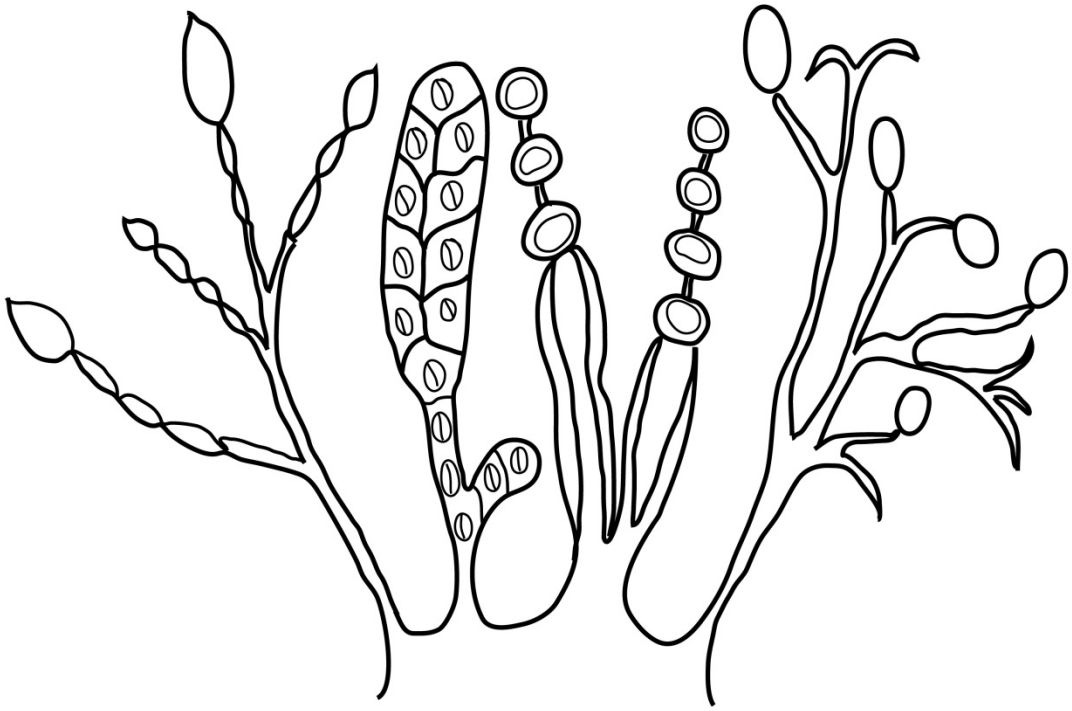
Oomycete Molecular Genetics Network Meeting

2nd July 2014 – 4th July 2014
The Top of the City, Jarrold Stand, Norwich City Football Club,
Carrow Road, Norwich, NR1 1JE, UK

Oomycete



Molecular Genetics

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The Oomycete Molecular Genetics Research Network (OMGN) was initially funded by an NSF Research Coordination Network grant in 2001 and continued to receive funding from the NSF for many years. More recently, the Network has received funding from the USDA-AFRI program (grant 2011-68004-30104). The purpose of our annual meeting is to promote communication and collaboration, and minimize the duplication of effort within the oomycete molecular genetics community. Our community now numbers well in excess of 100 Laboratories from around the world, and research on oomycetes attracts considerable attention from outside the community as well as within. The OMGN annual meeting alternates between Asilomar, CA, and a venue outside of the USA. This year it is being hosted in Norwich, UK, and the meeting will cover some of the latest research in the areas of Effectors, Oomycete Biology, Genomics/Transcriptomics/Population Genomics and Plant Response/Resistance Mechanisms. With over 140 registrants we expect a dynamic and thought-provoking meeting with plenty of opportunity for developing existing collaborations, establishing new ones or just meeting with old friends. Finally, remember, "Bats are not birds, dolphins are not fish and oomycetes are not fungi".

Committee Chairs:

Dr Mark J Banfield
Dept. of Biological Chemistry
John Innes Centre
Norwich Research Park
Norwich NR4 7UH

Prof Sophien Kamoun
The Sainsbury Laboratory
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Meeting Logistics:

Gemma Wallace
Department Administrator
John Innes Centre
Norwich Research Park
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Cover Art: Artemis Giannakopoulou & Khaoula Belhaj

Meeting Sponsors:

OMGN 2014 Program

All talks will be held in the "Top of the City", Jarrold Stand.
The poster session will be in the "Club 101" room, Jarrold Stand.

Wednesday 2nd July 2014

08:30 – 09:00	REGISTRATION

09:00 – 09:05	WELCOME

09:05 – 10:50	**Effectors I - Chair: Edgar Huitema**
09:05 – 09:20	Petra Boevink Differential targeting of PP1c isoforms by an RXLR effector
09:20 – 09:35	John McDowell An oomycete RXLR effector triggers antagonistic plant hormone crosstalk to suppress host immunity
09:35 – 09:50	Lennart Wirthmueller Oomycete effector HaRxL106 interacts with Arabidopsis RADICAL INDUCED CELL DEATH1 and suppresses NPR1-dependent and -independent immune pathways
09:50 – 10:05	Xiangzi Zheng Functionally Redundant RXLR Effectors from *Phytophthora infestans* Act at different Steps to Suppress Early flg22-triggered immunity
10:05 – 10:20	Guanghui Kong *Phytophthora sojae* Avirulence Effector Avr3b activated by plant cyclophilin
10:20 – 10:35	Yasin F. Dagdas An effector from the Irish potato famine pathogen *Phytophthora infestans* usurps a selective autophagy cargo receptor to enhance virulence
10:35 – 10:50	Richard K. Hughes Structural and biochemical characterisation of *P. infestans* RXLR effector protein PexRD54 and its interaction with the host autophagy protein ATG8

10:50 – 11:15	BREAK

11:15 – 12:45	**Effectors II – Chair: Eric Kemen**
11:15 – 11:30	Helen McLellan Two *P. infestans* nuclear localized RxLR effectors interact with targets involved in different host processes
11:30 – 11:45	Suomeng Dong How host jumps and host specialization impact effector evolution in the Irish potato famine pathogen lineage
11:45 – 12 Noon	Yu Du Subcellular localization of *Phytophthora infestans* RXLR effector AVR1 and its cognate resistance protein R1
12 Noon – 12:15	Michiel J.C. Pel Functional analysis of crinkler effectors from the plant pathogenic oomycete *Aphanomyces euteiches*
12:15 – 12:30	Stephan Wawra Characterisation of the RxLR-effector AVR3a from *Phytophthora infestans*
12:30 – 12:45	Brett M. Tyler Binding to Phosphatidylinositol-3-Phosphate Mediates Entry of Oomycete Effectors into Plant Cells During Natural Infection

12:45 – 14:15	LUNCH

14:15 – 15:30	**Oomycete Biology I - Chair: Sebastian Schornack**
14:15 – 14:30	Christine Strullu-Derrien Early terrestrial oomycetes. What do we know from the fossil record?
14:30 – 14:45	Anna Asman Insights into the functions of *P. infestans* Argonaute proteins
14:45 – 15:00	Mark Gijzen Genetic analyses of Avr gene silencing in *Phytophthora sojae*
15:00 – 15:15	Klaas Bouwmeester A novel in vitro infection system to study Phytophthora-host interactions.
15:15 – 15:30	Harold Meijer The actin cytoskeleton in *Phytophthora infestans* in free living mycelium and infection structures

15:30 – 16:00	BREAK

16:00 – 17:15	**Oomycete Biology II – Chair: Laura Grenville-Briggs**
16:00 – 16:15	Svante Resjo Proteomics and phosphoproteomics of *P. infestans* and potato
16:15 – 16:30	Laetitia Poidevin Turning on and off gene expressions in *Phytophthora infestans*
16:30 – 16:45	Maria Maistrou Role of the pectic enzymes in the oomycetes pathogenesis
16:45 – 17:00	Alyssa Burkhardt Alternative splicing in the obligate biotrophic oomycete pathogen, *Pseudoperonospora cubensis*
17:00 – 17:15	Theerapong Krajaejun The elicitin-like glycoprotein, ELI025, is secreted by the pathogenic oomycete *Pythium insidiosum* and evades host antibody responses

Thursday 3rd July 2014

09:00 – 10:30	**Genomics/Transcriptomics/Population Genomics I – Chair: Stephen Wawra**
09:00 – 09:15	H.A. Burbano Oomycete genomics using historic and modern samples: challenges and perspectives
09:15 – 09:30	Eric Kemen Multitrophic microbe-microbe interactions determine the leaf microbiome
09:30 – 09:45	R.J. Gil Functional Genomics of Bremia lactucae
09:45 – 10:00	Aurélien Tartar Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens
10:00 – 10:15	Lida Derevnina Comparative genomics of temperate and tropical downy mildews

10:30 – 11:00	BREAK

11:00 – 12:30	**Genomics/Transcriptomics/Population Genomics II – Chair: Shiv Kale**
11:00 – 11:15	H. Kokko Assembly and comparison of *Aphanomyces astaci* mt-genomes illustrate high diversity among genotypes
11:15 – 11:30	Laurence Godiard Comparative analysis of expressed CRN and RXLR effectors from two Plasmopara species causing grapevine and sunflower downy mildew
11:30 – 11:45	R.L. McDougal Enabling technologies for future trees free from *Phytophthora* disease
11:45 – 12 Noon	Kyle Fletcher Phylogentic investigation of marine holocarpic eukaryotic pathogens (stramenopiles) of filamentous algae
12 Noon – 12:15	Giovanna Danies A novel recombinant population of *Phytophthora infestans* in northeastern USA?

12:30 – 14:00	LUNCH

14:00 – 15:30	**Keynote Address: Nick Talbot, University of Exeter - Chair: Mark Banfield** Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

15:30 – 17:30	**Poster Session**
15:30 – 16:00	TEA/COFFEE

18:00	ORGANISED TRANSPORT TO JIC FOR CONFERENCE DINNER

18:30	PRE-DINNER DRINKS. CHATT ATRIUM, JIC

19:00	CONFERENCE DINNER

21:30	BUS BACK TO NORWICH CITY FOOTBALL GROUND/CITY CENTRE

Friday 4th July 2014

09:00 – 10:30	**Plant Response to Oomycetes/Resistance Mechanisms – Chair: Nicolas Champouret**
09:00 – 09:15	Guido Van den Ackerveken DMR6 and DMR6-like oxygenase 1 are partially redundant but distinct negative regulators of immunity in Arabidopsis
09:15 – 09:30	Kevin Fedkenheuer Effector-Directed Breeding to Improve Resistance against *Phytophthora sojae* in Soybean
09:30 – 09:45	Khaoula Belhaj The *Solanum bulbocastanum* Rpi-blb2 immune receptor recognizes a second effector AVRblb2b that is deleted in a partially virulent isolate of *Phytophthora infestans*
09:45 – 10:00	Oliver J. Furzer Elucidation of the gene-for-gene relationships of white rust on Arabidopsis
10:00 – 10:15	Thomas Rey Symbiotic plant processes involved in development of root interactions with detrimental microbes
10:15 – 10:30	Veronika Pleskova RNA-Seq analysis of BABA-induced resistance to *Phytophthora parasitica* in tomato emphasizes a hyper-responsive plant status

10:30 – 11:00	BREAK

11:00 – 12:30	**Plant Response to Oomycetes/Resistance Mechanisms – Chair: Klaus Bouwmeester**
11:00 – 11:15	Mahmut Tor Presence of heterozygosity in effectors allows avoidance of recognition by R-genes
11:15 – 11:30	Patricia Manosalva MORC1 Modulates Plant Immunity against *Phytophthora infestans* in Solanaceae
11:30 – 11:45	Sultana Jahan RNA silencing, a sensitive new tool in the fight against the potato late blight disease
11:45 – 12 Noon	Sophie Rommel microRNA signaling in tomatoes during *Phytophthora infestans* infections
12 Noon – 12:15	Pauline S M Van Weymers Mapping and cloning of the tomato late blight resistance genes Rpi-Ph2 and Rpi-Ph3 and their cognate *Phytophthora infestans* effectors Avr-Ph2 and Avr-Ph3.
12:15 – 12:30	Ruth Le Fevre Understanding colonisation mechanisms of monocot tissues by the oomycete pathogen *Phytophthora palmivora*

12:30 – 12:45	WRAP UP

Meeting Close.

ORAL PRESENTATIONS

Presenting Author(s) underlined.

Effectors I - Wednesday 2nd July 2014, 09:05 – 10:50

Differential targeting of PP1c isoforms by an RXLR effector

Petra Boevink[1,2], Wang X[1,4], Bukharova T[1,3], McLellan H[1,3], Armstrong, M[1,3] & Birch PRJ[1,3]

[1]Dundee Effector Consortium, [2]Cell and Molecular Sciences, The James Hutton Institute, Dundee, UK; [3]University of Dundee, Division of Plant Sciences, Dundee, UK, [4]Heilongjiang Academy Agricultural Sciences Harbin, China.

Protein phosphorylation and de-phosphorylation is a fundamental regulatory mechanism that occurs throughout the cell. We have recently published work describing how *Phytophthora infestans* RXLR effectors target kinases, specifically the MAPK cascades to suppress pattern triggered immunity and thereby tip the balance in favour of infection. I will present work indicating that a different RXLR effector targets protein phosphorylation by association with protein phosphatase type 1 catalytic subunit (PP1c). The effector strongly associates with several isoforms of the PP1c in yeast-2-hybrid and co-immunoprecipitation assays. Co-expression of fluorescent protein-tagged effector and the PP1c isoforms modifies the localisation of the latter. The effector provides a strong boost to *P. infestans* growth when over-expressed in a leaf that is subsequently infected. Mis-localising the effector abolishes this boost to infection and the ability of the effector to modify the location of PP1c. Similarly mutation of the conserved PP1c-interaction motif in the effector abolishes the interaction and the effector activity.

An oomycete RXLR effector triggers antagonistic plant hormone crosstalk to suppress host immunity

Devdutta Deb#, John Withers, Ryan G. Anderson#, Sheng Yang He*, and John M. McDowell#*

#Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24061, USA; *Howard Hughes Medical Institute, DOE Plant Research Lab, and Michigan State University, East Lansing, MI 48823, USA

Oomycete plant pathogens maintain large families of RXLR effector proteins that enter plant cells. The mechanisms through which these effectors promote virulence are largely unknown. We will present evidence that the HaRxL10 effector protein from the *Arabidopsis* pathogen *Hyaloperonsopora arabidopsidis* (*Hpa*) targets a Jasmonate-Zim Domain (JAZ) protein that represses responses to the phytohormone jasmonic acid (JA). This manipulation activates a regulatory cascade that reduces accumulation of a second phytohormone, salicylic acid (SA), and thereby attenuates immunity. This virulence mechanism is functionally equivalent to but mechanistically distinct from activation of JA-SA crosstalk by the bacterial JA mimic coronatine. These results reveal a new mechanism underpinning oomycete virulence and demonstrate that the JA-SA crosstalk is an Achilles' heel that is manipulated by unrelated pathogens through distinct mechanisms.

Oomycete effector HaRxL106 interacts with Arabidopsis RADICAL INDUCED CELL DEATH1 and suppresses NPR1-dependent and -independent immune pathways

Lennart Wirthmueller[1#], Shuta Asai[1], Ghanasyam Rallapalli[1], Georgina Fabro[1], Michael Wrzaczek[2], Jaakko Kangasjärvi[2], Jan Sklenar[1], Frank Menke[1], Mark J. Banfield[3], Jonathan D. Jones[1]

[1] The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK; [2] University of Helsinki, Division of Plant Biology, FIN-00014, Finland; [3] Dept. of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

The oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) causes downy mildew on Arabidopsis. We focus on *Hpa* effector proteins that are targeted to the host cell nucleus and we are aiming to understand how

these effectors suppress immunity. *Hpa* effector HaRxL106 renders plants more susceptible to biotrophic pathogens when expressed as a transgene in Arabidopsis. Transcriptome profiling of transgenic lines expressing HaRxL106 revealed that NPR1 target genes are underexpressed compared to wild type. Under short day conditions, non-infected *35S::NPR1-GFP* plants show a stunted morphology and constitutively express *PR* genes. These hallmarks of constitutive defense activation are suppressed by co-expression of HaRxL106. NPR1-GFP protein accumulation and localization are not affected by HaRxL106, suggesting that the effector may interfere with *PR* gene expression at the chromatin level. HaRxL106 expression also reverts NPR1-independent constitutive defense gene expression and stunted growth in the *snc1* mutant. Therefore, HaRxL106 likely targets nuclear defense regulators that function downstream of snc1 and NPR1. HaRxL106 interacts with Arabidopsis RADICAL INDUCED CELL DEATH1 (RCD1) and a mutant variant of HaRxL106 that does not bind RCD1 fails to suppress immunity. The cellular NPR1 pool distributes between disulfide-linked oligomers and the defense activating monomeric form[a]. Knocking out *RCD1* results in elevated NO levels and therefore might affect NPR1's redox status. Consistently, we find that NPR1 target genes are under-expressed in an *rcd1* mutant. Therefore, RCD1 is a novel regulator of NPR1 activity. We will present our work addressing how *Hpa* effector HaRxL106 modifies RCD1 function and how this suppresses plant immunity.

[a] Mou et al., Cell 2003

Functionally Redundant RXLR Effectors from *Phytophthora infestans* Act at different Steps to Suppress Early flg22-triggered immunity

Xiangzi Zheng[1 #], Hazel McLellan[2], Malou Fraiture[1], Xiaoyu Liu[1], Petra C Boevink[3], Eleanor M. Gilroy[3], Ying Chen[1], Kabindra Kandel[2], Guido Sessa[4], Paul R.J. Birch[2,3], Frédéric Brunner[1]

[1]Department of Biochemistry, Centre for Plant Molecular Biology, Eberhard Karls University, Auf der Morgenstelle 32, D-72076 Tübingen, Germany. [2]Division of Plant Sciences, University of Dundee (at James Hutton Institute), Errol Rd, Invergowrie, Dundee DD2 5DA, UK. [3]Cell and Molecular Sciences, The James Hutton Institute, University of Dundee, Errol Rd, Invergowrie, Dundee DD2 5DA, UK. [4]Department of Molecular Biology and Ecology of Plants, Tel Aviv University, 69978 Tel Aviv, Israel

Genome sequences of several economically important phytopathogenic oomycetes have revealed the presence of large families of so-called RXLR effectors that are thought to manipulate host cellular activities to the benefit of the pathogen. However, less is known about the molecular mechanisms underlying the modes of action of these effectors in planta. Perception of highly conserved pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs), such as flg22, triggers converging signaling pathways recruiting MAP kinase cascades and inducing transcriptional re-programming, yielding a generic anti-microbial response. We used a highly synchronizable, pathogen-free protoplast-based assay to screen a library of 33 Phytophthora infestans RXLR effectors (PiRXLRs) for their ability to suppress flg22-triggered defense signaling. Eight effectors, called Suppressors of early Flg22-induced Immune response (SFI), significantly reppressed a flg22-depedent reporter gene under control of a typical MAMP-inducible promoter (pFRK1-Luc) in tomato protoplasts. We extended our analysis to Arabidopsis thaliana, a non-host plant species of *P. infestans*. From the aforementioned eight SFI effectors, three appeared to share similar functions in both Arabidopsis and tomato by suppressing transcriptional activation of flg22-induced marker genes. A further three effectors interfere with MAMP signaling at, or upstream of, the MAP kinase cascade in tomato, but not in Arabidopsis. Transient expression of the SFI effectors in *Nicotiana benthamiana* enhances susceptibility to *P. infestans*. This study provides a framework to decipher the molecular mechanisms underlying the manipulation of host MAMP-triggered immunity (MTI) by *P. infestans* and to understand the basis of host versus non-host resistance in plants towards *P. infestans*.

Phytophthora sojae Avirulence Effector Avr3b activated by plant cyclophilin

Guanghui Kong, Yao Zhao, Jin Yang, Weixiao Yin, Maofeng Jing, Suomeng Dong, Wenwu Ye, Wenbo Ma, and Yuanchao Wang

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

Avr3b was identified as a putative Nudix hydrolase. The expressed Avr3b in *N. benthamiana* has Nudix hydrolase activity and the enzymatic activity is essential for virulence. However Avr3b expressed in *E. coli* have no enzymatic activity. We propose Avr3b require host factor for activation. Thus Y2H system was used to screen a constructed soybean cDNA library to find interactors of the Avr3b. A cyclophilin protein was

identified and further confirmed using pull-down *in vitro*. In addition, none of the other 11 homologous cyclophilins(9 from soybean, 2 from P.sojae) could interact with Avr3b in yeast system, which demonstrated a specificity between Avr3b-cyclophilin interaction. Activation of Avr3b could be inhibit by cyclosporine A, a inhibitor of the PPIase activity of Cyclophilin, suggesting that the activation of Nudix hydrase depends on PPIase activity. Substitution of several amino acids in Avr3b led to loss of activities in inducing cell death on *Rpa3b*-soybean. CsA could inhibit the recognition of *Rps3b*-soybean to Avr3b.These results suggest that the interaction between Avr3b and cyclophilin is necessary for recognition and PPIase activity is essential for the activation of Avr3b virulence function. Like type Ⅲ effectors, oomycete RxLR effector also require host factor for activation. This mechanism can keep effectors function be locked before entering in host cell.

An effector from the Irish potato famine pathogen *Phytophthora infestans* usurps a selective autophagy cargo receptor to enhance virulence

Yasin F. Dagdas[1], Khaoula Belhaj[1], Abbas Maqbool[2], Richard Hughes[2], Mark J Banfield[2], Tolga O. Bozkurt[1,3] & Sophien Kamoun[1]

[1]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK. [2]Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK. [3]Imperial College London, Department of Life Sciences, London, UK.

Autophagy is a protective catabolic process involved in cellular adaptation to dwindling nutrient resources. It involves sequestration of a part of the cytosol within vesicles named autophagasomes. Additionally, a form of autophagy, known as selective autophagy, can specifically degrade toxic substances such as invading pathogens. Selective autophagy functions through autophagy cargo receptors that confine the cargo within a special set of autophagasomes. Although there is documented evidence for the involvement of autophagy in response to animal pathosystems, the role of autophagy in plant-microbe interactions is unclear and somewhat controversial. Here, we discovered that a secreted RXLR-WY type effector of *Phytophthora infestans*, named PexRD54, binds the autophagy marker protein ATG8C. We identified an **A**TG8 **I**nteracting **M**otif (AIM) in PexRD54. Mutations in the AIM motif prevented both *in vivo* and *in vitro* PexRD54-ATG8C interactions. Consistently, overexpression of PexRD54 increased the number and size of GFP-ATG8C labeled autophagasomes and enhanced stability of ATG8C protein. To investigate the biological function of PexRD54, we studied the autophagy cargo receptor Joka2, which interacts with ATG8C. Overexpression of Joka2 *in planta* limited *P. infestans* infection, suggesting a role for Joka2/ATG8C selective autophagy in response to oomycete infection. Remarkably PexRD54, but not the AIM mutant of PexRD54, was able to out compete Joka2 for binding to ATG8C and restore full pathogen virulence. Our findings point to a model in which an RXLR-WY effector from *P. infestans*, out competes a selective autophagy cargo receptor to enhance pathogen virulence.

Structural and biochemical characterisation of *P. infestans* RXLR effector protein PexRD54 and its interaction with the host autophagy protein ATG8

Richard K. Hughes[1], Abbas Maqbool[1], Tolga Bozkurt[2,3], Yasin Dagdas[2], Khaoula Belhaj[2], Sophien Kamoun[2] & Mark J. Banfield[1]

[1]Deptment of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK; [2]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK. [3]Imperial College London, Department of Life Sciences, London, SW7 2AZ, UK.

The WY-domain of RXLR-type effectors from oomycete plant pathogens is now well established as a conserved structural unit found in many of these proteins. This fold can adopt different conformations, including a four-helix bundle and two completely different oligomeric states. Multiple WY-domains can also be encoded in a single RXLR effector. This versatility underpins the hypothesis that the WY-domain acts as a scaffold to support functional diversification of the virulence activities of effectors, and to evade the plant innate immune system. An important, as yet unanswered, question is how the WY-domain engages with virulence targets or immune receptors in the plant cell at the structural level. To this end, we have been studying the interaction of PexRD54 with the host selective autophagy protein ATG8c *in vitro* using biochemical and structural approaches. We have solved the crystal structure of PexRD54 in the presence of ATG8c. PexRD54 has five structurally distinct WY-domains and a C-terminal, disordered ATG8-interacting

("AIM") motif. We have used Small Angle X-ray Scattering (SAXS) to characterise the structure of the complex in solution. We have also characterised the interactions between PexRD54 and ATG8c (and variants) by Gel Filtration, Isothermal Titration Calorimetry and Surface Plasmon Resonance. To our knowledge, this is the first report of a structure of an RXLR effector in the presence of its host target protein and a study of RXLR effector/host target interactions *in vitro* using purified proteins. Our studies offer new insights into how RXLR effector proteins evolve and interact with their targets to perturb host cell physiology.

Effectors II - Wednesday 2nd July 2014, 11:15 – 12:45

Two *P. infestans* nuclear localized RxLR effectors interact with targets involved in different host processes

McLellan H[1,2], Wang X[2,3], Boevink PC[2,3], Armstrong MA[1,2] & Birch PRJ[1,2,3]

[1]Division of Plant Sciences, University of Dundee (at JHI); [2]Dundee Effector Consortium; [3]Cell and Molecular Sciences; all at James Hutton Institute (JHI), Errol Road, Invergowrie, Dundee DD2 5DA UK.

Oomycete plant pathogens such as *Phytophthora infestans* possess a large number of candidate RxLR effector proteins. These effectors are thought to interact with target proteins inside the host plant in order to suppress defences and alter metabolism to benefit the pathogen. However, comparatively little is known about the types of proteins these effectors target and how these targets are manipulated.

Here we demonstrate that two unrelated *P. infestans* RxLRs which share a similar nucleolar localization interact with target proteins involved in distinct host processes. The RxLR effector Pi06087 has been previously shown to alter PAMP Triggered Immunity (PTI) in a screen using tomato protoplasts to identify RxLRs which suppress early PTI responses, whereas Pi04089 does not. However, both RxLR effectors are able to increase *P. infestans* ability to colonize its host when expressed *in planta*. A Yeast 2 Hybrid (Y2H) approach has identified a host protein containing both Ubox and kinase domains as a candidate target of Pi06087, while Pi04089 was found to interact with a KH RNA binding protein. Similar proteins are known to have roles in mRNA processing. Fluorescent protein fusions of both Pi06087 and its target are observed to co-localise in a ringed structure around the plant nucleolus. In contrast, the RxLR Pi04089 accumulates in the nucleolus when expressed alone but co-localises to nuclear speckles in the presence of the KH RNA binding protein.

How host jumps and host specialization impact effector evolution in the Irish potato famine pathogen lineage

Suomeng Dong[1], Remco Stam[1], Liliana M. Cano[1], Jan Sklenar[1], Kentaro Yoshida[1], Jing Song[2], Tolga O. Bozkurt[1], Ricardo Oliva[1], Miaoying Tian[2], Joe Win[1], Mark J. Banfield[3], Alexandra M. E. Jones[1], Renier A. L. van der Hoorn[4], Sophien Kamoun[1]

[1]The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK. [2]Department of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, USA. [3]Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK. [4]The Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, UK.

Plant pathogens, including those in the lineage of the Irish potato famine organism *Phytophthora infestans*, evolve by host jumps followed by specialization. Genome analyses of the sister species of *P. infestans* uncovered many genes, especially effector coding genes, located in dynamic repeat-rich genome, that have rapidly diverged and display signatures of adaptive evolution. However, how adaptive evolution impacted the biochemical activities of these effectors remains unknown. We hypothesized that following a host jump, some effectors would be under selective pressure to adapt to targets in their new host. To test this hypothesis, we identified a number of effector genes that are induced during infection and show adaptive evolution in *P. infestans* and it's sister species *Phytophthora mirabilis*, a pathogen of four o'clock flower. For functional analysis, we first focused on the EPIC1 cysteine protease inhibitor of *P. infestans* that targets the *Solanum* cysteine protease RCR3. In *P. mirabilis* , the orthologous gene of *epiC1* encodes a cysteine protease inhibitor PmEPIC1 that is under marked positive selection. We discovered that the inhibitors are better adapted to targets from their host plants and mapped key amino acid residues that determine specialization. The results highlight the occurrence of functional specialization in a pathogen effector following host jump, shedding light on how host plants drive pathogen effector diversification and speciation.

Subcellular localization of *Phytophthora infestans* RXLR effector AVR1 and its cognate resistance protein R1

Yu Du, Klaas Bouwmeester & Francine Govers

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

Phytophthora infestans is a devastating plant pathogen that causes late blight on potato and tomato. To colonize host plants, *P. infestans* secretes effectors that can modulate host defence. Well-known are the RXLR effectors, which are able to translocate into host cells to manipulate the cell machinery. However, to counteract the pathogen potato has a set of immune receptors known as nucleotide-binding leucine-rich repeat (NLR) proteins that confer resistance against *P. infestans*. NLR-conferred resistance is mediated by recognition of RXLR effectors, with each NLR protein (or R protein) having its own cognate RXLR effector (or AVR protein). The mechanisms underlying NLR-mediated resistance are still poorly understood. In this study we focussed on the *P. infestans* RXLR effector AVR1 and its cognate potato NLR R1 and addressed the question in which subcellular compartment effector perception and defence activation takes place. We determined the subcellular localization of both AVR1 and R1. We also fused Nuclear Localization Signals (NLS) and Nuclear Export Signals (NES) to R1 and AVR1, as well as mutated NLS and NES, and used these constructs for artificial subcellular targeting of R1 and AVR1. This allowed us to determine the subcellular localization that is required to elicit R1-mediated immunity and AVR1-mediated host defence suppression.

Functional analysis of crinkler effectors from the plant pathogenic oomycete *Aphanomyces euteiches*

Michiel J.C. Pel[1,2], Diana Ramirez-Garcés[1,2], Laurent Camborde[1,2], Hélène San-Clemente[1,2], Bernard Dumas[1,2] and Elodie Gaulin[1,2]

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Microbial pathogens translocate effectors inside host cells to subvert cellular functions and suppress immune responses. Oomycetes, which are fungal-like eukaryotic microorganisms that cause some of the most destructive plant diseases in the world, secrete several different kind of effector proteins. Two large groups of these effectors are the RXLR and the CRN (Crinkler) proteins. RXLRs and CRNs are modular proteins with conserved N-termini and highly diverse C-terminal effector domains. We recently obtained the genome sequence of the legume root pathogen *Aphanomyces euteiches (ATCC201684, AphanoDBv2.0; https://www.polebio.lrsv.ups-tlse.fr/aphanoDB/)*. This data revealed the absence of RXLR effectors and the presence of over 150 putative CRN effectors in the genome of this pathogen*. Aphanomyces* sp. CRNs are characterized by the presence of an LYLALK translocation motif, and although many CRNs have been identified data on CRN function and targets is still limited. We started the functional analysis of these CRN effectors to gain insights in the virulence mechanisms of *A. euteiches* and to identify possible targets for disease control. We have been able to show that one of the CRN effectors, CRN13, localizes in the plant nucleus where it triggers cell death. Further, we found that the CRN13 ortholog of the fungal amphibian pathogen *Batrachochytrium dendrobaditis* is able to cause a similar response in both plant and amphibian cells. Additionally, we demonstrated that both CRN13s are able to bind DNA *in vitro* and cause DNA damage *in vivo*. Altogether, this work reveals that CRNs produced by plant and animal pathogens bind DNA to interfere with host responses.

Characterisation of the RxLR-effector AVR3a from *Phytophthora infestans*

Stephan Wawra[1,2], Ian Davidson[3], Uwe Linne[4], Pieter van West[1]

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Plant pathogenic oomycetes have a large set of secreted effectors that are directed into their host cells during infection. One group of these effectors are the RxLR-effectors found in plant pathogenic oomycetes. These RxLR-effectors are defined as putative secreted proteins that contained a conserved tetrameric amino acid

sequence motif, Arg-Xaa-Leu-Arg. This motif has to be within 40 amino acids C-terminal of the predicted cleavage sites of canonical signal peptides. Often this sequence is followed by a Glu-Glu-Arg (EER) motif. To date, one of the best characterised RxLR-effectors is AVR3a from the late blight "fungus" *Phytophthora infestans*. Here, we will report our latest findings regarding the biochemical characterization of this effector.

Binding to Phosphatidylinositol-3-Phosphate Mediates Entry of Oomycete Effectors into Plant Cells During Natural Infection

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Oomycete and fungal pathogens cause billions of dollars of damage to crops each year and threaten global food security. These pathogens secrete effector proteins that can enter plant cells to modify the physiology of their hosts. A major class of effectors produced by oomycetes contains RXLR motifs that mediate entry of these effectors into plant cells. Oomycete genomes encode hundreds of RXLR effector proteins, although only a minority of these are expressed and make major contributions to virulence. We previously showed that RXLR effectors, and also some fungal effectors that carry "RXLR-like" motifs, can enter host cells in the absence of any pathogen-encded machinery. Furthermore, these effectors can bind to specific lipids including phosphatidylinositol-3-phosphate (PI3P). PI3P-binding requires the RXLR motif, plus in some cases, C-terminal regions of the protein. Previously we showed that PI3P binding is required for the effectors to enter into host cells when the purified proteins are introduced into root or leaf tissue. Furthermore, entry of the proteins could by blocked by competing PI3P-binding proteins. In order to validate that PI3P-binding mediates host cell entry in planta, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RXLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. We have also produced stable transgenic cacao, rice and switchgrass plants that secrete PI3P-binding proteins in order to block effector entry. These plants show elevated resistance to diverse oomycete and fungal pathogens.

Oomycete Biology I - Wednesday 2nd July 2014, 14:15 – 15:30

Early terrestrial oomycetes. What do we know from the fossil record?

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Oomycetes are microscopic eukaryotes that are common saprophytes and parasites of plants, animals and fungi. Some species are known to cause serious diseases, and several have great economic impact. Oomycetes are an ancient group, but their evolutionary history is mostly inferred from molecular phylogenetic studies of living species. A mean age of 936 Ma (range: 1150 - 770Ma) has been suggested for the split between oomycetes and Bacillariophyta (diatoms) but internal calibration of the oomycetes molecular phylogeny has not yet been attempted. Fossil evidence of oomycetes is relatively rare, and where fossils have been documented their affinities are difficult to establish. Recent work, however, provides compelling evidence for oomycetes in Palaeozoic and early Mesozoic ecosystems. To date three groups of fossil vascular plants (lycophytes, ferns and seed ferns) are known to host oomycetes and only one form from the Carboniferous (ca 300 Ma) has been identified as a parasite. The oldest fossil oomycetes come from the 407 Myr old Rhynie Chert (Scotland, UK). This fossil site of exceptional preservation contains the remains of an early terrestrial ecosystem, including plants (non vascular and very simple vascular plants), arthropods, fungi and other microorganisms. Plants grew on sandy substrates or on sinter surfaces close to a river system with associated ephemeral ponds and small lakes. We will present an overview of the fossil record of oomycetes. Our recent research on the Rhynie Chert is documenting the diversity of oomycetes and their interactions with other organisms in the earliest well-preserved terrestrial ecosystem.

Insights into the functions of *P. infestans* Argonaute proteins

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Eukaryotic RNA-based silencing mechanisms act in gene regulation and in defense against endogenous and exogenous nucleic acids (transposons and viruses). Key components of these regulatory circuits are small RNAs (sRNAs) and their associated Argonaute (Ago) proteins. While the sRNA pathways of animals, plants and fungi are well studied, gene silencing in oomycetes is still to a large extent unexplored. Studies of sRNAs and sRNA-based silencing mechanisms in this organism group will therefore contribute to the knowledge of the evolution of eukaryotic RNA silencing. Moreover, identifying the sRNAs and proteins specifically influencing the silencing efficiency of different targets will improve RNAi as a tool in oomycete molecular biology research. The objective of the current project is to characterize the four Ago proteins of *Phytophthora infestans*. This pathogen is known to exploit RNA silencing to regulate effector-encoding genes and to suppress the activity of its abundant transposable elements. By expressing the PiAgo proteins as fusions to GFP, the subcellular localization of each protein will first be studied by confocal microscopy. Next, by co-immunoprecipitation of the PiAgos, the sRNAs and proteins specifically associated with each individual protein will be identified. A key question is whether these homologous proteins are functionally specialized in terms of their sRNA binding preferences. Sequecning of interacting proteins and analysis of co-purified sRNAs are in progress and the results will be presented.

Genetic analyses of *Avr* gene silencing in *Phytophthora sojae*

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Agriculture and Agri-Food Canada, London, ON, Canada

By performing outcrosses between naturally occurring strains of *Phytophthora sojae* we previously demonstrated transgenerational gene silencing of the *Avr3a* effector gene. We now test the stability of *Avr3a* gene silencing by tracking expression in sexual and asexual progeny, and after passage through host plants. From a cross of ACR10 x P7076, we developed recombinant inbred lines of *P. sojae* to the F_5 generation. Silencing of the *Avr3a* gene is maintained in all lines. The parental strain ACR10 was tested after passage through soybean, or after asexual (zoospore) or sexual (oospore) reproduction. Results indicate that silencing is maintained in all conditions. We tested whether *Avr3a* from the *P. sojae* reference strain P6497 can be silenced through outcrossing. First, heterozygous F_1 individuals with the genotype *Avr3aACR10/Avr3aP7076* and phenotypically silenced for *Avr3a* expression were crossed to the reference strain (*Avr3aP6497/Avr3aP6497*; positive for *Avr3a* expression). All progeny from this test cross (*Avr3aACR10/Avr3aP6497* and *Avr3aP7076/Avr3aP6497*) express *Avr3a* transcripts. Second, we performed a direct cross between ACR10 x P6497. In this case, hybrid progeny (*Avr3aACR10/Avr3aP6497*) display both phenotypes, *Avr3a*-expressing and *Avr3a*-silenced, in an approximate 1:1 ratio. The parentage of the F_1 progeny from ACR10 x P6497 was analyzed using mitochondrial markers, to determine whether maternal or paternal effects influence silencing of *Avr3a* in this cross. Results show no association between parentage and *Avr3a* silencing. Overall we conclude that there is interplay between conventional- and epi-genetic variation in *P. sojae* and suggest that silencing of effector genes is conditional on epistatic loci that are polymorphic among strains.

A novel *in vitro* infection system to study *Phytophthora*-host interactions.

Klaas Bouwmeester, Charikleia Schoina & Francine Govers

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One of the most devastating plant diseases world wide is late blight on potato and tomato caused by the oomycete pathogen *Phytophthora infestans*. During the early biotrophic phase of infection, *Phytophthora* penetrates host tissue and thereafter forms specialized feeding structures called haustoria. Here, effectors produced by the pathogen, are transferred into the host cells to manipulate the host cell machinery thereby suppressing plant defense. Therefore, studying the interface between the host and the pathogen at the early stages of infection is of great interest. An important drawback when studying the *Phytophthora*-host

interaction in leaves is the lack of synchronization of the infection process. For this purpose, a new *in vitro* infection system was established, in which MsK8 tomato cell suspensions were challenged with zoospores of different *Phytophthora* species. Here we show that *P. infestans* infects MsK8 cells in a similar fashion as tomato leaf tissue. In contrast, other *Phytophthora* species that are not pathogenic on tomato could not penetrate the MsK8 cells. Expression analyses of *Phytophthora* effector and tomato defense genes and various histological assays were performed to monitor *Phytophthora*-MsK8 interactions in more detail. The use of this novel infection system allows simplification and synchronization of the infection process, and is expected to provide a more detailed insight into *Phytophthora*-host interactions.

The actin cytoskeleton in *Phytophthora infestans* in free living mycelium and infection structures

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The actin cytoskeleton is a dynamic but well organized intracellular framework that is indispensable for proper functioning of eukaryotic cells. The plethora of functions ranges from facilitating transport of vesicles, muscle contraction, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. To visualize the dynamics of the actin cytoskeleton in the oomycete *Phytophthora infestans* we generated transformants expressing the actin binding peptide Lifeact-eGFP as an *in vivo* marker. Fluorescence microscopy revealed that all actin structures in *P. infestans* hyphae, including actin filament cables and actin filament plaques, are cortically localized. The actin filament plaques are distributed over the hyphae but their presence in the hyphal tip is related to hyphal tip growth. They are nearly immobile with an extremely long lifespan when compared to that of actin patches in other eukaryotes and their disassembly is not accompanied with internalization and the formation of endocytic vesicles as is the case with actin patches in yeast. We also investigated the *in vivo* actin cytoskeleton dynamics during growth in a low nutrient environment and during early stages of pathogenesis such as appressorium formation and infection. Our observations suggest that the actin cytoskeleton performs a variety of regulatory roles during distinct life stages of *P. infestans*. Future efforts will focus at identifying interactors and key regulators of the actin cytoskeleton and pinpoint features in the actin network that are unique for oomycetes.

Oomycete Biology II - Wednesday 2nd July 2014, 16:00 – 17:15

Proteomics and phosphoproteomics of *P. infestans* and potato

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We are using proteomics to study the underlying processes that govern pathogenicity and development in *Phytophthora infestans* and resistance to *P. infestans* in potato. In a large-scale phosphoproteomics study of six life stages, we obtained quantitative data for 2922 phosphopeptides. Life stage-specific phosphopeptides include ABC transporters and an appressorium specific kinase. We also identified 2179 phosphorylation sites and deduced 22 phosphomotifs. In addition, we detected tyrosine phosphopeptides that are potential targets of tyrosine kinases. Among the phosphorylated proteins are members of the RXLR and Crinkler effector families. The latter are phosphorylated in several life stages, in sites that are conserved between different members of the Crinkler family. This indicates that proteins in the Crinkler family have functions beyond their putative role as (necrosis-inducing) effectors. In a proteomic study of the same life stages, we have identified a number of proteins with expression patterns that are specific for life stages involved in (pre) infection. Among these are both proteins previously identified as being specifically expressed in these stages, as well as transport proteins, RXLR proteins, kinases and proteins putatively involved in cell wall synthesis. We are now analysing a selection of these proteins further. In addition, we have also used proteomics to study the apoplastic proteome of potato cultivars with different degrees of resistance to *P. infestans* in response to infection, identifying potential effector targets and proteins involved in pathogen resistance.

Thus, we show that proteomic techniques are valuable tools to elucidate novel mechanisms of pathogenicity and resistance in oomycete-host interactions.

Turning on and off gene expressions in Phytophthora infestans

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Phytophthora infestans transformation is a well-established procedure. However, transgenic lines are often unstable, losing transgene expression over time, probably due to silencing events. To develop new tools for expressing genes, we tested three ribosomal genes showing a strong and constant expression level during all *P. infestans* life stages. We assayed as well their counterparts from *P. capsici*, in order to avoid potential deleterious effects due to silencing of the native gene. A GUS reporter system allowed us to evaluate the strength and stability of transgenes expressed from the ribosomal promoters. Based on two years of data, a *P. capsici* promoter was identified as superior and used to generate a new general-purpose expression vector. The *ham34* promoter remains the strongest promoter assayed so far, however the new promoter allows more flexibility in producing complex constructs or sets of plasmids for co- transformation.
In addition, we established an artificial microRNA approach for generating knock-down lines in *Phytophthor*a for reverse genetics. So far, no evidence of microRNA silencing mechanism has been described in *Phytophthora* or Oomycota. We targeted an abundant *P. infestans* secreted protein, the elicitin INF1, using artificial miRNA with *P. sojae* or *P. infestans* bioinformatically predicted miRNA backbones. Silencing was observed with both of them and we could detect the expected mature amiRNA* by sequencing small RNAs from a silenced *P. infestans* transformant. These results open the road for an easy and efficient silencing of genes or gene families in *Phytophthora*.

Role of the pectic enzymes in the oomycetes pathogenesis

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The plant cell wall, the main structural element that a pathogen needs to overcome in order to penetrate and colonize the plant tissue, is organized as a complex network of polysaccharides such as cellulose, hemicellulose and pectin. Cell Wall degradation is accomplished from a wide array of Cell Wall Degrading Enzymes (CWDEs) produced by phytopathogenic microorganisms. One of the first enzymes to be secreted are the polygalacturonases (PG), a class of pectolytic enzymes, which facilitate the invasion of the plant tissue and release nutrients to be used as carbon source from the pathogen. PGIPs (polygalacturonase-inhibiting proteins) are plant cell wall proteins that specifically modulate the activity of PGs. The PG–PGIP interaction retards the hydrolysis of pectin but also favor the accumulation of oligogalacturonides (OGs) that activate the plant defense responses. Our aim is investigate the role of PGs in the pathogenesis of two different oomycetes, *Phytophthora nicotianae* and *Phytophthora capsici* on both tobacco and tomato plants. Here we show a comparative study based on phylogenetic analysis of large PG families found on the two oomycetes and, the results of the infections carried on transgenic plants that overexpressing the specific inhibitor PGIP. Finally we present our latest results in trying to study PG effector function in *Phytophthora* during the infection cycle.

Alternative splicing in the obligate biotrophic oomycete pathogen, *Pseudoperonospora cubensis*

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*Co-first authors, Corresponding authors: JHC (computational queries), BD (biological queries)

Alternative splicing (AS) is a conserved mechanism of eukaryotic gene regulation, which influences the proteome and timing of gene expression across several groups of eukaryotes, including the oomycete, *Pseudoperonospora cubensis*. Deep transcriptome sequencing analysis of *Ps. cubensis,* the causative agent of cucumber downy mildew, was completed for both the sporangia of *Ps. cubensis* and inoculated cucumber tissue collected over a time course from 1-8 days after inoculation. Data analysis pipelines for Illumina RNA-Seq data were optimized to re-annotate the genome and were used to predict splice variants. Nearly 30% of the expressed genome and nearly 58% of the intron-containing genes were predicted to be alternatively spliced. The predominant mechanism for AS was found to be intron retention; some evidence for alternative 3′ and alternative 5′ exon splicing was also observed. The types and frequencies of predicted AS in *Ps. cubensis* are consistent with its placement on the evolutionary tree near plants. Alternatively spliced isoforms for predicted non-effector-, secreted-, and effector protein-encoding genes were validated using real-time PCR and reverse transcriptase PCR. In some cases, the frequency of a specific splicing event in *Ps. cubensis* was found to change during sporangia development or during the time course of infection on the cucumber host. AS is hypothesized to have a regulatory role in the timing of gene expression during pathogen development and host infection. The descriptive data of splicing in *Ps. cubensis* will be discussed at the 2014 OMGN meeting; future work will focus on bioinformatics related to host resistance.

The elicitin-like glycoprotein, ELI025, is secreted by the pathogenic oomycete *Pythium insidiosum* and evades host antibody responses

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Pythium insidiosum is a unique oomycete that can infect humans and animals. Patients with the infection caused by *P. insidiosum* have high rate of morality and mortality. The pathogen resists conventional antifungal drugs. Information on biology and pathogenesis of *P. insidoisum* is limited. Many pathogens secrete proteins, so called effectors, which facilitate the infection process. These proteins include elicitin, CBEL, and RXLR. Recently, we report some elicitin-encoding genes from *P. insidiosum* transcriptome. Elicitins form a large protein family, and have been found only in *Phytophthora* and some *Pythium* species. In plant-pathogenic oomycetes, elicitins are pathogen-associated molecular patterns (or PAMPs), can binds sterol, and stimulate plant host defense. Function of elicitins in human host is unknown. This study aims at characterizing the elicitin protein ELI025 of *P. insidiosum*. Recombinant ELI025 protein (rELI025; 12 kDa in size) was successfully expressed in *Escherichia coli.* Rabbit anti-rELI025 antibody captured the native ELI025 in culture media, and cytoplasm. Deglycosylation reaction showed that the native ELI025 is glycosylated. *P. insidiosum* from different phylogenetic clade showed different glycosylation pattern. The rELI025 had none or very limited immunoreactivity against sera from pythiosis patients. Homology modeling predicted that ELI025 can bind some sterols and host immune mediators. In conclusion, ELI025 is a secreted small glycoprotein that could evade host humoral immune response. Among human pathogens (fungi and parasites), elicitins have been found only in *P. insidiosum*. This suggested that ELI025 could be a good candidate for development of diagnostic and therapeutic markers for pythiosis.

Genomics/Transcriptomics/Population Genomics I - Thursday 3rd July 2014, 09:00 – 10:30

Oomycete genomics using historic and modern samples: challenges and perspectives

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DNA is preserved and can be retrieved from unusual sources such as fossilized tissue, archaeological remains and museum collections. Independent of its age, this DNA is called "ancient" because it changes biochemically with time i.e. its fragments are shorter than 100 base pairs and it suffers post-mortem damage. Retrieving DNA from different time periods opens a window to the past and could thus provide direct insights into the history and evolution of a given organism. Natural history museums store millions of dissecated plant material in their herbaria. Infected herbaria samples are of particular importance, since they can allow to study the dynamics of past epidemics and the co-evolution of plant-pathogen interactions. Recently it has been shown that complete genomes of the Irish potato famine pathogen *Phytophthora infestans* can be sequenced from historic specimens. Challenges and perspectives on oomycete genomics combining historic and modern samples will be discussed using *P. infestans* as an example.

Multitrophic microbe-microbe interactions determine the leaf microbiome

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Eukaryotic plant symbionts and pathogens coevolve with the host plant microbiome and can acquire the ability to communicate and even collaborate. Obligate pathogens of the genus Albugo, the causal agents of white rusts on Brassicacea, cause broad-spectrum suppression of innate immunity and enable non-host pathogens to grow. Growth of non-host pathogens likely causes a massive change to the host microbiota. We hypothesize, that Albugo sp. not only manipulate plant immunity, but re-structure the microbiome of their ecological niche to their advantage through microbe-microbe interactions.

We have used 16S and ITS amplicon sequencing to identify the impact of Albugo sp. infection on host microbiota. Using Illumina sequencing technique we could show that Albugo sp. significantly reshape their host microbiome from a stochastic to a structured pathogen determined community. To identify why certain bacteria such as Pseudomonas sp. specifically enrich in the plant apoplast upon infection, we performed a metagenome approach. Based on this approach, we hypothesize that Albugo sp. specifically enrich for bacteria that have less cell wall degrading enzymes and are therefore compatible with the biotrophic lifestyle of the pathogen itself. An apoplastic metaproteomics approach further revealed insights into how pathogens interact with the host and associated microbes.

Our findings suggest, that Albugo sp. reshape their host microbiota not only through host immune suppression but through direct microbe-microbe interactions. We are currently investigating the impact of the host microbiome and pathogen associated microbes on pathogen fitness. Our findings will have a significant impact on understanding pathogen epidemics.

Functional Genomics of *Bremia lactucae*

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Downy mildew, caused by the biotrophic oomycete *Bremia lactucae* is the most important disease of lettuce worldwide. The disease is controlled by the use of resistant cultivars and fungicide applications. However, *B. lactucae* rapidly overcomes resistance genes and develops fungicide insensitivity. We are using functional genomic approaches to understand the molecular basis of variation in interactions between *B. lactucae* and lettuce. RNAseq is being used to characterize gene expression in *B. lactucae* during its life cycle and for

comparative genomics of a diverse set of 40 isolates from different geographical and temporal origins. These have been sequenced and their genomes mined for candidate effectors and other pathogenicity related genes using sequence homology, string searches, and HMMs. *B. lactucae* has a vast effector repertoire of genes encoding RxLR proteins. The majority of candidate effectors are present in only a few isolates, while others are present in most isolates of *B. lactucae*. A few are present in other oomycete species. A subset of these genes were selected on the basis of their conservation and expression patterns for functional analysis using *Agrobacterium*-mediated transient *in planta* expression to assay for their ability to induce necrosis on a differential set of resistant cultivars as well as suppression of basal defenses and inhibition of cell death induced by co-infiltration with HR inducers. These studies will provide tools for screening for resistance genes as well as for detailed population genomics that will provide data for the rational deployment of resistance genes and enhance the durability of resistance.

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens

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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 known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, a transcriptome analysis was initiated for *L. giganteum*, using various Next Generation Sequencing technologies (454 and PacBio platforms). Homology searches revealed a full complement of plant pathogenic oomycete effector orthologs. The characterization of full-length transcripts corresponding to Cellulose Binding Elicitor Lectin (CBEL), Crinkler, and elicitin proteins demonstrated that *L. giganteum* is the first described animal pathogenic oomycete to secrete canonical Crinkler and CBEL effectors. In addition, phylogenetic analyses identified a Glycoside Hydrolase 5 (subfamily 27; GH5_27) as a putative virulence factor. Genome mining indicated that GH5_27 orthologs are shared by entomopathogenic oomycetes and fungi, but virtually absent in all other oomycetes and fungi. Using PCR, GH5_27 fragments were amplified and sequenced from additional entomopathogens, suggesting that oomycete and fungi underwent convergent evolution and that GH5_27 proteins may play a crucial role in insect/microbe pathosystems. Detailing the molecular basis of entomopathogenicity may allow for the use of *L. giganteum* as control agents, and improve the environmental sustainability of mosquito control strategies.

Comparative genomics of temperate and tropical downy mildews

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Downy mildews (DMs) are obligate biotrophic oomycete pathogens that cause diseases on a wide range of plant species. They have narrow host ranges and exhibit high degrees of host specialization. We utilized next-generation sequencing to generate genome assemblies and studied evolutionary relationships and effector repertoires within and between diverse species of DM. We have sequenced the genomes of geographically and temporally separated isolates of lettuce DM (*Bremia lactucae*), tobacco DM (*Peronospora tabacina*), sorghum and maize DM (*Peronosclerospora sorghi*), and pearl millet DM (*Sclerospora graminicola*). These DMs have been assembled, annotated, and examined for their variation in genome size, repeat content, gene content, level of heterozygosity, and their repertoire of genes encoding candidate effectors and other pathogenicity-related proteins, as well as their mitochondrial structure and variation. They were shown to differ greatly in genome size and possess varying levels of heterozygosity, indicative of inbreeding, clonal, and outbreeding populations. Effector repertoires were diverse both between species and between isolates of the same species. Many effector-encoding genes appear to be species specific. A total of 3,640 RXLR encoding genes have so far been identified in 28 isolates of *B. lactucae*. These were highly divergent and could be grouped into approximately 900 clusters at 80% amino acid identity. Their distribution across isolates was bimodal, with majority present in only one or two isolates. Nearly all were private to *B. lactucae*; with only a

few detected in other DMs or *Phytophthora* spp. This distribution pattern was not observed when analyzing CRNs and NLPs, which are more conserved.

Genomics/Transcriptomics/Population Genomics II - Thursday 3rd July 2014, 11:00 – 12:30

Assembly and comparison of *Aphanomyces astaci* mt-genomes illustrate high diversity among genotypes

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Oomycetes are a diverse group of organisms that have adapted to a wide range of hosts in different life styles and environments. The model species *Aphanomyces astaci*, commonly known as crayfish plague in crustaceans, has been without doubt the most harmful pathogen of the native European crayfish species. After its first introduction during the 1860's it has been spreading through Europe destroying native crayfish populations and altering aquatic ecosystems. We have assembled the mitochondrial genome of *A. astaci* isolate AP03 (origin of subtropical crayfish *Procambarus clarkia* from South USA) and compared it to other more studied *A. astaci* isolates. The assembly has been generated from whole genome shotgun sequence reads from the NCBI Sequence Read Archive, sequenced by the Broad Institute (project SRP018895). The comparison and SNP analysis was done using RNA-seq reads from cultured hyphae. The reads were mapped against the generated mitochondrial genome using Bowtie 0.12.8 short read aligner and SNPs were discovered using Geneious software. The mitochondrial genome is 49,489 bp long containing two inverted repeat regions (12,570 bp). These contain large and small ribosomal subunits and eight protein coding genes. The mitochondrial genome codes 38 different proteins (three of these putative) and has 33 tRNAs coding for 19 amino acids. The SNP analyses showed an unexpectedly high diversity (a total of 158 SNPs) between AP03 and other host related isolates UEF8866-2 (origin of North American signal crayfish), Evira6462 and UEFT2B (isolated from Finnish noble crayfish). The analysis also identified 20 SNPs among the Finnish isolates.

Comparative analysis of expressed CRN and RXLR effectors from two *Plasmopara* species causing grapevine and sunflower downy mildew

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Plasmopara halstedii and *Plasmopara viticola* are the causative agents of downy mildew respectively on sunflower and grapevine, two economically important crops. These phylogenetically related oomycetes are obligate biotrophs belonging to Peronosporales but have different pathological profiles. We performed parallel transcriptome sequencing from *P. halstedii* and *P. viticola* and released a *Plasmopara* species cDNA database containing 46 000 clusters. In oomycetes, two classes of effectors are translocated into the host: RXLRs and CRNs. We screened the *Plasmopara* species database in order to identify the repertoire of expressed effectors used by both *Plasmopara*. About 50 putative RXLR and 60 CRN were identified for each *Plasmopara* species. We compared these effectors within both *Plasmopara* and with 7 sequenced species of oomycetes representative of Peronosporales (3 *Phytophthora* species, *Pseudoperonospora cubensis*,

Hyaloperonospora arabidopsidis and *Pythium ultimum*) and Albuginales (*Albugo candida*). Sequence analyses revealed the presence of 55 RXLR families, 12 of them shared by both *Plasmopara*, and 19 showing amino acid conservation with predicted peptides from at least one oomycete species. Analyses of *Plasmopara* sp. CRN C-terminal variable regions revealed sequence conservation inside *Plasmopara* sp. and across oomycetes, excepting *H. arabidopsidis*. Finally, our analyses confirmed the presence of 8 CRN C-terminal domains described in *P.infestans* and uncovered putative functions for CRN effectors. Transient expression in sunflower of *P. halstedii* selected effectors fused to GFP is currently done to study their functional role *in planta.*

Enabling technologies for future trees free from *Phytophthora* disease

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Phytophthora diseases in tree species are becoming more prevalent internationally. In New Zealand there are several *Phytophthora* species that are impacting native and exotic forests, and the horticultural industry. These include *Phytophthora pluvialis,* causal agent of the disease red needle cast in *Pinus radiata*, *Phytophthora* taxon *Agathis* which is causing severe disease in a native tree species, *Agathis australis* (kauri), and *Phytophthora cactorum* which has a long-standing record of disease in apple. A new research programme at Scion is taking a systems biology approach to address these issues. The overarching aim of the six year programme is to look for distinguishing features of pathogenicity and/or resistance across different pathogen species and hosts. We are assessing the potential for utilising genetic, gene expression and/or metabolite signatures for tree breeding, improving disease management and advancing current knowledge of *Phytophthora*-tree interactions. The primary focus will be *Pinus radiata,* with the analysis model also applied to kauri and apple. Host-pathogen interactions will be examined using disease expression from both field observations and artificial inoculations. Analysis will target different stages of infection by pre-screening with H1-NMR, with selected tissues further examined using histopathology, gas/liquid-chromatography-mass spectrometry, and genomic and transcriptomic analyses. Six *Phytophthora* species will be examined in New Zealand, and another two species examined in collaboration with international researchers. These species were chosen to be representative of *Phytophthora* diversity, pathogenicity on woody hosts and broad host range. An overview of the research programme will be discussed, with specific emphasis on genomic and transcriptomic analyses.

Phylogenetic investigation of marine holocarpic eukaryotic pathogens (stramenopiles) of filamentous algae

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Current understanding of pathogens of algae is very limited, with aquatic viruses being the most intensively studied area, yet the pathogen diversity is expected (and partially known) to be large with a host of organisms from viruses through bacteria, archaea and eukaryotic fungi and stramenopiles already described, but not intensively studied. Algaculture is becoming a global business, having expanded from Asian countries where food consumption and phycocolloid production was the main motivation for farming to other parts of the world such as Europe and desert regions of America and Australia (the latter specifically for bio-fuels). As algaculture increases the potential impact of these unexplored pathogens increases, unless algal strains are specifically bred against these unknown pathogens or algal immunity can otherwise be induced. Currently under investigation are four pathogens which infect marine filamentous algae; an *Olpidiopsis* sp., designated as an oomycete, an economically significant class of stramenopiles and three *Anisolpidium* sp. which morphologically are designated as belonging to the class Hyphochytridiomycota, stramenopiles closely related to the oomycetes, but much less well studied. It is hoped that this study will provide fundamental molecular knowledge in an under-sampled area of the stramenopile diversity for future investigation of these parasites through high-throughput techniques. Using several markers we establish the position of *Olpidiopsis* in the oomycetes, closely related to another group of holocarpic oomycetes which infect marine crustaceans and surprisingly place *Anisolpidium* within this clade causing some controversy due to established morphological differences.

A novel recombinant population of Phytophthora infestans in northeastern USA?

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Phytophthora infestans, the causal agent of late blight disease, has been reported in North America since the mid-nineteenth century. In the United States the lack of, or very limited sexual reproduction has resulted in largely clonal populations of P. infestans. In 2010 and 2011, but not in 2012 or 2013, 20 rare and diverse genotypes of P. infestans were detected in a region that centered around central New York State. The ratio of A1 to A2 mating types among these genotypes was close to 1:1. These genotypes were diverse at the glucose-6-phosphate isomerase locus, differed in their microsatellite profiles, showed different banding patterns in a restriction fragment length polymorphism assay using a moderately repetitive and highly polymorphic probe (RG57), and were polymorphic for four different nuclear genes. This diversity is consistent with a recombinant population, and two tests (the index of association and the pairwise homoplasy index) failed to reject the hypothesis that these individuals were a recombinant population. These new genotypes were monomorphic in their mitochondrial haplotype. Through parentage exclusion testing using microsatellite data as well as sequences of four nuclear genes, recent dominant lineages US-8, US-11, US-23, and US-24 (but not US-22) were excluded as possible parents for these genotypes. We conclude that US-22 could be a parent of some, but not all, of the new genotypes found in 2010 and 2011. There were at least two other parents for this population and the genotypic characteristics of the other parents are identified.

Keynote Address - Thursday 4th July 2014, 14:00 – 15:30

Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, which is focused as mechanical force to breach the rice cuticle and facilitate entry of the fungus into plant tissue. Re-polarisation of the appressorium requires a hetero-oligomeric septin GTPase complex for re-organisation of a toroidal F-actin network at the base of the appressorium. This allows host cuticle and leads to invasion of epidermal cells by biotrophic invasive hyphae of *M. oryzae*. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species occurs within the appressorium. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. The appressorium pore is the site of polarised exocytosis during plant infection and the octameric exocyst complex localises to the pore in a septin-dependent manner and is essential for cytoskeletal regulation. Both cell cycle and pressure-mediated checkpoints appear to be necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion. One tissue is invaded the fungus undergoes differential expression and secretion of a large repertoire of effector proteins that are destined either for the apoplastic space which surrounds invasive hyphae, which are bounded by the plant plasma membrane, or are directed instead into plant cells. How this delivery is achieved in not known, but it does appear to involve a specialised strcuture known as the biotrophic interfacial complex (BIC), a plant membrane-rich body where effectors accumulate. Secretion of effectors appears to be regulated distinctly, depending on their final destination. Initial characterisation of the effector gene families present in *M. oryzae* will be preented.

Poster Session - Thursday 3rd July 2014, 15:30 – 17:30

Conference Dinner (JIC) - Transport at 18:00.

Plant Response to Oomycetes/Resistance Mechanisms I - Friday 4th July 2014, 09:00 – 10:30

DMR6 and dMR6-like oxygenase 1 are partially redundant but distinct negative regulators of immunity in Arabidopsis

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Arabidopsis mutants lacking a functional *DMR6* gene are resistant to infection by the downy mildew *Hyaloperonospora arabidopsidis*. Here we show that resistance is also effective against the bacterium *Pseudomonas syringae* and the oomycete *Phytophthora capsici*, and is accompanied by enhanced defense gene expression, suggesting DMR6 is a negative regulator of plant immunity. In contrast, transgenic Arabidopsis lines overexpressing *DMR6* are enhanced susceptible to *H. arabidopsidis, P. capsici* and *P. syringae*. Phylogenetic analysis of the superfamily of 2OG oxygenases of 19 plant species revealed a subgroup of DMR6-like oxygenases (DLOs). Within Arabidopsis, DMR6 is most closely related to the DMR6-like oxygenases 1 and 2 (DLO1 and DLO2). Overexpression of *DLO1* and *DLO2* in the *dmr6* mutant resulted in loss of resistance indicating the DLOs act as negative regulators of defense, similar to DMR6. *DLO1*, but not *DLO2*, is highly co-regulated with *DMR6*, showing strong activation during pathogen attack or salicylic acid treatment. *DMR6* and *DLO1* differ in their spatial expression pattern in leaves of Arabidopsis infected with downy mildew; *DMR6* is mostly expressed in cells that are in contact with hyphae and haustoria of *H. arabidopsidis*, whereas *DLO1* is expressed mainly in the vascular tissues near the infection. Strikingly, the *dmr6-3_dlo1* double mutant, that is completely resistant to *H. arabidopsidis*, showed a strong growth reduction that was associated with high levels of salicylic acid. We conclude that DMR6 and DLO1 act redundantly as important negative regulators of plant immunity, but also have distinct activities based on their differential localization of expression.

Effector-Directed Breeding to Improve Resistance against Phytophthora sojae in Soybean

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Phytopthora sojae is the causal agent of soybean root and stem rot. This disease is estimated to cause $1-3 billion in crop losses worldwide. Naturally occurring resistance genes have been utilized against this disease, but their effectiveness is being compromised by pathogen co-evolution. We are using *P. sojae* RxLR effector genes as probes to screen for novel resistance genes in accessions of cultivated soybean (*Glycine max*). The screening system uses *Pseudomonas fluorescens* (EtHAN) to transiently deliver a *P. sojae* effector into soybean cells. If the effector is recognized by a resistance gene, a macroscopic cell death lesion (hypersensitive response, HR) appears on the leaf. To improve signal intensity, we co-infiltrate with *Pseudomonas syringe pv. glycinea* race 4 (*Psg*) which increases the growth of EtHAN *in planta*. Three *P. sojae* effectors were used to validate this system. All three effectors produced a macroscopic HR on cultivars that contain the cognate *R* gene, thereby validating the system. We used this system to screen for novel *R* genes against three *P. sojae* effectors that are conserved, highly expressed, and essential for *P. sojae* virulence. *R* genes that recognize these effectors are expected to be durable and to recognize a broad range of *P. sojae* isolates. We screened 31 *G. max* lines that were pre-selected for resistance against three resistance-breaking isolates of *P. sojae*. 19 lines responded to one or more of these three effectors. Furthermore, we have finished preliminary screens with seven additional effectors, and we have identified additional putative resistance genes. Our goal is to breed these resistance genes into elite cultivars for commercial use.

The *Solanum bulbocastanum* Rpi-blb2 immune receptor recognizes a second effector AVRblb2b that is deleted in a partially virulent isolate of *Phytophthora infestans*

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Understanding how plant pathogens evolve to evade immune recognition is critical for developing efficient strategies to genetically engineer disease resistance in plants. The potato late blight pathogen *P. infestans* is infamous for its remarkable ability to rapidly overcome plant resistance. Recently, an emerging *P. infestans* strain, NL07434, was discovered to partially colonize plants carrying Rpi-blb2, a broad-spectrum CC-NBS-LRR type immune receptor. Rpi-blb2 was previously shown to recognize several members of an RXLR effector family, known as AVRblb2, but the sequence and expression level of the canonical AVRblb2 were unchanged in NL07434. To understand how NL07434 partially overcomes *Rpi-blb2*-mediated resistance, we first reanalyzed the genome of avirulent *P. infestans* strain T30-4 for *Avrblb2*-like effectors. These effectors were differentially recognized by Rpi-blb2 when expressed *in planta*. One *Avrblb2*-like paralog was expressed at early stages of infection and activated *Rpi-blb2*. Interestingly, this paralog was deleted in the NL07434 genome although intact copies of other *Avrblb2* paralogs were present. We named this gene *Avrblb2B* and addressed its avirulence activity by complementation in *P. palmivora,* a readily transformable *Phytophthora* species*. P. palmivora* transformants carrying *AVRblb2B* triggered hypersentivity on Rpi-blb2 plants confirming the avirulence activity. Live cell imaging of an N-terminal GFP fusion of AVRblb2B revealed a nucleo-cytosolic pattern unlike AVRblb2, which localizes to the plasma membrane. However, similar to AVRblb2, AVRblb2B accumulated at the host pathogen interface inside infected plant cells. Our work reveals a novel perihaustorial effector with avirulence activity and points to complex gene-for-genes mechanisms of plant immune recognition of filamentous pathogens.

Elucidation of the gene-for-gene relationships of white rust on Arabidopsis

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Albugo sp, ("White rusts") are obligate biotrophic oomycete plant pathogens that infect plant species of the family *Brassicaceae*. *Albugo laibachii* (*Al*) specifically infects *Arabidopsis thaliana* (*At*). Since *Al* can colonise ~90% of *At* accessions and suppress effector-triggered-immunity to other pathogens we postulated that *Al* secretes effector proteins. In order to verify our hypothesis we decided to identify "avirulence genes" (*AVR*): effectors that are recognised and trigger strong defence response by the immune system of some host accessions. Using the opportunity to also learn about the natural diversity of *Al*, we undertook the genomic sequencing and comparison of six *Al* isolates. Using differential phenotype information to guide a genome wide association study in the pathogen, and our postulated expectations of the allelic diversity of *AVR* genes, we identified a novel recognised effector. This effector, a short secreted protein named "AlSSP16", is recognised by the *At* accession HR-5. SSP16 protein variants from isolates virulent on HR-5 do not trigger defence efficiently. We then used classical genetic mapping to identify the corresponding resistance locus in HR-5, which contains three putative CC-NB-LRR class R-protein encoding genes. Our current work is to establish which of these genes encodes the cognate receptor for AlSSP16/AVRRAL4. We have demonstrated the utility of combined genomics approaches to identify recognised effectors without known motifs. The identification of the first *Avr-R* gene pair from the *Al-At* pathosystem will pave the way for further dissection of the molecular interactions in this relationship.

Symbiotic plant processes involved in development of root interactions with detrimental microbes

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Plant resistance to microbial diseases has been extensively investigated in the last decades and achievements in understanding the molecular actors underlying such interactions have been obtained. However, most of these studies were carried out on above-ground plant organs largely ignoring the wealth of plant-microbe interactions occurring in soil and leading either to parasitism or mutualism.

Among these below-ground associations, root infection by oomycete pathogens such as *Phytophthora palmivora* as well as colonisation by beneficial Arbuscular Mycorrhiza (AM) fungi has been a recent focus of interest because of their agronomical relevance. Albeit plant and microbe genes underlying these interactions are starting to be uncovered, the degree of commonalities in the development of beneficial and detrimental interactions has not been addressed. Here we present the screening of a collection of 22 *Medicago truncatula* mutants affected in nitrogen fixing symbiosis and/or arbuscular mycorrhizal symbiosis for their resistance to *P. palmivora*. A combination of visual, cytological and molecular markers was used to pinpoint altered development of the interaction in the mutant background. In addition we also took benefit of the Medicago HAPMAP project to study the diversity of the interaction between *M. truncatula* and the oomycete and investigate a potential correlation with colonization by mycorrhizal fungi. Altogether, this research will allow sharpening our understanding of common and specific plant functions controlling interactions with beneficial and detrimental microbes.

RNA-Seq analysis of BABA-induced resistance to *Phytophthora parasitica* in tomato emphasizes a hyper-responsive plant status

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The non-protein amino acid BABA (b-aminobutyric acid) induces resistance in tomato towards downy mildew and root knot nematodes (Cohen *et al.*, 1994; Oka *et al.*, 1999). The aim of our work is to decipher gene networks that can explain induced resistance of tomato to pests, in order to further evaluate molecules that might be used for sustainable crop protection. BABA is unadapted for field applications (phytotoxicity) though the understanding of its mode of action remains of prime interest. BABA induces a strong resistance to *Phytophthora parasitica* (symptoms reduced by 90%). To monitor the BABA-induced changes in transcript accumulation, we collected 3 independent tomato leaves samples , 24 h after treatment. Transcript profiling was performed with NGS (Solid™). Sequencing depth was about 100-fold the genome size and reads were mapped to the tomato genome (www.solgenomics.net). We found that more than 1,300 genes were up-, and almost 200 genes were down-regulated upon treatment ($padj < 0.01$). Many up-regulated genes encode proteins involved in signal perception (membrane-bound and intracellular receptors), in regulation of transcription (ERF, WRKY, and MYB factors), and in defense (PR-proteins). BABA also triggers a noteworthy up-regulation of genes encoding tomato defense proteases that are targeted by Avr2 (Rooney *et al.*, 2005) and *P. infestans* effectors (Song et al., 2009). We also show that the coordinated regulation of enzymes from the secondary metabolism is fully supported by analytical results. Our data let us conclude that BABA responses are mainly governed by ethylene and that this molecule promotes pathogens perception.

Cohen Y, *et al*. 1994. *Plant Physiol.* **104**, 59-66. **Oka Y, *et al***. 1999. *Phytopathology* **89**, 1138-1143. **Rooney HC, *et al***. *Science* **308**, 1783-1786. **Song J, *et al***. 2009. *Proc Natl Acad Sci U S A* **106**, 1654-1659.

Plant Response to Oomycetes/Resistance Mechanisms II - Friday 4th July 2014, 11:00 – 12:30

Presence of heterozygosity in effectors allows avoidance of recognition by *R*-genes

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The *Arabidopsis thaliana-Hyaloperonospora arabidopsidis* system has been a very useful model to understand the relationship between downy mildews and their host plants at the molecular level. We had found that *Hyaloperonospora arabidopsidis* isolates, Noks1 and Cala2, were virulent on an Arabidopsis accession RMX-A02, but when these two isolates were crossed, a proportion of the subsequent F_2 generation was avirulent on the Arabidopsis accession. The segregation ratio indicated that a single dominant gene, designated *HAC1* (***H****yaloperonospora* ***a****rabidopsidis* ***c****ryptic1*), determined this 'cryptic' avirulence. We sequenced the genome of the parent isolates and, using a map-based cloning strategy based on bulk segregant analysis and SNP calling, we have identified the genomic interval for *HAC1* and cloned the candidate genes. It became clear that *HAC1* is non-functional in Noks1, and originates from Cala2. However, the locus in the Cala2 isolate is heterozygous with two different functioning alleles. Genetic and molecular evidence enabled the proposal of a mechanism for activation or suppression/avoidance of plant defence, in which the heterozygous nature of Cala2 plays a key role. Further investigations revealed that heterozygosity is maintained in other loci as well as in other isolates and may be crucial for maintaining pathogenicity. The genetic evidence suggests that the pathogen evolves towards favoring the gene that avoids recognition.

MORC1 Modulates Plant Immunity against *Phytophthora infestans* in Solanaceae

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The Microrchidia (MORC) proteins, a subset of the GHKL ATPase superfamily, have been recently described as components involved in transcriptional gene silencing in Arabidopsis. In addition, we have previously shown that this *MORC* family functions in multiple layers of plant immunity including non-host resistance against *Phytophthora infestans* in Arabidopsis. We have assessed the role of MORC1 during resistance against this oomycete pathogen in Solanaceae by altering the expression of the corresponding *MORC1* homologs in potato, tomato, and in *Nicotiana benthamiana.* Our results indicate that MORC1 modulates basal and in some cases *R* gene-mediated resistance in a species-specific manner. While silencing potato *MORC1* reduced resistance, silencing *MORC1* in tomato and in *N. benthamiana* enhanced resistance. Interestingly, silencing tomato *MORC1* further enhanced resistance to *P. infestans* conferred by two *R* genes (*Ph2/Ph3*). Moreover, altering the expression of *MORC1* in Solanaceae has an effect on resistance against bacterial pathogens. In order to assess whether this species specificity is due to small differences in the MORC1 proteins, we transiently express StMORC1, SlMORC1, and NbMORC1 proteins in *N. benthamiana* and determine their effects on the cell death induced by infestin 1 (INF1). Transient expression of these proteins also has opposite effects on the INF1-induced cell death. Domain-swapping experiments indicate that these species-specific effects map to the C-terminal region of these proteins. We found that this C-terminal region is required for protein dimerization, is phosphorylated, and display signaling activity. Together, these findings argue that MORC1 is an important component for broad-spectrum resistance in Solanaceae.

RNA silencing, a sensitive new tool in the fight against the potato late blight disease

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Phytophthora infestans is an oomycete that causes late blight disease on potato. This plant pathogen is notorious for its ability to rapidly evolve to overcome resistant potato varieties. Here, an RNA silencing strategy was evaluated to clarify if small interfering RNA homologous to selected genes in *P. infestans* could be targeted from the plant host. As a proof-of-concept a hairpin construct using the *GFP* marker gene was designed and introduced in potato. At 72 hpi a 75-fold reduction of the signal intensity of a corresponding GFP-tagged *P. infestans* strain on leaf samples of transgenic plants compared to wild-type potato was detected. This suggests that dsRNA in the potato host could be processed and target a transcript of the pathogen. Three genes important in the infection process of *P. infestans PiGPB1, PiCESA2, PiPEC,* and *PiGAPDH* taking part in basic cell maintenance were subsequently tested using an analogous transgenic strategy. Out of these gene candidates, a hairpin construct targeting the *P. infestans* G protein ß-subunit *PiGPB1* indispensable for pathogenicity resulted in the most restricted disease progress and malformed sporangia. Illumina sequencing of inoculated transgenic potato leaves revealed sRNAs of 24/25 nt size homologous to the *PiGPB1* gene in the transgenic plants indicating post-transcriptional silencing of the target gene. The work demonstrates that a host-induced gene silencing approach is functional against *P. infestans.* This approach broadens the arsenal for strategies to control the disease, and reduce the chemical burden of the intensive fungicide management deployed.

microRNA signaling in tomatoes during *Phytophthora infestans* infections

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Phytophthora infestans is an aggressive and devastating pathogen infecting different species in the Solanaceae. A main line of defense to this pathogen relies on breeding in resistance genes (*R* genes) into crop species from wild close relatives. However this resistance is often short-lived and can be overcome by the pathogen. Recentyl microRNAs (miRNAs) of the miR482/2118 family were found to target and transcriptionally regulate *R* genes. Since regulation of this gene family can be a critical linchpin in this interspecies interaction, we would like to evaluate how regulation of this family either enhances resistance in plants or is high-jacked by pathogens. We are studying regulation of this small gene family in *Solanum lycopersicum* and its sister species *Solanum pimpinellifolium* upon infection by different strains of *P. infestans*. Quantitative expression studies revealed that three members of the miR482/2118 family showed differential regulation during the pathogen infection. Strain specific differences are detected at both the level of transcription and processing of miR482 transcripts. Our next steps will focus on how mature miRNA expression correlates with the target expression and how different *P. infestans* strains influence the miRNA processing pathway. Evolutionary genetic analyses of this miRNA gene family in the Solanaceae indicate that differences in host response are caused mainly by sequence divergence of individual family members, rather than variation in gene number. Therefore, lineage specific co-evolution of miRNA and host targets could provide another angle to understand defense to this important plant pathogen.

Mapping and cloning of the tomato late blight resistance genes *Rpi-Ph2* and *Rpi-Ph3* and their cognate *Phytophthora infestans* effectors *Avr-Ph2* and *Avr-Ph3.*

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Tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) are two important food crops from the *Solanaceae* family. Both species are hosts to the oomycete late blight pathogen *Phytophthora infestans*.

Resistances found in some wild *Solanaceae* species provide environmentally-benign means of restricting late blight infections. Numerous resistance genes have been identified within wild potato accessions. In contrast, little is known about the resistances against *P. infestans* in tomato. *Rpi-Ph2* and *Rpi-Ph3* are two examples of diverse resistances used in tomato breeding. However, the genes underpinning these resistances have not yet been cloned and the avirulence proteins associated with the resistances remain elusive. The INRA Institute in Avignon, France hosts diverse tomato lines that carry *Rpi-Ph2* and *Rpi-Ph3*, respectively, alongside susceptible tomatoes. These plants are used in an effector-recognition screening to identify the cognate *P. infestans* effectors *Avr-Ph2* and *Avr-Ph3*. A suitable *P. infestans* effector expression system in tomato has been setup and comprises the *Agrobacterium tumefaciens* strain GV3101 to deliver a PVX-based expression vector. Utilising transient expression of cloned effectors in the *Rpi-Ph2* and *Rpi-Ph3* association panel, a candidate gene for *Avr-Ph3* has been identified. In order to fine-map and/or clone *Rpi-Ph2* and *Rpi-Ph3*, the association panel as well as segregating populations will be used for a Resistance gene enrichment and Sequencing (RenSeq) strategy. A new bait library has been designed that includes all 755 annotated NB-LRRs from potato, 397 annotated NB-LRRs from tomato and a set of 53 functionally validated *R* genes.

Understanding colonisation mechanisms of monocot tissues by the oomycete pathogen *Phytophthora palmivora*

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The genus *Phytophthora* comprises some of the most agriculturally and economically important plant pathogen species, which devastate many different crops worldwide. *Phytophthora palmivora* in particular has an extremely wide host range, infecting roots and leaves of many monocot and dicot species, and is a recurrent problem affecting tropical crops - such as rubber, durian, cocoa and coconut (Drenth and Guest 2004). To better understand the general mechanisms of infection of this pathogen we are elucidating genes underlying resistance to infection in roots and leaves of the monocot cereal crop barley (*Hordeum vulgare*). We are currently undertaking fine mapping of a dominant locus conferring susceptibility to root colonisation in a wild barley accession, *H. vulgare* ssp *spontaneum*. To characterise the level at which resistance or susceptibility occurs we are investigating the timing, morphology and cell biology of infection of *P. palmivora* in resistant and susceptible barley roots and leaves using fluorescently labelled *P. palmivora* strains. *P. palmivora* resistant and susceptible barley accessions are also being assessed for their ability to form mycorrhizal associations to investigate links between colonisation by beneficial and pathogenic microorganisms. This work will enable us to draw comparisons or elucidate differences between infection of monocot and dicot plant species and uncover important information regarding the fundamental mechanisms of colonisation by filamentous microbes.

Drenth, A., & Guest, D. I. (2004) *Phytophthora* in the tropics. In: Diversity and Management of *Phytophthora* in Southeast Asia. Australian Centre for International Agricultural Research (ACIAR) Monograph No. 114. ACIAR, Canberra.

POSTER PRESENTATIONS

Effectors and their plant targets as leads for downy mildew resistance breeding in lettuce

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Infection of susceptible lettuce plants by the downy mildew pathogen *Bremia lactucae* leads to major crop losses. Protection provided by classical resistance genes is usually rapidly broken by constantly evolving races of *B. lactucae*. Hence there is a growing need for more durable alternatives in resistance breeding. Oomycetes, comprising the downy mildews, translocate effectors, e.g. RXLR proteins, into plant cells. Effectors are best known for their suppression of plant immunity by modifying and interfering with cellular host processes, thereby allowing for and promoting infection. Recently, we cloned 34 RXLR-like effectors from *B. lactucae* including three GKLR effectors with a deviant RXLR motif. Two of these GKLR effectors were shown to be specifically recognized in a variety of lettuce breeding lines, with BLG03 specifically being detected in lines carrying the resistance gene *Dm2* (Stassen *et al.*, 2013). Although RXLR effectors are ubiquitously encoded in oomycete genomes, for only few their plant targets are known. Insight into the molecular mechanisms underlying effector mediated immunity suppression may provide new leads for resistance breeding. In our research project we aim to identify plant targets of *Bremia* effectors using the yeast-two-hybrid (Y2H) system. *Bremia* effectors are screened against a cDNA library prepared from differently treated lettuce leaves. To ensure sufficient coverage of transcripts upregulated during infection lettuce was either mock treated, inoculated with a compatible or incompatible *Bremia* strain, or BTH (salicylic acid analog) treated. The resulting potential targets will be further validated for interaction and functionally analysed to reveal their role in plant immunity and disease susceptibility.

Stassen *et al.,* (2013) Specific in planta recognition of two GKLR proteins of the downy mildew Bremia lactucae revealed in a large effector screen in lettuce. *Molecular Plant-Microbe Interactions*, *26*(11), 1259–70.

Preliminary bioinformatic and functional analysis of genes encoding Glycoside Hydrolase family 3 CAZymes in *Phytophthora sojae*

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Carbohydrate-Active Enzymes (CAZymes) metabolize complex carbohydrates, many of which comprise the structure of plant cell walls. Glycoside hydrolases (GH) constitute a superfamily of CAZymes that hydrolyze the glycosidic bonds in complex sugars. GH family 3, in particular, includes enzymes with β-glycosidase, xylan 1,4-β-xylosidase, glucan 1,3-β-glycosidase, and glucan 1,4-β-glycosidase activities. Since xyloglucan and many glucose derivatives are major components of plant cell walls, it is hypothesized that GH family 3 may play a role in *Phytophthora sojae* infection process. Using bioinformatic approaches, we identified 27 gene sequences belonging to GH family 3 in the *P. sojae* genome. The majority of sequences were located on scaffolds 1, 2 and 8; on average, these sequences were 2200 bp in length and many contained 1 or 2 introns. Although all of these sequences retained some sequence similarity with typical glycoside hydrolases archived in the CAZY database, some of them were clearly artifacts, either missing the consensus sequence motif for the active site or having introns largely exceeding the average intron length in *Phytophthora* spp. Phylogenetic analysis indicated that most sequences can be classified in three major clades, and bootstrap values support the occurrence of multiple paralogous genes. Four GH family-3 genes were chosen for expression analyses in mycelium grown *in vitro* and during plant infection. qPCR revealed differences in their level of expression at different time points during the infection process when compared to mycelial expression, suggesting a potential role for GH family 3 enzymes in the infection of soybean plants by *P. sojae*.

Investigations into phosphate and sulphate dependencies of Avr3a of *Phytophthora infestans*

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AVR3a is an effector protein secreted by *Phytophthora infestans,* which is an oomycete pathogen of potato. It weakens the hosts' defenses by, for instance, inhibiting plant apoptosis induced in response to an infection. In the past, the stability of AVR3a was found to require the presence of sulphate or phosphate, which are

implicated to be of relevance in the translocation process of AVR3a. Our research aimed at investigating the site in AVR3a, which is responsible for these anion interactions. After isolation from inclusion bodies with matrix associated refolding, characterization was performed using a thermal shift assay. SYPRO Orange, a dye that changes its fluorescence emission when bound to hydrophobic residues of proteins, which becomes exposed during thermal unfolding, was used to measure the thermal stability in a series of specific buffers. A mutant protein of AVR3a was found to have very similar baseline stabilities as the wild-type AVR3a protein, but failed to stabilize in the presence of sulphate or phosphate. Additionally stabilizing effects of wild-type AVR3a with other anions of similar character to sulphate and to some extend with chloride, were discovered. Hence the mutation appears to have disrupted the ion-binding pocket without destabilizing other parts of the protein. Implications of our findings will be discussed.

Phytophthora infestans RXLR effector AVR3a associates with DRP2, a GTPase involved in endocytosis

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Phytophthora infestans, the causal agent of potato late blight, secretes effector proteins to overcome plant immunity. The essential RXLR effector, AVR3a, suppresses the cell death induced by *P. infestans* INF1 elicitin, a conserved secreted protein with features of pathogen-associated molecular patterns (PAMPs) and induces R3a-mediated resistance. Mutants of AVR3a that fail to suppress INF1 cell death such as AVR3a^{Y147del}, but still activate R3a, suggest that distinct amino acids condition the effector activities. Activation of plant basal defense responses relies on perception of PAMPs by cell surface localized pattern recognition receptors (PRRs), a process known as PRR–triggered immunity (PTI). The receptor-like kinase BAK1/SERK3 a co-regulator/co-receptor of several cell surface receptors is required for INF1-mediated cell death and immunity against *P. infestans*. We investigated the extent to which AVR3a affects early immune signaling responses that require BAK1/SERK3 using the *N. benthamiana* experimental system. We found that both alleles of AVR3a (AVR3a^{KI} and AVR3a^{EM}) and the AVR3a^{Y147del} mutant suppressed reactive oxygen species production (ROS) and induction of gene expression induced by the bacterial PAMP flagellin-derived peptide flg22, whereas BAK1-independent ROS production triggered by the fungal PAMP chitin was not affected. Moreover, all variants of AVR3a partially inhibit the internalization of the activated flagellin receptor FLS2 but did not interfere with its inactivated plasma membrane localization. This led us to hypothesize that AVR3a might target cellular trafficking. Using co-immunoprecipitation and mass spectrometry analysis, we identified DRP2, a plant GTPase implicated in endocytosis that associates with AVR3a. Further work is underway to dissect the underlying mechanisms.

First insight into the effectors of strawberry crown rot pathogen *Phytophthora cactorum* using RNA-Seq

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Phytophthora cactorum is a hemibiotrophic pathogen infecting numerous plant species, including rhododendron, apple and strawberry. In strawberry, it attacks fruits and crown/root tissues and causes stunting and wilting of plants. Pathogenicity and virulence of *P. cactorum* varies considerably between different host species and isolates. We have developed an *in vitro* infection system, in which sterile plant material can be challenged with *P. cactorum* in the absence of other microorganisms. In this system we have studied *P. cactorum* isolates, which are able to colonize strawberry roots. To reveal *P. cactorum* effectors, we have generated transcriptomes of three *P. cactorum* isolates (E11-0115599 from strawberry, 407 and 440 from rhododendrons). RNA was extracted either from pure mycelia or from woodland strawberry (*Fragaria vesca)* roots after 2-day challenge with *P. cactorum* zoospores of isolates 407 or 440. These two isolates differ in their virulence towards garden strawberry (*Fragaria × ananassa*) in greenhouse conditions. The diploid *F. vesca* (Hawaii 4) was used as a host plant because its full genome is available and data analysis is easier than with octoploid garden strawberry. Illumina HiSeq2000 sequencing yielded 5,5 and 9,6 million paired-end reads from 407 and 440 mycelium samples, respectively. The first insight into the RxLR effectors of *P. cactorum* will be presented in the meeting.

Towards synthetic disease resistance genes

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Plants and pathogens are engaged in a co-evolutionary arms race, in which plants deploy various defense mechanisms and pathogens develop ways to modulate host processes and immunity. An important component of the plant defense machinery involves intracellular immunoreceptors of the nucleotide-binding leucine-rich repeat-containing protein family (NB-LRR). NB-LRRs typically recognize pathogen effector proteins with avirulent activities, and activate a response known as NB-LRR-triggered immunity (NTI). R3a and I2 are orthologous NB-LRRs from potato and tomato that recognize effectors of the late blight oomycete pathogen *Phytophthora infestans* and the wilt causing ascomycete *Fusarium oxysporum f. sp. lycopersici* (FOL) respectively. However, particular races of these pathogens have evolved stealthy effectors that evade recognition by R3a and I2. Our goal is to create synthetic NB-LRR mutants with expanded pathogen recognition specificities to develop broad-spectrum solutions to important plant pathogens. In a previous study we identified 8 single-residue mutations in the R3a protein that conferred expanded response to *Phytophthora sp.* effectors. We investigated whether these mutations alter the response profile of I2, and recovered I2 mutants with expanded spectrum of effector response from diverse pathogens. We are currently evaluating whether this expanded response correlates with a broader resistance spectrum using both transient and stable transgenic systems. These results could lead to new insights into the molecular interactions underlying pathogen perception by plants, building up our knowledge on basic and applied plant pathology.

Microscopic investigation of cell (re)-entry assays with AVR3a and AvrM effectors

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Phytophthora infestans AVR3a and *Melampsora lini* AvrM-A effector proteins contain N-terminal domains that are thought to mediate entry into plant cells. Functional analyses of these domains was based in part on cell re-rentry assays; proteins secreted out of a cell translocate back into the cytosol of the same cell. Here, we tested a variant of this assay, in which we monitored cell entry into cells neighboring transformed cells but that do not express the proteins. AVR3a and AvrM-A cell entry domains were fused in frame with the C-terminus of the *Nicotiana tabacum* PR1 secretory leader and in frame with the N-terminus of various fluorescent proteins. The fusions were transiently expressed in epidermal leaf cells of *Nicotiana benthamiana*, and protein localisation was determined by live cell imaging. AvrM-A and AVR3a fusions produced a strong and weak fluorescent signal in the apoplast, respectively, confirming secretion of the fusion proteins out of transformed cells. However, we could not detect any intracellular signal in non-transformed pavement or guard cells that are surrounded by apoplast saturated in secreted proteins. Interestingly, AvrM-A and AVR3a showed strong accumulation in the secretory pathway, notably in the Golgi and large endoplasmic reticulum (ER) aggregates, respectively. Such accumulation in the secretory pathway correlated with a weak cytosolic accumulation. In conclusion, although our results are inconclusive regarding the cell entry activity of AVR3a and AvrM-A, they support a revised interpretation of cell re-entry assays, in which effector proteins are retro-translocated into the cytosol before being secreted out of the plant cell.

Mining the *Phytophthora infestans* genome for metalloproteases

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Phytophthora infestans is a notorious plant pathogenic oomycete that causes late blight of potato and tomato. The molecular mechanisms exploited by *P. infestans* in order to circumvent host defence are not fully understood. In several pathogens, proteases are known that can modify host proteins and in this way, play an important role in virulence. Metalloproteases (MPs) are named after the presence of a metal ion at their catalytic site. In mammalian systems, members of the Matrix Metalloprotease (MMP) family and A Disintegrin And Metalloprotease (ADAM) family of MPs have been reported to modify transmembrane proteins during a process called ectodomain shedding. In order to study the potential role of MPs in *Phytophthora* virulence, an inventory of *P. infestans* MPs was made based on genome mining. The predicted gene models were verified using ESTs and transcriptomics data. This resulted in a total of 105 *P. infestans* MP genes that could be classified into 20 different MP families. However, none of the *P. infestans* MPs could be grouped into either the ADAM or MMP family. Further analyses of the domain compositions and phylogenetic relationships will give

insight into the evolution of MPs in *P. infestans* and related oomycetes. Expression profiles of *P. infestans* MPs during various growth and infection stages of *P. infestans* will be obtained from existing transcriptomics data in order to select candidates for functional analysis.

NRB1, a NB-LRR protein required for multiple immune receptors in solanaceous plants

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Most plant resistance genes encode nucleotide-binding leucine-rich repeat (NB-LRR) immune receptors that recognize effectors secreted by pathogens. NB-LRR mediated effector recognition can provide valuable disease resistance against important crop pathogens, and thus understanding how NB-LRRs function is of great interest. However, our basic knowledge of NB-LRR function is mostly limited to Arabidopsis, with little information about NB-LRRs involved in important crop pathosystems. *Phytophthora infestans* is a notorious oomycete plant pathogen that causes late blight on potato and tomato. Rpi-blb2, an NB-LRR protein from the wild potato *Solanum bulbocastanum*, recognizes the RXLR type effector AVRblb2 from *P. infestans,* and confers late blight resistance in potato and the model solanaceous plant *Nicotiana benthamiana*. In this study, we found that an additional NB-LRR protein, we termed NRB1, is required for the function of Rpi-blb2 as well as some other immune receptors. Using immunoprecipitation and mass spectrometry, we identified that Rpi-blb2 associates with NRB1 in *N. benthamiana* leaf lysates. The association between Rpi-blb2 and NRB1 was validated by *in planta* co-immunoprecipitation. By using gene silencing, we showed that NRB1 is genetically required for Rpi-blb2-mediated resistance and cell death. Remarkably, NRB1 is also required for the activities of two additional NB-LRR proteins, potato blight resistance protein R1 and nematode resistance protein Mi. Furthermore, tomato and potato NRB1 homologs complemented NRB1 function in *N. benthamiana*. We conclude that NRB1 is a signaling hub for a subset of NB-LRR immune receptors in solanaceous plants.

Investigating the virulence functions of *Hyaloperonospora arabidopsidis* effectors targeted to the host cell nucleus

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The interaction between *Hyaloperonospora arabidopsidis* (*Hpa*), a downy mildew pathogen, and the model plant *Arabidopsis* has proven a very useful system to understand the molecular mechanisms of plant immunity against oomycete pathogens. Pathogen effectors subvert host immunity to successfully colonize the plant. Genomics revealed ~ 140 *Hpa* effector candidates that carry a signal peptide and an RxLR motif, suggesting effector translocation into the host cell from haustoria. These HaRxL effectors are thought to manipulate diverse aspects of host physiology for the benefit of the pathogen. Elucidating how HaRxLs function will enhance our understanding of the molecular mechanisms employed by oomycete pathogens to suppress plant immunity and sustain a biotrophic interface with the host. In previous studies, several HaRxLs were shown to suppress plant immunity. Approximately one third of the HaRxLs localize exclusively to the plant cell nucleus and several candidates interact with transcription factors in yeast-two-hybrid assays. Ectopic expression of some nuclear HaRxLs in Arabidopsis leads to developmental phenotypes suggesting that nuclear-localized effectors might manipulate general regulatory components of host transcription. Based on previous screenings we selected 11 nuclear HaRxLs for further characterization. We are aiming to identify the host targets of these HaRxLs and we will test how they affect early and late read-outs of plant immunity.

Screening of RXLR-EER effectors from the oomycete *Phytophthora palmivora*

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Phytophthora palmivora is a soil-borne oomycete originating from tropical countries, where it infects both monocots and dicots, including economically important crops such as tobacco, cacao, coconut, papaya, rubber and oil palm. Spreading outside of its native geographical area by through international trade, *P. palmivora* now also causes disease in temperate countries. It is for example responsible of dieback on olive trees in Morocco and Italy. This plant pathogen is thus considered as a major concern in many countries worldwide. In order to gain insights on the molecular mechanisms that allow *P. palmivora* to infect such a large number of plant species, two isolates harvested in Colombia and Indonesia have been sequenced and expression data

for proteins secreted during infection of tobacco leaves has been obtained. Among these secreted proteins, we identified a set of RXLR-EER effectors that show no homology with known effectors from other *Phytophthora* species. We hypothesized that these effectors may account for *P. palmivora*'s ability to infect a large number of plant species by targeting general plant processes such as hormone physiology and development. To address this question, these effectors were screened for classical features such as cell death induction and cell death suppression. Their subcellular localization was determined in roots and leaves and their interference with plant development was investigated using Tobacco Rattle Virus (TRV)-induced systemic expression on *Nicotiana benthamiana*. Taken together, these data will help to select some of them for deeper analysis.

Enhanced resistance in *Theobroma cacao* against oomycete and fungal pathogens by secretion of phosphatidylinositol-3-phosphate-binding proteins

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The internalization of oomycete and fungal pathogen effectors into host plant cells has been reported to be blocked by proteins that bind to the effectors' cell entry receptor, phosphatidylinositol-3-phosphate (PI3P). This finding suggested a novel strategy for disease control by engineering plants to secrete PI3P-binding proteins. In this study we have tested this strategy using the chocolate tree *Theobroma cacao*, as a proof of concept crop model system. Transient expression and secretion of four different PI3P-binding proteins in detached leaves of *T. cacao* greatly reduced infection by two oomycetes, *Phytophthora capsici* and *Phytophthora palmivora,* that cause black pod disease. Lesion size and pathogen growth were reduced by up to 85%. Resistance was not conferred by proteins lacking a secretory leader, by proteins with mutations in their PI3P-binding site, or by a secreted PI4P-binding protein. Stably transformed, transgenic *T. cacao* plants expressing two different PI3P-binding proteins showed substantially enhanced resistance to both *P. capsici* and *P. palmivora*, as well as to the fungal pathogen *Colletotrichum theobromicola,* as measured by detached leaf pathogenicity assays. These results demonstrate that secretion of PI3P-binding proteins is an effective way to increase disease resistance in *T. cacao*, and potentially in other plants, against a broad spectrum of pathogens.

Towards the biochemical characterisation of the host targeting protein SpHtp1 from *Saprolegnia parasitica* in complex with its putative cell surface receptor

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Saprolegnia parasitica is a fish pathogenic oomycete that secretes putative effector proteins that can enter salmonid cells. We are interested in the early interactions of *Saprolegnia* and its hosts. How do these proteins enter the host cell? The host-targeting protein (SpHtp1) seems to use host transport mechanisms by binding to O-sulfated receptor molecules at the surface of fish cells. We are trying to identify the receptor by pull-down- co-immunoprecipitation experiments followed by mass spectroscopy analysis. For a deeper understanding of the translocation process we will characterize the protein-protein interaction between the effector protein and its receptor on atom scale with NMR spectroscopy as well as secondary structure changes with CD spectroscopy and some affinity studies with fluorescence anisotropy/ITC. Here we will present our latest findings.

Functional analysis of conserved RxLR effectors of the late blight potato pathogen *Phytophthora infestans*

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The ability of *Phytophthora infestans* to cause the late blight disease of potato is determined by pathogen virulence factors. One class of such secreted factors contains a conserved RxLR amino acid motif that mediates the translocation into host cells. The RxLR effectors aim to suppress plant innate immunity via interaction with specific host targets. However, the precise function of these effectors is not well -known. Our research aims to better understand the mode of action of the RxLR effectors which are conserved between different *Phytophthora* species. High interspecies sequence conservation suggests that homologous effectors have host targets shared between Arabidopsis and potato. Our strategy is to use the model pathosystem of Arabidopsis-*P. brassicae* to analyze RxLR effectors which share high similarity between *P. infestans* and *P. brassicae*. The aim of the project is the identification of conserved RxLR effectors, their target proteins in the host plants and their effect on plant disease phenotype and host immune response. We have identified six conserved RxLR effectors and are now in the process to identify their host targets via co-immunoprecipitation followed by mass spec analysis.

Phytophthora's Alternate Phospholipase C

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With the release of the genomes of *Phytophthora sojae, P. ramorum* and *P. infestans*, it was intriguing that there was no apparent gene for phospholipase C (PLC) - a protein thought to be essential for all life. PLC is a key signal transduction enzyme that catalyzes the cleavage of a membrane-bound lipid, PIP_2 (phosphatidylinositol (4, 5) bisphosphate), into two second messengers, IP3 (inositol 1, 4, 5 trisphosphate) and DAG (diacylglycerol). We have used structural bioinformatics to uncover a gene from *P. sojae* that encodes a protein with domains required for PIP_2 cleavage. This protein has no sequence homology but is structurally and functionally analogous to "classical PLC"; we call this analogue alternate phospholipase C (AltPLC). *AltPLC* is also present in the genomes of *P. infestans*, *P. ramorum*, *P. capsici* and *P. cinnamomi.* Bacterially expressed AltPLC from *P.sojae,* when challenged with PIP_2 *in vitro,* yielded IP3 but 1-monoacylglycerol (MAG) and a free fatty acid (FFA) were produced instead of DAG. We propose that this additional enzymatic function of AltPLC over PLC - the production of MAG and FFA - serves to increase fluidity in the *Phytophthora* plasma membrane; we are employing electrochemical methods to test this idea.

Evaluating *Avr-vnt1* sequence diversity within Irish *Phytophthora infestans* populations in the presence/absence of *Rpi-vnt1*

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Late blight caused by *Phytophthora infestans* is a major threat to potato cultivation. In the EU, control of this disease is estimated to cost nearly a billion euros per year. A control system integrating population monitoring, environment monitoring, fungicide development and resistance breeding has been proposed to sucessfully manage the disease. The breeding for resistance remains an on-going investment as the pathogen is highly adaptable to its host. To date, resistance breeding has be achieved via mainly traditional, but also cisgenic approaches. As part of the FP7-funded 'Amiga' project, cisgenic plants of potato cv. Desiree containing the *Rpi* gene *Vnt1* from *Solanum venturii* are being field assessed in both Ireland and The Netherlands. We hypothesise that exposure of the *P. infestans* to this cisgenic potato line will drive evolutionary change in the coding sequence of avirulence gene *Avr-vnt1*. For this, we sampled *P. infestans* from non-GM potato plots close to the plots containing the cisgenic line for further analysis. Using next generation sequencing we can detect coding sequence variations in *Avr-vnt1* at a low frequency. As expected, initial datasets indicate that the *Avr-vnt1* gene is conserved in the Irish *P. infestans* population. This supports

the observed strong resistance conferred by *Rpi-vnt1* to date. Continued monitoring of the *P. infestans* population will allow us to detect changes in the *Avr-vnt1* gene and allele frequencies. Our approach contributes to a better understanding of how *P. infestans* can evolve following exposure to novel *R* genes from wild *Solanum* species.

In planta interactor screens of oomycete RXLR effectors reveal vesicle trafficking components

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Oomycete plant pathogens secrete RXLR effectors that are translocated inside plant cells to alter plant cellular processes to establish infection. Effectors interact with plant proteins including their cognate targets and other helpers to achieve this. This study aims to identify the plant proteins interacting with RXLR effectors and ultimately the function of these effectors *in planta*. We selected 75 validated effectors for initial screen. We expressed FLAG-tagged effectors in *Nicotiana benthamiana* by agroinfiltration of effector constructs cloned in the high-expression vector pTRBO. Effector proteins and associated host proteins were co-immunoprecipitated with anti-FLAG resins. Bound proteins were eluted and subsequently identified by LC-MS/MS spectrum matching against *N. benthamiana* proteome database. Out of 75 effectors, 53 were expressed to sufficient levels for co-IP and subsequent MS identification of precipitated proteins. GO annotation of effector associated plant proteins revealed the plant processes that might be usurped by the effectors. Interconnections between the effectors and the associated plant proteins were visualized by Cytoscape. We have confirmed more than 20 effector-plant protein associations. We found that the PexRD12 family of effectors was associated with components of vesicle trafficking machinery including vesicle-associated membrane proteins, VAC14, coatomers, vesicle fusing ATPase, syntaxin, exocyst complex, and Ras-related protein RAB variants. In the presence of PexRD12 effectors, the number of endosomal membrane compartments within plant cells were increased indicating that the effectors are targeting this process. Co-localization of the effectors and trafficking components were confirmed by fluorescence microscopy. We will discuss these findings and their implications on plant-oomycete interactions.

Identification and monitoring of effector proteins in the spinach downy mildew pathogen *Peronospora farinosa*

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Peronospora farinose f. sp. *spinaciae* (*Pfs*, also known as *P. effusa*) is an obligate biotrophic oomycete pathogen of spinach (*Spinacia oleracea*). This downy mildew is a destructive disease of spinach worldwide as it affects the harvested parts of the plant (leaves). Infection can already start at the cotyledon stage resulting in discoloured and distorted plant tissue covered with grey sporangiospores. Although resistant cultivars are being bred, new *P. farinosa* races rapidly break the employed resistance genes. This recently initiated project will start with the sequencing and assembly of a high quality reference genome of *P. farinosa* race 1 (*Pfs:1*), followed by the sequencing of a number of additional other *Pfs* races. By comparing the different *Pfs* races we expect to follow the evolution and repertoire of effector genes. Also the sequencing of *Pfs* mRNA in spores and during infection will help to identify gene models and select genes that are highly induced during infection. Comparative genomics of the *Pfs* genes with those of related downy mildews will reveal conserved and species-specific effector genes that can be used to screen breeding material for new sources of resistance. Furthermore, the function of conserved downy mildew effector genes will be analyzed in the Arabidopsis-downy mildew system to identify their mode of-action and host targets. We will report on our initial experiments to identify important downy mildew effectors. The increased knowledge on effectors, the molecules that are used by downy mildews to evade immune response in the plant, can contribute to the development of new innovative ways to generate resistant spinach cultivars.

Phytophthora infestans PiGK4 is a membrane bound GPCR-PIPK that functions as a PI4P5-kinase and has a role in virulence.

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Signalling networks with heterotrimeric G-proteins and phospholipids as key players lie at the base of many cellular processes in eukaryotes. Oomycetes possess a family of novel proteins called GPCR-PIPKs (GKs) that are composed of a G-protein coupled receptor (GPCR) domain fused to a phosphatidylinositol phosphate kinase (PIPK) domain. Based on this domain structure GKs are anticipated to link G-protein and phospholipid signalling but their functions and biochemical activities are currently unknown. Previously we analysed the function of one of the twelve GKs in the potato late blight pathogen *Phytophthora infestans* by gene silencing and overexpression and showed that PiGK4 is involved in spore development, sporangial cleavage, hyphal elongation and virulence (Hua, Meijer et al. 2013, Mol. Microbiology). Overexpression of subdomains of PiGK4 fused to a fluorescent protein further revealed that the GPCR domain targets PiGK4 to membranes surrounding certain cellular compartments. To determine the enzymatic activity of the PIPK domain in PiGK4 we make use of the temperature sensitive yeast mutant *mss4^{ts}* that can be complemented with the full-length PiGK4 gene. Here we will present a more detailed analysis of the function of the various conserved motifs and domains in PiGK4 by complementation essays in yeast *mss4^{ts}* using modified versions of PiGK4 generated by deleting and swapping domains.

Novel oomycete species from the Falkland Islands

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Oomycetes are well known economically important pathogenic organisms, affecting many plant and animal species within both cultured and natural environments. The Falkland Islands are a relatively unexplored area of high biodiversity, both native and introduced. It is therefore highly likely that potential novel oomycete species are present. Different baits were used to the collected water samples from different sites during the Islands spring and summer in 2011 to 2013. Grown isolates were taxonomically identified by sequencing the internal transcribed spacer (ITS) region of the rRNA gene. Analysis of the ITS sequences confirmed a number of known and unique oomycete species present on the Falkland Islands, which are placed distinctly in a maximum likelihood tree. Detailed morphological characterization of the sexual and asexual structures of the novel isolates also strengthens results of the molecular data.

Genome evolution in an asexual lineage of *Phytophthora infestans*

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Phytophthora infestans, an oomycete capable of both sexual and asexual reproduction, rapidly adapts to new resistant potato cultivars. Successive emergence of asexual clonal lineages is a threat to potato cultivation. However, we know little about genome evolution in asexual plant pathogen lineages. In particular, the frequency at which genetic or epigenetic variation takes place in clonal lineages is unknown. The clonal lineage EC1 dominates South American populations of *P. infestans*. The *P. infestans* isolate EC1-3636 overcomes plant immunity via silencing of the RXLR effector gene *Avrvnt1*. In contrast, a sibling isolate EC1-3527 expresses *Avrvnt1* and is avirulent on *Rpi-vnt1* potatoes. This finding suggests that asexual strains can evade effector-triggered immunity through modulation of RXLR effector gene expression. To determine which mechanisms drive adaptation of asexual *P. infestans* to host plants, we tested nucleotide diversity and expression polymorphisms of EC1 lineages in Ecuador and Peru using Illumina transcriptome sequencing. Gene expression of 31 genes showed presence/absence gene expression polymorphisms between EC1 strains. These gene expression polymorphisms independently and multiply happened in the EC1 strains, indicating successive on/off switching of gene expression. In addition, the genes with on/off expression are enriched in RXLR effectors. Modulation of gene expression of these RXLR effectors might explain adaptation to host plants.

Genome sequencing and expression profiling of emerging strains of *Phytophthora infestans*

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P. infestans genotype US22 caused a massive epidemic on tomato in Eastern USA and Canada during the summers of 2009 and 2010. To fully characterize the genetic basis for the success of this genotype, we performed genome and gene expression analyses. Although US22 *P. infestans* strain P17777 is more aggressive on tomato when compared to the reference strain T30-4, we found that the majority of genes in P17777 were induced in both potato and tomato. Our results from these gene expression analyses indicate that the P17777 strain may be "host blind" and that without management US22 genotype could also become a threat to potato. Our findings illustrate how pathogen genome analyses can assist with the management of destructive plant disease epidemics.

Identification of two Arabidopsis nonhost resistance genes that confer immunity to soybean pathogens, *Phytophthora sojae* and *Fusarium virguliforme*.

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The soybean root and stem rot pathogen, *Phytophthora sojae,* can penetrate single cells of the Arabidopsis *penetration deficient mutant*, *pen1-1*. A mutant screen was undertaken in *pen1-1* to identify mutants that are infected by *P. sojae*. At least 70 progenies of each of the 3,556 M_2 families generated by treating *pen1-1* seeds with ethyl methane sulfonate were inoculated with *P. sojae* zoospores in 24-well microtiter plates to identify possible *P. sojae*-susceptible (*pss*) mutants by microscopy. Thirty putative *pss* mutants showing visible leaf necrosis following inoculation with *P. sojae* zoospores were identified. Twenty-three of the 30 mutants were inoculated with *Fusarium virguliforme* which causes sudden death syndrome in soybean. Fifteen of the 23 *pss* mutants are also infected by *F. virguliforme.* In both *pss1* and *pss30* mutants, *P. sojae* can complete its life cycle suggesting that they are new adaptive hosts for this oomycete pathogen. Characterization of a few additional *pss* mutants suggested that there are at least three additional *PSS* genes, *PSS6*, *21* and *25*, in the Arabidopsis genome that confer nonhost immunity to both *P. sojae* and *F. virguliforme*. *PSS1* and *PSS30* encode a glycine-rich-protein and a folate transporter, respectively. Susceptibility of T-DNA mutants (*PEN1*) for these two genes and segregation of *PEN1* alleles among $F_{2:3}$ families, homozygous for *pss1* and *pss30* alleles, suggested that *PEN1* is epistatic to neither *PSS1* nor *PSS30*. *PSS1* confers resistance to both *P. sojae* and *F. virguliforme* in soybean. We conclude that *PSS1* and *PSS30* encode two novel nonhost disease resistance mechanisms.

Molecular mechanism in Phytophthora RxLR effectors suppressing plant immunity

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The genome of the soybean pathogen *Phytophthora sojae* encodes nearly 400 candidate effectors carrying the host cell entry motif RxLR-dEER. In previous studies we revealed transcriptional programming and functional interactions within such RxLR effector repertoire, but their detail molecular mechanisms to suppress plant immunity are still largely unknown. Recently, we silenced the genes and found several effectors, which could suppress PTI and/or ETI, were essential for the virulence of *P. sojae*. Varied subcellular localization of effectors, which are CD-inducer or PTI/ETI suppressors, were observed when expressed in *N. benthamiana* and/or soybean, including the cytoplasm, plasma membrane, and/or cell nucleus. Interestingly, one effector, which could suppress INF1- and effector-triggered PCD, localized to the microtubule in host cell. Localization of effectors around the haustoria in infected plant cells were also observed. In addition, large scale screening of effector host targets were in progress.

Functional characterization of the *Phytophthora infestans* RXLR effector AVR2

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AVR2 is an RXLR effector from *Phytophthora infestans* that is recognized in some potato cultivars by the resistance protein R2 resulting in effector-triggered immunity (ETI). AVR2 was shown to interact with the potato ortholog of BRI1 SUPPRESSOR1 (BSU1)-LIKE1 (BSL1), a ser/thr phosphatase that is known to act as a positive regulator of brassinosteroid (BR) signaling in *Arabidopsis*. Very recently, several groups have build up evidence that the activation of the BR pathway is inhibiting MAMP-triggered immune responses. To what extent the interaction between BSL1 and AVR2 confers an advantage to the pathogen and contributes to host susceptibility is unknown. Using a cell-based system in *Arabidopsis* we have identified a strong interaction between AVR2 and BSL1-3 but not BSU1, a brassicacea specific member of this protein family. The main objective of this study is to exploit the large existing molecular, genetic and genomic resources in *Arabidopsis* to determine the virulence function of AVR2 and assess its impact on plant immunity and development. Our studies show that although AVR2 interacts with BSU1-like phosphatases, it is not affecting typical BR responses such as BR-dependent activation of BES1/BZR2 transcription factor and BR-regulated gene expression. AVR2 also does not affect MAMP-dependent early immune responses in *Arabidopsis* such as MAP kinase activation, reactive oxygen species (ROS) production or immune gene induction upon flg22 treatment. However, AVR2 impacts plant resistance and AVR2-expressing *Arabidopsis* plants are more susceptible to the hemi-biotrophic pathogen *Pseudomonas syringae* but more resistant to the necrotroph *Alternaria brassicicola*.

Development of rapid characterization tools for *Phytophthora infestans* and *Phytophthora ramorum* using real-time PCR and microsatellites from a genomic resource

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Phytophthora infestans (Mont.) de Bary and *Phytophthora ramorum* Werres, De Cock & Man in't Veld are pathogenic oomycetes with devastating impact worldwide, and more DNA-based tools are needed to detect, identify and characterize these pathogens. The objectives of our study were 1) to develop Allele Specific Oligonucleotide-PCR (ASO-PCR) assays using real-time PCR to detect and characterize Canadian strains of *P. infestans* as well as the four lineages of *P. ramorum*, and 2) to develop microsatellites to better assess *P. ramorum* genetic diversity mostly in the NA2 lineage, where fewer markers are currently available. Mining of the *P. infestans* genome revealed several regions containing SNPs, both within genes and flanking sequences of microsatellite loci. Nine ASO-PCR assays were developed from these SNPs, allowing the unambiguous identification of the five dominant *P. infestans* Canadian genotypes from the most recent outbreaks. For *P. ramorum*, two new ASO-PCR assays in the cellulose binding elicitor lectin (CBEL) region were combined with two existing assays within the same gene region to allow for identification of all four lineages of this pathogen, including the EU2 lineage. Mining of *P. ramorum* NA2 multiple genomic sequences also revealed microsatellite loci that could detect polymorphism within this lineage, currently lacking highly polymorphic loci. These DNA-based tools will contribute to the genomic toolboxes available to assess the genetic diversity of these oomycete pathogens from the species to the intra-lineage level.

Transcriptional diversity in *Phytophthora infestans*: a playground for functional variation

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The late blight pathogen *Phytophthora infestans* rapidly evolves to overcome resistant plants. Its genome shows striking potential for genetic change and the genes encoding secreted disease effector proteins are mostly located in highly dynamic genomic regions. Some of these effectors can be recognized by plant

immunoreceptors and are targets for disease resistance breeding. Previous results show that different *P. infestans* isolates express distinct sets of effectors. Our goal is to establish a comprehensive overview of expression polymorphisms in genes of *P. infestans* with an emphasis on effectors, and to further define a set of core *in planta*-induced effectors. To achieve this we performed RNAseq transcriptome sequencing of ~15 diverse isolates, including different genotypes from Europe and South America, selected on the basis of relevant characteristics such as virulence spectra and aggressiveness. The results of this transcriptomic survey will be presented, with special emphasis on the genotype 6_A1 (pink 6) and the clonal lineage EC1. In recent years, 6_A1 has become the predominant genotype in UK populations, displacing the highly aggressive 13_A2 (blue 13). EC1 dominates South American populations and effector transcriptional polymorphisms within this clonal lineage led to the defeat of an important source of resistance. Overall, these results will provide information on which resistance genes could be effective against different *P. infestans* genotypes and the set of core effectors defined can be used to screen germplasm, giving insights into the likely durability of newly identified resistances and allowing to combine resistances that recognize distinct alleles of essential effectors.

RNA-seq analysis to identify potential PsAvh172 targets

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Phytophthora sojae encodes hundreds of host-translocated RxLR effector proteins, many of which are expressed while establishing stem and root rot in soybean. Functional genomics analysis in yeast, a model eukaryote, has previously been used to identify conserved biological pathways targeted by bacterial effectors. We first tested eight P. sojae RxLR effectors for their ability to inhibit yeast growth when overexpressed in yeast. The ability of each effector to inhibit yeast growth suggests that each targets a biological target conserved among eukaryotes. RNA---seq analysis subsequently identified dozens of yeast genes that are differentially expressed in response to overexpression of the PsAvh172 effector domain. To develop hypotheses about probable targets of the PsAvh172 effector, pathway analysis is currently being conducted to identify biological pathways common to these differentially---expressed yeast genes. Probable targets of the PsAvh172 effector can then be further tested in yeast and ultimately in planta.

High-Throughput Screening of *Glycine soja* germsplasm for Novel *R* Genes against *Phytophthora sojae*

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Phytophthora sojae is the causal agent of soybean root and stem rot. In 2006, soybean crop losses were estimated at 1.46 million metric tons in the US and over 2.32 million metric tons worldwide. This was a loss of nearly 2% of all soybean produced that year in the US. Attempts to control this pathogen have been centered on the use of resistance genes (*R* genes) that recognize pathogen effector proteins. Our aim is to identify *R* genes against essential effectors in *P. sojae* resistant cultivars. We hypothesize that a resistance gene against an essential effector will provide durable resistance against a broad range of *P. sojae* isolates. We further hypothesize that *Glycine soja*, a sexually compatible relative of cultivated soybean, is a productive source of new R genes against *P. sojae*. We are using an effectoromics approach to identify *R* genes against *P. sojae* effectors. Using three essential effectors as probes, we have found several *G. soja* cultivars that produce effector-specific hypersensitive responses, suggestive of *R* genes that recognize these pathogens. Additionally, we have completed preliminary screening with 8 newly identified effector candidates, and these screens have yielded additional cultivars with putative *R* genes. We are currently breeding selected lines which contain putative *R* genes to validate their functions and map the resistance loci. Several *G.soja* lines have been shown to have additional resistance to *P. glycinea* race 4 and *Cercospora* leaf blight. We are working to optimize a similar system in *Phaseolus vulgaris*.

Phylogeny of the *Pythium irregulare* complex inferred from multilocus approach

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Pythium irregulare is a prevalent pathogenic species of numerous plant hosts worldwide. Within *P. irregulare* sensu lato exist high levels of genetic diversity. Two cryptic species, *P. irregulare* sensu stricto (s.s.), and *P. cryptoirregulare* have been described. However, additional groups have been tentatively identified within the *P. irregulare* complex. Therefore, further analyses for resolving the phylogenetic status of this group of oomycetes are needed. Ninety six isolates from various geographical origins were collected and compared based on DNA sequence data of ß-tubulin (Btub), heat shock protein 90 (HSP90), cytochrome oxidase subunit I, including the cox I-II spacer (coxI-II) and the internal transcribed spacer regions of the ribosomal DNA including the 5.8S unit (ITS). To further assess the genetic diversity of this complex and define species boundaries, a subset of samples were analyzed using SSR and ISSR markers. Analyses of DNA sequence data and DNA fingerprinting revealed four discrete clades within the complex, supporting the species status of *P. irregulare* s.s. and *P. cryptoirregulare*, and of two additional groups, previously reported as P. 'vipa' I and P. 'vipa' II. Phylogenetic relationships of these species and other members of *Pythium* clade F will be discussed.

Polyamine metabolism and transport in oomycetes.

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Polyamines are small metabolites that are found in all organisms, and are essential for life. In plants, polyamines are of particular importance, because the plants that are able to change polyamine levels quickly, are also then able to survive environmental stresses such as drought or freezing or salt. Previous work in our lab has shown that zoospores actively take up polyamines, but zoospores are generally believed not to acquire other nutritional sources from the environment. RNA-seq analyses have shown that seven of eight predicted polyamine uptake transporters are overexpressed by zoospores of P. *parasitica*. A second class of highly expressed transporters with predicted localization to the vacuole may be involved in the storage of polyamines acquired from the environment. Yeast functional assays will be used to confirm the activity of these transporters. Metabolite analyses will also be employed to follow the transition of polyamine levels from the swimming zoospore stage to hyphal germlings.

Quantitative transcriptomics of the *P. capsici* – host interaction

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Plant-microbe interactions feature dynamic interplay between pathogen encoded virulence factors and host (defence) signalling components. Despite major advances in host-microbe interaction research, little is known about the processes that result in pathogen virulence or host susceptibility. *Phytophthora capsici* is a hemibiotrophic plant pathogen that infects important crop plants such as pepper, tomato and cucumber. The *P. capsici* infection cycle features a dynamic developmental programme, driven by stage specific gene expression in both pathogen and the host. A detailed understanding of the transcriptional changes associated with susceptible interactions thus has the potential to unveil processes associated with virulence and immunity. Previously, we have shown that during infection, transcriptional shifts occur and appear coordinated in tomato and *P. capsici.* These shifts coincide with the early infection phase (after germination) and the switch from biotrophy to necrotrophy and suggest modulation of host immune signalling by both organisms. Here we expand on the transcriptional profiling of *P. capsici*-tomato interactions by means of RNA sequencing of plant and pathogen using Illumina technology. For this purpose we performed a detailed timecourse experiment (0, 4, 8 16 and 24 hours after inoculation) on both infected and mock inoculated tomato leaves. Here we will report on our latest progress on these experiments and the new information arising from downstream analyses.

Getting closer to unravelling the underlying mechanism of Avr3a recognition?

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In order to colonise host tissue, the potato late blight pathogen *Phytophthora infestans* secretes an array of effector proteins to suppress plant immune reactions. However, the mode-of-action of how effectors promote disease development by targeting specific host targets and how this is monitored by resistance proteins, remains largely unknown. We have previously shown, that in the presence of the recognised Avr3a effector form the potato resistance protein R3a relocalises to endosomal versicles which is required for the immune response. The host E3-ubiquitin ligase CMPG1 has been described as an interactor of Avr3a, although its presence is not required for the R3a hypersensitivity. Here we show, that Avr3a can interact in yeast and in planta with several other host proteins. One of them, named KIPI30, is a pyruvate kinase-like protein and localises to chloroplasts. Silencing KIPI30 doesn't result in a dramatic decrease of the R3a hypersensitivity, indicating that activation of R3a and downstream signalling is independent of KIPI30. However, the absence of KIPI30 causes an extreme elevation of *Phytophthora infestans* sporulation suggesting a role of KIPI30 in plant defence. In the presence of Avr3aKI, the exocyst component Sec3, another host interactor of Avr3a, undergoes a relocalisation from the cytoplasm to the same endosomal vesicles to which R3a relocalises. We also found that silencing Sec3 dramatically reduces the development of R3a-mediated HR, indicating a potential role of exocyst components as R3a guardees. Additionally, the co-localisation of R3a with autophagosomes in the presence of the recognised Avr3a effector form suggests the involvement of autophagy in this particular immune response.

Current Progress on the Detailed Biochemical Characterization of RXLR Phospholipid Binding Properties of PsAvr1b, PsAvr1d, and PiAvr3a

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Oomycete plant pathogens utilize a cadre of diverse mechanisms to facilitate infection. One such mechanism is the deployment of small-secreted proteins that are believed to facilitate various aspects of pathogenesis. A large subset of these effectors contains a conserved N-terminal signature, RxLR-dEER, which mediates translocation into host cells. We had previously shown that this translocation may occur in the absence of the pathogen through receptor-mediated endocytosis via the interaction of the N-terminal canonical RxLR region and newly discovered cell surface phospholipid PtdIns-3-P. A debate has emerged on how and where RxLR effector proteins bind phospholipids and whether or not this interaction is specific. Here we present our latest findings on our rigorous and detailed biochemical characterization of the RxLR-phospholipid interaction via surface plasmon resonance spectroscopy, isothermal titration calorimetry, and circular dichroism spectroscopy of *Phytophthora sojae* Avr1b, *P. sojae* Avr1d, and *P. infestans* Avr3a. Our analysis indicates these effectors bind specifically to PtdIns-3-P via their RxLR-dEER region and do so in a very unique manner. These findings provide fundamental insight into what we postulate will be a unifying model for RxLR-phospholipid binding.

Discovering the mode of action of a novel anti-oomycete compound

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A new active ingredient was found to have high biological efficacy and selectivity against oomycete plant pathogens under glasshouse and field conditions. It was therefore highly desirable to know the mode of action. Methods crossing many disciplines are used when trying to find the mode of action of a novel anti-oomycete compound. Studies of uptake and incorporation of various radiolabelled substrates (^{14}C-acetate, ^{14}C-uridine, ^{14}C-phenylalanine) were used to test the involvement of major biochemical pathways. Microscopy and culturing studies were used to see changes to cellular physiology. Forward genetics mutants were generated and mutations were separated from underlying genomic heterozygosity. Several individual resistant strains carrying mutations in the same open reading frame confirmed the target of the active ingredient and provided invaluable mode of action evidence.

The function of RAM2 in development of late blight pathogen infection structures

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The formation of appressoria in both beneficial arbuscular mycorrhizal (AM) fungi and pathogenic oomycetes is dependent upon the presence of plant cutin monomers. Recent research using *Medicago truncatula* mutants found that plants which were defective in a GPAT (glycerol-3-phosphate acyltransferase), encoded by the gene *RAM2*, have disrupted cutin monomer composition. *RAM2* mutant plants are much more resistant to colonisation by both *Phytophthora palmivora* (oomycete pathogen) and *Rhizophagus irregularis* (AM fungi) while their overall development is unaffected. Our hypothesis is that the function of *RAM2* may be conserved in other plant species with regard to their interaction with filamentous microbes. If this is true, it may be possible to transfer this resistance principle to important crop species, i.e. resistance to *Phytophthora infestans*, which causes late blight, in tomato and potato. So far we have identified multiple homologues of *MtRAM2* in tomato, potato and *Nicotiana benthamiana* and are utilising knockdown, CRISPR/Cas nuclease genome editing and overexpression techniques to investigate the effect of these homologues on *P. infestans* infection whilst also checking for developmental effects. *MtRAM2* is upregulated at the site of infection, but intriguingly also in the flowers of *M. truncatula*, suggesting it plays a role in whole-plant signaling during colonisation. A similar pattern of expression in the flowers and fruit is observed with *RAM2* homologues in tomato and potato. I am aiming to understand the physical and biochemical basis of resistance to filamentous microbes provided by defects in *RAM2* and whether different levels of expression or truncations of the gene lead to differences between AM fungi and pathogenic oomycetes in their ability to penetrate and colonise plant cells.

Unveiling and exploiting *P. capsici* nuclear effector functions

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Plant pathogens are a major agricultural concern hampering food production worldwide. The highly destructive oomycete *Phythophtora capsici* threatens a diverse range of economically important crops such as pepper, tomato, eggplant, snap, lima beans as well as many cucurbits. *Phythophtora capsici* secretes a vast arsenal of proteins (effectors) that are thought to be crucial for its virulence. Work in our lab has identified a suite of *P. capsici* effectors that localise to the host nucleus, nonetheless little is known about the nuclear processes they target. The aim of my project is then to unveil the activities of *P. capsici* effectors responsible for reprogramming the host nucleus during infection. An interdisciplinary approach that combines Yeast two hybrid (Y2H), proteomics, transcriptomics and functional analyses will be employed to study the functions of effectors and their targets *in vivo*. By Y2H screening two protein interactors of Crinkler 83_152 (CRN83_152), a nuclear effector from *P. capsici,* were identified: SUMO ligase and endonuclease. Here I will present my results regarding the confirmation and characterization of CRN83_152 interactions.

Rapid detection of *Phytophthora* species on plant samples using recombinase polymerase amplification

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Recently several isothermal amplification techniques have been developed that are extremely tolerant towards inhibitors present in many plant extracts. Recombinase polymerase amplification (RPA) assays (a form of isothermal amplification) for the genus *Phytophthora* have been developed which comprise a simple and rapid method to macerate suspect plant tissue and detect target DNA using primers and a labeled probe. Four RPA assays were developed, a *Phytophthora* genus specific assay, two species-specific assays (*P. ramorum* and *P. kernoviae*) and a plant internal control. Assays were tested for sensitivity (ranging from 10 ng to 1 fg of DNA) and specificity using DNA extracted from more than 90 *Phytophthora* spp., 22 *Pythium* spp., and several plant species. The lower limit of linear detection using purified DNA was 1 pg of DNA in all pathogen RPA assays. Six different extraction buffers were tested for use during plant tissue maceration and the assays were validated in the field by collecting 222 symptomatic plant samples from over 51 different hosts. Ninety samples were positive using the *Phytophthora* genus specific RPA test and the same samples were also positive using TaqMan PCR and traditional isolation techniques. A technique for the generation of sequencing templates from positive samples to confirm species identification has also been developed. These

RPA assays have added benefits over traditional technologies because they are rapid, do not require extensive training to accomplish, require less expensive equipment and are significantly more specific than current immunologically based methods.

Golden Gate Cloning Vectors for *Phytophthora* transformation

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The Golden Gate (GG) cloning method relies on type IIs restriction enzymes that cut outside of their recognition sequence and allows fast and convenient assembly of multiple genetic elements, such as promoters, terminators and open reading frames. Here we report a GG vector, pPTV1, for transforming *Phytophthora sp*. As part of the vector system, we created GG cloning modules encoding the NPTII selectable marker, Ham34 promoter and Ham34 terminator flanked by BsaI restriction sites as well as a destination vector carrying the *LacZ* marker for the blue/white screening of positive colonies. The vector system is compatible with GG modules encoding protein tags (HA, FLAG etc.) and open reading frames (GFP, YFP etc.) described by Weber et al.[1] and Engler et al.[2]. It allows maximum flexibility as selectable markers, promoters or terminators can be easily added as new cloning modules. We validated the vector by transforming *Phytophthora palmivora* with constructs expressing YFP and tdTomato. In both cases stable transformed lines have been recovered.

[1]Weber E, Engler C, Gruetzner R, Werner S, Marillonnet S.(2011). A modular cloning system for standardized assembly of multigene constructs. *PLOS One* 6(2):e16765 [2]Engler C, Youles M, Grützner R, Ehnert T-M, Werner S, Jones JDG, Patron NJ, Marillonnet S (2014) A Golden Gate Modular Cloning Toolbox for Plants. *ACS Synth. Biol.* DOI: 10.1021/sb4001504

The Arabidopsis *WRR4* gene provides a "one-gene stack" that confers recognition of multiple *Albugo candida* effectors

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The oomycete *Albugo candida* that causes white blister rust is one of the most important diseases of Brassica crop species. Some *A. candida* races can also infect various Arabidopsis accessions and this enabled the cloning of a resistance gene *WRR4* (encoding a TIR-NB-LRR R-protein) from Arabidopsis accession Col-0. *WRR4* provides resistance to multiple *A. candida* races. In order to identify the *A. candida* effector recognised by *WRR4*, we determined genome sequences for seven *A. candida* races including a recently identified race that can partially overcome *WRR4*-mediated resistance. We carried out comparative and association genomics with the recently discovered novel class of CHxC (reclassified as CX2CX5G and abbreviated to CCG) Albugo effector family and secreted proteins, and identified strong candidates for *Avr-WRR4*. We transiently co-expressed *WRR4* with several CCG candidates as well as an additional suite of CCGs in tobacco. We identified four CCGs (CCG28, CCG30, CCG33 and CCG71) that specifically triggered a *WRR4*-dependent HR. One of these, CCG28, confers the strongest HR with *WRR4* and has a premature stop codon in the *A. candida* race that breaks the resistance conferred by *WRR4*. Unusually, we found that the N-terminal parts of the CCGs are recognised by WRR4. Thus, our genomics analysis and assay systems enabled us to identify several CCGs from *A. candida* that are specifically recognized by *WRR4* and potentially explains the broad-spectrum resistance conferred by *WRR4*.

Lectin receptor kinases: potential immune receptors against *Phytophthora* pathogens

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Phytophthora pathogens cause devastating diseases on a broad range of plant species. In this study we focus on legume-like (L-type) lectin receptor kinases (LecRKs), a sub class of receptor-like kinases that could be employed as novel types of immune receptors to control *Phytophthora* pathogens. Previously, we found that Arabidopsis LecRK-I.9 functions as a *Phytophthora* resistance component (Bouwmeester et al., 2011, *PLoS Pathogens*). LecRK-I.9 belongs to a family consisting of 45 members in Arabidopsis. The function of other LecRKs was determined by infection assays on Arabidopsis T-DNA insertion mutants with various *Phytophthora* spp. as well as the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Alternaria brassicicola*. Several of the LecRKs were found to play a role in resistance to *Phytophthora* but not to other pathogens. Overexpression of one of these, here named *LecRK-α,* enhanced *Phytophthora* disease resistance and also induced cell death in Arabidopsis. However, further analysis of the functional domains and

downstream signaling components indicated that the LecRK-α mediated *Phytophthora* resistance is independent of the cell death phenotype. Mass spectrometry of proteins that are in a complex with LecRK-α upon co-immunoprecipitation led to the identification of LIP1, a so-called **L**ecRK-α-**I**nteracting-**P**rotein-1, which was confirmed to interact with LecRK-α *in planta*. Similar to *lecrk-α* lines, *LIP1* T-DNA insertion mutants showed compromised *Phytophthora* disease resistance. Further analysis on the interaction between LecRK-α and LIP1 will provide a better insight into how LecRK-α mediates resistance to *Phytophthora* pathogens.

Understanding the virulence mechanism of PopP2, a bacterial effector

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Genome and proteome information from oomycetes has allowed rapid prediction of secreted proteins that are likely to be effectors. A current challenge is to characterise the function of these effectors. Identifying the virulence targets of effectors will provide insight into how effectors suppress immunity and how they are recognised by hosts as avirulence factors. Here, we will present our most recent results from a proteomic analysis to identify the virulence function of PopP2, a bacterial effector from *Ralstonia solanacearum*. The *Arabidopsis thaliana* resistance mechanism against PopP2 is well studied and the system contains allelic variation that makes it ideal for high-throughput comparative proteomics. We aim to establish a framework for the identification of virulence targets of oomycete effectors that function through post-translational modification of host factors. Identifying the targets of effectors will help develop our understanding of oomycete pathogenicity and host resistance mechanisms.

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