OMGN 2013 Program

Location: Talks will be held at Kiln on Sunday Morning and at Fred Farr for all other sessions. Poster sessions will be held at Kiln.

Saturday, March 9, 2013

5:30-6:30	Registration, Main Lobby

Sunday, March 10, 2013

8:00-9:00	Registration
9:00-9:15	Opening remarks
9:15-10:00	**Keynote address: Sarah Grant** The *Pseudomonas syringae* HrpL regulon: Beyond effectorome
10:00-12:00	**Effectors I - *Chair: Paul Birch***
10:00-10:20	*Lars Löbach* Host-targeting protein 3 (SpHtp3) from the oomycete *Saprolegnia parasitica* translocates specifically into fish cells in a pH and tyrosine O-sulfate-dependent manner
10:20-10:40	*Stephan Wawra* Biochemical characterisation of *Phytophthora infestans* AVR3a
10:40-11:00	*Shiv Kale* Characterization of several oomycete RxLR effectors phospholipid binding properties and their role in cell entry
11:00-11:20	*Brett Tyler* Structural basis for interactions of *Phytophthora sojae* RxLR effectors with phosphatidylinositol 3-phosphate and for host cell entry
11:20-11:40	*Qunqing Wang* How oomycete pathogens exploit PI3P to target secreted RxLR effectors into host cells
11:40-12:00	*Mark Banfield* Structure-led studies of oomycete RXLR effectors
12:00-12:15	**Group Photo**
12:15-1:30	**Lunch**
1:30-2:15	**Keynote address: Jeff Dangl** Effector-host interactome: getting to the hub of the matter
2:15-3:15	**Effectors II - *Chair: Brad Day***
2:15-2:35	*Guido Van den Ackerveken* Nep1-like proteins of the downy mildew *Hyaloperonospora arabidopsidis* trigger immunity, but not necrosis, in the *Arabidopsis* host
2:35-2:55	*Mark Gijzen* Bulk segregant analysis and next generation sequencing to map the *Phytophthora sojae Avr1c* gene
2:55-3:15	*Edouard Evangelisti* Penetration-specific effectors from *Phytophthora parasitica* favour plant infection
3:15-3:40	**Break**
3:40-5:00	**Effectors III - *Chair: Guido Van den Ackerveken***

3:40-4:00	*Wenbo Ma* *Phytophthora* effectors promote infection by suppressing host RNA silencing
4:00-4:20	*Marie-Cecile Caillaud* Mechanism of nuclear suppression of host immunity by HaRxL44, an *Arabidopsis* downy mildew effector
4:20-4:40	*Hazel McLellan* Two potato NAC transcription factors are the targets of a *Phytophthora infestans* effector
4:40-5:00	*Adam Steinbrenner* How do oomycete effectors trigger resistance in plants? New findings from the RPP1-ATR1 battlefront of *Arabidopsis* infection by *Hpa*

7:30- 10:00	**Reception and Poster Session I** *Omar Harb* Oomycete resources in FungiDB demo - during poster session

Monday, March 11, 2013

9:00-10:20	**Genomics I - *Chair: Elodie Gaulin***
9:00-9:20	*Richard Michelmore* Comparative genomics of Downy Mildews
9:20-9:40	*Franck Panabières* Comparative genomics of host range in *Phytophthora parasitica*
9:40-10:00	*Sucheta Tripathy* EuMicrobeDB-M: A light weight oomycete genome database based on Mysql with C++ API
10:00-10:20	*Wenwu Ye* De novo sequencing of the litchi downy blight pathogen *Peronophythora litchii* reveals an orphan between *Phytophthora* and *Peronospora*

10:20-10:40	**Break**

10:40-12:20	**Genomics II - *Chair: Yuanchao Wang***
10:40-11:00	*Elodie Gaulin* Genome-wide analysis of Crinklers effectors in *Aphanomyces euteiches*
11:00-11:20	*Anandi Reitmann* Identification of genes involved in *Phytophthora cinnamomi* pathogenicity
11:40-12:00	*Laetitia Poidevin* Novel core promoter elements in the oomycete *Phytophthora infestans* and their influence on expression pattern detected by genome-wide analysis
12:00-12:20	*Alyssa Burkhardt* Alternative splicing provides a possible mechanism for *Pseudoperonospora cubensis* transcriptome regulation

12:20-1:30	**Lunch**

1:30-2:50	**Evolution and Population Genetics I - *Chair: Jaime Blair***
1:30-1:50	*Eric Salomaki* The genomes of free-living *Thraustotheca clavata* and parasitic *Achlya hypogyna* implicate a role for gene family expansion and gene transfer in the adoption of a parasitic lifestyle
1:50-2:10	*Harri Kokko* Rapid influx and numerous epidemics of *Aphanomyces astaci* strains are altering the European crayfish distribution and biodiversity

2:10-2:30	*Svetlana Rezinciuc* PCR-AFLP as a method for genotyping the crayfish plague pathogen *Aphanomyces astaci*
2:30-2:50	*Andres Espindola* Strain-identification of *Pythium aphanidermatum* in metagenomic samples from 454 pyrosequencing

2:50-3:10	**Break**

3:10-4:30	**Evolution and Population Genetics II - *Chair: Sucheta Tripathy***
3:10-3:30	*Dylan Storey* - Global effector diversity in *Phytophthora capsici*
3:30-3:50	*Jian Hu* - Genetic diversity of *Phytophthora capsici* on pepper in China
3:50-4:10	*Mohammad Nasif Sarowar* Detailed characterisation of two novel species within the Oomycete order of the *Saprolegniales*
4:10-4:30	*Lien Bertier* Why are there so many *Phytophthora* species: does polyploidy play a role? A case study of *Phytophthora* clade 8b

6:00-7:30	**Banquet**

8:00- 10:00	**Reception and Poster Session II** *Omar Harb* - Oomycete resources in FungiDB Demo - during poster session

Tuesday, March 12, 2013

9:00-10:20	**Oomycete Biology I - *Chair: Steve Whisson***
9:00-9:20	*Howard Judelson* High-throughput prediction and functional validation of promoter motifs regulating gene expression during *Phytophthora* development
9:20-9:40	*Sebastian Schornack* - Haustoria-forming *Phytophthora palmivora* to study commonalities and differences of pathogenic and symbiotic infection processes in roots
9:40-10:00	*Hugo Mélida* Deciphering cell wall structure and biosynthesis in oomycetes using carbohydrate analyses and plasma membrane proteomics
10:00-10:20	*Laura J. Grenville-Briggs* Cell wall biology to illuminate mechanisms of pathogenicity in *Phytophthora infestans*

10:20-10:40	**Break**

10:40-12:20	**Oomycete Biology II - *Chair: Laura Grenville-Briggs***
10:40-11:00	*Bernard Dumas* *Phytophthora*-Associated Molecular Patterns-triggered immunity and resistance to the root pathogen *Phytophthora parasitica* in *Arabidopsis*
11:00-11:20	*Carol Davis* Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers
11:40-12:00	*Steve Whisson* Involvement of RNA polymerase I in mefenoxam insensitivity in *Phytophthora investans*

11:40-12:00	**Closing Remarks – Brett Tyler** **OMGN 2014 Announcement – Mark Banfield**

Tues afternoon	Omar Harb Workshop on Oomycete Resources in FungiDB (90 min). Location: Residence room adjacent to Fred Far

Oral Presentations

Presentations organized by appearance in program.
Presenting author(s) underlined.

Keynote Address

The *Pseudomonas syringae* HRPL regulon: Beyond effectorome

Sarah Grant

(Biology Department, University of North Carolina at Chapel Hill, 4258 Genome Sciences Building, Chapel Hill, NC 27599)

Pseudomonas syringae is a Gram-negative bacterial plant pathogen with high phylogenic diversity responsible for worldwide disease on many crop species. HrpL is the master regulatory transcription factor of *P. syringae* that controls expression of the genes encoding the structural and functional components of the type III secretion system (T3SS), essential for virulence, and expression of the type three secreted effectors (T3Es) which are mainly encoded on pathogenicity islands that are variable between strains. HrpL also regulates expression of some genes for non-type III secreted proteins including toxin producing exzymes and proteins not previously associated with virulence. We implemented and refined transcriptional analysis methods using cDNA derived high-throughput short read sequencing data (RNA-seq) to identify the HrpL-regulated genes for six isolates of *P. syringae,* that represent the diversity of the species in order to identify putative novel virulence factors and broaden our understanding of the evolution of *P. syringae* virulence genes. Comparative analysis of the HrpL-regulons highlighted strain-specific variability not only for secreted type III effectors but also for the genes for non-type III secreted HrpL-regulated proteins. We identified new effectors and characterized operons of non-secreted enzymes which produce secondary metabolites involved in pathogenicity. Overall, we highlight the advantage of next generation transcriptomics to identify putative virulence factors, but also the power of integrating genomic, transcriptomic, and phylogenetic information to gain better insight into pathogenicity.

Effectors I – Sunday 10AM-Noon

Host-targeting protein 3 (SpHtp3) from the oomycete *Saprolegnia parasitica* translocates specifically into fish cells in a pH and tyrosine O-sulfate-dependent manner.

Lars Löbach[1], Stephan Wawra[1], Irene de Bruijn[1], Aleksandra D. Toloczko[1], Tim Rasmussen[2], Christopher J. Secombes[3], Pieter van West[1]

([1]Aberdeen Oomycete Laboratory, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK, [2]University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK, [3]Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, Scotland, UK)

The success of eukaryotic oomycete pathogens depends largely on effector proteins, molecules which manipulate or interfere with host defence mechanisms in the extracellular space or inside their host cells. One economical important oomycete parasite is the fish pathogen *Saprolegnia parasitica* (*Spa*), which is the causal agent of the disease Saprolegniosis. *Spa* is responsible for devastating losses in the aquaculture industry worldwide. In order to effectively fight any pathogen it is crucial to understand the key molecular mechanisms that lead to the disease. With the focus on putative effector proteins we screened the genome of *Spa* in the present study for potential effector candidates. Analysis identified a novel putative secreted *Spa* effector protein, which we named host-targeting protein 3 (SpHtp3). Gene expression analyses showed that mRNA levels of SpHtp3 are highest in mycelium, sporulating mycelium and during the later stages of infection. Recombinant SpHtp3 was able to translocate specifically into fish cells in a tyrosine O-sulfate and pH dependent manner. SpHtp3 was found in vesicular structures inside fish cells and was released from these upon infection of the cells with *Spa* Interestingly, SpHtp3 possesses an N-terminal RTLR tetra-peptide sequence at a similar location as found in RxLR-effectors from plant pathogenic oomycetes. However, this RTLR-sequence was not required for the fish cell translocation property of SpHtp3. These findings suggest that SpHtp3 from *Spa* is a novel intracellular protein that might play an important role in Saprolegniosis.

Biochemical characterisation of *Phytophthora infestans* AVR3a.

Stephan Wawra[1*], Armin Djamei[2], Isabell Küfner[3], Thorsten Nürnberger[3], Justin A. Boddey[4], Stephen C. Whisson[5], Paul R.J. Birch[5], Regine Kahmann[2], Pieter van West[1]

([1]Aberdeen Oomycete Laboratory, University of Aberdeen, Aberdeen, AB25 2ZD, UK, [2]Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Germany, [3]Department of Plant Biochemistry, University Tübingen, Germany, [4]Department of Medical Biology, University of Melbourne, Australia, [5]Cell and Molecular Sciences, James Hutton Institute, Dundee, UK)

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. It has been shown, in a few cases, that the RxLR-motif is important for the delivery of these proteins into host cells. However, how these proteins translocate into the cytoplasm of their host is currently the object of intense research activity and debate. One model suggests that the RxLR-leader sequences of these effectors are sufficient to translocate the respective effectors into eukaryotic cells through binding to surface exposed PI-3-P. However, analysing the translocation behaviour of the RxLR-leaders from *Phytophthora infestans* avirulence protein 3a (AVR3a) and *P. sojae* avirulence protein 1b (Avr1b) we were unable to obtain conclusive evidence for specific RxLR-mediated translocation. Importantly, we confirm that the reported phospholipid binding properties of AVR3a and Avr1b are not mediated by their RxLR-leaders. In addition, we will present data showing that the observed phospholipid interaction of the AVR3a effector domain is attributable to a weak association with denatured protein molecules, and is therefore most likely physiologically irrelevant.

Characterization of Several Oomycete RxLR Effectors Phospholipid Binding Properties and their Role in Cell Entry.

Shiv D. Kale[1], Gloria E. Trivitt[1,2], Furong Sun[2], Tian Zhou[1,2], Kelly Drews[1,2,3], Helen Clark[1,3], Hugo F. Azurmendi[2], Dan Li[2], Amanda, Rumore[1,2], Christopher B. Lawrence[1,2], Daniel G. Capelluto[2], Brett M. Tyler [4]

([1]Virginia Bioinformatics Institue, Virginia Tech. Blacksburg, VA 24060, USA; [2]Department of Biological Sciences, Virginia Tech. Blacksburg, VA 24060, USA; [3]Department of Biochemistry, Virginia Tech. Blacksburg, VA 24060, USA; [4]Center for Genome Research and Biocomputing & Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA)

The Oomycetes are a grouping of predominantly plant and animal pathogens resembling fungi in their mechanism of pathogenesis yet closely related to stramenopiles such as brown algae and diatoms. Several oomycete species, thus far showcased in genus *Phytophthora*, contain large reservoirs of candidate effector proteins sharing a conserved N-terminus motif known as RxLR-dEER. Members of this super family contain known intracellular avirulence proteins such as *Phytophthora sojae* Avr1b, Avr3a, Avr3b, Avr3c, Avr1a, Avr4/6 and Avr1k and *P. infestans* Avr3a, Avr2, Avr4. The translocation of several effectors into host cells have been shown to require the RxLR-dEER motif and can occur in the absence of any pathogen encoded machinery. Interestingly a C-terminal patch of residues have shown to be important in the phospholipid binding properties of PiAvr3a and PsAvh5. The C-terminal patch has also been shown to play an important role in the cell entry activities of PsAvh5 into soybean root cells and airway epithelial cells. Here we present our latest findings on the phospholipid binding properties and cell entry activity of several oomycete RxLR effectors from these important pathogens.

Structural Basis for Interactions of *Phytophthora sojae* RxLR Effectors with Phosphatidylinositol 3-phosphate and for Host Cell Entry.

Furong Sun[1,3], Shiv D. Kale[2], Hugo F. Azurmendi[3], Dan Li[1], Brett M. Tyler[2,4], and Daniel G. S. Capelluto[1,3]

([1] Protein Signaling Domains Laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, United States; [2] Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061, United States.; [3] Department of Chemistry, Virginia Tech, Blacksburg, VA 24061, United States; [4] Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331, United States)

Oomycetes, such as the soybean pathogen *Phytophthora sojae*, employ effector proteins that enter plant cells to facilitate infection. Specific cell entry by oomycete effectors can occur independently of the pathogen into tissues as diverse as soybean roots and wheat leaves, or even human epithelial cells. Entry of a large class of effector proteins is mediated by RxLR motifs in the effectors and phosphatidylinositol 3-phosphate [PI(3)P] resident in the host plasma membrane. There have been differing reports regarding the regions on RxLR effectors involved in PI(3)P recognition. We have structurally and functionally characterized the *P. sojae effector*, Avh5, which is a paralogs of Avr1b. Using NMR spectroscopy, we demonstrate that Avh5 is helical in nature with a long N-terminal disordered region. NMR titrations of Avh5 with the PI(3)P head group, inositol 1,3-bisphosphate, directly identified the ligand-binding residues. A C-terminal lysine-rich helical region (helix 2) was the principal lipid-binding site, with the N-terminal RxLR (RFLR) motif playing a more minor role. Mutations in the RFLR motif affected PtdIns(3)P binding, while mutations in the basic helix almost abolished it. Mutations in the RFLR motif or in the basic region both significantly reduced protein entry into plant and human cells. Both regions independently mediated cell entry via a PtdIns(3)P-dependent mechanism. Based on these findings, we propose a model where Avh5 interacts with PtdIns(3)P through its C-terminus, and by binding of the RFLR motif, which promotes host cell entry.

How Oomycete Pathogens Exploit PI3P to Target Secreted RxLR Effectors into Host Cells.

Qunqing Wang[1], Sarah Ferrer[1], Justin Carlough[1], Felipe S. Arredondo[1], Shiv D. Kale[2], Brett M. Tyler[1,2]

([1]Center for Genome Research and Biocomputing and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, US; [2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24060, US)

Effector proteins from diverse oomycetes and fungi can enter plant cells to facilitate infection. Recent research suggests that phosphoinositides (PIPs) resident in the host plasma membrane such as phosphatidylinositol 3-phosphate (PI3P) mediate the entry of some oomycete RxLR effectors. The PIP recognition domain of these effectors is still controversial. Current evidence shows that either the RxLR domain or positive residues in the C-terminal domains (Ct) of some effectors such as the *P. sojae* effectors Avr1b and Avh5 can bind PI3P, it has been unresolved which of these domains, if either, or both, are involved in cell entry during natural pathogen infection. Here we have used heterologous PI3P-binding proteins, such as the yeast VAM7p PX domain to replace the RxLR or Ct domains of Avr1b in *P.sojae* transformants. Our results reveal that the VAM7p PX domain can replace the RxLR domain of Avr1b in carrying the C-terminal domain of Avr1b into soybean cells, conferring an avirulent phenotype on the transformants. Mutations which abolish the binding of VAM7p to PI3P substantially reduce but do abolish avirulence conferred by the construct. Mutations in the PI3P-binding residues of the Avr1b Ct also substantially reduce avirulence, while the double mutant cannot confer avirulence at all. These results strongly support the hypothesis that PI3P-binding is essential for Avr1b cell entry during natural infection, and further suggest that efficient entry by Avr1b may require two PI3P binding sites.

Structure-led studies of oomycete RXLR effectors.

Stuart RF King[1], Richard K Hughes[1], Hazel McLellan[2], Petra C Boevink[2], Miles Armstrong[2], Joe Win[3], Sophien Kamoun[3], Paul Birch[2] & Mark J Banfield[1]

([1]Dept. of Biological Chemistry, John Innes Centre, Norwich NR4 7UH UK; [2]James Hutton Institute & University of Dundee Invergowrie Dundee DD2 5DA UK; [3]The Sainsbury Laboratory Norwich NR4 7UH UK)

In my Laboratory we have determined the crystal structures of three *Phytophthora* RXLR-type effector proteins, AVR3a11, PexRD2 and PexRD16. These proteins are unrelated in primary amino acid sequence. Through structure determination we discovered unexpected similarities in the folds of these proteins that suggest a common evolutionary origin. This 'WY-fold' is predicted to be widely distributed in oomycete RXLR-type effectors may act as a stable structural scaffold to promote functional diversification. The *Phytophthora infestans* RXLR effector protein PexRD2 has been shown to adopt a homodimeric 3D structure incorporating the WY-fold. PexRD2 has also been shown to induce a weak cell death activity when expressed in *N. benthamiana*. With the protein structure available, we were interested in learning more about the functional activity of PexRD2 and how this effector might promote pathogenesis in solanaceous plants. A Y2H screen identified an interaction with a host protein known to be involved in plant immunity-related signaling. We have been using combined biochemical, structural, and in planta approaches to understand the significance of this interaction and I will present a summary of these studies. We are also actively pursuing methods for heterologous expression of other effectors from plant pathogenic oomycetes, and relevant host cell proteins, for protein biochemistry and structural studies. I will provide an overview of current progress.

Keynote Address

Effector-host interactome: getting to the hub of the matter.

Jeff Dangl

(Biology Department, University of North Carolina at Chapel Hill, 4260 Genome Sciences Building, Chapel Hill, NC 27599)

Pathogen virulence effectors have evolved to manipulate host cellular machines, thereby disrupting or subverting normal host physiology to benefit the pathogen. In turn, host targets either evolve to avoid pathogen effector-mediated manipulation, or to 'recognize' that manipulation as a trigger for efficient immune responses. Our pathogen models are the Gram-negative bacteria, *Pseudomonas syringae* (Psy) and an obligate biotrophic eukaryotic oomycete pathogen called *Hyaloperonospora arabidopsidis* (Hpa). These species have virulence systems evolved independently over ~2 billion years and different mechanisms of infection. Each has evolved to suppress host defense responses by specifically redirecting normal hormonal control of development. We discovered, surprisingly, that a specific family of host TFs called TCP proteins are common targets of evolutionarily divergent pathogen effectors. Different TCPs were recently shown to be targeted by effectors from feeding insects. Given the well characterized roles of TCPs in normal growth and development, our results provide an experimental model with which to understand the integration of developmental and pathogenic cues into signaling networks that regulate the growth versus defense equipoise. We focus on a particular set of pathogen effectors and the specific sub-network of plant TCP TFs with which they interact. We described altered infection phenotypes for these TFs. We will define the molecular mechanisms by which effectors from divergent pathogens manipulate the normal developmental function of particular TCPs to alter host immune function. We will take advantage of our effector repertoire to dissect the developmental program governed by highly redundant gene families.

Effectors II & III – Sunday 2:15-5 PM

Nep1-like proteins of the downy mildew *Hyaloperonospora arabidopsidis* trigger immunity, but not necrosis, in the Arabidopsis host

Stan Oome[1,2], Adriana Cabral[1], Simon Samwel[1], Tom Raaymakers[1], and Guido Van den Ackerveken[1,2]

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The genome of the downy mildew pathogen *Hyaloperonospora arabidopsidis* encodes several necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLP). The *HaNLPs* constitute a family of 12 genes and 15 pseudogenes, most of which form a species-specific cladeseparate from NLPs of related *Phytophthora* species. The secreted HaNLPs were found to be nontoxic when tested on Arabidopsis or tobacco, in contrast to known necrosis-inducing NLPs, e.g. the *P. sojae* PsojNIP protein that is cytolytic and induces a strong cell death response in dicot plant tissues. Even HaNLP3, which is most similar to necrosis-inducing NLP proteins of other oomycetes, and which contains all amino acids that are known to be important for necrosis-inducing activity, did not induce necrosis. Chimeras constructed between HaNLP3 and the necrosis-inducing PsojNIP protein demonstrated that most of the HaNLP3 protein is functionally equivalent to PsojNIP, except for an exposed domain that prevents necrosis induction.

The *HaNLP* genes are mostly expressed early during infection, suggesting an alternative function of noncytolytic NLP proteins during biotrophic infection of plants. To investigate if HaNLP production in the host affects susceptibility to infection, transgenic Arabidopsis plants were generated. Surprisingly, overexpression of HaNLP3, 5, 6, 9, and 10 resulted in plants with severely reduced growth. To be able to monitor NLP-effects on pathogen infection, in the absence of growth reduction, an Arabidopsis line with an estradiol-inducible *HaNLP3* construct was generated. DNA microarray analysis revealed that plant immune responseswere strongly activated upon estradiol-induced *HaNLP3* expression. Furthermore, resistance to *H. arabidopsidis* infection was activated, suggesting that the plant is able to recognize the pathogen-associated HaNLP3 protein and mount an effective immune response. Our research is now focused on determining how Arabidopsis is able to respond to the HaNLPs and how the downy mildew pathogen can suppress the host immune response triggered by non-toxic NLPs.

Bulk segregant analysis and next generation sequencing to map the *Phytophthora sojae* Avr1c gene.

Ren NA[1&2], Dan YU[3], Patrick CHAPMAN[1], Ryan AUSTIN[1], Jun ZHAO[2], Mark GIJZEN[1]

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The soybean and *Phytophthora sojae* interaction is governed by host resistance (Rps) and pathogen avirulence (Avr) genes. The aim of this work is to map and identify the Avr1c gene. A total of 26 F2 progeny from a cross of *P. sojae* strains ACR10 x P7076 were scored for virulence on plants carrying Rps1c. Results indicate that Avr1c segregates as a single dominant gene. Candidate genes for Avr1c were selected from an inventory of predicted RXLR effectors generated from previous genome sequencing of *P. sojae* strains. Testing of 24 candidate genes for co-segregation with Avr1c in the F_2 progeny did not result in any positive hits. We resorted to de novo mapping of Avr1c by combining bulk segregant analysis (BSA) with next generation sequencing. We created two pools of genomic DNA using six F_2 individuals for each pool. The avirulent (pool1) and virulent (pool2) samples were deeply sequenced, generating more than 150 million reads per sample. The reads were aligned to the reference genome and single nucleotide polymorphisms (SNP) were identified for each pool using software. High quality SNPs were filtered to select for positions where SNP frequency was close to expected values for pool1 (0.33) and pool2 (1.0). Only three SNP positions fit all requirements, and these occurred in close proximity (92 kb) in the reference genome assembly. Further genetic mapping in the F_2 progeny validated that the three SNPs occur in the vicinity of Avr1c.

Penetration-specific effectors from *Phytophthora parasitica* favour plant infection.

Edouard Evangelisti [1], Benjamin Govetto [1], Naïma Minet-Kebdani [1], Marie-Line Kuhn [1], Agnès Attard [1], Michel Ponchet [1], Franck Panabières [1], Mathieu Gourgues [1]

(INRA/CNRS/Université de Nice Sophia Antipolis, UMR Institut Sophia Agrobiotech, Sophia Antipolis, France)

Oomycetes are crop pests which cause million dollar losses every year. A better understanding of plant-oomycete interactions will help to propose new strategies for crop protection. Using a transcriptional analysis, we identified a set of penetration-specific effectors (PSE) bearing a RXLR motif. Here we report the functional analysis of three of these genes, referred to as PSE1, PSE2 and PSE3. All of them abolished plant defense responses when transiently expressed in *Nicotiana* plants. Constitutive expression of PSE1 and PSE3 in *A. thaliana* led to an enhanced susceptibility to *P. parasitica* infection, suggesting a role for these proteins in *P. parasitica* pathogenicity. Transgenic Arabidopsis lines accumulating PSE1 protein showed developmental perturbations, including coiled roots, associated with altered auxin physiology. Root growth inhibition assays showed that auxin signaling pathway is not altered by PSE1 accumulation. Nevertheless, the coiled-root phenotype and the enhanced susceptibility of PSE1-expressing lines to *P. parasitica* were reverted by synthetic auxin 2,4-D or auxin efflux inhibitor TIBA, suggesting that a reduced auxin accumulation is responsible for these phenotypes. This hypothesis was confirmed by a reduced activity of the pDR5 auxin sensitive promoter at the root apex. The alteration of the expression pattern observed for the auxin efflux carriers PIN4 and PIN7 suggests that a perturbation of auxin efflux could be responsible for the PSE1 associated defects. Inoculation of HS::AXR3NT-GUS auxin reporter line with *P. parasitica* resulted in modulation of the auxin content in infected cells. We proposed that PSE1 could favour *P. parasitica* virulence by locally interfering with auxin content. Our results show that penetration specific effectors can modulate general plant functions to facilitate plant infection. Perturbation of hormone physiology was previously reported for other plant pathogens, including nematodes and bacteria, supporting the hypothesis that infection strategies from distant pathogens species could converge onto a limited set of plant targets.

Phytophthora effectors promote infection by suppressing host RNA silencing.

Yongli Qiao[1,2], Qin Xiong[1,3], James Wong[1,2], Howard Judelson[1,2], Yuanchao Wang[3], Wenbo Ma[1,2]

([1] Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521; [2]. Center for Plant Cell Biology, University of California, Riverside, CA 92521; [3]. Department of Plant Pathology, Nanjing Agriculture University, Nanjing, China 210095)

Effectors are essential virulence proteins produced by a broad range of parasites. Upon entry into host cytoplasm, pathogen effectors manipulate specific physiological processes or signaling pathways to subvert host immunity. However, the majority of effectors, especially those produced by eukaryotic pathogens, remain functionally uncharacterized. Recently, we reported that two RXLR effectors from *Phytophthora sojae* can suppress RNA silencing in plants. Furthermore, these *Phytophthora* Suppressors of RNA silencing (PSRs) as well as some Viral Suppressors of RNA silencing (VSRs) promote *Phytophthora* infection. These data demonstrate that *Phytophthora* pathogens have evolved effectors that target host RNA silencing processes for the benefit of disease development. These findings are also consistent with a role of small RNAs in host defense against *Phytophthora*. Here, we will report our recent progress on the mechanistic analysis of the RNA silencing suppression activities and virulence functions of PSRs. We will also discuss the role of small RNAs in regulating plant defense during *Phytophthora* infection.

Mechanism of nuclear suppression of host immunity by HaRxL44, an Arabidopsis downy mildew effector

Marie-Cecile Caillaud[1], Shuta Asai[1], Lennart Wirthmueller[1,2], Sophie J. M. Piquerez[1], Georgina Fabro[1,3], and Jonathan D. G. Jones[1]

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Plants are continuously exposed to microbial pathogen attacks but usually remain healthy because they are able to perceive microbes and then activate defence. Nonetheless, pathogens have evolved to overcome plant immunity by expressing effectors that increase host susceptibility, resulting in disease. Recent studies suggest that effectors may manipulate host transcription or other nuclear regulatory components for the benefit of the pathogen. However, the specific mechanisms by which these effectors promote susceptibility remain unclear. Out of two recent screenings, we identified 15 nuclear localized Hpa effectors (HaRxLs) that enhance plant susceptibility to pathogens. When stably expressed in planta, nuclear-HaRxLs cause diverse developmental phenotypes which highlight their interference with fundamental plant regulatory mechanisms. Results obtained in the large-scale yeast two-hybrid screen highlight that nuclear-HaRxLs interact preferentially with transcriptional regulators. In particular, HaRxL44 interacts with a component of Mediator, a major integrator of biotic stress responses, essential for plant nuclear immunity. We will present recent advances in our understanding on how HaRxL44 effector activates JA signalling at transcriptional level via its interaction with Mediator, in order to promote Hpa virulence.

Two potato NAC transcription factors are the targets of a *Phytophthora infestans* effector.

McLELLAN, H.[1], ARMSTRONG, MA.[1], BOEVINK, PC.[2] & BIRCH, PRJ.[1]

([1]Division of Plant Science, College of Life Sciences, University of Dundee (at JHI), Invergowrie, Dundee DD2 5DA, UK; [2] Cell and Molecular Science group, JHI, Invergowrie, Dundee DD2 5DA, UK)

The potato late blight pathogen *Phytophthora infestans* secretes a vast array of effector proteins which are thought to act in its hosts by disarming defences and promoting an environment conducive to pathogen growth and replication. However, very little is known to date about the host targets of these effectors and how they are manipulated by the pathogen. This work describes the identification of two putative membrane bound NAC transcription factors (TF) as the host targets of the RxLR effector Pi03192. The interaction takes place at the ER membrane where these proteins are localised. Silencing of NAC1 and 2 in Nicotiana benthamiana is shown to compromise resistance to *P. infestans* establishing the role of these genes in defence. Transcription of NAC1 and 2 is rapidly induced by *Phytophthora* PAMP treatment and StNAC1 and 2 proteins are released from the endoplasmic reticulum (ER) membrane on PAMP treatment and are rapidly turned over in the nucleus by the proteasome. The virulence mechanism of Pi03192 is to block the translocation of StNAC1 & 2 into the nucleus, this is a novel mode of action for a plant pathogen effector.

How do oomycete effectors trigger resistance in plants? New findings from the RPP1-ATR1 battlefront of Arabidopsis infection by Hpa.

Steinbrenner, Adam D [1] Goritschnig, Sandra Staskawicz, Brian J

(Department of Plant and Microbial Biology UC Berkeley Berkeley, CA 94720 USA)

ATR1 is one of over 100 effector proteins predicted to be secreted by the oomycete pathogen *Hyaloperonospora arabidopsidis* (Hpa). It is recognized by Arabidopsis in a race-specific manner by a cognate resistance protein, RPP1, one of ~150 NLR (nucleotide-binding leucine-rich-repeat) receptors encoded in the host genome. ATR1 alleles from the Cala2, Maks9, and Emoy2 strains are differentially recognized by the RPP1 alleles NdA (from the Nd-1 Arabidopsis ecotype) and WsB (from Ws-0): Cala2 by neither allele, Maks9 by RPP1-WsB only, and Emoy2 by both alleles. We carried out a random mutagenesis screen for mutations conferring recognition capability to the unrecognized ATR1-Cala2 allele. Out of over 2000 mutants screened, two sets of "gain-of-recognition" mutations conferred recognition by RPP1-NdA. Interestingly, these mutants were not recognized by RPP1-WsB, demonstrating non-overlapping specificity of recognition in two alleles of a single NLR. These results also complement recent findings that single amino acid substitutions in ATR1 can alter recognition specificity by RPP1. The recently solved crystal structure of ATR1 allows us to place these mutations in the context of ATR1's large solvent-exposed surface, as well as structurally conserved WY domains common across oomycete effectors for which structural data are available. We will report the avirulence function of these gain-of-recognition ATR1 mutants in Arabidopsis ecotypes containing RPP1, as well as preliminary data on the molecular determinants of their differential recognition by alleles of RPP1.

Genomics I & II – Monday 9AM-12:20PM

COMPARATIVE GENOMICS OF DOWNY MILDEWS.

Richard Michelmore, Joan Wong, Lida Derevina, Juliana Gil, Tadeusz Wroblewski, Lutz Froenicke, Bertrand Perroud, Keri Cavanaugh, Sebastian Reyes Chin-Wo, Huaqin Xu.

(The Genome Center, University of California, Davis, CA 95616, USA)

We are sequencing multiple isolates of several downy mildews to enable comparative genomics across the *Peronosporaceae*. Our primary focus has been on *Bremia lactucae*, the most important pathogen of lettuce. This has been challenging due its high level of heterozygosity. A series of hybrid assemblies have been made using sequences from several technologies. A draft genome sequence has now been completed. Candidate genes encoding over 130 effectors and other pathogenicity-related proteins have been identified. Functional analysis of 45 effectors has been made using *Agrobacterium* mediated transient in planta expression and yeast two-hybrid analysis of protein-protein interactions. Expression of *B. lactucae* effectors in a set of lettuce cultivars expressing all 25+ known Dm resistance genes failed to detect any that elicited a necrotic reaction, reflecting the adaptation of B. lactucae to its host, similar that observed with bacterial pathogens. Yeast two-hybrid analysis identified interactions consistent with the idea that effectors from diverse pathogens target common points of vulnerability within defense pathways of plants. We are also sequencing several other downy mildews, including *Sclerospora graminicola* and *Peronosclerospora sorghi*, to characterize the effector repertoires of these important tropical downy mildews. These and publicly available genome sequences will be used to elucidate taxonomic and evolutionary relationships, study genome architectures, fluidity, and synteny, and characterize expression and variation of effector repertoires.

Comparative genomics of host range in *Phytophthora parasitica*.

Franck Panabières, on behalf of the *P. parasitica* genome initiative [1], Weixing Shan [2] Carsten Russ [3] Brett Tyler [4]

([1] Sophia Agrobiotech Institute, INRA 1355 / CNRS 7254 / UNS 06903 Sophia Antipolis Cedex, France; [2] Northwest Agricultural & Forestry University, Yangling, China; [3] Broad Institute, Cambridge, MA, USA; [4] Oregon State University, Corvallis OR, USA)

Among oomycetes, plant pathogens of the genus *Phytophthora* cause devastating diseases and limit agriculture worldwide. These microorganisms display diverse lifestyles, and their host range varies from a single host to thousands of plant species. Several *Phytophthora* genomes have been sequenced, and vary in size and content, ranging from 43 Mb to 250 Mb. These sequencing projects yielded insights into the molecular basis of pathogenicity, but evolutionary mechanisms governing host range diversity are still largely unknown. *P. parasitica* is a very broad host range pathogen that attacks numerous crops, including solanaceous plants, cocoa and citrus, a wide variety of fruit trees, nursery and ornamental plants. While *P. parasitica* as a species possesses a very broad host range, many isolates are pathogenic to a single, or a restricted number of hosts. The comparison of the genome sequences obtained from isolates with various origins and hosts may help to identify mechanisms underlying host specificity. We generated a whole genome shotgun sequence assembly of a broad host range *P. parasitica* isolate (INRA PP310) as a reference sequence. In addition, we generated Illumina data from five additional *P. parasitica* isolates collected on various hosts. This will contribute to identify conserved and rapidly diversifying genes, in order to enable a comparative genomic analysis of genes that determine host range in *P. parasitica*. The 20,501 gene repertoire of the *P. parasitica* reference strain was compared with three sequenced *Phytophthora* species, including *P. infestans*, which is evolutionary close to *P. parasitica*. First results will be presented, that will assist in identifying genes associated with general pathogenicity and host specificity in oomycete pathogens.

EuMicrobeDB-M: A light weight Oomycete Genome Database based on Mysql with C++ API

Sucheta Tripathy[1], Akash Gupta[1], Brett M Tyler[2,3]

([1]Indian Institute Of Chemical Biology, 4, Raja, S.C Mullick Road, Kolkata, India; [2] Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, USA; [3] Center for Genome Research and Biocomputing, Oregon State University, Corvallis, Oregon, USA)

EuMicrobeDB.org (formerly known as VMD; Tripathy et al, Nucleic Acids Research 2006), was created as a comprehensive research portal for oomycetes genomes with community annotation features. Eumicrobedb.org is based on the Genome Unified Schema (GUS). We decided to move the contents of eumicrobedb into FungiDB that is also based on GUS. While FungiDB has a number of powerful user interfaces, the architecture is designed for completed genome projects. For ongoing projects involving draft genomes and assemblies, most of the features that are available through FungiDB are not useful. GUS has been adapted widely by portals as part of the EuPathDB project. The GUS schema is fairly robust and accommodates diverse data types. However, GUS is not easily portable due to its architecture and dependence on Oracle. Also the complexity of this database system restricts its portability across different research labs. We therefore have created a light weight mysql based version of EuMicrobeDB carrying the same usability and features. GUS has a bioperl based data object layer that we replaced by a C++ API. The current release version 1.0 can be downloaded as a package including the front end tools. Release version 1.0 has genome and annotation data from two *Phytophthora sojae* assembly versions (i.e, version 1.0 and version 5.0), *P. ramorum* (1.0), *P. infestans* V4.0, *P. capsici* V 11.0, *Pythium ultimum* V4.0, *Hyaloperonospora arabidopsis* V8.3. The front end tools and the toolbox were directly ported from EuMicrobeDB, and hence the user experience remains the same. The package is much simpler and easier to handle, and installation time and complexity have been drastically reduced. The database is available at www.eumicrobedb.org.

De novo sequencing of the litchi downy blight pathogen *Peronophythora litchii* reveals an orphan between *Phytophthora* and *Peronospora*.

Wenwu Ye[1], Yang Wang[1], Delong Li[1], Danyu Shen[1, 2], Daolong Dou[1], Brett Tyler[2], Yuanchao Wang[1*]

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Peronophythora litchii, causes downy blight on litchi, is a transitional species between *Phytophthora* and *Peronospora* belonging to single family, single genus, and single species, because its the morphology feature is multideterminate sporangiophore, that is different to the indeterminate of *Phytophthora* and determinate of *Peronospora*. Its genome was de novo sequenced and the 38.2 Mb assembly contains 2,543 scaffolds and 13,155 predicted genes. 96% orthologs of 248 conserved single-copy eukaryotic genes could be identified indicating a good completeness of genome assembly. Thus, the genome size and number of predicted genes were the smallest and least among the sequenced oomycete plant pathogens. It could be explained by the fewer repeats (10.1%) and compacter gene to gene distance. A phylogenetic tree constructed by concatenated protein sequences of housekeeping genes showed that *Pe. litchii* was located between *Phytophthoras* and *H. arabidopsidis*. The predicted proteins of *Pe. litchii* had most hits, highest identities, and best synteny in *Phytophthoras*, then was *H. arabidopsidis*. Over 300 RxLR and a total of 30 NLP effectors were predicted, that were much equal to *Phytophthora* genomes but rather the *H. arabidopsidis* with much repressed number. In contrast, *Pe. litchii* had only 14 CRN effectors, that was much equal to the *H. arabidopsidis* but not *Phytophthoras* who contained much expanded gene number. Phylogenetic analyses of the NLPs and CRNs showed that the genes of *H. arabidopsidis* located in special clades of trees while the genes of *Pe. litchii* were closed to *Phytophthora*. Gene numbers of the carbohydrate-active enzymes were between *Phytophthora* and *H. arabidopsidis*. In *H. arabidopsidis*, enzymes for assimilation of inorganic nitrogen and sulfur were lost but not in *Pe. litchii*. All of these evident revealed that consistently to the feature of morphology, the genome component of *Pe. litchii* was also between *Phytophthora* and *Peronospora* but more closed to *Phytophthora*.

Genome-wide analysis of Crinklers effectors in *Aphanomyces euteiches*.

Diana Ramirez-Garcés, Laurent Camborde, Hélène San-Clemente, Juliette Lengellé, Bernard Dumas, Elodie Gaulin

(Plant Research Laboratory (LRSV) UM5546 CNRS-Univ Toulouse 3 31326 Castanet-Tolosan France)

Aphanomyces euteiches is an oomycete infecting roots of various legumes species such as pea, alfalfa and the model legume *Medicago truncatula*. The genus *Aphanomyces*, which belongs to the group of Saprolegniales, is phylogenically distant from the well known *Phytophthora* genus and comprises both animal pathogen and plant pathogen species. The first genome draft of *Aphanomyces euteiches* (ATCC201684, 57 Mb) will be soon released and zoo- and phytopathogen species are being sequenced in our lab. *A. euteiches* genome miming revealed the expansion of CRNs (Crinkling and Necrosis) genes, initially identified in *Phytophthora infestans*. These modular proteins contain a conserved N-terminal characterized by the presence of a LFLAK amino acid motif implicated in the protein translocation from the pathogen to the host cell, whereas the modular C-terminal effector domain is highly diverse. The proposed role of the CRNs effectors is to suppress plant defense or to modulate other host cell processes that increase susceptibility and enhance pathogen virulence. In *A. euteiches*, the active translocation LYLAK motif was detected (Schornack et al., 2010), and conserved, as well as original effector subdomains, were identified. Functional studies conducted on two types of A. euteiches CRNS, AeCRN5 and AeCRN13, showed that both proteins are highly induced during infection of *M. truncatula* roots. In planta expression of both proteins revealed host nucleus localization and cell-death induction or alteration of roots architecture when expressed in plant cells. Such observations suggest that *A. euteiches* CRNs are virulence proteins exerting their function through the interaction with nuclear compounds. Latest results regarding the putative function of AeCRNs will be presented.

Identification of genes involved in *Phytophthora cinnamomi* pathogenicity.

A Reitmann[1], DK Berger[2], N van den Berg[1]

([1]Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South-Africa; [2]Department of Plant Science, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South-Africa)

Phytophthora cinnamomi is an economically important pathogen that is able to successfully infect more than 3000 plant species. Every plant pathogen interaction is very specific. The host possesses a repertoire of genes enabling it to protect itself from a range of stresses, including pathogen attack. In a similar way the pathogen requires proteins that are able to surpass the host defence system and facilitate infection. We aimed to identify the arsenal of pathogenicity related genes of the oomycete *P. cinnamomi*. We produced the first cDNA library of the geminating cyst developmental stage of *P. cinnamomi* using the Illumina sequencing platform. Over 70 000 unigenes were identified from 225 049 contigs that were assembled from 13 million paired end reads. More than 3000 unigenes had a putative role in pathogenesis. Cell wall degrading enzymes and genes involved in the protection against oxidative stress were most abundant among them. This included cutinase, cellulase and reductase enzymes. We found many ABC transporters that aid in detoxification and metabolite transport. A preliminary search revealed putative RXLR effector genes, together with elicitin-like and necrosis-inducing peptide transcripts. This work opens the door to understanding the molecular basis that is responsible for *P. cinnamomi's* success as a wide host-range pathogen.

Novel core promoter elements in the oomycete *Phytophthora infestans* and their influence on expression pattern detected by genome-wide analysis.

Laetitia Poidevin, Sourav Roy, Tao Jiang, and Howard S. Judelson

(Department of Plant Pathology and Microbiology and Department of Computer Science, University of California, Riverside, California 92521 USA)

The core promoter is the region flanking the transcription start site (TSS) that directs pre-initiation complex formation. While core promoters have been studied intensively in mammals and yeast, little is known about more diverse eukaryotes, like oomycetes. Prior studies of a small collection of cloned genes proposed that oomycete core promoters contain a 19-nt block bearing both an Initiator-like sequence (INR) and a novel 3' sequence named FPR, but this has not been extended to whole-genome analysis. We used expectation maximization to find over-represented motifs near TSSs of *Phytophthora infestans*, the potato blight pathogen. The motifs corresponded to INR, FPR, and a new element found 25-nt downstream of the TSS called DPEP. Assays of DPEP function by mutagenesis were consistent with its role as a core motif. Genome-wide searches found a well-conserved combined INR+FPR in only 13% of genes after correcting for false discovery, contradicting prior reports that INR and FPR are adjacent to each other in most genes. INR or FPR were found alone near TSSs in 18% and 7% of genes, respectively. Promoters lacking the motifs had pyrimidine-rich regions near the TSS. The combined INR+FPR motif was linked to higher than average mRNA levels, developmentally-regulated transcription, and functions related to plant infection, while DPEP and FPR were over-represented in constitutive housekeeping genes. The motifs were all detected in other oomycetes including *Hyaloperonospora arabidopsidis*, *Phytophthora sojae*, *Pythium ultimum*, and *Saprolegnia parasitica*, but only INR seemed present in a non-oomycete stramenopile. The absence of a TATA box and presence of novel motifs show that the architecture of oomycete core promoters has diverged from that of model systems, which likely explains failures in prior heterologous expression studies. The association of the INR+FPR motif with developmentally-regulated genes shows that oomycete core elements influence stage-specific transcription in addition to regulating pre-initiation complex formation.

Alternative splicing provides a possible mechanism for *Pseudoperonospora cubensis* transcriptome regulation.

Alyssa Burkhardt[1], Jason Cumbie[2,3], Jeff Chang[2,3,4], and Brad Day[1,5]

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Pseudoperonospora cubensis (downy mildew) is a foliar pathogen of cucurbits – cucumber, melon, squash and pumpkin – that threatens agriculture production around the world. In recent years, *Ps. cubensis* has become a resurgent problem for cucumber growers in the United States, with ~50 years of resistance being overcome in 2004. In order to better understand the virulence of *Ps. cubensis*, including the genetic basis of host specificity and resistance within its host, recent work has investigated the molecular interactions between *Ps. cubensis* and *C. sativus* (cucumber). Previous work from our laboratory has generated a draft genome sequence of *Ps. cubensis*, and has begun to characterize several candidate effector proteins. Recent work from our group has generated extensive transcriptomic data from *Ps. cubensis* and *C. sativus*, respectively, over the course of infection. From this data, we have identified alternative splicing as a potential mechanism to broaden or regulate the pathogen proteome (Savory et al., 2012). From this initial finding, we expanded our research and now present current data detailing the role of alternative splicing of both effector and non-effector genes in *Ps. cubensis*. Alternative splicing of *Ps. cubensis* genes were bioinformatically predicted using Illumina Hi-Seq data from an inoculation time course of sporangia and infected leaves at 1, 2, 3, 4, and 8 days after inoculation; alternatively spliced genes were validated using reverse-transcriptase PCR and real-time PCR. Real-time PCR data collected over a time course indicates that each alternatively spliced gene has a specific splicing profile over an infection time course. Future studies will functionally characterize these alternatively spliced genes, providing a comprehensive overview of alternative splicing in *Ps. cubensis*, as well as a potential mechanism for gene regulation and transcriptome and proteome expansion.

Evolution and Population Genetics I & II – Monday 1:30-4:30PM

The genomes of free-living *Thraustotheca clavata* and parasitic *Achlya hypogyna* implicate a role for gene family expansion and gene transfer in the adoption of a parasitic lifestyle.

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([1]Dept. Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom; [2]The Sainsbury Laboratory, Norwich Research Park , Norwich NR4 7UH, United Kingdom)

Plant pathogens are a continuous threat to global food security. The oomycetes are an important class of filamentous eukaryotic pathogens that delivers an arsenal of effector proteins into plant cells to promote infection and perturb plant immune systems. Hundreds of effector genes have been identified in the genomes of plant pathogenic oomycetes including *Phytophothora* and *Hyaloperonospora*. RXLR effectors make up a large family of cytoplasmic effectors and the effector protein is defined by a secretion signal peptide followed by an N-terminal domain defined by the RXLR (Arg-Xaa-Leu-Arg) consensus sequence and a C-terminal effector domain. Once inside the cell, many effector domains are thought to interact with the host proteins to promote disease. The structural basis for such interactions remains elusive and the molecular details of such interactions is poorly understood. In the present study we aim to understand the molecular details of how these effectors interact with the plant proteins and modulate plant immunity. Specifically, we aim to determine the three-dimensional structures of the effectors, their identified plant targets and possibly the complexes. Heterologous expression of RXLR effectors and their plant targets in E.coli has proven to be a challenge due to problems related to solubility and stability. By employing various expression vectors and expression hosts we could successfully express and purify some effector proteins. The recombinant protein will be used for structural and biophysical characterization of effector proteins and their host targets.

Rapid influx and numerous epidemics of *Aphanomyces astaci* strains are altering the European crayfish distribution and biodiversity

Harri Kokko[1], Jenny Makkonen[1], Daniel Blande[1], Anssi Vainikka[2], Raine Kortet[2], Arto Vesterbacka[1], Lars Granlund[1] and Japo Jussila[1]

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The crayfish plague caused by the oomycete *Aphanomyces astaci* (Apa) causes extinctions of native European crayfish species and reduces their distribution. Crayfish plague started its European invasion from the River Po valley, Italy, in 1859 and since then has killed billions of crayfish and destroyed numerous populations of all the native European crayfish species. We carried out infection trials to study the virulence of several *Apa* isolates by monitoring symptoms and mortality of different noble crayfish (*Astacus astacus*) populations. The studied As-genotypes, isolated from the noble crayfish, expressed variable virulence while the PsI-genotype, isolated from more recently introduced signal crayfish (P*acifastacus leniusculus*), caused rapid and full mortality among the tested noble crayfish populations. In some cases the As-genotype infected crayfish did not show increased mortality in relation to the control group. So far all the tested noble crayfish stocks have had better resistance and tolerance towards the As-genotypes compared to the PsI-genotype. We have also shown that there are clear differences in virulence among different *Apa* genotypes and also within main genotype groups. During the infection experiments and field studies we have observed increased resistance in different noble crayfish populations towards tested *Apa* isolates. The rapid evolution of *Apa* after several host jumps, selection and adaptation is visible in the chitinase gene sequences and transcriptome of different *Apa* isolates. We have compared the partial chitinase gene sequences of 28 *Apa* isolates and the transcriptome of selected isolates with different virulence. Based on the results, we have already observed that *Apa* has adapted to its new host species. We will show and summarise these results and discuss theoretical and practical outcomes of the studies.

PCR-AFLP as a method for genotyping the crayfish plague pathogen *Aphanomyces astaci* (Oomycetes)

Rezinciuc, S. and Diéguez-Uribeondo, J.

(Mycology Dept. Royal Botanic Garden Madrid, E-28014 Spain)

Aphanomyces astaci (Oomycetes) is a specialized parasite of freshwater crayfish responsible for the so-called crayfish plague. Due to its devastating effect and rapid spread it is listed among the one hundred of the world's worst invasive alien species (Lowe 2004). So far, studies on its genetic diversity have been only carried out by using PCR-RAPD analysis. Because of the limitations of this technique, we have tested the application of another DNA-finger printing markers based on PCR-AFLP. For this purpose, we checked sixteen different primer combinations on selected isolates that represent the distinct RAPD-genotype groups so far identified. The analysis yielded a total of three hundred fifty two polymorphic loci. Analyses using UPMGA clustering and likelihood inference showed that the tested isolates could be separated into four distinct genogroups that were similar to those previously identified using PCR-RAPD. Thus, our results indicate that AFLP markers can be successfully applied to *A. astaci* system for conducting large-scale population genetic studies.

Strain-identification of *Pythium aphanidermatum* in metagenomic samples from 454 pyrosequencing.

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Pythium aphanidermatum (*Pap*) is a soilborne plant pathogen causing root rots and damping off in a wide range of hosts grown in greenhouses, nurseries, and the field in warm regions. The early and accurate detection of the pathogen allows timely management. *Pap* is good model system for development of strain typing because it is a monophyletic species with relatively low genetic diversity. Next Generation sequencing allows to sequence multiple genomes in few hours after the sample has been pre-processed. This study uses E-probe Diagnostic Nucleic acid Analysis (EDNA), a newly developed bioinformatics diagnostic approach, to discriminate among *Pap* isolates/strains from infected plant metagenomic data. EDNA for strain identification uses strain specific e-probes designed from three molecular markers of *Pap* (ITS, beta-tubulin [TUB] and cytochrome oxidase II [COXII]). Three different isolates of *Pap* (NBRC100101, MAFF305568, and P36-3) were utilized for the e-probe design. The final number of unique e-probes for *Pap* was 42 for COXII [MAFF305568], 27 for COXII [P36-3] and 27 from COXII [NBRC100101]. The e-probe length was selected based on the highest number of e-probes designed for all three genes. For TUB gene,, e-probes length selection kept 184 e-probes to the isolate P2, 128 for the isolate P36.3 and 128 for the isolate NBRC100101. Finally, for ITS gene, the e-probe number ranged from 150 to 47.Mock sequencing databases (MSD) were used for the validation of the EDNA approach in silico. The pathogen was detected to the strain level in MSDs of 140,000 total reads. EDNA incorporates to its algorithm: % identity; e-value and e-probe hit depth to account for high quality matches.

Global Effector Diversity in *Phytophthora capsici*.

Dylan Storey, Kurt Lamour

(Genome Sciences and Technology University of Tennessee Knoxville TN USA)

Phytophthora capsici is a serious pathogen of vegetables causing significant economic loss worldwide. The species contains an impressive amount of genetic variation and our goal was to assess diversity in the following three families of *P. capsici* effectors; CRN, RxLR and NEP. Eighteen *P. capsici* isolates were re-sequenced using Illumina single-end technology, assembled to the *P. capsici* reference genome and haplotypes estimated using read backed phasing. Haplotypes were translated in all 6 frames and putative effectors identified from amino acid sequence >70 in length. The RxLR family was highly diverse with 1,936 loci detected across all isolates , 1,051 loci had 3 or more haplotypes. Overall, loci are relatively dissimilar to each other with only 374 loci able to be linked into 118 groups at greater than 60% identity. The CRN and NEP families contained considerably fewer loci with less divergence and different cluster patterns. Specific results and potential implications will be discussed.

Genetic diversity of *Phytophthora capsici* on pepper in China

Jian Hu[1,2], Xili Liu[1], Kurt Lamour[2]

(Department of Plant Pathology, College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, China, [2]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville TN 37996)

Phytophthora capsici causes significant losses to pepper production in China. Little is known about the population structure and our goal was to assess the genetic diversity of *P. capsici* across the entire country. From 2006 to 2012, a total of 237 isolates were collected from 27 of the 34 Chinese provinces. Isolates were analyzed using 24 single nucleotide polymorphism (SNP) markers. A total of 82 multi-locus genotypes were resolved with 61 being unique and the other 21 comprising clonal lineages of between 2 to 69 members. There was no correlation between genetic distance and physical distance and some clonal lineages were spread widely and persisted multiple years. The overall population structure appears unique compared to other areas of the world because clonal lineages persist on a wide scale over long periods in the midst of sexually-derived genotypic diversity. The implications for management of *P. capsici* are discussed.

Detailed characterisation of two novel species within the Oomycete order of the *Saprolegniales*.

Mohammad Nasif Sarowar[1,*], Ariane Willems[1,*], Jose Vladimir Sandoval Sierra[3], David Belo[1&2], Paul Brickle[4], Steve Woodward[2], Javier Diéguez-Uribeondo[3] and Pieter van West[1]

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The order of *Saprolegniales* contains some of the most invasive and devastating oomycete animal pathogens, however the real extend of families and genera within this order remains largely unknown. Recently, we collected oomycetes from the Falkland Islands and Northeast Scotland by baiting fresh water samples with sterilized green pepper sections, hemp seeds, rice seeds and flies. Oomycete isolates were characterised by sequencing of the internal transcribe spacer (ITS) and large ribosomal subunit 28S (LSU) genes and subsequent BLAST search of the Genbank database. This led to the identification of three individual isolates representing two novel species that form a distinctive branch in a phylogenetic tree of the Saprolegniales, suggesting the presence of an entire new clade and possibly a new genus. Both species grow well on solid and liquid medium. Mycelium is sparingly branched with a similar growth rate on potato dextrose agar plates (0.7±0.1 mm/h (average over 96 h±SEM) at 18°C; n=3 each). The strains from the Falkland Islands readily form sporangia, which are highly melanised and tubular with mainly saprolegnoid and occasionally aplanoid release of zoospores. Rarely oogonia were observed, which are spherical with a tubulate/papillate outer cell wall. The strain from Scotland seldom sporulates with sporangia hardly distinguishable from the mycelium. However, oogonia were formed abundantly and are spherical with tubulate/papillate outer cell wall, which are very similar to the ones found in the Falkland Island strains. Generally, the oogonia contain one single oospore. Anteridial branches are androgynous with the cells apically appressed. In order to test for potential pathogenicity, we challenged salmon eggs (*Salmo salar*) with both species. Both species were able to infect however, the Falkland strains appeared to be less pathogenic when compared to the Scottish species. Moreover they were both significantly less pathogenic when compared to isolates of the known fish pathogen *Saprolegnia diclina*, suggesting that these novel species may have another more suitable host in their aquatic habitats or have a predominantly saprophytic lifestyle.

Why are there so many *Phytophthora* species: does polyploidy play a role? A case study of *Phytophthora* clade 8b.

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In the past, polyploidy has already been shown in *Phytophthora infestans*, the infamous potato late blight pathogen. It was hypothesized that polyploidy might offer an advantage for the pathogen to adapt to cooler environments. This has never been proven, and with the advent of the genomic era, research efforts aimed at understanding polyploidy in *Phytophthora* diminished and disappeared almost completely.

Our research focuses on *Phytophthora* clade 8b species. Recently, we have described three new species in this group (Bertier et al. 2013, accepted with revisions), bringing the total number of species in this clade to six (*P. porri*, *P. primulae*, *P. brassicae* and the newly described *P. dauci*, *P. cichorii* and *P. lactucae*). This is a host-specific, cold-loving clade, containing a group of pathogens specifically adapted to cause disease at low temperatures in a range of important agricultural crops.

Moreover, we detected three different types of interspecific hybrids by ITS heterozygosity, mtDNA sequencing (coxI and nadh1) and DNA content estimation using flow cytometry. We found large DNA content variation among the 12 hybrid isolates and have proof of ongoing homeologous recombination (recombination between chromosomes of the different parental species).

We also detected that polyploidy is a common and distinctive feature among the species of clade 8b, with most species being polyploid in some way (auto- and allopolyploidy, aneuploidy, mixoploidy as well as diploidy were found).

Our results, together with the observations in *P. infestans*, indicate that polyploidy might play an important role in *Phytophthora* evolution. Next to adaptation to new ecological niches, polyploidy could enhance host adaptation by neofunctionalization of excess gene copies. Polyploidy also offers an enormous potential for possible recombination of effector genes, especially in homothallic species like the ones in clade 8b.

Oomycete Biology I & II – Tuesday 9AM-Noon

High-throughput prediction and functional validation of promoter motifs regulating gene expression during *Phytophthora* development

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Filamentous pathogens such as *Phytophthora infestans* have complex life cycles in which gene expression networks orchestrate the formation of stages specialized for dissemination, survival, infection, and host colonization. Microarray studies have shown that major shifts in mRNAs occur during such life-stage transitions. To help identify the transcriptional networks regulating development, we used several search algorithms to discover about 100 motifs that are over-represented in promoters of genes up-regulated in hyphae, sporangia, sporangia undergoing zoosporogenesis, swimming zoospores, or germinated cysts forming appressoria. Most of the putative stage-specific transcription factor binding sites (TFBSs) thus identified had features typical of TFBSs such as position or orientation bias, palindromy, and conservation in *P. ramorum* and *P. sojae*. Each of six motifs tested in *P. infestans* transformants using the GUS reporter gene conferred the expected stage-specific expression pattern, and were shown to bind nuclear proteins in gel-shift assays. Several motifs linked to the appressoria-forming stage were over-represented in promoters of genes encoding effectors and other pathogenesis-related proteins. To understand how promoter and genome architecture influence expression, we also mapped transcription patterns to the *P. infestans* genome assembly. Adjacent genes were not typically induced in the same stage, including genes transcribed from a small shared promoter region. Analyses of global expression, however, demonstrated that co-regulated gene pairs occurred more than expected by random chance. Studies of core promoter architecture also identified known and novel motifs preferentially associated with developmentally-regulated genes. These data help illuminate the processes regulating development and pathogenesis, and will enable future attempts to purify the cognate transcription factors.

Haustoria-forming *Phytophthora palmivora* to study commonalities and differences of pathogenic and symbiotic infection processes in roots.

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Do filamentous pathogens, such as *Phytophthora*, share common mechanisms with mutualistic symbionts, such as mycorrhiza, to colonize their hosts? We established a system for comparative inter-organismic interaction studies based on isolates of a haustoria-forming *Phytophthora* species and its biotrophic root interaction with Mycorrhiza-host plants. Our system is amenable to transformation and fluorescent tracer isolates have been generated. We are currently addressing Mycorrhiza mutants in different host backgrounds for their ability to block *Phytophthora* infection and have preliminary data, that a key element of *Medicago* symbiosis signalling plays a role in the response towards *P. palmivora*. Microarray studies revealed a set of genes specifically induced in biotrophic *Phytophthora* infection and Myccorhization but not necrotrophic infections. Mycorrhiza fungi and *Phytophthora* induce formation of cellular inter-organismic interfaces in roots, termed arbuscules and haustoria, respectively. During *Phytophthora* infection, several membrane-associated plant proteins are altered in their spatial distribution in haustorium-containing cells including the selective absence of immune receptors at perihaustorial membranes. In line with a supposed alteration of key host processes by *Phytophthora* effector proteins, we recently described host-translocated *P. infestans* effectors which localize to haustoria and likely contribute to their development and composition. Furthermore *Phytophthora* effectors can also localize to and interfere with Mycorrhiza arbuscules. We employ them as molecular probes to highlight common and contrasting processes in cells with arbuscules and haustoria.

Deciphering cell wall structure and biosynthesis in oomycetes using carbohydrate analyses and plasma membrane proteomics

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Some oomycete species are severe pathogens of economically important animals or plants. Proteins involved in cell wall metabolism represent excellent targets for disease control. The objective of our work was to determine the fine cell wall polysaccharide composition of selected species and identify the corresponding membrane-bound biosynthetic enzymes and other proteins involved in cell wall remodeling. In the first instance, we performed a detailed carbohydrate analysis of the mycelial cell walls of 11 oomycete species from 2 major orders, the *Saprolegniales* and *Peronosporales*. We then selected the fish pathogen *Saprolegnia parasitica* for in-depth proteomics analysis. Our results indicate the existence of 3 clearly different cell wall types. This biochemical distinction is in agreement with the taxonomic grouping based on molecular markers of the species studied. The 3 cell wall types are distinguishable by their cellulose content and the fine structure of their 1,3-β-glucans. Furthermore, unique features were found in each case. Type I cell walls (e.g. *Phytophthora*) are devoid of N-acetylglucosamine (GlcNAc) but contain glucuronic acid and mannose; type II (e.g. *Achlya*, *Dictyuchus*, *Leptolegnia* and *Saprolegnia*) contain up to 5% GlcNAc and residues indicative of cross-links between cellulose and 1,3-β-glucans; type III (e.g. *Aphanomyces*) are characterized by the highest GlcNAc content (> 5%) and the occurrence of unusual carbohydrates that consist of 1,6-linked GlcNAc residues. Analysis of the recently sequenced genome of S. parasitica was combined with quantitative mass spectrometry-based proteomics (label-free and iTRAQ) to characterize the plasma membrane proteome of hyphal cells. This strategy allowed us to experimentally identify a total of 677 plasma membrane proteins, including several key cell wall polysaccharide synthases, e.g. cellulose, 1,3-β-glucan and chitin synthases, some of which are specifically enriched in plasma membrane microdomains similar to lipid rafts in animal cells.

Cell wall biology to illuminate mechanisms of pathogenicity in *Phytophthora infestans*.

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The cell wall is a dynamic extracellular compartment protecting the cell, providing rigidity, and playing an essential role in the uptake of molecules and signalling. In pathogenic organisms, the cell wall is at the forefront of disease, providing contact between the pathogen and host. Using a multidisciplinary approach, we seek to understand the role of the cell wall in oomycete disease, both as a communication centre with the host organism and as a compartment that is continually reshaped and strengthened throughout the lifecycle, to penetrate and colonise the host. Understanding these mechanisms in more detail will pave the way for better control of oomycete diseases. We are combining novel chemical genomics approaches with state-of-the-art biochemistry and biophysics to study the cell wall and to develop new anti-oomycete drugs. *Phytophthora infestans* (*Pin*) produces a variety of spores and infection structures that are essential for disease development throughout its lifecycle. In particular thick-walled sporangia release wall-less motile zoospores that rapidly synthesise a cell wall upon contact with host plant cells. These cysts further differentiates to produce appressoria which build up turgor pressure and act as a focal point for cell wall degrading enzymes to penetrate the host cell. A highly strengthened cell wall is thus essential for the onset of infection. Here we present the results of our detailed biochemical analyses, using GC-MS and methylation analysis to determine the neutral sugar composition and glycosidic linkages of the cell wall structural carbohydrates present at these key points in the lifecycle. Having previously established an essential role for a cellulosic cell wall in appressorium production and infection of potato by *Pin* (Grenville-Briggs et al 2008), we are now working to elucidate the precise functions of the individual cellulose synthase (CesA) enzymes. Silencing each CesA using RNAi reveals overlapping functions with subtle differences in phenotype. These results will be presented. Since the genome of *Pin* also contains a putative chitin synthase, but hyphal cell walls are devoid of measurable chitin we are also investigating the role of this gene in the *Pin* cell wall and in pathogenicity and here we present our latest findings.

Phytophthora-Associated Molecular Patterns-triggered immunity and resistance to the root pathogen *Phytophthora parasitica* in *Arabidopsis*.

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The perception of pathogen-associated molecular patterns (PAMPs) contributes to plant resistance to pathogens. To get a deeper insight into the role of PAMP responses in plant-*Phytophthora* interactions, we used the *P. parasitica* PAMP Cellulose Binding Elicitor Lectin (CBEL) to identify *Arabidopsis thaliana* accessions and mutants impaired in CBEL responses. Upon screening an *A. thaliana* ecotype collection, a CBEL-responsive line (Col-0) and CBEL-insensitive lines (Ws-4, Oy-0 and Bla-1) were selected for further analyses. The three insensitive lines were unable to mount a necrotic response after CBEL treatment and Ws-4 and Oy-0 were also impaired in the production of an NADPH oxidase-dependent oxidative burst and expression of defense genes, while Bla-1 was only partially affected in these responses. CBEL-induced responses were also analyzed in the NADPH oxidase double mutant Atrboh D/F as well as in the bak1-4 line, mutated in the LRR-receptor –like BAK1 gene which was shown to be a central regulator of plant immunity. These two lines were still able to develop a necrotic response upon CBEL treatment but were severely affected in the production of ROS and induction of defense genes. Root infection assays were performed using the compatible *P. parasitica strain* Pp310 and the incompatible strain Ppn0. While all the lines tested were equally well colonized by the Pp310 strain, AtrbohD/F and bak1-4, but not Ws-4 and Oy-0, displayed a significant increase in *P. parasitica* Ppn0 susceptibility. Overall, our results show that CBEL-triggered immunity required the BAK1 and AtRBOH genes which also control resistance to the non-adapted *P. parasitica* strain Ppn0 strain. However, natural variability in CBEL responses is not correlated to the outcome of the interaction suggesting that other mechanisms are involved in *Phytophthora* resistance.

Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers.

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Phytophthora infestans is the causative agent of late blight and was responsible for the Irish famine in the 1840's. Today it still continues to be a global problem and in the USA it has been reported that the economic loss on potato crops alone exceeds $6 billion per year. A successful phytopathogenic relationship depends on the ability of the organism to adapt its metabolism during infection on various nutritional substrates (e.g., plant versus tuber) and at different times throughout infection when nutrients may be limiting. Investigation of this metabolic adaptation is key to understanding how *P. infestans* succeeds as a pathogen. To do this, tomato plants and potato tubers were infected with zoospores using a "dipping" method. RNA was extracted at 3 days post inoculation (dpi) and 6 dpi and subsequently used in library preparation. Following this, the libraries were quality checked by analysis on a Bioanalyzer using a high sensitivity DNA chip. Using Illumina technology (50 bp, paired-end reads) RNA Sequencing was performed. For each sample an average of 262 million reads was obtained. As a reference for the in planta data, RNASeq was also performed on defined and complex media. Mining of the data shows that the expression profiles of some pathways change, such as glycolysis and gluconeogenesis from the carbohydrate metabolic pathway, and fatty acid elongation and metabolism from the lipid metabolic pathway. Learning how metabolic adaptation occurs will prove useful in the development of novel control strategies for this plant pathogen.

Involvement of RNA polymerase I in mefenoxam insensitivity in *Phytophthora infestans*

Stephen C. Whisson[1], Eva Randall[1], Vanessa Young[1], Paul R.J. Birch[2], David E.L. Cooke[1], Michael Csukai[3]

([1]Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee, United Kingdom; [2]Division of Plant Sciences, College of Life Sciences, University of Dundee at the James Hutton Institute, Invergowrie, Dundee, United Kingdom; [3]Syngenta Jealott's Hill International Research Center, Bracknell, United Kingdom)

Metalaxyl, containing the active compound known as Mefenoxam or Metalaxyl-M, has been a significant compound for controlling oomycete-incited plant disease for over 30 years. Insensitivity to this chemical was recorded early in its usage history, but the underlying mechanism has proven difficult to determine. Here, genetic crosses between mefenoxam sensitive and insensitive isolates of *Phytophthora infestans*, the potato late blight pathogen, were established. F1 progeny showed the expected semidominant phenotypes for mefenoxam insensitivity. However, the small F1 progeny size precluded use of positional cloning for identifying the gene conditioning insensitivity to mefenoxam. Instead, a candidate gene strategy was used, based on previous observations that the primary effect of mefenoxam was to inhibit ribosomal RNA synthesis. The subunits of RNA polymerase I (RNApolI) were sequenced from sensitive and insensitive isolates and F1 progeny. Single nucleotide polymorphisms (SNPs) specific to insensitive isolates were identified in the gene encoding the large subunit of RNApolI (RPA190), with one SNP, showing an 86 % association with Mefenoxam insensitivity. Isolates not showing this association belonged predominantly to one *P. infestans* genotype. Transfer of the 'insensitive' allele of *RPA190* to a sensitive isolate yielded transgenic lines that grew slower than the non-transgenic wild-type, but were insensitive to Mefenoxam. These results demonstrate that sequence variation in RPA190 contributes to insensitivity to Mefenoxam in *P. infestans*.

Poster Presentations

Presentations organized alphabetically
Presenting author(s) underlined.

Novel quantitative trait loci for partial resistance to *Phytophthora sojae* mapped in two recombinant inbred soybean populations

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Stem and root rot, the second major yield reducing disease in soybean, is caused by the oomycete pathogen Phytophthora sojae. With the emergence of new pathogen races, managing the disease by planting cultivars with single-gene mediated resistance is becoming ineffective. Therefore, incorporation of partial resistance (PR) that provides broad-spectrum, low level of root resistance against all physiological races of the *P. sojae*, would provide a more durable form of disease management. The main objectives of this study were to identify molecular markers linked to quantitative trait loci (QTL) for PR to *P. sojae* , and to validate the rice method as a more objective screening method for PR. Two recombinant inbred line populations developed by crossing the plant introduction, PI399036 (high PR), with two germplasm lines, AR2 and AR3 (low PR), were screened for PR using the rice method. Two potentially novel QTL were identified in the chromosomes 5 and 13, which accounted for 30% and 34% of the phenotypic variation in the AX20931 population, respectively. Another QTL that accounted for 12% of the phenotypic variation was mapped in the same population, to a region in chromosome 15 where a previous QTL has been reported. One potentially novel QTL in chromosome 11, which accounted for 8% of the phenotypic variation was detected in the AX20925 population.

Cdc14 association with basal bodies in the oomycete *Phytophthora infestans* indicates potential new role for this protein phosphatase.

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The dual-specificity phosphatase Cdc14 is best known as a regulator of cell cycle events such as mitosis and cytokinesis in yeast and animal cells. However, the diversity of processes affected by Cdc14 raises the question of whether its cell cycle functions are truly conserved between species. Analyzing Cdc14 in the lower eukaryote *Phytophthora infestans*, a member of the oomycete group of the Stramenopile kingdom, should provide further insight into the role of Cdc14 since this organism does not exhibit a classical cell cycle. Prior study in this organism already revealed differences in Cdc14. For example, instead of being post-translationally regulated like its fungal and metazoan relatives, PiCdc14 appears to be mainly under transcriptional control. It is absent in vegetative hyphae where mitosis occurs and expressed only during the spore stages of the life cycle which are mitotically quiescent. Since transformants overexpressing PiCdc14 exhibit normal nuclear behavior, the protein likely does not play a critical role in mitotic progression although PiCdc14 is known to complement a yeast Cdc14 mutation that normally arrests mitosis. Further investigation into the role of PiCdc14 uncovered a novel role. Subcellular localization studies based on fusions with fluorescent tags showed that PiCdc14 first appeared in nuclei during early sporulation. During the development of biflagellated zoospores from sporangia, PiCdc14 transits to basal bodies, which are the sites from which flagella develop. A connection between Cdc14 and flagella is also supported by their phylogenetic distribution, suggesting an ancestral role of Cdc14 in the regulation of basal bodies or development of flagellated cells. Further studies such as colocalization with known basal body/centrosome markers and the identification of its interacting partners might help unravel the link between PiCdc14 and the flagellar apparatus.

Comparative Analysis of Avr1a Effector Locus in the Oomycete Plant Pathogen, Phytophthora sojae to Characterize Variation in Copy Number, Expression, and Sequence Diversity Among Isolates from Iowa and Ohio.

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Phytophthora sojae has reemerged as a prominent pathogen in some areas of the Midwest due to the pathogen's ability to adapt to many of the resistant (*Rps)* genes, deployed in soybean cultivars. Prior research identified several *Avr* genes namely, *Avr1a*, *Avr3a* and *Avr3c*, and showed they belong to the RXLR family of effectors. Several mechanisms by which these effectors may contribute to changes in virulence in pathogen populations were proposed including the copy number variation of avirulence genes, differential regulation of the transcription of the genes and changes in amino acid composition of the proteins. However, this research only evaluated a few standard isolates. We evaluated diversity within the *Avr1a* locus across more than 220 isolates of *P. sojae* recovered from diseased soybean plants in Iowa and Ohio. The number of copies of *Avr*1a ranged from zero to two among the isolates. Furthermore different levels of expression of the gene were detected among isolates. To determine the variability around the *Avr1a* gene region, gene specific primers and a protocol for long-range amplification of the gene were developed. Four and 21 isolates of *P. sojae* from OH and IA, respectively, were selected to do a targeted re-sequencing in 10kb covering the *Avr1a* gene. A clearer understanding of the evolution of *Avr* genes to escape recognition by the corresponding *Rps* gene will lead to improved disease management practices to reduce losses due to this economically important pathosystem.

Molecular phylogentic analysis of *Phytophthora nicotianae* isolated from ornamentals in Florida

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Various *Phytophthora* species infect a variety of foliar and woody ornamental plants and cause significant yield and quality loss. In this study, we have investigated genetic variability and fungicide resistance of a spatio-temporal population, infecting diverse genera of plants. These analyses show that a vast majority (74%) of these strains belong to *P. nicotianae*, followed by *P. tropicalis* (8.8%), *P. palmivora* (8.8%), *P. capsici* (6 %) and *P. cinnamomi* (3%). ITS-based molecular phylogenetic analyses indicate that *P. parasitica* isolates recovered from ornamental plants represent a highly diverse population. For managing fungicide-resistance, it is important to determine prevalence of *Phytophthora* isolates that are insensitive to mefenoxam, a commonly used fungicide for controlling *Phytophthora* diseases. In mefenoxam sensitivity assays, we found moderate to complete insensitivity in several isolates. In addition, several isolates were insensitive to additional fungicides with different mode of actions with some insensitive to multiple fungicides. These studies suggest that a genetically diverse *Phytophthora* population infect ornamental crops and that the build up of fungicide-insensitive *Phytophthora* populations raises concerns about disease management in ornamentals. Mitigating the issue of fungicide resistance will require prudent fungicide resistance management strategies including rotation of chemicals with different modes of actions.

Optimizing protoplast production and transformation of *Phytophthora cinnamomi*.

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Phytophthora cinnamomi is a soil-borne plant-pathogenic oomycete, and is responsible for Phytophthora Root Rot (PRR) and stem cankers in *Persea americana* (avocado) and other crops. Tolerant avocado rootstocks are the major control measure used to limit the devastating effects of PRR on avocado production world-wide. The mechanism underlying tolerance or susceptibility in rootstocks is still unknown, and thus an improved understanding of the interaction between *P. cinnamomi* and its host will aid the identification of resistance mechanisms in tolerant rootstocks. Pathogens tagged with fluorescent proteins are widely used to study host-pathogen interactions in planta. A fluorescently-tagged strain of *P. cinnamomi* will serve as a useful tool to aid in unravelling this complex interaction. The aim of this study was to optimize protoplast production and transformation of *P. cinnamomi*. The growth conditions of cultures prior to digestion (cultures were starved for one day prior to digestion), digestion conditions (increases in digestion time and mannitol concentration in enzyme digestion buffer) and steps involved in harvesting the protoplasts were optimized for *P. cinnamomi*. This allowed for the successful release of protoplasts from mycelia of *P. cinnamomi*. A PEG-and Lipofectin-based protocol was used to transform protoplasts with plasmid DNA containing the Green Fluorescent Protein (GFP) gene. Following incubation of regenerated protoplasts, fifty colonies appeared on the selection plates. A GFP-specific PCR confirmed the presence of the GFP gene in two of these colonies, and these colonies will further be characterized for levels of fluorescence and stable maintenance of the transgenes.

Measuring oomycete biodiversity in aquatic, forest, and agricultural ecosystems: culture-based and metagenomic approaches.

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The fungal-like oomycetes include a number of devastating pathogens, such as the notorious potato late blight agent, *Phytophthora infestans*, and the cause of rare human pythiosis, *Pythium insidiosum*; it is not surprising then that most research has focused on host-pathogen interactions, with relatively little study concerning the natural diversity of saprotrophic species. This knowledge gap has important ramifications on our estimates of oomycete biodiversity, as well as on our understanding of the evolutionary history of this important group. The goal of this study is to estimate and compare oomycete biodiversity from several distinct habitats, including aquatic environments, undisturbed forest soils, and highly managed agricultural settings. Our on-going culture-based surveys have relied on various baiting techniques and have revealed a high level of diversity in aquatic and agricultural environments. However, it has been shown that baiting methods can lead to biased estimates of biodiversity as they tend to favor fast-growing organisms and those producing motile zoospores. We have therefore developed a complementary metagenomic approach using massively parallel pyrosequencing to more thoroughly sample species diversity from the different environments. We are currently verifying the mitochondrial cytochrome c oxidase subunit 1 (cox1) locus as our sequencing target; previous studies have shown that this locus is able to discriminate among closely related species, and provide phylogenetic signal at a number of taxonomic levels. We expect that this combination of culture-based and sequence-based identification methods will enhance our understanding of oomycete ecology and evolution, and perhaps give us more insight into the roles of certain species in the outbreak of disease.

Artificial miRNA constructs for *Phytophthora sojae* transformation.

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Phytophthora, a genus of fungal-like oomycetes, contains some of the most devastating plant pathogens, causing multi-billion dollar damage to crops, ornamental plants, and natural environments. The genomes of five *Phytophthora* species, including the soybean pathogen *P. sojae*, have recently been sequenced, with many more species soon to be completed. Gene regulation by small RNA pathways is highly conserved among eukaryotes, although little is known about small RNA pathways in the Stramenopile kingdom. Two Dicer homologs, DCL1 and DCL2, and one RDR homolog were cloned and annotated from *P. sojae*, and gene expression analysis revealed only minor changes in transcript levels among different lifestages and infection timepoints. At this point, the role of the two oomycete Dicer homologs are only speculated. This study aims to down-regulate DCL1 and DCL2 expression in order to analyze the contribution of each homolog to small RNA biogenesis. Traditional RNAi, such as overexpression of RNA complementary to a target mRNA transcript, has been used to knockdown gene expression in *Phytophthora*, although the effect is most often short-lived. Dicer homologs are involved in the RNAi pathway, therefore this method may not be effective, especially for the homolog involved in the siRNA pathway. Artificial miRNAs, designed from endogenous miRNAs, have recently been used to target transcripts such as these. We designed artificial miRNA constructs based on the conserved *Phytophthora* miRNA found in *P. sojae*, targeting both DCL1 and DCL2 as well as the effector *Avr1k*, the histidine biosynthesis enzyme HISG, and GFP for controls. Analysis of transformants is currently underway.

Alternative splicing provides a possible mechanism for *Pseudoperonospora cubensis* transcriptome regulation.

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RxLR effector proteins are secreted by oomycete pathogens into plant cells to manipulate host cellular processes. The genome of *Hyaloperonospora arabidopsidis* (Hpa, downy mildew of Arabidopsis) contains at least 134 candidate RXLR effector genes. Only a small subset of these genes is conserved in related oomycetes from the *Phytophthora* genus. We are undertaking a comparative functional characterization of the Hpa RXLR effector gene HaRxL96 and a homologous gene, PsAvh163, from the soybean pathogen *Phytophthora sojae*. HaRxL96 and PsAvh163 are induced during early stages of infection and carry a functional RXLR motif that is sufficient for protein uptake into plant cells. Both effectors can suppress immune responses in soybean. HaRxL96 suppresses immunity in *Nicotiana benthamiana*, while PsAvh163 induces an HR-like cell death response in *Nicotiana* that is dependent on RAR1 and Hsp90.1. Transgenic Arabidopsis plants expressing HaRxL96 or PsAvh163 exhibit elevated susceptibility to virulent and avirulent Hpa as well as decreased callose deposition in response to non-pathogenic *P. syringae*. Both effectors interfere with defense marker gene induction, but do not affect salicylic acid biosynthesis. Together, these experiments demonstrate that evolutionarily conserved effectors from different oomycete species can suppress immunity in plant species that are divergent from the source pathogen's host. We will present data from experiments to establish the subcellular site of action and virulence targets of these proteins.

Chromatin immunoprecipitation (ChIP) for *Phytophthora sojae*.

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Modifications of histone tails are known to play an important role in gene regulation. Chromatin immunoprecipitation (ChIP) is one of the powerful assays used to identify specific protein–DNA interactions in vivo. ChIP involves fixing cells with formaldehyde and then precipitating transcription complexes using antibodies. Once the cross-link between proteins and DNA is broken, DNA fragments can be subjected to PCR in order to identify specific regions that interact with proteins of interest. When combined with microarray or high throughput sequencing, establishment of the genome-wide location of histone modifications can be acquired. In this study, we have developed an improved protocol that is simple, rapid and highly efficient in probing multiple proteins that interact directly and indirectly to DNA in a single experiment.

Effect of heterotrophic bacteria communities on *Pythium* spp. in recycled irrigation water

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Pythium species are among the most damaging pathogens in herbaceous ornamental plants causing damping-off, root and stem rots. They are ubiquitous in nature. A main concern in commercial greenhouses is the harboring of *Pythium* species in recycled irrigation water that may spread throughout the production facility into susceptible crops. Occasionally, pathogenic *Pythium* spp. are isolated from recycled irrigated water in commercial greenhouses in PA. This raises the question of possible microbial suppression of *Pythium* present in recycled irrigation water. An understanding of the interaction among these oomycetes and the microbial community present in this ecosystem is vital for establishing a long term management strategy. Few studies have examined the impact that *Pythium* spp. have on the bacteria communities in water. Changes in bacterial community composition may be associated with the presence of *Pythium* species in the water. The present study focused on the deleterious effect that microbial communities in recycled irrigation water had in the development of *Pythium aphanidermatum*, *P. irregulare* and *P. cryptoirregulare*. In addition, automated ribosomal intergenic spacer analysis (ARISA) was employed to examine the impact that the presence of *P. aphanidermatum*, *P. irregulare*, or *P. cryptoirregulare* had on bacteria diversity in recycled irrigated water. Results provided evidence that microbial communities residing in recycled irrigation water had a deleterious effect on *Pythium aphanidermatum*, *P. irregulare* and *P. cryptoirregulare*. In addition, profiles obtained using ARISA indicated that a shifting of community composition was *Pythium*-species specific. Results suggested that *Pythium* presence influenced bacterial community composition.

Cloning and expression of the pyrimidine enzymes aspartate transcarbamoylase and uridine monophosphate Synthase of an oomycete plant pathogen.

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Current control of *Phytophthora infestans* is difficult since the pathogen quickly gains resistance against traditionally used chemicals. Due to the existence of common characteristics and phylogenetic relationships shared between apicomplexan parasites and oomycetes, our hypothesis is that druggable targets which are used to control these parasites might also work on *P. infestans*. Since inhibition of de novo pyrimidine biosynthesis has been shown to be a good target for apicomplexan parasites, our aim is to study this pathway in *P. infestans* and *S. tuberosum*, to determine which enzymes could be suitable for the design of inhibitors. Phylogenetic analyses suggested that the best candidate for inhibitor development is uridine monophosphate synthetase (UMP) because it appeared to have different evolutionary origins for *P. infestans* and *S. tuberosum*. In *P. infestans*, the UMP gene sequenced was duplicated in the genome and showed an inversed order when compared with *S. tuberosum* or human UMP. Although active site residues were predicted to be the same in the orotidine monophosphate decarboxylases (ODC) there were apparent differences in the active site residues of the orotate phosphoribosyltransferases (OPRT) from *P. infestans* and *S. tuberosum*. A full length UMP sequenced was cloned. Expression of this sequence and sequences coding for individual OPRT and ODC domains were found to be insoluble under a number of induction conditions. On the other hand, aspartate transcabamoylase (ATC) seems to be a less promising target, because phylogenetic analyses show no major differences for pathogen and host ATCs. Nevertheless, there appear to be differences near the catalytic sites and PiATC appears to have a predicted ATP binding site while StATC does not. We cloned and overexpressed the enzymes and found that the recombinant enzymes migrated near 38 kDa for PiATC and 45 kDa for the StATC. Preliminary measurements of the PiATC enzymatic kinetics show values of Vmax=435μmol.min^{-1}.mg^{-1} and Km=0,3mM.

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals plant pathogen-like effectors.

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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, Expressed Sequence Tags (EST) were generated using 454 pyrosequencing. To date, a total of 58,931 'Titanium chemistry' reads have been produced, and homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins. A full complement of plant pathogenic oomycete effector orthologs were identified. Rapid Amplification of cDNA Ends (RACE) PCR reactions were used to obtain the full-length cDNA sequences of selected crinkler, elicitin and CBEL transcripts. Computational analyses predicted that the selected *L. giganteum* effector proteins are secreted and have similar domains than the Phytophthora spp. effectors, indicating that they may be involved in the pathogenicity process. In particular, the CBEL (Cellulose Binding Elicitor Lectin) orthologs contain the alternating Cellulose Binding and PAN/APPLE modules that have been associated with attachment to host tissue. The crinkler orthologs are characterized by a modular organization that includes the conserved LxLYLAR/K and HVLVxxP N-terminal motifs previously described for *Pythium ultimum* crinkler proteins. The roles of these effectors in the oomycete-insect host pathosystem are under investigation.

DNA Methylation in *Phytophthora sojae*.

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DNA methylation is a common epigenetic mechanism associated with transcriptional gene silencing in eukaryotes. Treatment of *Phytophthora sojae* with 5-aza-2'-deoxycytidine, a DNA methyltransferase inhibitor, showed alteration in hyphal and zoospore behaviors as well as pathogenesis on soybean host plant. Evidence of DNA methylation using bisulfite sequencing and immunoprecipitation methods will be presented.

The Arabidopsis pathogen *Hyaloperonospora arabidopsidis* manipulates the jasmonic acid signaling pathway.

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Plant pathogens have evolved mechanisms to subvert plant immunity and colonize their hosts. In response, plants have co-evolved sophisticated surveillance mechanisms to recognize invading pathogens that are dependent on hormone signaling to effectively mount a defense response. In general, salicylic acid (SA) mediated-defense confers resistance to biotrophic pathogens, including oomycetes and bacteria, while jasmonic acid (JA) confers immunity to necrotrophic pathogens. In some pathosystems, SA and JA are antagonistic. Previous studies indicate that certain bacterial and fungal plant pathogens exploit this antagonism to promote virulence. The oomycete *Hyaloperonospora arabidopsidis* (Hpa) is an obligate biotrophic pathogen of *Arabidopsis thaliana*. We studied the importance of JA biosynthesis and signaling for Hpa virulence. Hpa growth was reduced in mutants compromised in JA signaling, suggesting that JA signaling is necessary for full Hpa pathogenicity. Furthermore, searches in a protein interaction network revealed that the JA regulator JAZ3 is targeted by an Hpa RXLR effector. We will describe data that validate this interaction and suggest that effector-mediated manipulation of JAZ proteins is utilized by Hpa to hijack JA signaling and suppress SA-mediated defenses.

Effectoromics to Improve Resistance against *Phytophthora sojae* in Soybean

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Oomycete pathogens cause billions of dollars in crop losses each year. *Phytophthora sojae*, the causal agent of soybean root and stem rot, is responsible for soybean losses estimated at $1-3 billion worldwide and $420 million in the US (2008). These losses are rising because resistance (R) genes, which have been utilized for decades in soybean cultivars, are being defeated by pathogen co-evolution. In this project, we are using a high-throughput, effector-directed breeding strategy, pioneered against *P. infestans*, to identify and breed R genes against *P. sojae* into elite soybean cultivars. The disadvantages of R gene mediated resistance are that resistance is often race-specific and that avirulence loci often encode effectors that can be modified or discarded with little or no impact on virulence. To overcome these problems, we aim to identify R genes that recognize essential effectors which cannot be discarded without a fitness penalty to the pathogen. As a first step in this screen, we are developing a system to transiently deliver effector proteins to the interior of soybean cells. To test different delivery systems, we are delivering known *P. sojae* avirulence effectors into soybean leaves that contain a corresponding R gene. The goal is to produce a visual hypersensitive response (HR) to the delivered effector. Our most candidate system uses a strain of *Pseudomonas fluorescens* called EtHAN to deliver effectors through a genetically engineered type III secretion system. To improve the consistency and intensity of the visual signal, we co-infect soybean leaves with *P. syringe pv glycinea* (helper strain) to disrupt plant PTI responses that inhibit the growth of EtHAN. We are currently using this system for high-throughput screens of *G. max* germplasm to identify resistance genes against essential effectors. Our ultimate goal is to breed these genes into elite soybean cultivars.

Effector Directed Breeding to Identify Robust Resistance Genes Against *Phytophthora Sojae* in Legumes.

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Soybean root and stem rot causes billions of dollars in crop losses worldwide. This disease thrives in hot, wet climates and is caused by the oomycete pathogen *Phytophthora sojae*. Root and stem rot caused an estimated 420 million dollars in crop losses as of 2008, and these losses are rapidly increasing due to pathogen co-evolution. Resistance (R) genes against *P. sojae*, currently employed in the field, are being quickly defeated by the pathogen. Unfortunately, there are very few R genes available and most known R genes were produced by years of traditional breeding. The goal of this project is to discover new and robust R genes against *P.sojae* in *G. max* relatives and non-host legumes. Ultimately, we would like to clone these resistance genes into elite soybean cultivars. We have developed a high-throughput screening system in *G. max* for identification of resistance genes against *P. sojae*. This system uses a strain of *Pseudomonas fluorescens* called EtHAN to deliver effectors through a genetically engineered type III secretion system. Co-infiltration with *Pseudomonas syringe pv glycinea* (helper strain) improves throughput in our system by disrupting plant PTI responses which inhibit the growth of EtHAN. Currently, we are applying this system for use in *Glycine soja*, *Phaseolus vulgaris*, and *Medicago sativa*. After adapting our high throughput screening system for use in these legumes, we will look for resistance genes against "essential" *P. sojae* effectors. Essential effectors are monomorphic, highly expresses, and they cannot be discarded without a fitness penalty to the pathogen. By looking outside of the *P. sojae* host range, we hope to identify durable resistance genes that prevent *P. sojae* infection.

A hypotaurocyamine kinase from the protozoan *Phytophthora sojae* : Implications for the evolution of the phosphagen kinase family.

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Phosphagen kinases (PKs) are a family of enzymes that are distributed throughout the animal kingdom as well as some protozoan and bacterial species. In animal cells, PKs have been shown to play a critical role in energy homeostasis by catalyzing the reversible transfer of a high-energy phosphoryl group from ATP to an acceptor molecule containing a guanidinium group. Using sequence homology searches, a putative PK gene was identified in a protozoan, the oomycete *Phytophthora sojae*, that was predicted to encode a dimeric enzyme. The recombinant PK exhibited taurocyamine kinase activity and a complex quaternary structure. This is the first report of a nonarginine-substrate specificity within the protozoa and has implications for the evolution of substrate specificity and quaternary structure in the PK family. Specifically, these data suggest that dimeric PKs evolved much earlier than previously thought and indicates that protozoan PKs may exhibit diversity in substrate specificity that was previously thought to exist only in annelida. Finally, the taurocyamine knase seen in all Phytophthora represent potential targets for pest control strategies.

Characterization of genetic diversity of three species of *Pythium* from forest nursery soils in Oregon and Washington.

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Pythium species include some of the most important soilborne pathogens associated with damping-off, limiting conifer seedling production in forest nurseries of the Pacific Northwest US. The aim of this study was to assess the genetic diversity of *Pythium* [*P. irregular* (*Pir*)*, P. sylvaticum* (*Psy*)*, and P. ultimum* (*Pul*)] isolated from soil at three forest nurseries in OR and WA. Two molecular marker methods were used: Simple Sequence Repeats (SSR), and Amplified Fragment Length Polymorphisms (AFLP). AMOVA/ PhiPT, based on geographic distribution suggest that *Pir and Psy* have significant genetic diversity, whereas no significant differences among *Pul* populations were found. *Psy* was the most genetically diverse while *Pir* and *Pul* were moderately and weakly diverse, respectively. This is consistent with their sexual reproduction: *Psy* is heterothallic and individuals are self-incompatible whereas *Pir* and *Pul* are homothallic and self-compatible. The low genetic differentiation index suggests that *Pul* is highly clonal and its presence in nurseries from OR and WA may be the result of a recent introduction. The allelic richness, including private alleles, and the average number of migrants between populations per generation (Nm), which are congruent among methods, suggest that there is genetic flow between populations within species for *Pir* and *Psy*. In these species, isolate distribution by Principal Coordinate Analysis and UPGMA were consistent among methods, but provided no clear evidence of geographically-defined populations in either species. Instead, significant intraspecific variation, unrelated to nursery of origin, was observed in *Pir* (2 groups), *Psy* (3 groups), and *Pul* (2 groups). This study indicates that either SSRs or AFLPs markers could serve as a useful tool in the characterization of genetic diversity and help us determine the population structure of *Pythium* spp. Local strains of *Pir* and *Psy* may be present but further analyses are needed to distinguish native populations from recently-introduced strains in the three nurseries studied.

Cell-death: a conserved resistance mechanism of marine brown algae against the oomycete *Eurychasma dicksonii*.

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The hypersensitive response (HR) has been the cornerstone of plant pathology for over a century. In contrast, the innate immunity of brown algae (*Phaeophyta*) is poorly known. This phylum encompasses key primary producers of temperate and cold coastal seas, and diverged from plants and animals over a billion years ago. Here we show that across ten brown algal species, disease resistance against the oomycete pathogen *Eurychasma dicksonii* is mediated by hypersensitive cell death. It is accompanied by cell-wide b1-3 glucan and fluorescent metabolites deposition, expression of a programmed cell death (PCD) marker, and often followed by tissue regeneration according to a defined developmental program. Our findings reveal striking similarities of brown algal innate immunity with other distantly related eukaryotes. They also have wide ranging implications for understanding the dynamics of marine ecosystems, as well as for the exponentially-growing seaweed aquaculture industry.

Host spectrum determination of closely related legume-root infecting *Phytophthora species* and correlation with zoospore chemotaxis.

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Oomycetes are fungal like organisms; some of them cause destructive diseases in different economically important crops. Although some species have a broad host range, others show host specificity. *Phytophthora sojae* and *P. vignae* are reported to be specific on soybean and vignabean, respectively. *P. pisi* and *P. niederhauserii* are putative novel species, which and their host range among legume is unknown. Zoospores of some *Phytophthora* species are chemotactically attracted to the isoflavones that are secreted by their host plants. The focus of the current study was to determine the host spectrum of the closely related root-infecting *Phytophthora* species among legume plants and to find the correlation to zoospore chemotaxis. The pathogenicity of *P. sojae*, *P. vignae*, *P. pisi* and *P. niederhauserii* to pea, soybean, vignabean, fababean (variety Laura and Tatto), lentil and clover was tested. Two criteria, root symptoms and dried shoot weight reduction, were used to assess the disease severity. *P. sojae, P. vignae and P. pisi* showed high specificity towards soybean, vignabean and pea respectively, whereas P. niederhauserii has a broader host range. The zoospore chemotaxis of *Phytophthora* species against prunetin, genistein and daidzein was tested. *P. pisi* and *P. niederhauseii* showed attraction to the pea-specific compound prunetin, whereas, zoospores of *P. sojae, P. vignae* and *Aphanomyces euteiches* showed attraction to all tested isoflavones. These results suggest that the differential chemotactic response of zoospores to plant isoflavones might be a factor affecting host specificity.

Characterization of a member of the Polyamine Uptake Transporter family of *Phytophthora parasitica*.

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In the soil environment, polyamines and other metabolites are released as a consequence of lysis of bacteria by phage, but their concentration in this environment may be low due to their rapid acquisition by other organisms. In earlier work (Chibucos and Morris 2006) zoospores from *Phytophthora sojae* were shown to acquire the polyamines putrescine and spermidine from the surrounding medium. While PA are essential metabolites for all organisms it is not understood why zoospores would specifically deploy selective uptake transporters for these compounds. Phylogenetic analysis of *Phytophthora parasitica* transporters has identified eight transporters that form a clade with characterized polyamine uptake transporters from *A. thaliana* and rice. Notably, seven of the eight genes were up-regulated in the zoospores relative to transcript levels in mycelia and infected tissue. From this family, PPTG_00424 has been selected to test whether it also has polyamine uptake transporter activity. PPTG_00424 has been codon optimized for expression in yeast. This gene will be expressed in a yeast mutant AGP2. A combination of toxicity and radioisotope uptake assays will be used to assess the transport activity of this gene.

Diverse classes of small RNAs originating from genomic hotspots, tRNA and the mitochondrial genome in *Phytophthora infestans*

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Phytophthora infestans is causing late blight disease on potato and tomato. *P. infestans* is notorious for its ability to evolve to overcome resistant potato varieties. The genome of this pathogen has been sequenced and revealed vast numbers of transposon sequences, and hundreds of disease-promoting effector proteins. We are aiming at understanding gene-silencing mechanisms in *P. infestans* including deciphering roles of small non-coding RNAs. In our previous work we have shown that *P. infestans* has an active RNA silencing pathway (Vetukuri et al. 2011). We also performed deep sequencing of sRNAs from *P. infestans* and knocked down the genes encoding the RNA silencing components Argonaute and Dicer in order to investigate their roles in sRNA biogenesis (Vetukuri et al. 2012). Here, we describe the distribution of genomic sites from which sRNAs originate. Genome-wide analysis of sRNAs revealed diverse classes mapping to genomic sources such as tRNAs, rRNAs, genomic sRNA 'hotspots', and the mitochondrial genome. Most tRNA-derived RNA fragments (tRFs) mapped to the sense strand of the 5'-halves of mature tRNAs and peaked at 27 and 30 nt lengths. In accordance with reports from other organisms (Franzén et al. 2011), the tRFs mapped to different tRNA isoacceptors with unequal frequencies, the Ile_tRNA_Cluster_0 showing the highest proportion of mapping sRNAs. We are presently using our Dicer knockdown transformants to investigate the tRF biogenesis mechanism. Another interesting group of sRNAs are those that map to transposons that have close-by neighboring RXLR-effector genes, indicating that RNA silencing may play a role in regulation of this important effector gene. Over a hundred additional predicted genes were found to be sRNA hotspots in our data: Crinkler effector genes, arrays of duplicated genes, potentially antisense overlapping transcripts, and genes containing transposon insertions. Our present task is to reveal the role that sRNAs might play in their regulation.

Cell wall biology to illuminate mechanisms of pathogenicity in *Phytophthora infestans*.

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Isolates of *Phytophthora ramorum* belonging to the NA1 clonal lineage display a large variation in colony morphology and virulence. Isolates originating from oaks (*Quercus* spp.) are less virulent on oak seedlings and more likely to show irregular growth phenotype than isolates from California bay laurel (*Umbellularia californica*). Furthermore, derepression of hundreds of transposable elements (TEs) and down-regulation of Crinkler effector homologs were observed in the majority of oak isolates, but this expression pattern was rare in isolates from bay laurel. Because (1) no genetic subdivision has been detected between bay laurel and oak isolates, and (2) oak is a dead-end host and infection on oak is initiated from asexual propagules produced on nearby foliage host such as bay laurel, we hypothesized that *P. ramorum* derived from a foliar host undergoes host-dependent genetic/epigenetic alterations inside an oak host. The observed phenomenon may be described as host-induced phenotypic diversification (HIPD); stable phenotypic changes of the pathogen can occur inside host plants and the rate of change is host species dependent. We are currently cross-examining the genome, transcriptome and phenotypes of an isolate originating from bay laurel and those of the same isolate but which was artificially inoculated into and recovered from oak trunks. Nine months post inoculation, 20 percent of re-isolates (n=67) from oak showed colonies with altered morphology resembling to those seen in isolates from naturally infected oaks. Global mRNA profiling confirmed that some of the re-isolates growing in culture showed expression patterns typical for those isolated from oak trunks (i.e., derepression of TEs and repression of genes belonging to Crinkler effector family). HIPD can thus be experimentally reproduced, however further study is needed to uncover molecular mechanisms underlying the phenomenon as well as evolutionary implications of HIPD.

Investigating the function of a putative chitin synthase from *Phytophthora infestans*

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Phytophthora infestans is a plant pathogenic oomycete that causes potato late blight, a devastating disease associated with tremendous economic losses. In contrast to true fungi, oomycetes are traditionally described as cellulosic microorganisms. Indeed, in addition to other β-glucans, cellulose is a major polysaccharide in the mycelial cell wall of *P. infestans* while chitin and other N-acetylglucosamine (GlcNAc)-based carbohydrates are absent from hyphal walls. However, a putative chitin synthase gene (chs) is present in the genome. Bioinformatic analysis identified the C-terminal region of the predicted protein to be highly similar to glycosyltransferase family 2 proteins, such as fungal chitin synthases, while the N-terminal domain is more divergent. Orthologous putative chs genes are present in all sequenced oomycete genomes and phylogenetic analysis shows the oomycete gene products form a new clade separate from the fungal lineage. The *P. infestans* chs transcript is highly abundant in older mycelium. However, no chitin synthase activity was detectable in microsomal fractions assayed with radioactively-labeled UDP-GlcNAc, the natural substrate of chitin synthase. Suprisingly, hyphal growth was severely retarded in the presence of low micromolar concentrations of the chitin synthase inhibitor nikkomycin Z, a structural analogue of UDP-GlcNAc. Microscopic analysis of nikkomycin Z-treated hyphae revealed frequent tip swelling and bursting. Similarly, transient RNA-mediated silencing of the chs gene resulted in severely reduced growth, and hyphae showed a hyper-branched morphology with swollen tips. As a first step to determine the precise function of the *P. infestans* chs gene, we have cloned and expressed it in *Saccharomyces cerevisiae*.

Gene Networks in the *P.capsici S. lycopersicum* Pathosystem

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In order to dissect the molecular mechanisms of infection in the *P. capsici* x *S. lycopersicum* pathosystem we are leveraging high throughput sequencing technologies combined with cutting edge computational graph theory algorithms. In order to determine the effects of amino acid changing SNPs on these interactions we are employing a diverse panel of *P. capsici* isolates and sampling total transcriptomes at the biotrophic and necrotrophic stages of infection. Clique centric algorithms have been successful for dissecting molecular interactions in microarray data, however they have not yet been applied to RNA-seq experiments. RNA-seq data and microarray data collection is fundamentally different however the numerical representations of the data are synonymous. These considerations along with the experimental design including highly variable genotypes necessitates the development of a robust pipeline for pre processing prior to application of clique centric algorithms. Here we discuss current progress and results.

The signal recognition particle, PsSRP54, is involved in sexual development and soybean infection of oomycete plant pathogen *Phytophthora sojae*

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To successfully infect hosts, plant pathogens secrete effector proteins to suppress plant immunity. These secreted proteins should be translocated into the ER, which is the first step of the eukaryotic secretory pathway. Translocation of the polypeptide into the ER lumen can occur either cotranslationally or posttranslationally. Essential to this type of translocation is the presence of the signal recognition particle (SRP), that binds signal sequences by SRP54. However, the mechanism by which oomycete pathogens deliver effector proteins during plant infection remains unknown. PsSRP54 is a multidomain protein with ahelical bundle, a GTPase domain and a methionine-rich domain bound the signal sequence. PsSRP54 was up-regulation in five infection stages (1.5, 3, 6, 12, and 24 h post inoculation) of *P. sojae*, that indicated it may play an important role during host infection. PsSRP54 was not indispensable in growth, formation of sporangium and release of zoospores. Interestingly, the silenced mutants not only affected oospores development, but also reduced activities of extracellular enzymes. Silenced mutants were impaired in invasion of susceptible soybean, however these mutants were found an inability to elicit the hypersensitive reaction (HR) soybean differential hosts. To further determine whether silenced PsSRP54 affects secretion of effector proteins, we silenced PsSRP54 by dsRNA in Avr1b-overexpressed strain and found transformants of PsSRP54 greatly impaired Avr1b-mediated resistance to susceptible soybean, presumably due to a defect in secretion of the Avr1b protein. These results demonstrate the importance of PsSRP54 to the virulence of *P. sojae* and extracellular protein secretion.

Fine-scale population dynamics of *Phytophthora capsici* on Long Island, New York.

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Recent studies with *Phytophthora capsici* suggest mating type and virulence may vary in clonally propagated field isolates or sexual progenies that experience loss of heterozygosity (LOH). The mating-type switch appears to be primarily from the A2 to the A1 mating type. In this study, zoospore progeny from 7 A2 and 3 A1 isolates from the USA and China were analyzed for mating type changes. Between 24 and 100 single zoospore progeny were isolated and tested for mating type for each isolate. For the A2 isolates, three different mating types (A1, A2 and A1A2) were identified in the zoospore progenies of 6 isolates and one had progenies with the A1 and A2 mating type. None of the A1 zoospore progeny exhibited a mating type switch. The proportion of progenies with A1, A2 and A1A2 mating types differed for the A2 isolates and these preliminary results are congruent with the hypothesis that homozygous and heterozygous alleles control A1 and A2 mating type respectively, and LOH spanning mating type region during mitotic recombination might be responsible for mating type switch for A2 isolates. Further studies employing specific molecular markers are underway and the results will be discussed

Structure and function of RXLR effectors and their host targets.

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Plant pathogens are a continuous threat to global food security. The oomycetes are an important class of filamentous eukaryotic pathogens that delivers an arsenal of effector proteins into plant cells to promote infection and perturb plant immune systems. Hundreds of effector genes have been identified in the genomes of plant pathogenic oomycetes including *Phytophothora* and *Hyaloperonospora*. RXLR effectors make up a large family of cytoplasmic effectors and the effector protein is defined by a secretion signal peptide followed by an N-terminal domain defined by the RXLR (Arg-Xaa-Leu-Arg) consensus sequence and a C-terminal effector domain. Once inside the cell, many effector domains are thought to interact with the host proteins to promote disease. The structural basis for such interactions remains elusive and the molecular details of such interactions is poorly understood. In the present study we aim to understand the molecular details of how these effectors interact with the plant proteins and modulate plant immunity. Specifically, we aim to determine the three-dimensional structures of the effectors, their identified plant targets and possibly the complexes. Heterologous expression of RXLR effectors and their plant targets in *E. coli* has proven to be a challenge due to problems related to solubility and stability. By employing various expression vectors and expression hosts we could successfully express and purify some effector proteins. The recombinant protein will be used for structural and biophysical characterization of effector proteins and their host targets.

A survey of *Pythium* species affecting corn and soybean in Iowa.

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Cold, wet soils at planting increase the risk of stand loss in corn and soybean due to seedling disease. In order to maximize yield potential, farmers in Iowa are planting earlier each growing season before soils warm up. Furthermore, with more widespread use of minimum tillage and consequently more surface crop residue prolong the period that soils remain cold and wet. Not surprisingly, the prevalence of seedling disease in both crops has increased. Since there is limited data on what species cause seedling blight in Iowa, two surveys were done during the 2011 and 2012 growing seasons, to determine the causal organisms. Symptomatic corn and soybean seedlings were collected from 30 and 16 locations in Iowa, respectively, and root and mesocotyl pieces were plated on PARP-B. A total of 89 isolates representing 9 *Pythium* species were recovered from corn. The most prevalent species were *P. torulosum* (85 percent of the isolates) followed by *P. sylvaticum* (4 percent) and *P. lutarium* (3 percent). From soybean, 350 isolates were recovered representing 19 *Pythium* species. On soybean the three most prevalent species recovered were *Pythium oopapillium* (48 percent), *P. dissotocum* (10 percent), and *P. lutarium* (8 percent). Furthermore, *Pythium schmitthenneri*, which was described in 2011, was recovered from corn and identified using morphological characteristics and comparing the ITS region with species within clade E1 of the genus *Pythium*. The most prevalent *Pythium* spp. (*Pythium torulosum, Pythium oopapillium, Pythium lutarium, Pythium sylvaticum*) recovered from both surveys are currently being screened for pathogenicity on corn and soybean and fungicide sensitivity.

Mechanisms of nutrient transport from plants to biotrophic pathogens.

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Obligate biotrophic pathogens are absolutely dependent on living plant tissue for nutrient acquisition and cannot live apart from their hosts. Obligate oomycete pathogens have lost genes for assimilation of nitrogen and sulfur compared to their free-living relatives, suggesting that they acquire these nutrients in organic forms from their hosts. Reduced carbon acquisition is also critical for pathogens, and recent studies revealed that expression of glucose exporters of the SWEET family is increased in response to diverse pathogens, and some SWEET genes are directly induced by bacterial effectors. This project aims at investigating mechanisms of nutrient acquisition by pathogens, using the interaction of Arabidopsis with its naturally occurring, obligate pathogen *Hyaloperonospora arabidopsidis* (Hpa). We hypothesize that plant-encoded transporters of sugars and amino acids are co-opted by Hpa to facilitate nutrient acquisition. The first stage of the project consists of a reverse genetic approach to identify sugar and amino acid transporters necessary for colonization of Arabidopsis by Hpa. Mining of transcript data reveal large scale reprogramming of nitrogen metabolism and transport genes during Hpa colonization, and identified candidate transporter genes for reverse genetic tests. Several loss-of-function mutants, in amino acid transporter genes and SWEET genes, displayed reduced susceptibility to Hpa compared to wild-type plants. In addition, A SWEET-GFP protein re-localized to the haustorium, suggesting that it is involved in sugar transfer to the pathogen. These preliminary data validate the utility of our reverse genetic approach to identify transporter genes that are important for colonization by Hpa. We hope to lay groundwork for long-term strategies to test the utility of engineering pathogen non-responsive alleles of transporter genes that contribute to colonization.

Dynamics of the actin cytoskeleton in *Phytophthora infestans*

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The actin cytoskeleton is conserved among all eukaryotes and plays essential roles during many cellular processes. It forms an internal framework in cells that is both dynamic and well organised. The plethora of functions ranges from facilitating cytoplasmic streaming, muscle contraction, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. Oomycetes are filamentous organisms that resemble Fungi but are not related to Fungi. The two groups show significant structural, biochemical and genetic differences. One prominent lineage within the class of oomycetes is the genus *Phytophthora*. This genus comprises over 100 species that are all devastating plant pathogens threatening agriculture and natural environments. The potato late blight pathogen *Phytophthora infestans* was responsible for the Irish potato famine and remains a major threat today. Previously the actin organization has been studied in several oomycetes. Next to the common F-actin filaments and cables, cortical F-actin containing patches or plaques have been observed as in Fungi. However, only a static view was obtained. Here, we use an in vivo actin binding moiety labelled to a fluorescent group to investigate the actin cytoskeleton dynamics in hyphae of *P. infestans*. Our results provide the first visualisation of the dynamic reorganization of the actin cytoskeleton in oomycetes. In the future, this line will provide insight in the role of the actin cytoskeleton during infection.

Subcellular localization and kinase activity of GK4, a *Phytophthora infestans* GPCR-PIPK involved in actin cytoskeleton organisation

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For dispersal and host infection plant pathogens largely depend on asexual spores. Pathogenesis and sporulation are complex processes that are governed by various cellular signaling networks including G-protein and phospholipid signaling. Oomycetes possess a family of novel proteins called GPCR-PIPKs (GKs) that are composed of a seven trans-membrane spanning (7-TM) domain fused to a phosphatidylinositol phosphate kinase (PIPK) domain. Based on this domain structure GKs are anticipated to link G-protein and phospholipid signalling pathways. Our studies in the potato late blight pathogen *Phytophthora infestans* revealed involvement of one of twelve GKs (i.e. PiGK4) in spore development, hyphal elongation and infection. Moreover, ectopic expression in *P. infestans* of subdomains of PiGK1 and PiGK4 fused to a fluorescent protein showed that the GPCR domain targets the GKs to membranes surrounding different cellular compartments. To further elucidate the function of the PIPK domain we tested kinase activity of PiGK4 both in vivo and in vitro and analysed the relationship between PiGK4, phosphoinositide signaling and the organisation of the actin cytoskeleton using complementation in yeast combined with various live-cell markers.

Exploring *Phytophthora* RXLR effector functions using a yeast model.

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Phytophthora species include some of the most devastating pathogens of plants. During infection, these oomycete pathogens secrete effector proteins, many of which seem to be directed into the host cell by virtue of a N-terminal RXLR-dEER domain. Bioinformatics analysis has revealed that *Phytophthora* genomes may encode hundreds of these RXLR effectors. Outside of the N-terminal signal domain, however, RXLR effectors rarely exhibit amino acid similarity to each other or to previously characterized proteins; consequently, their roles in infection remain largely unknown. To complement the in planta screens for avirulence and virulence activities of RXLR effectors being conducted by others, we have implemented a yeast functional genomics approach, previously used to explore the function of bacterial effectors. We previously reported that a subset of *P. infestans* RXLR effectors inhibit yeast growth when overexpressed in yeast, presumably by targeting a conserved eukaryotic pathway. Expanding our previous screen, we are now assaying a panel of *P. sojae* RXLR effectors for growth inhibition in yeast. Subsequently, yeast functional genomic screens, including transcriptome analysis, can be used to identify the targets of *Phytophthora* effectors in yeast, and presumably the natural plant hosts.

Comparative analyses suggest that transposable elements may differently impact the *P. parasitica* and *P. infestans* genomes

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Phytophthora species are responsible for important plant diseases on a wide range of cultivated crops, forestry and natural ecosystems worldwide. They greatly differ in their physiology, genetics and pathogenic strategies. They also diverge in their genome size and predicted gene repertoire, with a wide variation in repetitive DNA content, essentially in the form of transposable elements (TEs). In order to evaluate the impact of TEs on the structure, expression and evolution of *Phytophthora* genomes, we estimated the TE content of *P. infestans* T30-4 and a broad host range *P. parasitica* isolate (INRA-PP310). These two species display different lifestyles, but have close phylogenetic affinity. We first compared *P. infestans* and *P. parasitica* TE libraries. The ratio of retroelements to DNA transposons is different between species, being significantly lower in INRA-PP310. *P. parasitica* appears to lack any intact retrotransposon, although evidence of transcription is inferred from annotation of cDNA libraries. The under-representation of retrotransposons in *P. parasitica* was supported by pfam domain enrichment analyses. *P. parasitica* comprises a large set of DNA transposons, organized in several families. Their relative abundance varies between *P. parasitica* and *P. infestans*. Several of them appear to contain intact, potentially functional copies. Meanwhile, we observed an enrichment of DNA transposon-related pfam domains in *P. parasitica* and identified several transcripts in cDNA libraries generated from different situations, including various infection stages. The nature of the integration sites of these elements is currently investigated in the genome of five additional *P. parasitica* isolates collected on various hosts in different geographical areas, and to a larger extent in other *Phytophthora* genomes.

Pathotype and Genetic Shifts in in a Population of *Phytophthora sojae* under Soybean Cultivar Rotation

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Changes in the pathotype population of *Phytophthora sojae* (Kaufmann & Gerdeman) have been attributed to deployment of specific mayor resistant genes (*Rps*), which exert selection pressure and favor the development of resistant pathotypes. An alternative way to prolong the life of a resistance gene in the field is to generate disruptive selection by rotating major *Rps* genes through time and space or, by rotating cultivars with single gene resistance with cultivars with high partial resistance. This strategy has been proven to be effective in the tobacco-*Phytophthora nicotianae* pathosystem. The goal of this research was to determine if cultivar rotation could be used to effectively minimize pathotype shifts in a population of *P. sojae*. A four year soybean-cultivar rotation in microplots on a *P. sojae*-free site was established and inoculated with isolate PR1, which is virulent on *Rps 7*. Treatments consisted of sequences of cultivars with various *Rps* genes and/or different levels of partial resistance. A total of 121 isolates of *P. sojae* were recovered from the soil throughout the experiment and diversity was assessed using eight microsatellite markers and conventional pathotype characterization. Less than half of the recovered isolates had the same pathotype and multilocus genotype (MLG) as PR1. A total of 14 pathotypes and 21 MLGs were recovered during the four year experiment, and 31% of the isolates had at least one new allele. We found that *P. sojae* has the potential to shift pathotypes and evolve new genotypes very quickly, and that cultivar rotation affected the genetic structure of the population. We were unable to demonstrate, however, that pathotype shifts in the population were a function of cultivar rotation.

Diversity of oomycetes associated with soybean seedling disease in the U.S.

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Soybean (*Glycine max*) is the second most important crop in the U.S. and yield is often reduced by diseases affecting stand establishment and root health. Oomycete pathogens are commonly associated with seedling damping off and root rot under conducive environmental conditions. However, limited information exists regarding the frequency and diversity of oomycetes species across the major soybean producing states. In order to determine the distribution and diversity of oomycete species associated with seedling diseases, a survey was conducted across 11 states over two years. In 2011, a total of 2400 isolates were collected on the semi-selective medium (CMA-PARP+B), approximately 300 isolates per state from five fields. The survey was repeated in 2012 and included a second semi-selective medium (V8+RPBH), but due to drought conditions and limited number of symptomatic fields the number of recovered isolates was reduced to 1100 isolates in total. Isolates were identified by sequencing of the ITS region of rDNA and BLASTn analysis to a local curated database. In 2011, preliminary sequence results distinguished a total of 55 *Pythium* and 4 *Phytophthora* spp., with *Py. sylvaticum* (16%) and *Py. oopapillum* (12%) being the most frequent. In 2012, a total of 59 *Pythium* spp., 6 *Phytophthora* spp., and 3 *Phytopythium* were found, with *Py. sylvaticum* (15%) and *Py. heterothallicum* (13%) species being recovered most frequently. However, *Py. sylvaticum* was the most abundant species across the sampled region in both years, abundance of the other species fluctuated considerably. Oomycete species composition frequency was noted to fluctuate greatly between sampling locations with a significant ($P<0.05$) gradient due to latitude with significant ($P=0.02$) differences in composition by state. These data provides valuable information on abundance and prevalence of oomycetes associated with diseased soybean seedlings across different geographical locations and climatic conditions. Pathogenicity and aggressiveness will be evaluated for representatives of each species. This study will serve as the foundation for the development of diagnostic tools and the improvement of soybean seedling and root health.

Identification and characterization of the chitin synthase genes in the fish pathogen *Saprolegnia parasitica*

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The oomycete *Saprolegnia parasitica* is a fungus-like microorganism responsible for fish diseases and huge losses in aquaculture. The analysis of the cell wall composition of the microorganism and the characterization of key enzymes involved in cell wall biosynthesis may facilitate the identification of new target proteins for disease control. The cell wall of hyphal cells of *S. parasitica* consists mainly of cellulose, β-(1→3)- and β-(1→6) glucans, whereas chitin is present in minute amounts only. The main objective of this work was to test the effect of nikkomycin Z, a competitive inhibitor of chitin synthase (CHS), on the growth of *S. parasitica*. Genome mining allowed the identification of six different putative chs genes whose actual occurrence in the genomic DNA of the microorganism was confirmed by Southern blot analysis. The expression of the chs genes in the mycelium was analyzed using Real-Time PCR. The results revealed a higher expression level of four of the six genes while the two others exhibited undetectable levels of expression in the mycelium. This suggests that the latter genes are most likely primarily involved in chitin formation at a different developmental stage. The presence of nikkomycin Z increased the expression level of one of the genes, chs3, suggesting that the corresponding product is involved in forming the abnormal branching structures in the hyphae exposed to the inhibitor. The capacity of the mycelium to synthesize chitin was demonstrated by performing in vitro synthesis reactions using cell-free extracts. CHS activity was measured in intact cell membranes as well as in detergent-extract of membranes. The polysaccharide synthesized in vitro was characterized by enzymatic hydrolysis with a specific chitinase. Our data demonstrate that CHS represent promising targets of anti-oomycete drugs, even though the amount of chitin in the cell wall of *S. parasitica* does not exceed a few percent.

Oömycetes Protein Array Project

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Oömycetes are eukaryotes that outwardly resemble fungi, but are related to brown and golden-brown algae. The most destructive oömycete genus is *Phytophthora*, with over 80 species that collectively attack a wide range of plant species, causing damage to crops that is estimated in billions of dollars annually in the US. The goal of the Oömycetes Protein Array Project is to generate a collection of cloned proteins from 1440 predicted oömycete effector sequences, to use Gateway® technology to facilitate the easy transfer of clones into expression vectors, and to make the resulting clones available to the scientific community for further research. When the project is over, the final collection should include 390 clones from *P. sojae*, 550 clones from *P. infestans*, 370 clones from *P. ramorum*, and 130 clones from *H. arabidopsidis*.

Increased late blight resistance in HIGS potato lines targeting a *P. infestans* gene.

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Worldwide potato harvests are strongly diminished due the late blight disease caused by the oomycete *Phytophthora infestans*. The fungus-like eukaryote and its interaction with its host plants has been extensively investigated during the last decades whereas diverse research projects focus on its infection processes. *P. infestans* colonizes potato as well as tomato plants and thereby differentiates haustoria. Those barriers between host cells and invading pathogens are capable for exchange of nutrients, minerals but also of macromolecule like RNA molecules as shown for haustoria of parasitic plants. In oomycetes the exchange of effector protein from the pathogen to its host has been demonstrated. The movement of RNAi signals was shown in the interaction of parasites with their host plants and can be used to target not only plant genes but also genes of plant invading organism in a mechanism called host-induced gene silencing (HIGS). This technique has been applied for gene silencing in plant parasites as well as in nematodes and fungi. In oomycetes the RNA silencing is used as a standard method to characterize genes either by transient or by stable gene silencing and enzymes of the RNAi machinery have been identified. However, no efficient HIGS of oomycetes could be observed so far. We defined a *P. infestans* gene expressed during diverse developmental and infection stages of the oomycete as a HIGS target and could show that in planta expression of a HIGS hairpin construct targeting this particular gene in transgenic potato lines can be employed for late blight control. Our results present the appropriate processing of transformed HIGS hairpin constructs to siRNAs, their efficient function to silence the specific target gene sequence as shown in the reporter gene assays and subsequently reduced infection levels and diminished disease spreading on those transgenic HIGS potato lines in the field.

Phytophthora infestans effector Avr2 and the brassinosteroid pathway in potato.

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Phytophthora infestans is the oomycete pathogen responsible for late blight, the most devastating disease of potato crops globally. The pathogen secretes effector proteins into plant cells during infection - capable of modifying host processes to help establish disease. In some cases, plants have evolved corresponding resistance genes to these effectors, enabling the detection of pathogen invasion and initiation of plant defences.

PiAVR2 is one such effector protein, secreted by *P. infestans* and recognised by potato varieties expressing the resistance protein R2. A variant of this effector, PiAVR2-like, exists in the aggressive *P. infestans* strain Blue-13. This is currently one of the dominant UK isolates of the pathogen, with the ability to evade R2-mediated recognition.

Here, I present the interaction of both PiAvr2 and Avr2-like with a family of Ser/Thr phosphatases in the plant. These phosphatases are thought to play a role in signal transduction following perception of brassinosteroid hormones at the cell surface. The brassinosteroid pathway has well characterised links to plant growth, development and abiotic stress, and has more recently been linked to aspects of plant defence, with both positive and negative implications for disease resistance.

When host BSL1 is silenced, the recognition of PiAvr2 is compromised, suggesting that R2 requires these phosphatases for function and potentially 'guards' the brassinosteroid pathway in the plant. My PhD project aims to characterise how the effector PiAVR2 influences the brassinosteroid pathway in potato, the nature of the interaction with the host BSL phosphatases, and how this in turn is monitored by the resistance gene R2.

Identifying functional alleles of the resistance to *Phytophthora infestans* (Rpi) vnt1 genes in *Solanum okadae* accessions.

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The biggest threat to potato production world-wide is late blight disease caused by the oomycete pathogen *Phytophthora infestans*. Resistances found in some wild *Solanaceae* species provide environmentally-benign means of restricting late blight infections. The Commonwealth Potato Collection (CPC) is hosted at the JHI and contains more than 1800 accessions from 80 different wild potato species. This collection represents a unique resource for identifying and cloning novel resistances. The resistance gene Rpi-vnt1 from the wild potato species *Solanum venturii* recognises the cognate *P. infestans* effector Avr-vnt1 and provides broad spectrum resistance against various late blight isolates. Three functionally equivalent alleles of Rpi-vnt1 (Rpi-vnt1.1, Rpi-vnt1.2 and Rpi-vnt1.3) have been identified and share more than 98% sequence identity.

Some late blight resistant *S. okadae* accessions recognize Avr-vnt1 following transient expression via Agrobacterium tumefaciens and yield a hypersensitive-like response. The aim of this study is to assess the presence of functional Rpi-vnt1-like genes within these CPC accessions. A two pronged approach is used to assess functionality: (a) cloning and sequencing of Rpi-vnt1-like genes; (b) Avr-vnt1 recognition studies following transient co-expression of candidate Rpi-vnt1-like genes in *Nicotiana benthamiana*. So far, functional Rpi-vnt1.1 genes have been identified in three different accessions of *S. okadae*. Moreover, 13 different Rpi-vnt1 variants with unknown functions have been amplified.

Identifying essential effectors from the soybean pathogen *Phytophthora sojae*

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Breeding for resistance to plant pathogens is one of the most effective means of disease control. However, the ability of plant pathogens evolve new pathogenicity factors and evade host defense mechanisms drives the continual necessity to identify new resistance genes. We are exploiting genomic technologies in an effector-directed breeding approach that augments traditional breeding efforts against *Phytophthora sojae*, the casual agent of soybean root and seedling rot. This approach is founded on identifying monomorphic *P. sojae* effector genes that are essential for virulence, and using these genes as probes to identify new sources of resistance in soybean and related legumes. Our goal is to identify 10 essential RXLR effector genes. These essential effectors will make excellent candidates for screening for new, durable resistance to P. sojae, as these genes cannot be mutated or deleted without a significant fitness penalty. The majority of predicted *P. sojae* RXLR effector genes are polymorphic amongst sequenced isolates of *P. soja*e, however, a subset of *P. sojae* RXLR effectors displays little or no allelic diversity. We have established a workflow for transient gene silencing and quantitative virulence assays. To date, we have silenced and assessed the virulence contribution of 17 *PsAvh* genes. 12 of these effectors showed reduced virulence. Among these effectors, *Avh16*, *Avh180* and *Avh240* showed significantly reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. We are currently using these three effectors as candidates in a high throughput screen system utilizing Pseudomonas Type III secretion system to screen for new resistance genes against *P.sojae*.

Soybean resistance genes are regulated by small RNAs during *Phytophthora sojae* infection.

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Plant defense mechanisms are regulated in part by innate small RNAs. MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) expression change in plant hosts in response to pathogens. Certain small RNAs are involved in plant innate immunity against pathogen infections and potentially contribute to disease resistance. However, we have little understanding of the functions of these small RNAs during plant interactions with oomycete pathogens. In this study, we analyzed small RNA populations in soybean upon infection with *Phytophthora sojae*. Following high throughput Illumina sequencing and bioinformatic analysis, we have identified a few small RNAs including known/conserved miRNAs, potential novel soybean miRNAs and in phased secondary siRNAs (PhasiRNAs) that are differential expressed in *P. sojae*-infected soybean roots.

We have experimentally verified the differential expression of 11 miRNAs in soybean roots infected with *P. sojae*. Four differentially expressed miRNAs, miR1507, miR1509, miR2109 and miR482, target resistance genes that encode nucleotide-binding leucine rich repeat (NB-LRR) proteins. Recent findings have shown the induction of phasiRNAs by 22nt miRNA-mediated cleavage of target transcripts. We found the induction of phasiRNAs in *P. sojae*-infected roots with induced expression of the 22nt miR1507. The induction of these phasiRNAs largely reduce the abundance of target NB-LRR gene transcripts, consistent with a role of both the parent miRNAs and the phasiRNAs derived from these miRNAs in regulating NB-LRR gene expression during *P. sojae* infection. The conserved miRNA, miR393, regulates plant development and anti-bacterial immunity in Arabidopsis. We found that miR393 was induced by *P. sojae* infection in soybean roots and that reduction in miR393 levels leads to increased susceptibility of soybean to *P. sojae* infection. These data suggest that miR393 is a positive regulator of basal defense against *Phytophthora*.

Effectoromics for functional dissection of Oomycete effectors in host and non-host plants

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Genomes of several Oomycete plant pathogens have been sequenced and genomic sequences of many others will soon be available. Bioinformatic and functional approaches have identified from 130 to 550 RxLR effector-encoding genes per genome. Oomycete pathogens differ in their host ranges; it is unknown whether their compatibility with particular plant species is due to the presence of suitable host targets of effectors, to the absence of specific recognition events, or to a combination of both. Analysis of the huge repertoire of effectors in Oomycete plant pathogens requires high-throughput phenotyping technologies (phenomics). We have established a phenotyping platform for efficient functional analysis of effector proteins from Oomycetes and other plant pathogens. We are analyzing the reactions of multiple genotypes of *Arabidopsis* and several *Compositae* and *Solanaceous* crop species to numerous effectors using a Tobacco Rattle Virus (TRV)-based expression system. Phenotypes resulting from TRV-mediated expression of effectors are curated in a searchable database that includes phenotypic data on the induction or suppression of plant defenses, the effect on plant growth and development, and the effect on pathogens' virulence. Information on the determinants of effector recognition in non-host species will identify candidate resistance genes for agronomic use.

Virulence Function of *Phytophthora sojae* Effectors PSR1 and PSR2 During Infection.

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Effectors are essential virulence proteins produced by a broad range of parasites including viruses, bacteria, fungi, oomycetes, protozoa, insects and nematodes. We have previously identified two RXLR effectors from Phytophthora sojae that can suppress RNA silencing in plants. Here, we report an investigation on the virulence function of these *Phytophthora* Suppressors of RNA silencing (PSRs) during *P. sojae* infection of the natural host soybean and Phytophthora capsici infection of *Arabidopsis thaliana*. We firstly employed *Agrobacterium rhizogenes*-induced hairy root system to over-express PSR1 and PSR2 in the susceptible soybean cultivar Harosoy. Despite numerous trials, transgenic roots expressing PSR1-YFP could not be generated, presumably due to its profound impacts on small RNA biogenesis or cell death-triggering activity. On the contrary, we obtained hairy roots expressing PSR2-YFP, which were subsequently inoculated with zoospores of *P. sojae* strain P6497. Although no differences on *P. sojae* development were observed in YFP- and PSR2-YFP-expressing roots, the PSR2-YFP-expressing roots allowed significant enhancement in mycelial biomass and number of oospores compared to roots expressing YFP. We also examined the susceptibility of PSR1 and PSR2 transgenic Arabidopsis plants to *P. capsici* isolate LT263 using a recently developed pathosystem. Using three independent transgenic lines for each gene, we observed strikingly enhanced susceptibility of these Arabidopsis transgenic lines. These findings strongly suggest that PSRs are important virulence factors that promote Phytophthora infection in plants.

The nuclear import adaptor, importin α is essential to sexual and asexual development and pathogenicity in *Phytophthora sojae*.

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In eukaryotic cells, importin α is an essential carrier for transport proteins into nucleus. In Phytophthora sojae, a conserved importin α gene was identified and named as PsIMPA. It comprised a flexible N-terminal importin β binding (IBB) domain and a tandem series of ten armadillo (ARM) repeats representing NLS binding domain in C-terminal. PsIMPA is differentially expressed during life cycle of *P. sojae* with the highest expression level at 6 hours post inoculation. PsIMPA-silenced transformants exhibited multiple defects during the development stages and infection process. The PsIMPA-silenced transformants showed reduced growth rate and aberrant morphology with more branches and curling. The ability of forming oospores and sporangia were also severely affected, because of the significant reduced number and viability of oospores and the defect on forming sporangia. Inoculation assay revealed reduced pathogenicity of the PsIMPA-silenced transformant. DAB staining at 12 hours after inoculation and the test of tolerance H2O2 in vitro showed the ability of scavenging ROS reduced significantly for PsIMPA-silenced transformants. These results indicated that PsIMPA regulated multiple processes during the life cycle and point to the importance of PsIMPA in *P. sojae*.

Transcriptional and phylogenetic study of an expanded bZIP transcription factor family in oomycete plant pathogen *Phytophthora sojae.*

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The basic leucine zipper (bZIP) transcription factor (TF) family is one of the largest and most diverse families among several TF families and presents exclusively in eukaryotes. But little is known for the transcription factors in oomycete plant pathogens. In this study, a total of 71 bZIP TF candidates were identified by bioinformatics methods and manually checked in *Phytophthora sojae*, this number was higher than previous predicted numbers in relative databases and reports. For comparing, bZIPs in other oomycetes (*Phytophthora ramorum*, 60; *Phytophthora infestans*, 47; *Pythium ultimum*, 37; and *Hyaloperonospora arabidopsidis*, 25), two diatoms (*Thalassiosira pseudonana*, 20; *Phaeodactylum tricornutum*, 20), and two fungi (*Fusarium graminearum*, 25; *Magnaporthe oryza*e, 25) were also predicted by the same methods, and revealed the P*hytophthora* genomes encoded a larger bZIP TF family, that was because of a set of novel members containing bZIP domains with substitutions at the conserved DNA binding sites. These novel types bZIPs could not find expansion even presence in any other species. Phylogenetic analyses of the bZIPs from above mentioned species revealed that the novel types were in distinct evolution clades with extensive gene duplication, while the N-R bZIPs were likely to evolve from the common eukaryote ancestor but suffered gene lost and some duplication. Gene expression profiling of the *P. sojae* bZIPs revealed the novel types of bZIPs were expressed in lower level, but all bZIPs were predominantly associated with the transcription shifts during either zoospores-cysts or host infection stages. Transcription response to external H2O2 stress was also tested at 0, 0.5, 1, and 2 hours after treatment. The results showed that many bZIPs were induced in specific or all stages, more bZIPs induced during infection were also induced under oxidative stress, then were the zoospores-cysts induced bZIPs.

Mating type switch in asexual progenies of *Phytophthora capsici* with A2 mating type

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Recent studies with *Phytophthora capsici* suggest mating type and virulence may vary in clonally propagated field isolates or sexual progenies that experience loss of heterozygosity (LOH). The mating-type switch appears to be primarily from the A2 to the A1 mating type. In this study, zoospore progeny from 7 A2 and 3 A1 isolates from the USA and China were analyzed for mating type changes. Between 24 and 100 single zoospore progeny were isolated and tested for mating type for each isolate. For the A2 isolates, three different mating types (A1, A2 and A1A2) were identified in the zoospore progenies of 6 isolates and one had progenies with the A1 and A2 mating type. None of the A1 zoospore progeny exhibited a mating type switch. The proportion of progenies with A1, A2 and A1A2 mating types differed for the A2 isolates and these preliminary results are congruent with the hypothesis that homozygous and heterozygous alleles control A1 and A2 mating type respectively, and LOH spanning mating type region during mitotic recombination might be responsible for mating type switch for A2 isolates. Further studies employing specific molecular markers are underway and the results will be discussed.

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