transfer of effector candidates in the native system.
By combining *Albugo* transformation with *P. capsici – N. benthamiana* translocation assays, we have developed excellent tools for revealing indispensable virulence components and thus identification of important targets for durable resistance mechanisms.

I.8 - Evaluating the use of germinated zoospores for the identification of effectors from *Plasmopara viticola*, the causal agent of grapevine downy mildew

MESTRE P., PIRON M.C., AND D. MERDINOGLU

UMR 1131 INRA-UDS Santé de la Vigne et Qualité du Vin, 28 ru de Herrlisheim, BP507, 68021 Colmar, France.

Grapevine downy mildew caused by *Plasmopara viticola* is one of the most important diseases affecting *Vitis spp*. The current strategy of control relies on the use of chemical fungicides. An alternative to the use of fungicides is the use of downy mildew resistant varieties, which is cost-effective and environment friendly. The identification of pathogen effectors as putative avirulence genes is a necessary step to understand the biology of the interaction as well as to choose the most efficient combination of resistance genes in a strategy of pyramiding. Based on knowledge from other Oomycetes, *P. viticola* effectors can be identified using a candidate gene strategy based on data mining of genomic resources. Public genomic resources of *P. viticola* are very limited, not to say inexistent. A recent search at NCBI/EMBL databases produced 79 *P. viticola* entries (10 ESTs and 69 core nucleotides), the majority sequences of mitochondrial or ribosomal origin. As an obligate biotrophe, *P. viticola* can only grow on living tissues and the pathogen biomass in the invasive stages of infection is quite low compared to the plant biomass. Nevertheless, zoospores are easily obtained by washing off sporangia from infected leaves; furthermore, the first stages of pathogen development (growth of germinative tubes and vesicle formation) can be reproduced in vitro. To explore the suitability of such material for the identification of pathogen effectors, we created a cDNA library from in vitro germinated spores and obtained 1500 ESTs. Preliminary sequence analysis revealed the presence of 58 ESTs from genes putatively involved on pathogenicity. Detailed sequence analysis of the ESTs as well as the expression profile of candidate effector genes will be presented.

I.9 - Identification of *P. infestans* RXLR effectors inhibiting yeast growth

MORGAN W., ATASSI T., CHRISTIAN M. AND M. HEO

The College of Wooster, Wooster, Ohio, USA

Bioinformatics analysis has revealed that *Phytophthora* genomes may encode hundreds of RXLR effectors. Outside of the N-terminal signal domain, however, RXLR effectors rarely exhibit homology to each other or to previously characterized proteins; consequently, their roles in infection remain largely unknown. Recently researchers have demonstrated that the function of bacterial effectors that target conserved eukaryotic pathways can be elucidated using yeast functional genomic approaches. Exploiting this approach to discern the function of *Phytophthora infestans* effectors, we have recently identified several RXLR proteins that inhibit yeast cell growth. To elucidate the targets of RXLR action, we are now examining the transcription profiles of growth-inhibited cells in hopes of identifying up- and down-regulated gene networks. In a complementary strategy, we are developing yeast-based assays specific for pathways targeted by other pathogen effectors. To start, we are currently screening RXLR effectors for the ability to block the MAPK pathway, conserved throughout eukaryotes, using a simple reporter gene assay in yeast.

I.10 - AVRblb2 family members associate with different plant proteases suggesting functional diversification

OLIVA R., WIN J., BOZKURT T. O., SCHORNACK S., CANO L., JONES A., THINES M., AND S. KAMOUN

The Sainsbury Laboratory, Colney Lane, Norwich NR4 7UH, UK

Oomycete pathogens secrete a battery of effectors that modulate plant immunity. Host translocated protein effectors, such as RXLRs, are highly diverse and ubiquitous in *Phytophthora* genomes. In the potato late blight pathogen *Phytophthora infestans*, several RXLR effectors have evolved in clusters of related genes that occupy repeat-rich dynamic regions of the genome. That is the case of AVRblb2, a small-secreted protein that is recognized by the resistance protein Rpi-blb2. We identified at least 19 AVRblb2 family members in the genome of *P. infestans* strain T30-4. In this study, i) we describe a phylogenetic reconstruction of AVRblb2 family, and more importantly ii) we identified putative plant target proteins that associate with particular AVRblb2 family members. To achieve this, we used *in planta* co-immunoprecipitation assay. *Agrobacterium* binary vector constructs expressing N-terminally FLAG-tagged effector proteins were delivered in *Nicotiana benthamiana* by agroinfiltration. Protein complexes from plant extracts were immunoprecipitated with anti-FLAG resin and identified using mass spectrometry (LC-MS/MS). Preliminary results suggest that effectors of the AVRblb2 family associate with different plant proteases in such a way that some AVRblb2 family members have overlapping activities whereas others have acquired distinct functional properties. Overall, these results point to a model in which gene duplication in the AVRblb2 family resulted in both functional redundancy and diversification.

I.11 - Necrosis-inducing Like Proteins of the obligate biotroph *Hyaloperonospora arabidopsidis*

OOME S., CABRAL A., AND G. VAN DEN ACKERVEKEN

Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Necrosis Inducing Proteins (NIPs), such as NEP1 from *Fusarium oxysporum* and PsojNIP from *Phytophthora sojae*, act as toxins. These proteins rapidly kill dicot plant cells, and are found in bacteria, fungi and oomycetes. In the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis* (*Ha*) we have identified nine members of this protein family which we named NLPs (for Necrosis-inducing Like Protein). One does not expect cell death promoting proteins to be produced by an obligate biotroph such as the downy mildew pathogen of Arabidopsis. However in other downy mildews, e.g. *Bremia lactucae* and *Peronospora tabacina*, we also identified NLPs. As expected, none of the NLPs from *Ha* induce necrosis when transiently expressed in tobacco. Expression of the nine *HaNLP* genes is induced during the first 24 hours of infection. The only HaNLP which contains all amino acid residues known for necrosis-inducing activity (HaNLP3) is also the one closest related to the necrosis-inducing PsojNIP. We will report on our study of HaNLP3 made by comparing it *in silico* to NIPs with proven necrotic activity, by mutational analysis and by domain swaps with PsojNIP. The goal of this research is to determine what part of HaNLP3 is making it unable to induce necrosis. The fact that *Ha* has a species-specific expansion of several NLPs, together with their expression at early time point after inoculation suggests that the NLPs have an important alternative role during penetration or early colonization.

I.12 - Exploring the role of pathogen effectors in the suppression of defense signaling pathways

PEL M.J.C. [1], **CABRAL A.**[1], **BAUTOR J.** [2], **PARKER J.** [2], **VAN DER ENT S., PIETERSE C.M.J.** [1] , **AND G. VAN DEN ACKERVEKEN**[1]

[1] *Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, P.O.Box 800.56, 3508 TB Utrecht, the Netherlands*
[2] *Dept. Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Cologne, Germany*

Plants are equipped with an arsenal of defense mechanisms. These mechanisms enable the plant to defend itself against a multitude of attackers. Interestingly, specialized microbial pathogens have acquired the ability to manipulate the plants defense signaling pathways to evade host immunity. The downy mildew pathogen *Hyaloperonospora arabidopsidis* has a biotrophic life style and depends on the ability to suppress host immunity for its survival. *H. arabidopsidis* produces a large number of putative effector proteins, many of which contain an N-terminal RxLR motif. This motif is thought to target the effectors to the host cell. To investigate the hypothesis that RxLR-containing effectors of *H. arabidopsidis* play a role in the suppression of host defenses, we started to study transgenic Arabidopsis lines that overexpress these putative *H. arabidopsidis* effectors. These lines are being tested for reduction in PAMP-, pathogen- and hormone-triggered defense responses. The results of this study will be presented.

I.13 - Evolution of effector proteins in *Hyaloperonospora* during host species adaptation

SAUNDERS D.G.O.[1], CANO L.[1], KAMOUN S.[1], AND M. THINES [2]

[1]Sainsbury Laboratory, Colney Lane, Norwich NR4 7UH;
[2]University of Hohenheim, Institute of Botany 210, 70593 Stuttgart, Germany

Host jumps occur frequently in biotrophic pathogens, however, little is known about the impact of these events on the genome and effectorome of the respective organisms. Phylogenetic analyses of the *Hyaloperonospora arabidopsidis* cluster, formerly referred to as *Peronospora parasitica*, revealed several host jumps of the pathogens to multiple host species, e.g. to *Erophila* (now *Draba*), *Microthlaspi* and *Reseda* (Resedaceae). Therefore, this cluster includes host-specialized sister species that can be exploited experimentally for investigating the evolutionary fate of effector genes following a host jump. We have established single spores cultures of *H. thlaspeos-perfoliati* (*Htp*), an obligate pathogen of *Microthlaspi perfoliatum*, and are now in a position to develop this pathosystem for investigating the evolutionary processes associated with host jumps, host specificity and adaptation in obligate parasites. The aim of the current study is to sequence the complete genome and transcriptome of *Htp* using Illumina next generation sequencing technology. Here we report the initial genomic and transcriptome analysis of *Htp*, providing a novel insight into the impact of host jump events on the genome and effectorome of obligate phytopathogens

I.14 - *Phytophthora* species evolved related protease inhibitor effectors with differential specificities towards plant proteases

STAM R.[1], CANO L.M.[1], WIN J.[1], OLIVA R.[1], THINES M. [1], VAN DER HOORN R.H.L.[2], AND S. KAMOUN[1]

[1]*The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK*
[2]*The Max Planck Institute for Plant Breeding Research, Carl-von-Linneweg 10, 50869 Cologne, Germany*

The highlands of Central Mexico are the center of origin of the potato late blight pathogen *Phytophthora infestans* and home to sister species of *P. infestans* including *P. mirabilis* and *P. ipomoeae*. These and other species from so-called clade 1c, have evolved by host jumping/switching followed by adaptation and specialization on distinct hosts belonging to different botanical families.
Phytophthora infestans secretes several cystatin-like extracellular protease inhibitors (EPICs). Two of these, PiEPIC1 and PiEPIC2B, show differential activity on tomato papain like cysteine proteases (PLCPs) RCR3 and PIP1. Whereas PiEPIC2B inhibits both RCR3 and PIP1, PiEPIC1 only inhibits RCR3. We reconstructed the evolutionary history of the EPIC family in *P. infestans* and its sister species. All sequenced sister species contain orthologs of PiEPIC1. Sequence analysis of these EPICs revealed that most orthologs contain only a few polymorphisms and that *P.* mirabilis PmEPIC1 is under positive selection.
Therefore, EPICs provide an opportunity to study effector evolution as a consequence of host adaptation. To study their differential activities towards proteases from different hosts, we purified several secreted EPICs overexpressed in *E. coli* and used them in inhibition assays with DCG-04, a PLCP-specific probe that allows us to monitor cysteine protease activities.
Our findings suggest that EPICs are under selective pressure and are part of the arms race between the *Phytophthora* species on one side and their hosts on the other side.

I.15 - Identification of plant proteins targeted by oomycete RXLR effectors using *in planta* co-immunoprecipitation

WIN J., BOZKURT T.O., OLIVA R., CANO L., JONES A., AND S. KAMOUN

The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

Oomycete plant pathogens secrete RXLR effectors that are translocated inside plant cells to alter plant physiology and thereby establish infection. This study aims to identify both the target and function of oomycete effectors *in planta*. We selected 63 putative effectors, including the validated oomycete RXLR effectors and their homologs, and overexpressed each protein by using the high-expression vector pJL-TRBO, a binary plasmid containing a modified *Tobacco mosaic virus* with its coat protein gene replaced by cDNAs coding for FLAG-tagged mature oomycete effectors. Effector constructs were delivered into the leaves of *Nicotiana benthamiana* by agroinfiltration and effectors expressed under the viral coat protein promoter. Leaves were harvested 2-3 days post-infiltration and total proteins extracted. Effector proteins and their host targets were co-immunoprecipitated (co-IP) with anti-FLAG resins under non-denaturing conditions. Bound proteins were specifically eluted using 3X FLAG peptides, separated by SDS-PAGE and visualized by colloidal Coomassie blue staining. Protein bands were excised, digested with trypsin and identified by LC-MS/MS peptide ion spectrum matching. To date, we have expressed over 53 effectors to sufficient levels for co-IP and subsequent MS identification of precipitated proteins. Here we report the identified effector target proteins and corresponding alterations in plant immunity resulting from overexpression or virus-induced gene silencing of these targets (Please see posters by Bozkurt *et al* and Oliva *et al* for analysis of these targeted proteins).

II. Plant Immunity

II.1 - Microtubule-Associated Protein MAP65-3 function during the compatible interaction between the downy mildew pathogen and *Arabidopsis*

BAURÈS I., QUENTIN M., CAILLAUD M.C., LECOMTE P., MARFAING N., ABAD P., PANABIÈRES F., FAVERY B., AND H. KELLER

Plant-Oomycete and Plant-Nematode Interactions Groups, UMR-Plant Biotic Interactions and Plant Health, INRA1301-CNRS6243-Université Nice-Sophia Antipolis, 06903, Sophia Antipolis, France.

Downy mildew oomycete pathogens from the genus *Hyaloperonospora* are obligate biotrophs infecting crucifers and having devastating effects on brassicaceous crops. On *Arabidopsis*, *H. arabidopsidis* spores germinate on leaf surfaces and form appressoria, thus enabling interacellular hyphae to invade leaves. Hyphae develop haustoria within host cells, which are feeding structures required for the biotrophic lifestyle of the oomycete. To complete the infection cycle, the oomycete forms conidiophores through stomatal openings for asexual reproduction and propagation.
A promoter-trap strategy let to the identification of the *A. thaliana* Microtubule-Associated Protein, AtMAP65-3. AtMAP65-3 has been shown to be essential for the development of feeding giant cells, which are induced by the root knot nematode *Meloidogyne incognita.* AtMAP65-3 plays a critical role in organizing cytoskeleton microtubule arrays during mitosis and cytokinesis in all dividing cells (Caillaud *et al.*, 2008).
Upon infection with *H. arabidopsidis, AtMAP65-3* is expressed locally in cells harboring haustoria. In addition, a *map65-3* null mutation results in reduced oomycete asexual reproduction. We will present a detailed functional analysis, in order to show whether AtMAP65-3 plays a critical role in the establishment of the compatible interaction between *A. thaliana* and *H. arabidopsidis*

II.2 - Responses of *Vitis vinifera* to *Plasmopara viticola* after elicitation with different plant stimulators: efficacy, gene expression and stilben production

DUFOUR M-C., LAMBERT C., MÉRILLON J.M. AND CORIO-COSTET M-F

INRA, UMR Santé végétale, ISVV, IFR 103, BP 81, 33883 Villenave d'Ornon, France
GESVAB, ISVV, BP 81, *33883 Villenave d'Ornon, France*

Phosphonates are well known to possess powerful antifungal activity but also to stimulate the defences of plants. In this study, the efficiencies of two phosphonate derivatives (fosetyl-Al and a foliar fertilizer (PK2)) were evaluated on different phenotypes of grape downy mildews (*Plasmopara viticola*), in comparison to that found after elicitation with benzothiadiazole (BTH), which mimics salicylic acid in natural systemic acquired resistance. To verify their action mode as plant defence stimulating in grapevine, we quantified the level of gene expressions involved in potential plant defence pathways by RT-qPCR and the accumulation of phytoalexins (stilbens) by HPLC. In spite of downy mildew strains exhibited no difference in sensitivity to phosphonate derivatives depending on their phenotypes (resistant or sensitive to fungicides) in biological tests, grapevine modulated its defence induced respons. BTH induced over expression of CHIT3, PR1, GLU and LOX and GST while phosphonates induced over expression of CHI/CHS, PR1/PR10, CHIT4, PGIP, LOX and GST genes. A difference of expression pattern was also observed between these products in plant response against different downy mildew phenotypes. An assay of efficiency in vineyard was also realized, in strong parasitic pressure of mildew, by using a dose presenting at least 80 % of efficiency in laboratory. Significant effects are so obtained, leading us to hope their use in new alternative fight strategies to fungicides. We conclude on the role of diversity of the two pathogens on the efficacy of these chemical compounds and also on the effective stimulation of grapevine plant defences.

II.3 - A novel elicitor (PiPE) from *Phytophthora infestans* induces active oxygen species and the hypersensitive response in potato

FURUICHI N. AND K. YOKOKAWA

Graduate School of Sciences and Technology, Niigata University, Niigata 950-2181, Japan.

A novel elicitor (PiPE) from the oomycete *Phytophthora infestans (Pi)* stimulates the hypersensitive response (HR) in potato. The PiPE, purified by anion-exchange chromatography from a water-soluble extract of *Pi* caused cell death, characteristic of HR, and enhanced active oxygen species (AOS) generation in tuber tissues. The partial amino acid sequence was determined by using LC-Mass. The sequence of the PiPE cDNA derived by PCR had homologous domain to fructose 1,6 bisphosphate aldolase (FBA) genes. To demonstrate that the PiPE cDNA encodes an active elicitor, we expressed PiPE in *Echerichia coli*, and purified the recombinant protein.
His-PiPE induced HR, browning and generation of AOS in potato tissues. The PiPE was produced in the germination fluid from *Pi* and was existing in the cell wall of *Pi*. The role of PiPE peptides in the induction of HR in an incompatible interaction between *Pi* and potato cells is discussed. His-PiPE induced HR, browning and generation of AOS in potato tissues. The PiPEwas produced in the germination fluid from *Pi* and was existing in the cell wall of *Pi*. The role of PiPE peptides in the induction of HR in an incompatible interaction between *Pi* and potato cells is discussed.

II.4 - Host proteins that contribute to oomycete susceptibility in *Arabidopsis*

HOK S.[1], ALLASIA V.[1], ANDRIO E.[1], ATTARD A.[1], CLEMENT M.[2], PANABIÈRES F.[1], VOLL L.[3], AND H. KELLER[1]

[1]*Plant-Oomycete Interactions Group, UMR-Plant Biotic Interactions and Plant Health, INRA1301-CNRS6243-Université Nice-Sophia Antipolis, 06903, Sophia Antipolis, France.*
[2]*Laboratory of Plant Development Biology, UMR 6191 CNRS-CEA-Aix-Marseille II, CEA Cadarache, 13108 St Paul lez Durance, France.*
[3]*Lehrstuhl Biochemie, Department Biologie, Universität Erlangen-Nürnberg, 91058 Erlangen, Germany.*

Fungicide treatments and resistance breeding approaches are basically inefficient in managing oomycete diseases on agricultural crops and in natural ecosystems. To develop new and efficient methods to control the pathogen, it is essential to increase our knowledge on the molecular bases that govern the development of disease. In this context, our aim is to identify plant functions, which are manipulated by oomycete pathogens during the infection process, and which might be potential targets for novel plant protection strategies. We combined an analysis of the *Arabidopsis thaliana* transcriptome at different stages of a compatible plant-oomycete interaction with reverse genetics approaches. This approach revealed a subset of genes, which are activated by *Hyaloperonospora arabidopsidis,* and which are required for ordered oomycete development in plant tissues. One of them codes for a putative leucine-rich repeat receptor-like kinase (LRR-RLK). Transcription of this gene is locally activated in haustoria-harboring cells, and appears to positively affect oomycete development in plant tissues. A detailed functional analysis will be presented, which aimed at understanding the role of the LRR-RLK for normal plant development and for the compatible plant-oomycete interaction.

II.5- Partial resistance to *Aphanomyces euteiches* in *Medicago truncatula*: involved mechanisms and fine mapping of a resistance QTL

DJEBALI N.[1, 2], BOTTIN A.[2], DUMAS B.[2], AND C. JACQUET[2]

[1] Lab. Interactions Légumineuses Microorganismes, Centre de Biotechnologie à la Technopole de Borj Cedria, BP 901, 2050 Hammam-Lif, Tunisia ;
[2] UMR 5546 CNRS/UPS. Pôle de biotechnologies végétales, BP42617, Auzeville, 31326, Castanet-Tolosan, France

A pathosystem between *Aphanomyces euteiches*, the causal agent of pea root rot disease, and the model legume *Medicago truncatula* was developed to gain insights into mechanisms involved in resistance to this oomycete. The F83005.5 French accession and the A17-Jemalong reference line, respectively susceptible and partially resistant to A. euteiches, were selected for further analyses. Microscopy analyses of root thin sections revealed that a major difference between the two inoculated lines occurred in the stele which remained pathogen free in A17. Striking features were observed in A17 roots only, including i) frequent pericycle cell divisions, ii) lignin deposition around the pericycle and iii) accumulation of soluble phenolic compounds. Involvement of phenylpropanoid and flavonoid pathways were confirmed by transcriptome and mass spectrometry approaches that revealed both quantitative and qualitative differences in root phenolic compounds between the susceptible and partially resistant lines. Genetic analysis of resistance was performed with a F7 population of 139 recombinant inbred lines (RILs) and highlighted a major QTL, named *prAe1*, on the top of chromosome 3. The response to A. euteiches of near isogenic lines (NILs) confirmed the key role of this QTL in *M. truncatula* resistance. Fine mapping allowed the identification of a 130 kb sequenced genomic DNA region, rich in proteasome-related genes. qRT-PCR results showed that most of these genes were induced only in inoculated A17. Hypotheses about mechanisms possibly involved in the observed partial resistance will be discussed.

II.6- GRP-CD cell wall proteins in plant kingdom and their involvement in tobacco resistance to *Phytophthora parasitica*

PONCHET M.[1], MARAIS A.[1], ELMORJANI K.[2], INDUSTRY B.[1], SEASSAU A.[1], PANABIERES F.[1], MARION D.[2] AND E. GALIANA[1]

[1]Plant-Oomycete Interactions Group, UMR-Plant Biotic Interactions and Plant Health, INRA1301-CNRS6243-Université Nice-Sophia Antipolis, 06903, Sophia Antipolis, France.
[2]Laboratory of Plant Biopolymers Assembly, INRA, BIA, 44316 Nantes, France.

Glycine Rich Proteins "with a" Cysteine Domain (GRP-CD) are plant cell wall proteins, particularly abundant in xylem tissues. They were only described in tomato, tobacco and *Arabidopsis thaliana*. Analysing the transcriptome of more than 125 species, we demonstrate a very sparse repartition in *Viridiplantae* probably restricted to seed plants. This particular feature could not be connected to botanical and taxonomical classification.
A functional analysis of this multigenic family in tobacco was achieved by gene silencing. *GRP-CD* silencing targets the seven tobacco genes and does not affect plant development. Loss of function clearly impaired basal and elicitin-triggered Systemic Acquired Resistance towards *Phytophthora parasitica* by ca 50 %. This was not due SA pathway regulation by GRP-CD which was previously described in *Arabidopsis*. The tobacco recombinant GRP-CD1 drastically inhibits *P. parasitica* germination and growth and was found to accumulate in the oomycete cell wall. This protein also strongly binds to cellulose matrix.
This study points out the role of GRP-CDs in plant resistance to *Phytophthora*. These proteins may have a dual effect (i) inhibiting *Phytophthora* growth and (ii) reinforcing plant cell walls. From the evolutionary point of view, the sparse distribution of this gene family in seed plants raises the question of independent apparition and/or loss of genes. The functional divergence noted between tobacco and *Arabidopsis* also indicate probable independent evolution of GRP-CD functions among higher plants.

II.7 - R3a resistance protein mutants with extended effector recognition specificity

SEGRETIN M.E. AND S. KAMOUN

The Sainsbury Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UH, United Kingdom

Phytophthora infestans is one of the most devastating pathogens affecting potato production worldwide. One strategy to generate resistant cultivars is the introduction of resistance genes that are able to recognize *P. infestans* effector proteins with avirulence activities. R3a, a resistance protein discovered in potato, can trigger an hypersentive response upon the recognition of the avirulence effector $AVR3a^{KI}$ from *P. infestans* but cannot recognize $AVR3a^{EM}$, the product of another allele that is predominant in pathogen populations. To date, all the characterized *P. infestans* strains in nature carry at least one of these AVR3a proteins. The objective of this work is to extend R3a recognition specificity to $AVR3a^{EM}$. To accomplish this, we generated a library of R3a mutant variants obtained by random mutagenesis. The mutated molecules were cloned in a T-DNA binary vector and transformed into *Agrobacterium tumefaciens*. We screened the mutant clones by co-agroinfiltration with $AVR3a^{EM}$ in *Nicotiana benthamiana* plants, and evaluated the presence of HR-like phenotypes after 5 days. Of approximately 2200 evaluated clones, 20 triggered different degrees of HR-like responses and were subjected to new rounds of infiltrations to confirm the results. In parallel, the candidate clones were co-infiltrated with $AVR3a^{KI}$ and with a negative control plasmid to investigate the conservation of the original R3a recognition specificity and also to eliminate auto-active R3a mutants. In total, 17 clones were selected for further analyses, including sequencing and the construction of chimeric clones to investigate which mutations are responsible of the observed phenotypes. This works highlights how knowledge of pathogen effectors can be exploited for engineering novel resistance genes.

II.8 - Infection of *Arabidopsis thaliana* by *Phytophthora parasitica*

WANG Y.[1,2], MENG Y.[1], SUN Y.[1], BOUWMEESTER K.[2], GOVERS F.[2], AND W. SHAN[1]

[1]Biotechnology Center and College of Plant Protection, Northwest A & F University, 3 Taicheng Road, Yanglin, Shaanxi, 712100 P.R. China.
[2]Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

The genus *Phytophthora* consists of over 90 species that all cause devastating diseases on a wide range of agriculturally and ornamentally important plants, and in forests and natural ecosystems. The soilborne species *Phytophthora parasitica*, also known as *P. nicotianae*, is the causal agent of root and stem rot on a wide, diverse range of host plants. In this study, we infected the model plant *Arabidopsis thaliana* with *P. parasitica* and demonstrated that *A. thaliana* is highly susceptible to *P. parasitica*. Roots and detached leaves of *A. thaliana* were inoculated with *P. parasitica* zoospores, and the infection process was monitored from 30 minutes to 5 days post inoculation and examined by scanning electron microscopy and interference contrast microscopy. Following inoculation the *P. parasitica* zoospores encysted and germinated. Subsequently, appressorium-like swellings were formed, and epidermal host cells were penetrated directly at the junction between anticlinal cell walls and the stomata. After penetration, mycelia branched and developed numerous haustoria, as well as sporangiophores for generating abundant sporangia. Colonization of the leaves by *P. parasitica* caused water-soaked lesions within 3 days, while the root infection led to collapse of whole seedlings. Pathogenicity assays indicated the presence of specificities between *A. thaliana* and *P. parasitica*. The developed A. *thaliana-P. parasitica* pathosystem will facilitate a better understanding of processes underlying plant-*Phytophthora* recognition and *Phytophthora* pathogenicity

II.9 - Functional characterization of *Hyaloperonospora arabidopsidis* RxL(R)-type effectors in *Arabidopsis thaliana*

YATUSEVICH R.[1], CABRAL A [2], BAUTOR J. [1], KATOU S.[1], VAN DEN ACKERVEKEN G.[2], AND J. PARKER[1]

[1] Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany;
[2] Dept. Biology, Utrecht University, Utrecht, The Netherlands. yatusev@mpiz-koeln.mpg.de

Downy mildew disease of *Arabidopsis* is caused by the oomycete pathogen *Hyaloperonospora arabidopsidis (Hpa)*. As an obligate biotroph, *Hpa* is thought to deploy a battery of virulence proteins (effectors) to suppress host immune responses and establish a feeding relationship for growth and reproduction. The processes by which biotroph-derived effectors manipulate host cell metabolism and defense remain poorly understood. We have identified *Hpa* candidate effectors expressed during Ws *eds1-2* leaf infection with the virulent *Hpa* isolate Waco9. One group of predicted *Hpa*-secreted proteins with an appropriately positioned RxL(R) host cell-translocation motif has been cloned for functional studies. We used the bacterial Type III ('effector detector vector'; EDV) delivery system on a range of *Arabidopsis* accessions, as well as transient defence and cell death suppression assays to test for measurable virulence activities of individual RxL(R) proteins. We extended the assays to characterize allelic forms RxL(R) proteins that are polymorphic between different *Hpa* isolates. Through these approaches, we have identified several polymorphic or isolate-specific *Hpa* RxL(R) candidates which are able to suppress PTI (PAMP-triggered immunity) and/or ETI (effector-triggered immunity). *Arabidopsis* transgenic lines over expressing or conditionally expressing particular RxL(R) alleles have been made and are being screened for altered host immune responses. We are focusing on these candidates to define effector activities more precisely, identify potential host targets in *Arabidopsis* and explore mechanisms contributing to downy mildew pathogenesis and interception by host receptors.

III. Reproduction, Evolution and Epidemiology

III.1 - Resistance of *Plasmopara viticola* to QoI fungicides: origin, diversity and fitness

CORIO-COSTET M.F., DUFOUR M.C., CIGNA J., ABADIE P., MARTINEZ F., AND W.J. CHEN

INRA, UMR Santé végétale, INRA-Bordeaux, ISVV, IFR 103, BP 81, 3388, Villenave d'Ornon, France.

Since its introduction in Europe, *Plasmopara viticola* (grapevine downy mildew) has been controlled by chemicals, but resistant strains of the pathogen have emerged regularly, i.e. general resistance to Qoi fungicides. Analysis of the cytochrome b gene revealed a single mutation (G143A) as a major mechanism factor conferring QoI resistance. Phylogenetic analysis of mitochondrial DNA fragments (2281 pb) allowed the detection of four major haplotypes (IS, IR, IIS, IIR) belonging to two distinct clades, each of which contained a different QoI resistant allele [1]. The prevalence of mitochondrial haplotypes and resistant alleles was assessed by characterizing 839 isolates collected in 21 localities in a Bordeaux vineyard without QoI treatment. The most frequent haplotypes (IR, IS) were found in 74% of *P. viticola* populations. Resistant-allele frequencies ranged from low (0) to very high (0.75) with an average value of 0.29. At least two independent events led to the emergence of QoI resistance. By combining microsatellite markers [2] and selected markers, a temporal genetic structure of *P. viticola* populations was obtained in three localities in Bordeaux. The genetic variability was low and the genotypic richness was high. Based on determination of the fitness index (Fic), QoI-resistant strains did not exhibit a cost and often tended to have good fitness. To assess these results, competition assays with different mixtures of sensitive and resistant strains using biological and molecular (Q-PCR) tests were done.

[1] Chen et al., 2007, Appl. Environ. Microbiol. ***73***: 5162-5172
[2] Delmotte et al., 2006. Molecular Ecology Notes, 6**,** 379-381

III.2 - Monitoring *Phytophthora infestans* epidemics on potato in Tunisia using genetics and molecular tools

HAMADA W. AND K. HARBAOUI

Laboratory of Plant Genetics, Department of Plant Sciences and Biotechnology, National Agronomic Institute of Tunisia, Tunis, Tunisia.

Late blight caused by *Phytophthora infestans* in Tunisia is always present all the year around on potato or tomato since weather conditions and plant host are permitting. Sampling of several isolates from these plants, different locations and among various seasons was achieved. Molecular analysis performed on either pure isolates or infected material allowed us to highlight the presence of the A2 type in Tunisia with a stable frequency of 12% during three years of analysis. This could be explained by an asexual multiplication rather than the sexual one which is rare. These A2 strains exhibited high levels of resistance to the fungicide metalaxyl. They also showed more severe symptoms when inoculated on the host plant. The characterization of the avirulence genes achieved on differential set of *Solanum* revealed the presence of different races of the pathogen within the population analyzed. In addition, SSR molecular markers confirmed the presence of a large genotypic variation within the population and the dominance of a clone in different regions. On another hand, analysis of genes involved in plant - pathogen interaction revealed that expression level is different according to the origin plant host (potato or tomato) of the strain and the organ infected. Some adaptation of the pathogen to the plant material could be possible. All these characteristics of the pathogen are important to consider for more efficient control of late blight in Tunisia.

IV. Cell Biology and Genomics

IV.1 - Association of Cdc14 with the basal body in *Phytophthora infestans* suggests a new role for this canonical cell-cycle regulator

AH-FONG A.M.V., AND H.S. JUDELSON

Department of Plant Pathology and Microbiology, University of California, Riverside, California, USA

In model eukaryotes such as yeast and animal cells, Cdc14 is required for normal mitotic behavior during cellular proliferation. Here we propose additional functions based on studies of the *Phytophthora infestans* ortholog. PiCdc14 is not expressed during vegetative growth (where most mitoses occur) although it complements a yeast Cdc14 mutation that normally arrests mitosis. Transformants overexpressing PiCdc14 exhibit normal nuclear behaviour and development while such experiments in traditional models typically cause cell cycle defects. A diversification of Cdc14 function in *Phytophthora* is further supported by subcellular localization studies indicating that although some PiCdc14 is nuclear, during zoosporogenesis most transits to an extranuclear organelle which appears to be the basal body, which is the microtubule nucleation site from which flagella develop. An evolutionary association of Cdc14 with flagella also is supported by data from most eukaryotic kingdoms. To better understand how and where PiCdc14 associates with the basal body, colocalization studies with other basal body proteins such as SFA and DIP13 and microtubule binding experiments are being performed. While the purpose of PiCdc14 localization to basal bodies remains to be determined, other data suggest that the protein may have multiple functions during spore development. Analyses of the PiCdc14 promoter also suggest that multiple signalling pathways activate transcription of the gene at different stages of spore biology.

IV.2 - Zinc finger proteins for manipulation of gene expression in *Phytophthora infestans*

ANDREEVA K., AND H.S. JUDELSON

Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA

Improvements to the genetic tools available for manipulating genes in oomycetes would enhance studies of gene function in these organisms. To help accomplish this we are attempting to use two strategies based on custom engineered zinc finger proteins (ZFPs). It has been reported that in a variety of transgenic organisms, ZFPs fused to transcriptional repression domains inhibit transcription whereas ZFPs fused to nuclease domains create targeted mutations. We are testing these approaches in *P. infestans* using the *inf1* elicitor gene as a target. Using a modular assembly approach, ZFPs were designed that either target positions in *inf1* promoter region or its open reading frame. These were tested for their DNA binding activity and specificity using bacterial two-hybrid reporter system and EMSA assay approaches. One ZFP with high specificity and strong binding activity was then fused to a SID transcriptional repression domain, transformed into protoplasts of *P. infestans,* and the resulting transformants were assayed for the expression of both the ZFP-SID chimera and INF1. Preliminary results indicated that at least some of the transformants displayed a significant down-regulation of INF1 expression. Additionally, experiments are under way involving ZFPs fused to the nuclease domain of FokI, which are designed to targeted mutations to the inf1 coding sequences.

IV.3 - *Phytophthora ramorum* pathogenesis transcriptome analysis

BROADHURST B.[1], LEVESQUE A.[2], RIOUX D.[3] AND B. SAVILLE[1]

[1]Environmental & Life Sciences Graduate Program, Trent University, DNA Building, 2140 East Bank Dr, Peterborough, ON K9J 7B8, Canada;
[2]Agriculture and Agrifood Canada, Eastern Cereal Research Centre, KW Neatby Bldg, 960 ave Carling, Ottawa (ON), K1A 0C6 Canada;
[3]Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Stn. Sainte-Foy, Québec (Québec) G1V 4C7 Canada

The emerging pathogen *Phytophthora ramorum* has been responsible for substantial losses in the coastal forests of California and Southern Oregon. It has also been responsible for significant economic costs to nurseries in North America and Europe as well as regulatory agencies attempting to control its spread and prevent its establishment in new areas. The sequencing of the *P. ramorum* genome and the discovery of putative effector proteins in *Phytophthora* species are two recent advances that have enabled us to study the molecular aspects of *P. ramorum* pathogenesis. We have begun a transcriptome analysis that will utilize next generation sequencing technologies to determine *P. ramorum* gene expression changes during pathogenesis. In these investigations we will assess changes in gene expression resulting from different growth conditions of *P. ramorum* prior to infection and from growth on divergent host species. We will report on the initial experiments in this project, infections of *Rhodododenron catawbiense* with a *P. ramorum* NA2 genotype that is either: 1) cycled through the same host 6 times, 2) cycled a single time through the host or 3) used directly from the stock. Cycling is a common process used in *P. ramorum* pathogenicity studies and has been determined to increase the level of virulence. Our focus is to assess gene expression changes associated with this increased virulence. We will expand these investigations to compare gene expression among different *P. ramorum* lineages and for a given *P. ramorum* strain on multiple hosts. These transcriptome studies will provide substantial information on genes expressed during the infection process as well as providing additional data to improve gene identification and genome annotation.

IV.4 - PsSAK1, a stress-activated MAP kinase of *Phytophthora sojae,* is required for zoospore viability and infection of soybean

LI A., WANG Y., TAO K., DONG S., HUANG Q., DAI T., ZHENG X., YUANCHAO WANG

Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China

Mitogen-activated protein kinase (MAPK) pathways are universal and evolutionarily conserved signal transduction modules in all eukaryotic cells. In this study, PsSAK1, encoding a stress-activated mitogen-activated protein kinase of *Phytophthora sojae*, is identified. PsSAK1 is highly conserved in oomycetes, and it represents a novel group of MAPKs due to its pleckstrin homology domain. RT-PCR analysis showed that PsSAK1 expression was up-regulated in zoospores, cysts and during early infection. In addition, its expression was induced by osmotic and oxidative stress mediated by NaCl and H2O2, respectively. To elucidate the function, the expression of *PsSAK1* was silenced by using stable transformation of *P. sojae*. The silencing of *PsSAK1* did not impair hyphal growth, sporulation or oospore production but severely affected zoospore development, in that the silenced strains showed quicker encystment and lower germination ratio than the wild type. PsSAK1-silenced mutants produced much longer germ tubes and could not colonize wounded or unwounded soybean leaves. Our results indicate that PsSAK1 is an important regulator of zoospore development and pathogenicity in *P. sojae*.

Keywords: *Phytophthora sojae*, zoospores development, pathogenicity, MAPK

IV.5 - Role of a new glycoproteins family in *Phytophthora parasitica* biofilm formation

THEDORAKOPOULOS N.[1,2,3], GOVETTO B.[1,2,3], INDUSTRI B.[1,2,3], MURA C.[1,2,3], DELEURY E.[1, 2,3], MARAIS A.[1,2,3], ARBIOL G.[1,2,3], PANABIÈRES F.[1,2,3], PONCHET M.[1,2,3], AND E. GALIANA[1,2,3]

[1] INRA (Institut National de la Recherche Agronomique) ; [2] CNRS (Centre National de la Recherche Scientifique) ; [3] Université de Nice-Sophia Antipolis, Plant-Oomycete Group, Unité Mixte de Recherche Interactions Biotiques et Santé Végétale, F-06903 Sophia Antipolis, France

Alternatively to the single-cell behaviour (adhesion of a zoospore, encystment and germ tube penetration), the infection cycle of *Phytophthora parasitica* may involve cell population dynamics via the formation of biofilms. Initially, founder cells adhere to the host surface, aggregate, drive the migration of a second wave of zoospores leading to formation of structured microcolonies embedded in an exopolysacharridic matrix (Galiana *et al.*, 2008, Environ Microbiol 10: 2164).

Microarray analyses revealed that microcolony formation is mainly characterized by the coordinated up-regulation of genes encoding proteins involved in extracellular matrix elaboration or in the export/import of substrates. Two of the ten most up-regulated genes belong to the same gene family widespread in *Phytophthora,* with four to eight genes per species all of them annotated in sequenced genomes as coding for conserved hypothetical proteins. They encode secreted proteins organized in two domains. The first one is highly conserved and unrelated to any known functional domain. The second one is particularly enriched in Proline, Threonine, Serine residues and contains a variable number of repeats, supporting a mucin-like classification. The two *P. parasitica* (see above) recombinant proteins expressed in *Pichia pastoris* show indeed a high level of glycosylation in domain 2. These characteristics taken together with a specific mucin staining of microcolonies suggest that these proteins contribute to the elaboration of the exopolysacharridic matrix of the biofilm through extensive O-glycosylation.

IV.6 - Functional characterization of bZIP transcription factors in the asexual development of the plant pathogen *Phytophthora infestans*

GAMBOA-MELENDEZ H., AND H.S. JUDELSON

Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA

Transcription factors of the basic leucine zipper family (bZIP TFs) help control processes such as development, energy homeostasis, and stress response in all eukaryotes. bZIPs contain a leucine repeat motif domain responsible for TF dimerization and a basic region important for DNA binding. So far, only one bZIP, *Pibzp1*, has been identified in *Phytophthora infestans* which was shown to be essential for appressoria development and infection. Nine other bZIPs were found within its genome. To help characterize their roles, expression profiling using qRT-PCR revealed that one was induced strongly at cleaving sporangia stage, another in cysts, and three in the sporangia, cleaving sporangia and germinated cyst stages. Five others were highly expressed at both germinated and ungerminated cysts, and one more expressed strongly in mycelium, cysts and germinated cysts. Overexpression and gene silencing studies are currently being carried out to determine whether these regulate development in *P. infestans*.

To develop additional tools to study gene function in oomycetes, we are also developing a chemically-inducible gene expression system based on the ecdysone receptor (EcR) gene switch. Similar systems in plants have proved to be useful in either monopartite and two-hybrid formats. So far, we have tested the monopartite EcR system in *P. infestans* using the GUS reporter. Preliminary results indicate that the transactivator drives reporter expression, but with a high background in the absence of inducer. A two-hybrid system in which the DNA binding and activation domains are carried on different expression cassettes is now being tested.

IV.7 - Gene expression during the appressorium mediated penetration of *Arabidopsis thaliana* roots by *Phytophthora parasitica*

ATTARD A., MAGGIO C., KEBDANI N., DELEURY E., PONCHET M., PANABIÈRES F. AND M. GOURGUES

Plant-Oomycete Interactions Group : UMR Interactions Biotique et Santé Végétale, INRA1064-CNRS6192-Université Nice–Sophia Antipolis, France

Oomycetes from the genus *Phytophthora* are fungus-like pathogens that are devastating for agriculture and natural ecosystems. Due to their particular physiological characteristics, no treatment against these microorganisms is available. To develop such treatments, it appears essential to dissect the molecular mechanisms that determine the interaction between *Phytophthora* species and host plants. Available data are scarce, mainly concerning the early events of root penetration and colonization. The laboratory developed cDNA libraries corresponding to various developmental stages among which an appressorium-derived library. A custom oligo array containing 4700 *P. parasitica* uni-sequences was used to analyze the appressorium mediated penetration of plant roots through a transcriptome analysis. Samples were recovered from *A. thaliana* roots (see poster *A. thaliana* transcriptome) challenged with *P. parasitica* (4 time points from 2.5 to 30 hours after inoculation), from appressoria differentiated on onion epidermal layers (a simplified system used to enrich for pathogen sequences) and from mycelium and swimming zoospores as controls. We showed that 4200 sequences (89%) were expressed in at least one condition. 3788 sequences were significantly modulated (Anova, P. value < 0.05) and 1829 sequences (40%) showed a max fold chance higher than 4. K-mean clustering identified genes with highly regulated expression pattern. These first results show that a highly dynamic transcriptome occurs during the first hours of the interaction between a plant and a root infecting *Phytophthora* species. Notably, genes whose expression is modulated during appressorium mediated penetration of Arabidopsis roots were identified.

IV.8 - *Phytophthora capsici*: genome assembly of a polymorphic organism

LAMOUR K. H.[1], SCHMUTZ J.[2], MUDGE J.[3], KAMOUN S.[4], MILLER N.[3], RICHARDSON P.M. [5], PLATT D.[5], GRIGORIEV I.[5], KUO A.[5], FINLEY L.[1] , HURTADO-GONZALES O. [6], DONAHOO R.[7], SAVIDOR A.[8], CHERTKOV O.[9], HAN C. S.[9], DETTER C.[9], MAY G. D.[3], W. BEAVIS D.[10], AFFOURTIT J.[11], EGHOLM M. [11], KNIGHT J.[11], AND S.F. KINGSMORE[3]

[1] The University of Tennessee, Knoxville, Tennessee, USA
[2] Hudson Alpha Institute for Biotechnology, Huntsville, AL, USA,
[3] National Center for Genome Resources, Santa Fe, New Mexico, USA,
[4] The Sainsbury Laboratory, Norwich, UK,
[5] USA Department of Energy, Walnut Creek, California, USA,
[6] Pioneer Hybrid, Johnston, IA, USA,
[7] University of Florida, Immokalee, USA,
[8] Universit of Tel Avivy, Tel Aviv, Israel,
[9] Los Alamos, New Mexico, USA,
[10] Iowa State University, Ames, Iowa, USA,
[11] Roche, USA

Phytophthora capsici is a devastating oomycete pathogen of vegetable crops. Using 454 titanium and Sanger sequencing technology, we have sequenced and assembled a draft of this ~60 Mb eukaryotic genome. The assembly includes 15X shotgun coverage of 454 Titanium pyrosequencing sequencing averaging 338 nt, 4.5X coverage of 454 Titanium 20kb paired end sequencing averaging 155 nt, and 5X coverage of Sanger 6 or 36 kb paired end sequencing averaging 900 nt. We have assembled approximately 60 Mb into 2,000 scaffolds (scaffold N50 = 669kb). We recovered over 98% of full-length cDNAs with less than 1% represented in multiple positions. Approximately 3 Mb of repetitive DNA supported only by unanchored repetitive reads was excluded from the assembly to avoid repeat-induced misjoins. In addition, Solexa cDNA libraries from eight life stages and one infection stage have been sequenced to improve gene annotation, identify SNPs, and compare gene expression levels. The broad and expanding host range of this organism emphasizes the genomic adaptability of this organism. To start to understand the natural diversity within *P. capsici* we have sequenced reduced representation genomic samples (Floragenex's RAD technology and solexa sequencing) from eight field isolates and analyzed the resulting SNPs. The successes and challenges of doing a 454-based de novo genome sequencing project in an oomycete will be discussed.

IV.9 - Expression analysis of Carbohydrate-esterase coding genes in *Phytophthora infestans* grown *in vitro* and *in planta*

OSPINA-GIRALDO M., LAIRD E., AND C. MINGORA

Biology Department, Lafayette College, Easton, PA 18042, USA

Preliminary analyses of *Phytophthora infestans* genome have led to the identification of a group of 49 putative genes homologous to members of 8 families belonging to the Carbohydrate esterase (CE) gene superfamily. CE enzymes, such as the ones classified within the CE Family 5 (the "cutinase" family) may play a role in the infection process by targeting and degrading the cell wall. We have analyzed the expression a subgroup of CE-coding genes using reverse-transcription PCR (RT-PCR) and found that most genes are expressed in mycelium of *P. infestans* grown *in vitro*. To determine the level of expression of each of these genes, a quantitative PCR (qPCR) analysis was conducted. In addition, because of the potential importance of cutinase for *P. infestans* pathogenicity, a qPCR study was performed using plant tissue samples obtained at different stages of the infection process. Results of these investigations will be presented and discussed.

IV.10 - Signatures of adaptation to obligate biotrophy in the *H. arabidopsidis* genome

MCDOWELL J. M.[1], **ON BEHALF OF THE H. ARABIDOPSIDIS GENOME COALITION**[2]

[1]*Virginia Tech, and* [2]*other institutions in the US, Netherlands, France, Canada, China, and the United Kingdom*

Many plant pathogens extract nutrients exclusively from living plant tissue and cannot grow apart from their hosts (termed "obligate biotrophy"). Here we report the genome sequence of the oomycete *Hyaloperonospora arabidopsidis*, a natural downy mildew pathogen of *Arabidopsis thaliana* and a model for obligate biotrophy. Although downy mildews are uniformly obligate, they have evolved in evolutionary recent times from paraphyletic *Phytophthora* spp. that can exist as saprobes and destroy plant tissue after they have invaded their hosts. Thus, comparison of the *H. arabidopsidis* genomes with recently sequenced *Phytophthora* genomes provides an opportunity to understand how an obligate biotroph has evolved from a free-living ancestor that employed a very different pathogenicity strategy. Our comparisons revealed two striking themes. First, gene families encoding proteins with potential to damage host cells or otherwise trigger defense responses (cell wall-degrading enzymes, elicitins, necrosis-inducing proteins, RxLR effectors, and others) were dramatically reduced in *H. arabidopsidis* compared to *Phytophthora*, indicative of optimization for stealth inside the host. Second, genes involved in several metabolic pathways were absent from *H. arabidopsidis*, suggestive of metabolic dependency on the host. Some features of *H. arabidopsidis* gene space are paralleled in the genomes of non-obligate, biotrophic fungi (the plant smut pathogen *Ustilago maydis* and the ectomycorrhizal symbiont *Laccaria bicolor*), demonstrating that oomycetes and fungi have evolved similar molecular adaptations to a biotrophic lifestyle.

IV.11 - Effect of the cyclic lipopeptide MassA on *Phytophthora infestans* gene expression

MEIJER H. J.G., DE KEIJZER J., VAN DE MORTEL J., DEKKERS E., RAAIJMAKERS J. AND F. GOVERS

Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Phytophthora infestans and related oomycetes are notorious plant pathogens that are responsible for serious economic losses in agriculture worldwide. At present, control of oomycete pathogens relies mainly on frequent applications of agrochemicals but concerns about the adverse effects of agrochemicals on food safety and the environment are increasing. Hence, there is a high demand for novel, environmentally friendly control strategies preferably based on natural products. Cyclic lipopeptides (CLPs), a class of natural compounds produced by a variety of bacteria including *Pseudomonas* species, were shown to have a strong activity against *Phytophthora* and other oomycetes. The adverse effects of the CLP Massetolide A (MassA) on *P. infestans* are not limited to *in vitro* growth stages such as zoospores or mycelium, but are also observed when *P. infestans* is infecting and colonizing tomato leaves. We monitored genome-wide changes in gene expression in *P. infestans* mycelium grown *in vitro* and found several distinct genes that were up- or down-regulated upon exposure to MassA. For several of these genes, we are now analyzing their expression in a range of *P. infestans* isolates that vary in their sensitivity to MassA. Since MassA causes inhibition of lesion expansion on tomato leaves, we will also assess the effect of MassA on *P.infestans* gene expression during growth *in planta*. We expect that this study will reveal signaling pathways and/or structural components in *Phytophthora* that are targeted by CLPs and as such, may help in developing novel strategies to control *Phytophthora* diseases.

IV.12 - *SPACES* and the Oomycete undergraduate molecular genetics network: an internet resource for and by the oomycete community

OSPINA-GIRALDO M., AND C. BENTLEY

Biology Department, Lafayette College, Easton, PA 18042, USA

The oomycete community is rapidly expanding and researchers in multiple laboratories have created comprehensive websites that provide explanations of research projects, publications and/or links to published articles, novel techniques and basic protocols on (mostly) *Phytophthora* research. The Oomycete Molecular Genetics Research Collaboration Network also supports a website that constitutes an excellent source of up-to-date information on events, meetings, fellowships, and important news for the community. In addition, the databases maintained by the Broad Institute, the Joint Genome Institute, and the Virginia Bioinformatics Institute contain and share a wealth of resources focusing on recently sequenced genomes of several oomycetes. The increasing availability of the Internet has made these portals easily accessible to most members of the community. A complementary resource would be one that facilitates member interaction and becomes a forum that makes the exchange of ideas possible. The Oomycete Undergraduate Molecular Genetics Network (OUMGN) has been developed as a means to fill this need; although originally geared towards members of the OMGN research community affiliated with undergraduate institutions, it also welcomes individuals from major universities and other organizations. The OUMGN *SPACES* site is a place where OMGN members and non-members can post questions, comments, announcements, links of interest, etc. Details on its characteristics, advantages, and use will be presented.

IV.13- Modulation of the *Arabidopsis* transcriptome during root infection with the soilborn oomycete, *P. parasitica*

GOURGUES M., LE-BERRE J.-Y., KELLER, H., PANABIERES, F., AND A. ATTARD

Plant Oomycete Interactions Group, UMR-Plant Biotic Interactions and Plant Health, INRA1301-CNRS6192-Université Nice–Sophia Antipolis, 06903 Sophia Antipolis, France.

Oomycetes from the genus *Phytophthora* are devastating for crop plants. As to date no treatment against these microorganisms is available, it appears essential to dissect the molecular mechanisms that underlie the interaction between *Phytophthora* species and host plants. The laboratory developed a novel interaction system involving the model plant, *Arabidopsis thaliana* and *Phytophthora parasitica*, a soil-borne pathogen infecting a wide host range and thus representing the majority of *Phytophthora* species. A characteristic feature of the compatible *Arabidopsis*/*P. parasitica* interaction is an extended biotrophic phase, compared to infection of the natural host, tomato. Using this pathosystem we performed a genome-wide transcriptome analysis of both partners of the interaction during the onset of a compatible interaction (5 time points were assessed from 0 to 30hpi; see poster *P. parasitica* transcriptome). Among the genes represented on the *Arabidopsis* ATH1 chips, 76% appeared differentially expressed in at least one conditions, and 6,062 (26%) presented a fold change ratio that was higher than 2. K-mean clustering led to 5 main expression patterns, with 1,358 gene transcripts accumulating specifically in response to penetration or during the onset of infection. In addition, hierarchical clustering showed that the *A. thaliana* response to penetration involves a peculiar genetic program. These results show that modulations of the *Arabidopsis* transcriptome occur immediately upon contact with *P. parasitica*.

IV.14 -Analysis of EST sequences from the entomopathogenic oomycete *Lagenidium giganteum*

DOUMA L. G.[1], TYLER B.M. [2], AND A. TARTAR[1]

[1] *Nova Southeastern University, Fort Lauderdale, FL, USA*
[2] *Virginia Polytechnic Institute and State University, Blacksburg, VA, USA*

The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is know about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, a Expressed Sequence Tag (EST) project has been initiated. Following *in vitro* culture and mRNA extraction, cDNA was synthetized and ESTs were generated by 454 pyrosequencing technologies. High quality sequences were annotated and screened for pathogenicity-related candidate genes. Overall, the EST database was used to detail the molecular basis of *Lagenidium giganteum* pathogenicity towards mosquitoes, and initiate comparative genomics analyses between *L. giganteum* and the much better characterized plant pathogenic oomycetes (such as *Phytophthora* spp.)

IV.15- Germination dynamics and biology of *P. viticola* oospores

TOFFOLATTI S.L., AND A.M. VERCESI

DI.PRO.VE. Sez. di Patologia Vegetale, Università degli Studi di Milano, via Celoria 2, 20133, Milano, Italy

Grapevine downy mildew, caused by *Plasmopara viticola*, is a seriuos diseases affecting *V. vinifera* in temperate climates. *P. viticola* overwinters as oospores, which provide the inoculum for primary infections. Compared to the secondary cycles, the contribution of primary infections to the final disease incidence has been traditionally considered of lesser importance and limited to the first phases of grapevine growing season. Recently, genetic analyses, based on SSR markers, showed that new *P. viticola* genotypes appear in vineyard form late spring to summer, indirectly implying that oospore germination occurs throughout the period. Despite the key role of the oospores in the epidemics, however, limited information is available on the dynamics and on the biochemical mechanisms leading to the germination process. Germination assays, carried out at 20 °C on oospores overwintered in vineyard and in controlled conditions, show a positive influence of water availability on the oospore germination throughout the overwintering season. The higher viability of the oospores in winter than in summer, investigated by trypan blue, suggests that high temperatures have a negative effect on the structures which, in fact, do not germinate from the middle of June onwards. Finally, germination assays with inhibitors of different steps of the Ca^{2+}/calmodulin and calcium imaging, suggest the involvement of calcium signalling in macrosporangium formation. Taken together, the results show a complex relationship between the oospores and the environment which could be better elucidated if the genes involved in the germination process were known.

IV.16 - Towards the development of alternative control strategies against *Saprolegnia diclina* on salmonid eggs

VAN DEN BERG A.H.[1], PEARSON M.[2], SECOMBES C.J.[3], AND P. VAN WEST[1]

[1] *Aberdeen Oomycete Laboratory, University of Aberdeen, Aberdeen, Scotland, UK.*
[2] *Landcatch Ltd., UK*
[3] *Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, Scotland, UK.*

Saprolegnia diclina is one of the most important and devastating pathogens on salmonid eggs in fish hatcheries. It is responsible for millions of pound losses to the aquaculture business worldwide. At Landcatch Ltd. losses of 10% per year, representing £300K, are commonplace.
Up until the beginning of the century, *Saprolegnia* was kept under control with malachite green. This is an organic dye that is very efficient at killing the pathogen. However, the use of malachite green has been banned in the UK and around the world, due to its carcinogenic and toxicological effects.
As a result, new and effective strategies to control *S. diclina* infections on eggs are sought after. In this study, a chemical, a biological and a genetic approach are being investigated. All three different approaches will be discussed, giving an up to date overview of progress of each.

IV.17 - Molecular and genetic analysis of *Bremia lactucae*

WONG J., MATVIENKO M., KOZIK A., GIL J., AND R. MICHELMORE

The Genome Center, University of California - Davis, 451 Health Sciences Drive, Davis, CA 95616, USA

Bremia lactucae is an obligate biotrophic oomycete that causes the most serious disease of lettuce throughout the US and worldwide. In order to investigate the molecular basis of pathogenicity and host specificity in this pathosystem, we are sequencing the genome and characterizing the activities of effector proteins of *B. lactucae* and their plant targets using data mining and functional analyses to identify and characterize candidates.

We are currently assembling a high-coverage draft genome of *B. lactucae* using sequences from several types of libraries including BAC-end Sanger reads and single, paired-end, and mate-end Illumina reads of genomic DNA. The scaffolds will be validated, ordered, and oriented using BAC-end sequences and an ultra-high resolution genetic map generated by sequencing the gene space of segregating progeny. We will use sequence-based, multiplexed bulked segregant analysis to relate phenotypes to sequence. We will also sequence ESTs using RNAseq to aid in assigning gene models and annotating the genome. The novel combination of high-throughput sequencing and genetic validation of the assemblies will result in the efficient generation of a high quality genome sequence and enable efficient discovery of candidate effectors.

Bioinformatic analysis of cDNA and genomic sequence assemblies has so far identified several candidate genes with RXLR motifs, which are known to be involved in pathogenicity in other oomycetes. Candidate effectors are being functionally characterized for HR-eliciting activity by transient expression assays on a differential set of resistant lettuce cultivars, and for HR-suppressing activity during transient coexpression with known HR-elicitors.

Participants

AH-FONG Audrey
audreya@ucr.edu
University of California Riverside
Department of Plant Pathology
Boyce Hall
92521 Riverside
USA

ALBOUY Marie Ange
albouy@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

AMELINE Carine
carine.ameline-torregrosa@ensat.fr
Laboratoire SP2 INP-ENSAT
28 chemin de Borde Rouge Auzeville
31326 Castanet-Tolosan
FRANCE

ANDERSON Rayn
andersrg@vt.edu
Verginia Tech
536 Latham Hall Virginia Tech
24601 Blacksburg VA
USA

ANDREEVA Kalina
kalinaa@ucr.edu
University of California
Department of Plant Path & Microbiol Boyce Hall
92521 Riverside
USA

ARMSTRONG Miles
miles.armstrong@scri.ac.uk
Scottish Crop Reseach Institute
Errol Road Invergowrie
DD2 5DA Dundee Scotland
UNITED KINGDOM

AS-SADI Falah
falah.as-sadi@toulouse.inra.fr
UMR CNRS-INRA 2594/441 (LIPM)
Chemin de Borde-Rouge BP 52627 Auzeville
31326 Castanet Tolosan Cedex
FRANCE

ÅSMAN Anna
anna.asman@vbsg.slu.se
Swedish Univ. of Agricultural Sciences
Dept. of Plant Biology and Forest Genetics,
Box 7080, 750 07 Uppsala
SWEDEN

ATTARD Agnès
attard@sophia;inra.fr
INRA/CNRS/UNSA
400, route des chappes BP 167
06903 Sophia Antipolis
FRANCE

BADEL Jorge Luis
Jorge.Badel@sainsbury-laboratory.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

BANFIELD Mark
mark.banfield@bbsrc.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

BAUDIN Mael
baudin@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

BAURES Isabelle
ibaures@yahoo.fr
INRA/CNRS/UNSA
400, route des chappes BP 167
06903 Sophia Antipolis
FRANCE

BIRCH Paul
pbirch@scri.ac.uk
Scottish Crop Reseach Institute
Errol Road Invergowrie
DD2 5DA Dundee Scotland
UNITED KINGDOM

BLAIR Jaime
jaime.blair@fandm.edu
Franklin & Marshall College Dept of Biology
415 Harrisburg Ave
17603 Lancaster PA
USA

BONGSOO Park
bxp12t@psu.edu
Departement of Plant Pathology
1104 Smithfield St Apt3
16801 State College
USA

BORDAT Amandine
abordat@toulouse.inra.fr
LIPM CNRS-INRA
24, chemin de Borde Rouge
BP 52627 Auzeville
31326 Castanet-Tolosan
FRANCE

BORHAN Hossein
borhanh@agr.gc.ca
AAFC
107 Science Place
S7N 0X2 Saskatoon
CANADA

BOTTIN Arnaud
bottin@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

BOUWMEESTER Klass
klaas.bouwmeester@wur.nl
Wageningen University, Lab. of Phytopathology
Droevendaalsesteeg 4
6711 PB Wageningen
NETHERLANDS

BOZKURT Osman
osman@bbsrc.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

BREEN Osman
susan.breen@scri.ac.uk
Scottish Crop Research Institute
Errol Road Invergowrie
DD2 5DA Dundee Scotland
UNITED KINGDOM

BROADHURST Ben
Jamesbroadhurst@trentu.ca
Forensic Science Program Trent University
DNA Building 1600 West Bank Drive
K9J 7B8 Peterborough, ON
UNITED KINGDOM

BROWN Christian
brow@kth.se
KTH
AlbaNova University Centre
Roslagstullsbacken 21
SE-106 91 Stockholm
SWEDEN

BRUNNER Frédéric
frederic.brunner@zmbp.uni-tuebingen.de
ZMBP - Eberhard Karls University
Auf der Morgenstelle 5
72076 Tuebingen
GERMANY

BRURBERG May Bente
may.brurberg@bioforsk.no
Bioforsk - Norwegian Institute for Agricultural and Environmental Research
Hoegskoleveien 7
N-1432 Aas
NORWAY

BULONE Vincent
bulone@kth.se
KTH Biotechnology
SE-10691 Stokholm
SWEDEN

CANO Liliana
liliana.cano@bbsrc.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

CANONNE Joanne
Joanne.Canonne@toulouse.inra.fr
LIPM-CNRS-INRA
Chemin de Borde Rouge-Auzeville
31326 Castanet-Tolosan
FRANCE

CARBONNE Francis
carbonne@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

CHAPARRO-GARCIA ANGELA
angela.chaparro-garcia@sainsbury-laboratory.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

CHAUVIN Jean-Eric
Jean-Eric.Chauvin@rennes.inra.fr
INRA UMR APBV
Domaine de Kéraiber
29260 Ploudaniel
FRANCE

CHEN Xiaoren
xiaoren.chen@bioforsk.no
Bioforsk Plantehelse
Hoegskoleveien 7
1432 Aas
NORWAY

COOKE David
david.cooke@scri.ac.uk
Scottish Crop Reseach Institute
Division of Plant Science
Errol Road Invergowrie
DD2 5DA Dundee
UNITED KINGDOM

CORIO-COSTET Marie-france
coriocos@bordeaux.inra.fr
INRA bordeaux,
Rue E bourleaud, BP 81
33883 Villenave d'Ornon
FRANCE

COUDERC François
couderc@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

DANOUN Saïda
danoun@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

DE BRUIJN Irene
i.debruijn@abdn.ac.uk
University of Aberdeen
Institute of Medical Sciences
Foresterhill
AB25 2ZD Aberdeen
UNITED KINGDOM

DEB Devdutta
ddeb1@vt.edu
Virginia Tech
536 Latham Hall, Virginia Tech
24061 Blacksburg
USA

DELMOTTE François
demotte@bordeaux.inra.fr
INRA Bordeaux
Centre Bordeaux Aquitaine
71, Avenue Edouard Bourlaux BP 81
33883 Villenave d'Ornon cedex
FRANCE

DIEGUEZ-URIBEONDO Javier
dieguez@rjb.csic.es
Head of Molecular Systematics Lab Tenured Scientist Department of Mycology
Royal Botanic Garden Spanish Murillo 2
28014 MADRID
SPAIN

DONAHOO Ryan
rsdonahoo@gmail.com
University of Florida
UF IFAS SWFREC 2685 SR 29 N
34142 Immokalee
USA

DONG Suomeng
smdong@njau.edu.cn
Plant Protect College, Nanjing Agricultural University
No.1, Weigang
210095 Nanjing
CHINA

DOU Daolong
ddou@njau.edu.cn
Nanjing Agricutural University
#1 Weigang Rd,
210095 Nanjing
CHINA

DUFOUR Marie-Cécile
dufour@bordeaux.inra.fr
INRA bordeaux,
Rue E bourleaud, BP 81
33883 Villenave d'ornon
FRANCE

DUMAS Bernard
dumas@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

ENGELHARDT STEFAN
stefan.engelhardt@scn.ac.uk
Scottish Crop Research Institute
Errol Road Invergowrie
DD2 5DA Dundee Scotland
UNITED KINGDOM

ESQUERRE-TUGAYE Marie-Thérèse
esquerre@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

FABRO GEORGINA
geor.fabro@sainsbury-laboratory.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

FENG Bao Zhen
zhxg@sdau.edu.cn
Shandong Agricultural University
Daizong Street No.61
271018 Taian
CHINA

FLIEGMANN Judith
fliegmann@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

FOURNIER Sylvie
fournier@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

FRAITURE Malou
malou.fraiture@zmbp.uni-tuebingen.de
ZMBP Plant Biochemistry
Auf der Morgenstelle 5
72076 Tuebingen
GERMANY

FU Li
zhxg@sdau.edu.cn
Shandong Agricultural University
Daizong Street No.61
271018 Taian
CHINA

FURUICHI Naotaka
naotakafuruichi@nifty.com
Niigata Univ., Faculty of Agriculture
2-8050, Igarashi, Nishi-ku
950-8121 Nigata shi
JAPAN

GACHON Claire
cmmg@sams.ac.uk
Scottish Association for Marine Science
Scottish Marine Institute
Pa37 1QA Oban
UNITED KINGDOM

GALIANA Eric
galiana@sophia.inra.fr
INRA UMR IBSV 1301
400 routes des chappes BP 167
06903 Sophia Antipolis
FRANCE

GAMBOA-MELENDEZ Heber
heber70@hotmail.com
University of California
900 University Avenue
92521 Riverside
USA

GARAVITO Manuel Fernando
ma-garav@uniandes.edu.co
Universidad de los Andes
Carrera 1a No 18A-10
Edificio M-301
11001000 Bogota
COLOMBIA

GARCIA Leonor
le-garci@uniandes.edu.co
Universidad de los Andes
Carrera 1 No.18A-12
Edificio J-205 none
Bogota
COLOMBIA

GAULIN Elodie
gaulin@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

GIJZEN mark
mark.gijzen@agr.gc.ca
Agriculture and Agri-Food Canada
N5V4T3 London, Ontario
CANADA

GILROY Eleanor
eleanor.gilroy@scri.ac.uk
Scottish Crop Research Institute
Invergowrie
DD2 5DA Dundee Scotland
UNITED KINGDOM

GODIARD Laurence
Laurence.Godiard@toulouse.inra.fr
UMR CNRS-INRA 2594/441 (LIPM)
Chemin de Borde-Rouge
BP 52627 Auzeville
31326 Castanet Tolosan Cedex
FRANCE

GOURGUES Mathieu
mathieu.gourgues@sophia.inra.fr
INRA/CNRS/UNSA
400, Route des Chappes
06903 Sophia Antipolis
FRANCE

GOVERS Francine
francine.govers@wur.nl
Wageningen University, Lab of Phytopathology
Droevendaalsesteeg 1
6708 PB Wageningen
NETHERLANDS

GRENVILLE-BRIGGS Laura
l.grenville-briggs@abdn.ac.uk
University of Aberdeen
Institute of Medical Sciences Foresterhill
AB25 2ZD Aberdeen
UNITED KINGDOM

GUERRIERO Gea
gea@kth.se
KTH Biotehnology
SE-10691 Stockholm
SWEDEN

HAMADA Walid
w.hamada@yahoo.fr
Institut National Agronomique de Tunisie
43, avenue Charles Nicolle
1082 Mahrajène
TUNISIA

HARBAOUI kalthoum
harbaoui_kalthoum@yahoo.fr
Institut National Agronomique de Tunisie
43, avenue Charles Nicolle
1082 Mahrajène
TUNISIA

HUA Chenlei
chenley.hua@wur.nl
Wageningen University,
Lab of Phytopathology
Droevendaalsesteeg 3
6710 PB Wageningen
NETHERLANDS

HUITEMA Edgar
Edgar.Huitema@scri.ac.uk
Division of Plant Science
Scottish Crop Reseach Institute
Errol Road Invergowrie
DD2 5DA Dundee Scotland
UNITED KINGDOM

JACQUET Christophe
jacquet@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

JAENTSCH Christiane
christiane.jaentsch@zmbp.uni-tuebingen.de
ZMBP
Auf der Morgenstelle 5
72076 Tuebingen
GERMANY

JAOUANNET Maelle
maelle.jaouannet@sophia.inra.fr
INRA/CNRS/UNSA UMR IBS 1301
400, route des chappes BP 167
06903 Sophia Antipolis
FRANCE

JIANG Rays
rjiang@broadinstitute.org
The Broad Institute of MIT and Harvard
7 Cambridge Center
MA 02142 Cambridge
USA

JONES Jonathan
jonathan.jones@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

JOURNET Etienne Pascal
etienne-pascal.journet@toulouse.inra.fr
UMR CNRS-INRA 2594/441 (LIPM)
Chemin de Borde-Rouge BP 52627 Auzeville
31326 Castanet Tolosan Cedex
FRANCE

JUDELSON Howard
Howard.judelson@ucr.edu
University of California
Department of Plant Pathology and Microbiology
92521 Riverside, California
USA

KALE Shiv
sdkale@vbi.vt.edu
Virginia Bioinformatics Institute
Virginia Tech
1 Washington Street
24060 Blacksburg
USA

KAMOUN Sophien
sophien.kamoun@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

KELLER Harald
keller@sophia.inra.fr
INRA/CNRS/UNSA UMR IBS 1301
400, Route des chappes BP 167
06903 Sophia Antipolis
FRANCE

KEMEN Ariane
Ariane.Kemen@bbsrc.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR2 3BP Norwich
UNITED KINGDOM

KING Stuart
Stuart.King@bbsrc.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

KRAJAEJUN MD Theerapong
mr_en@hotmail.com
Department of Pathology Faculty of Medicine-
Ramathibodi Hospital Mahidol University
Bangkok,
THAILAND

KÜFNER Isabell
isabell.kuefner@zmbp.uni-tuebingen.de
University Tübingen
Auf der Morgenstelle 5
72076 Tübingen
GERMANY

LAMOUR Kurt
klamour@utk.edu
University of Tennessee
Rm 205 Ellington Plant Science, 2431
Joe Johnson Drive
37996 Knoxville
USA

LAPIN Dmitry
D.lapin@uu.nl
Utrecht University, Faculty of Science,
Department of Biology,
Plant-Microbe Interactions
PMI, W302 Padualaan 8
3584 CH Utrecht
THE NETHERLANDS

LARROQUE Mathieu
larroque@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

LEBA Louis-Jérôme
leba@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

MADOUI Mohammed-Amine
amine.madoui@gmail.com
CNRS/IRD/Université de Marseille
101, avenue Timone
13010 Marseille
FRANCE

MAGALON Hélène
helene.magalon@gmail.com
INRA UMR BIO3P
Domaine de la Motte
35653 Le Rheu Cedex
FRANCE

MARINO Daniel
daniel.marino@toulouse.inra.fr
LIPM UMR CNRS/INRA 2594/441
Chemin de Borde Rouge
31326 Castanet-Tolosan
FRANCE

McDOWELL John
johnmcd@vt.edu
Virginia Tech
536 Latham Hall,
24061 Blacksburg VA
USA

McLELLAN Hazel
mhazel.mclellan@scri.ac.uk
Scottish Crop Reseach Institute
Division of Plant Science
Errol Road Invergowrie
DD2 5DA Dundee
UNITED KINGDOM

MEIJER Harold
harold.meijer@wur.nl
Wageningen University, Lab of Phytopatho
Droevendaalsesteeg 2
6710 PB Wageningen
NETHERLANDS

MESTRE Pere
pere.mestre@colmar.inra.fr
INRA
28, rue de Herrlisheim BP 507
68021 Colmar
FRANCE

MINOR Kirsty
k.minor@abdn.ac.uk
University of Aberdeen
Institute of Medical Sciences
Foresterhill
AB25 2ZD Aberdeen
UNITED KINGDOM

MORGAN William
wmorgan@wooster.edu
College of Wooster
1189 Beall Ave.
44691 Wooster, OH
USA

MORRIS Paul
pmorris@bgsu.edu
Bowling Green State Univ
217 Life Sciences
43403 Bowling Green
USA

NARS Amaury
nars@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

NEEMA Claire
neema@agroparistech.fr
Agro Paris Tech
16, Rue Claude Bernard
75005 Paris
FRANCE

OLIVA Ricardo
Ricardo.Oliva@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

OOME Stan
s.oome@uu.nl
Utrecht University
Padualaan 8
3584 CH Utrecht
NETHERLANDS

OSPINA-GIRALDO Manuel
ospinam@lafayette.edu
Lafayette College
29 Kunkel Biology Department
18042 Easton, PA
USA

PANABIERES Franck
franck.panabieres@sophia.inra.fr
INRA/CNRS/UNSA UMR IBS 1301
400, route des chappes BP 167
06903 Sophia Antipolis
FRANCE

PARK Bongsoo
bxp12@psu.edu
Penn State University
1104 Smithfield St. APT3
16801 State College
USA

PEL Chiel
M.J.C.Pel@uu.nl
Utrecht University
Padualaan 8
3584 CH Utrecht
NETHERLANDS

PHUNTUMART Vipaporn
vphuntu@bgsu.edu
Bowling Green State University
455 LifeSciences
43403 Bowling Green, OH
USA

PONCHET Michel
ponchet@sophia.inra.fr
INRA/CNRS/UNSA UMR IBSV 1301
400, route des chappes BP 167
06903 Sophia Antipolis
FRANCE

PRITCHARD Leighton
lpritc@scri.ac.uk
Scottish Crop Reseach Institute
Division of Plant Science
Errol Road Invergowrie
DD2 5DA Dundee
UNITED KINGDOM

QUTOB Dinah
Dinah.Qutob@agr.gc.ca
Agriculture and Agri-Food
Canada, SCPFRC
1391 Sandford St.,
London ON
N6P1K9 London
CANADA

RAFFAELE Sylvain
Sylvain.Raffaele@bbsrc.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

RAMIREZ Diana
ramirez@scsv.ups-tlse.fr
UMR5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

REP Martijn
m.rep@uva.nl
University of Amsterdam
Science Park 904
1098XH Amsterdam
NETHERLANDS

REY Thomas
rey@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

RICKAUER Martina
martina.rickauer@ensat.fr
ENSAT-INP
18, chemin de Borde Rouge
B.P.32607 Auzeville
31326 Castanet-Tolosan
FRANCE

ROBERTS Pamela
pdr@ufl.edu
University of Florida
2686 SR 29 N
34142 Immokalee, FL
USA

ROBIDEAU Gregg
gregg.robideau@agr.gc.ca
Carleton University
Dpmt of Biology
Nesbitt Building Carleton
1125 Colonel By Drive
K1S 5B6 Ottawa, Ontario
CANADA

ROSSO Marie Noelle
rosso@sophia.inra.fr
INRA/CNRS/UNSA
UMR IBS 1301
400 route des chappes BP 167
06903 Sophia Antipolis
FRANCE

RUSS Carsten
carsten@broadinstitute.org
Broad Institut
10, Windsor Street
MA 01844 Methuen
USA

RZESZUTEK Elzbieta
Elsbieta.rzeszutek@biotech.kth.se
Royal Intitute of technology (KTH)
Roslagstulbacken 21
SE-106 91
Stockholm
SWEDEN

SAUNDERS Diane
diane.saunders@sainsbury-laboratory.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

SCHORNACK Sebastian
sebastian.schornack@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park, Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

SEGRETIN Maria Eugenia
maria.segretin@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

SEIDL Michael Franziskus
m.f.seidl@uu.nl
Utrecht University, Faculty of Science,
Department of Biology,
Plant-Microbe Interactions
PMI, W302 Padualaan 8
3584 CH Utrecht
THE NETHERLANDS

STAM Remco
remco.stam@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

STASSEN Joost
J.H.M.Stassen@uu.nl
Utrecht University,
Faculty of Science,
Department of Biology,
Plant-Microbe Interactions
PMI, W302 Padualaan 8
3584 CH Utrecht
THE NETHERLANDS

TARTAR Aurelien
aurelien@nova.edu
Nova Southeastern University
Parker Bldg- 370H
3301 College Avenue
33314 Fort Lauderdale, FL
USA

THINES Marco
thines@uni-hohenheim.de
University of Hohenheim
Institute of Botany 210
Garbenstr. 30
70599 Stuttgart
GERMANY

TOFFOLATTI Silvia Laura
silvia.toffolatti@unimi.it
University degli Studi di Milano,
DI.PR.VE. Sez di Patologia Vegetale
via Celoria 2
20133 Milano
ITALIA

TYLER Brett
bmtyler@vt.edu
Virginia Tech
VBI, One Washington St,
24060 Blacksburg, Virginia
USA

VALADE romain
romain.valade@versailles.inra.fr
INRA-Agro ParisTech
UMR 1290 BIOGER-CPP (bât 13)
Avenue Lucien Brétignières BP 01
78850 Thiverval-Grignon
FRANCE

VAN DAMME Mireille
mireille.vandamme@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

VAN DEN ACKERVEKEN Guido
g.vandenacherveken@uu.nl
Utrecht University,
Faculty of Science, Department of Biology,
Plant-Microbe Interactions
PMI, W302 Padualaan 8
3584 CH Utrecht
THE NETHERLANDS

VAN DEN BERG Albert Hendrik
ahvdberg@abdn.ac.uk
University of Aberdeen
Foresterhill
AB25 2ZD Aberdeen
UNITED KINGDOM

VERGNES Sophie
vergnes@scsv.ups-tlse.fr
UMR 5546 UPS/CNRS
24, chemin de Borde Rouge
BP 42617 Auzeville
31326 Castanet-Tolosan
FRANCE

VETUKURI Ramesh raju
Ramesh.Vetukuri@vbsg.slu.se
SLU Uppsala BioCenter
Dept. of Plant Biology and Forest Genetics
Univ. of Agricultural Sciences,
Box 7080
750 07 Uppsala,
SWEDEN

VICRE-GIBOUIN Maïté
maite.vicre@univ-rouen.fr
Plateforme de Recherche en Imagerie Cellulaire
Faculté des Sciences
Université de Rouen
76 823 Mont saint Aignan
FRANCE

VINCOURT Patrick
Patrick.vincourt@toulouse.inra.fr
INRA
24, Chemin de Borde-Rouge
BP 52627 Auzeville
31326 Castanet Tolosan Cedex
FRANCE

WANG Yuanchao
wangyc@njau.edu.cn
Plant Protection College,
Nanjing Agricultural University
No.1, Weigang
210095 Nanjing
CHINA

WANG He-Mei
zhxg@sdau.edu.cn
Shandong Agricultural University
Daizong Street No.61
271018 Taian
Shandong
CHINA

WANG Yan
wangyan@wur.nl
Wageningen University,
Lab of Phytopathology
Droevendaalsesteeg 2
6710 PB Wageningen
NETHERLANDS

WAWRA Stephan
s.wawra@abdn.ac.uk
University of Aberdeen
Foresterhill
AB25 2ZD Aberdeen
UNITED KINGDOM

WEIDE Rob
rob.weide@wur.nl
Wageningen University,
Lab of Phytopathology
Droevendaalsesteeg 2
6712 PB Wageningen
NETHERLANDS

WHISSON Steve
swhiss@scri.ac.uk
Scottish Crop Reseach Institute
Errol Road Invergowrie
DD2 5DA Dundee,
UNITED KINGDOM

WIN Joe
joe.win@tsl.ac.uk
The Sainsbury Laboratory
JIC, Norwcih Research Park,
Colney Lane
NR4 7UH Norwich
UNITED KINGDOM

WONG Joan
1silverleaf@gmail.com
UC Davis Genome Center
4212A GBSF
451 Health Sciences Dr.
95616 Davis
USA

YATUSEVICH Ruslan
yatusev@mpiz-koeln.mpg
Max Plank Institute for Plant Breeding Research
Carl-von-Linné-Weg 10
50829 Cologne
GERMANY