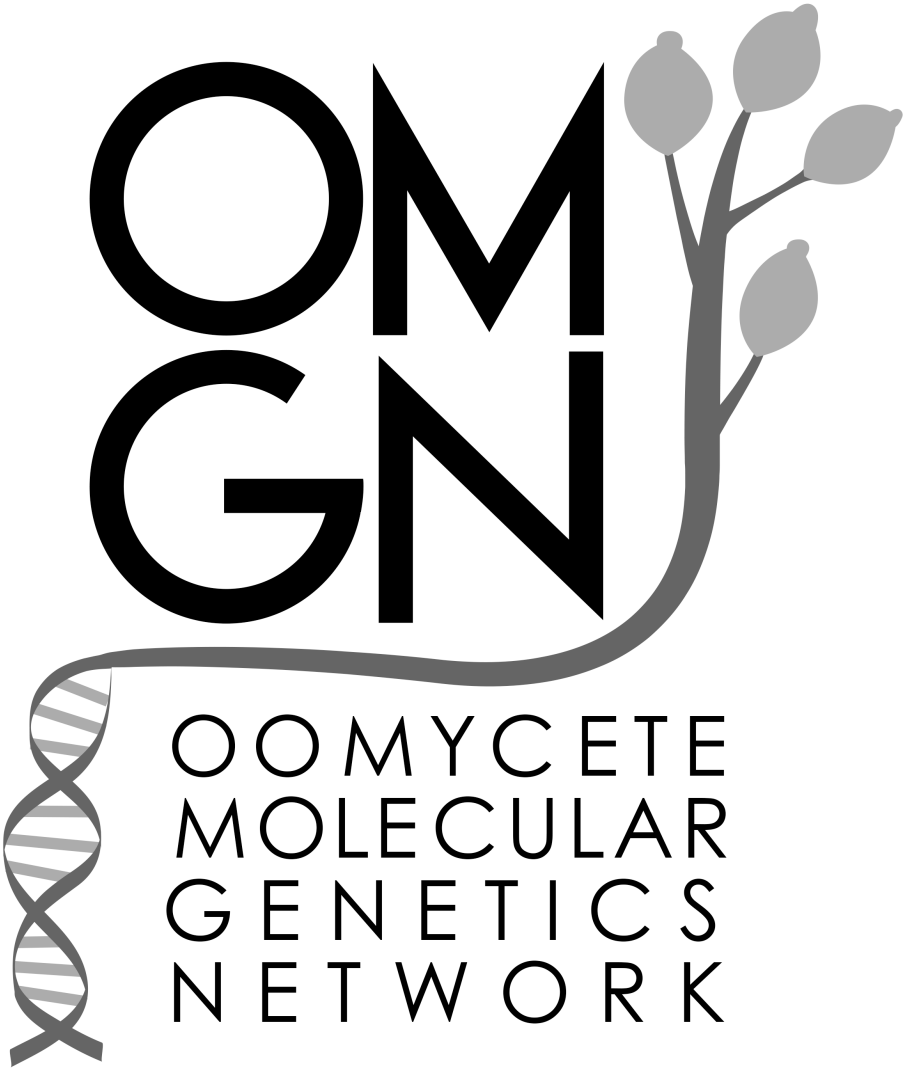

Pacific Grove, California, USA
March 14-17, 2015

Oomycete Molecular Genetics Network Meeting 2015

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Organizers

Scientific Program	Wenbo Ma and Howard Judelson, University of California, Riverside
Meeting logistics	Joel Shuman, Virginia Tech Paul Morris, Bowling Green State University

Acknowledgements

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Cover art: Kelsey Wood, University of California, Davis

PROGRAM

Oral presentations are in Chapel Hall. Reception/Poster sessions are in Kiln Hall.

Sunday, March 15th, 2015

8:30-9:00	**REGISTRATION**	
9:00-9:05	**WELCOME**	
9:05-10:25	**Genomics (I)**	**Chair: Wenbo Ma**
9:05-9:20	Frank Martin	Mitochondrial genomes of *Bremia lactucae* and development of haplotype markers for population and genetic studies
9:25-9:40	Takao Kasuga	Chromosomal aberrations underlie host-induced phenotypic diversification in the Sudden Oak Death pathogen *Phytophthora ramorum*
9:45-10:00	Meenakshi Kagda	Metabolic adaptations of *Phytophthora infestans* within host environments
10:05-10:20	Alyssa Burkhardt	Investigating resistance to *Pseudoperonospora cubensis* in *Cucumis sativus*
10:25-10:50	**BREAK**	
10:50-12:00	**KEYNOTE SPEECH**	**Chair: Howard Judelson**
	Bruce McDonald	Effector evolution and diversification in necrotrophic fungi
12:00-14:00	**LUNCH**	
14:00-15:20	**Effector (I)**	**Chair: Patricia Manosalva**
14:00-14:15	Francine Govers	R1-mediated immunity triggered by the *Phytophthora infestans* RXLR effector AVR1 is activated inside the nucleus
14:20-14:35	Bernard Dumas	Effector-driven adaptation of *Aphanomyces* species to animal or plant hosts
14:40-14:55	Adam Steinbrenner	Allele-specific immune recognition of the RXLR effector ATR1 can occur on both tandem WY-domains
15:00-15:15	Liliana Cano	Embracing discovery of effectors in the hop downy mildew plant pathogen
15:20-15:50	**BREAK**	
15:50-17:10	**Oomycete Biology (I)**	**Chair: Miaoying Tian**
15:50-16:05	Johan van den Hoogen	*Phytophthora infestans* GPCR-PIPK GK4 is a membrane localized PI4P5-kinase and is required for virulence
16:10-16:25	Yufeng Fang	Deciphering nuclear localization signals in *Phytophthora sojae*
16:30-16:45	Jasmine Pham	Co-regulation of gene expression in *Phytophthora*
16:50-17:05	Melania Abrahamian	Nitrate assimilation pathway contributes to pathogenicity of *Phytophthora infestans*
18:00-19:30	**DINNER**	
19:30-22:00	**RECEPTION/POSTER SESSION (odd numbers)**	

Monday, March 16th, 2015

9:00-10:20	**Effector (II)**	**Chair: Laura Grenville-Briggs**
9:00-9:15	Wenbo Ma	*Phytophthora* Suppressor of RNA silencing 1 (PSR1) targets a novel component of small RNA biogenesis in plants to promote infection
9:20-9:35	Elodie Gaulin	CRN13 from *Aphanomyces euteiches* trigger host DNA damage
9:40-9:55	Yasin Dagdas	An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor
10:00-10:15	Kevin Fedkenheuer	Effector directed breeding to improve soybean resistance against *Phytophthora sojae*

10:20-10:50 **BREAK**

10:50-11:50	**Oomycete Biology (II)**	**Chair: Shiv Kale**
10:50-11:05	Steven Kelly	Clotrimazole as a potent agent for treating the oomycete fish pathogen *Saprolegnia parasitica* through inhibition of sterol 14α-demethylase (CYP51)
11:10-11:25	Harri Kokko	Novel *Aphanomyces* sp. isolates from Lake Tahoe (USA) signal crayfish (*Pacifastacus leniusculus*)
11:30-11:45	Timothy Miles	Mitochondrial marker systems for studying *Phytophthora* and *Pythium* for community analysis
11:50-12:20	**GROUP PHOTO**	

12:20-14:00 **LUNCH**

14:00-15:20	**Plant Response**	**Chair: Takao Kasuga**
14:00-14:15	Francine Govers	Lectin receptor kinase; sentinels in defense against plant pathogens
14:20-14:35	William Holdsworth	Development of downy mildew resistant cucumbers for the Eastern U.S.
14:40-14:55	Emily Helliwell	Enhanced resistance in *Theobroma cacao* against oomycete and fungal pathogens by secretion of phosphatidylinositol-3-phosphate-binding proteins
15:00-15:15	Benjamin Hall	Manipulation of host proteins to generate resistance to *Phytophthora infestans* in solanaceous crops

15:20-15:50 **BREAK**

15:50-17:10	**Genomics (II)**	**Chair: Jaime Blair**
15:50-16:05	Jean Ristaino	Displacements and evolutionary origins of US lineages of *Phytophthora infestans*
16:10-16:25	Michael Martin	Population genomics of New World *Phytophthora infestans*
16:30-16:45	Javier Tabima	Genomic signatures of host jumping onto raspberry and strawberry in two *Phytophthora* sister taxa
16:50-17:05	Miaoying Tian	De novo assembly and analysis of transcriptome of *Peronospora belbahrii*
17:10-18:00	**COMMITTEE MEETING**	

18:00-19:30 **DINNER**

19:30-22:00 **REFRESHMENT/POSTER SESSION (even numbers)**

Tuesday, March 17th, 2015

9:00-10:00	**Oomycete Biology (III)**	**Chair: Bernard Dumas**
9:00-9:15	Francine Govers	Dynamics of the actin cytoskeleton in *Phytophthora infestans* hyphae and infection structures
9:20-9:35	Anne Njoroge	Displacement of US-1 clonal lineage by a new lineage of *Phytophthora infestans* on potato in Kenya and Uganda
9:40-9:55	Rebecca Lyon	Population structure of downy mildew on spinach

10:00-10:30 **BREAK**

10:30-11:50	**Genomics (III)**	**Chair: Liliana Cano**
10:30-10:45	Lina Quesada	Developing species-specific molecular diagnostics for cucurbit downy mildew
10:50-11:05	Michael Fedkenheuer	High-throughput screening of *Glycine soja* germplasm for novel R genes against *Phytophthora sojae*
11:10-11:25	Alejandro Rojas	The oomycete phytobiome, a case study of soybean roots
11:30-11:45	Kelsey Wood	Mining of a plant pathogen genome to identify candidate WY-domain containing pathogenicity proteins
11:50-12:30	**CLOSING REMARKS**	

12:30-14:00 **LUNCH/ADJOURN**

ORAL PRESENTATIONS (in order of presentation)

Mitochondrial genomes of *Bremia lactucae* and development of haplotype markers for population and genetic studies

Frank Martin[1], Juliana Gil[2], Lida Derevnina[2], Cayla Tsuchida[2], Richard Michelmore[2]
[1]*USDA-ARS, Crop Improvement and Protection Research Unit, Salinas, CA 93905;*
[2]*The Genome Center, University of California, Davis, CA 95616 USA*

Bremia lactucae, the causative agent of lettuce downy mildew, is the most important pathogen of lettuce in the US and worldwide. In order to identify cytoplasmic markers for use in population and genetic studies the reference mitochondrial genome of *B. lactucae* isolate SF5 was assembled from Illumina and Pacific Biosystems reads that had been generated for sequencing the nuclear genome. The SF5 mitochondrial genome is 40,513 bp in size, encodes the 2 rRNAs, 34 genes, 5 putative ORFs and tRNAs common in *Pythium* and *Phytophthora* spp.; it also has two unique putative ORFs, a 212 bp inverted repeat encoding duplicate *trnK* genes, and is estimated to have over 41 copies of an 80 bp repeat. An unusual feature of its organization is the position of the *rns* coding region; this is in a similar location as in *Phytophthora* spp. but on the strand opposite to the *rnl* gene. The mitochondrial genomes have been assembled for 49 isolates from geographically diverse origins with polymorphisms due to both SNPs and indels. The most significant length polymorphism among isolates is due to the varying number of 80 bp repeats (from one to over 41 copies). Network analysis grouped the isolates approximately paralleling their geographic distribution. The relationship between mitochondrial haplotypes and nuclear genotypes and virulence phenotypes will be discussed.

Chromosomal aberrations underlie host-induced phenotypic diversification in the Sudden Oak Death pathogen *Phytophthora ramorum*

Takao Kasuga, Jennifer Yuzon, Elizabeth Bernhardt, Tedmund Swiecki, Kamyar Aram, Liliana Cano, Sophien Kamoun, Joan Webber, Clive Brasier, Caroline Press, Niklaus Grünwald, David Rizzo, Matteo Garbelotto. *USDA-ARS, Davis, California and Corvallis, Oregon, USA; Univ. of California, Berkeley, USA; Sainbury Laboratory, UK; UK Forestry Commission.*

Rapid phenotypic diversification in clonal invasive populations is often observed, although the underlying genetic mechanisms remain elusive. The Sudden Oak Death pathogen *Phytophthora ramorum* is exclusively clonal, yet exhibits extensive phenotypic differences when obtained from specific bark hosts. This phenotypic variation is seen despite the fact that population genetic and host-specificity studies negate any host-driven population subdivision. We hypothesized that the bark host environment in oak (from genus *Quercus*) is responsible for the observed phenotypic diversification. When *P. ramorum* isolates from foliar host California bay laurel (*Umbellularia californica*) were inoculated and re-isolated from canyon live oaks (*Q. chrysolepis*) 20 and 40 weeks post inoculation, 39% and 60% of re-isolates, respectively, displayed diverse morphological phenotypes and transposon de-repression only seen in cultures from oaks infected naturally. High-throughput sequencing-based analyses identified major genomic

alterations in isolates from oaks included partial aneuploidy and copy-neutral loss of heterozygosity. These alterations were not identified in isolates from foliar hosts such as *U. californica*. Chromosomal breakpoints were found to be located at or near transposons, linking transposon de-repression caused by the chemical environment of oaks to structural genomic changes (Kasuga at al., 2012). We will discuss whether the host-dependent phenotypic diversification is an outcome of adaptive response or of damage inflicted by host defense chemicals, or possibly both.

Title: Metabolic adaptations of *Phytophthora infestans* within host environments

Meenakshi Kagda[1], Carol Davis[2], Howard Judelson[1,2]. *[1]Genetics Genomics and Bioinformatics Program, University of California, Riverside, CA 92521; [2]Dept. of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA*

Nutrient acquisition and metabolic adaptations are important characteristics of pathogen biology. Information about these aspects in the *Phytophthora infestans*-potato/tomato pathosystems will help us better understand disease progression. To do this, transcriptional profiling and live-cell imaging technique using gene fusions under native promoter with fluorescent proteins is being implemented. We performed an RNA-seq experiment on tuber infections, tomato leaf infections as well as different artificial media conditions. Also, we generated a comprehensive list of all the metabolic genes encoded by the *P. infestans* database using different bioinformatics approaches. When we analyzed the RNA-seq data for metabolic genes involved in different pathways, we found many to be differentially expressed when we compared plant infections with artificial media conditions. Our next step was aimed at answering the question: are some of these genes expressed in a spatial and a time-dependent manner? Based on this analysis, we have currently identified one metabolic protein participating within the carbohydrate metabolism pathway to be localized specifically within the *P. infestans* haustoria. To the best of our knowledge, this is the first report of a *Phytophthora* metabolic protein to have a haustorial specific localization.

Investigating resistance to *Pseudoperonospora cubensis* in *Cucumis sativus*

Alyssa Burkhardt, Brad Day. *Michigan State University, East Lansing, MI USA*

Pseudoperonospora cubensis (downy mildew) is an agriculturally relevant oomycete, which causes severe foliar damage of cucumbers. In the past, host resistance had been used to control downy mildew in cucumber; however, by 2004 this host resistance was overcome. Previous research has revealed potential new sources of resistance, including the plant introduction line PI 197088, which has resistant leaves but very poor fruit quality. The goal of this study is to identify genes that are involved in mediating resistance by comparing the transcriptomes of a resistant cucumber line, PI 197088, and a susceptible cucumber cultivar, Vlaspik. Two biological replicates each of mRNA and small RNA extracted from inoculated resistant and susceptible cucumber plants over 0-6 days post inoculation time course have been sequenced using 50 bp reads on the Illumina Hi-Seq. Trimmed reads and quality-checked reads were uniquely mapped to the cucumber genome using Bowtie and TopHat. The number of reads mapped to

each gene was counted using HTSeq and differentially expressed genes within and between time courses were determined using DESeq. A weighted genome correlation network analysis (WGCNA) was performed within and between plant line time courses to identify modules of coexpressed genes that were similarly or differentially expressed over time. Gene ontology (GO) analyses show that some transport-related genes are specifically down-regulated in PI 197088. Future work will further analyze the annotation and functional descriptions of genes that are differentially expressed or co-expressed within a unique module and will also examine the impact of small RNA on gene expression.

KEYNOTE TALK: Effector Evolution and Diversification in Necrotrophic Fungi

Bruce McDonald. *Plant Pathology, Institute of Integrative Biology, ETH Zürich, Switzerland*

The first population genetic analysis of a plant pathogen effector was conducted with the NIP1 protein of *Rhynchosporium commune* and published in 2004. This work established the global significance of both non-synonymous substitutions and deletions as important mechanisms for avoiding detection by host R-genes at the field scale. The NIP2 and NIP3 effectors of R. commune have also been well characterized in global populations and global diversity for three host-specific toxins in *Parastagonospora nodorum* (ToxA, Tox1, Tox3) has also been established. All six of these effectors have unique features, but all also share some general patterns of diversity. For example, deletions occur for all six effectors in natural field populations, but the frequency of the deletion varies according to the effector and the field population analyzed. It is clear that each effector is operating under evolutionary constraints likely due to fitness costs associated with different mutations. There is also compelling evidence for horizontal gene transfer for several of these effectors. We are now identifying effectors in Zymoseptoria tritici using a combination of RNAseq, QTL mapping and population genomics. Several candidate effectors have been identified and one has been functionally validated. Several more effector candidates are under functional investigation. We anticipate conducting detailed population genetic analyses of these effectors and also expect to track their evolutionary history based on comparisons with the wild sister species *Z. pseudotritici* and *Z. ardabiliae.*

R1-mediated immunity triggered by the *Phytophthora infestans* RXLR effector AVR1 is activated inside the nucleus

Yu Du, Klaas Bouwmeester, Jeroen Berg, Francine Govers. *Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands*

Phytophthora infestans is a devastating plant pathogen that causes late blight on potato and tomato. To colonize host plants, *P. infestans* secretes effectors that can modulate host defence. Well-known are the RXLR effectors that are translocated into host cells to manipulate the cell machinery. To counteract the pathogen, potato exploits nucleotide-binding leucine-rich repeat (NLR) immune receptors that confer resistance against *P. infestans* upon recognition of a RXLR effector, with each NLR protein (or R protein)

having its own matching RXLR effector (or AVR protein). The mechanisms underlying NLR-mediated resistance are still poorly understood. In this study we exploited fluorescent tags and nuclear localization and export signals (NLS/NES) for determining the subcellular localization of the potato NLR protein R1 and the *P. infestans* RXLR effector AVR1, and for targeting these proteins to nucleus or cytoplasm. Microscopic imaging revealed that both R1 and AVR1 occur in nucleus and cytoplasm, and in close proximity. Transient expression of NLS- or NES-tagged R1 and AVR1 in *Nicotiana benthamiana* showed that activation of R1-mediated hypersensitive response and resistance requires localization of the R1/AVR1 pair in the nucleus. However, AVR1-mediated suppression of cell death in absence of R1 is dependent on localization of AVR1 in the cytoplasm. A balanced nucleocytoplasmic partitioning of AVR1 seems to be a prerequisite.

Effector-driven adaptation of *Aphanomyces* species to animal or plant hosts

Elodie Gaulin, Michiel J.C. Pel, Sarah Courbier, Laurent Camborde, Hélène San Clemente, Bernard Dumas. *Laboratoire de Recherche en Sciences Végétales (LRSV), UM5546 CNRS-Université Paul Sabatier, 24 Chemin de Borde-Rouge, Pôle de Biotechnologie Végétale, 31326 Castanet-Tolosan; France*

Within the Saprolegniale lineage the genus *Aphanomyces* comprises highly specialized species pathogenic on plants, fishes and crustaceans. This diversity offers the opportunity to decipher mechanisms driving adaptation of related oomycete species to distinct hosts. To get insight in these mechanisms, we performed genome sequencing of 10 strains of *Aphanomyces* sp. representing the biological diversity of the genus. A combination of sequencing technologies led to the generation of a 61 Mb reference genome for an *A. euteiches* strain causing important damages on pea crops. Three genome sequences of strains of the crayfish pathogen *A. astaci* with low and high virulence on crayfishes have been obtained. A comparative genomics approach was developed to identify effector repertoires which could be involved in adaptation of *Aphanomyces* species to various hosts. This analysis revealed that animal and plant pathogenic species harbor specific effector repertoires which have been shaped by various evolutionary processes including expansion, gain or loss of gene families. Strikingly, plant pathogenic *Aphanomyces* species have expanded gene families coding plant-cell wall degrading enzymes, which are absent in animal pathogenic species, and acquired a specific family of small secreted proteins highly expressed during pathogenesis. In animal pathogens, expansion of secreted protease gene families occurred and a lineage-specific gene family coding secreted proteins harboring a chitin-binding module putatively involved in degradation of crustacean shell has been identified. Together, these data allowed the identification of new classes of pathogenicity effectors and give clues on evolutionary mechanisms shaping adaptation of pathogenic oomycetes to unrelated hosts.

Allele-specific immune recognition of the RXLR effector ATR1 can occur on both tandem WY-domains

Sandra Goritschnig, Adam D. Steinbrenner, Brian J. Staskawicz. *Dept. of Plant and Microbial Biology, University of California, Berkeley, CA 94720 USA*

Hyaloperonospora arabidopsidis is predicted to secrete ~140 effectors with an N-terminal RXLR motif, many of which have single or tandem copies of helical WY-domains. Direct interaction of one of these RXLR effectors, ATR1, with the Arabidopsis NLR receptor RPP1 activates a hypersensitive response (HR), and thus serves as a model for how these oomycete effectors can trigger plant immunity. Across a series of allelic combinations, ATR1-RPP1 interaction *in planta* correlates with HR activation. In combination with the ATR1 crystal structure, we previously identified ATR1 surfaces that are recognized by two RPP1 alleles, which occurred on either an N-terminal "head" region or the first of two tandem WY-domains. To further explore the molecular basis of ATR1 recognition, we have cloned two additional alleles of RPP1 from Arabidopsis ecotypes displaying unique ATR1 recognition spectra. The complex *RPP1* loci from Estland (Est-1) and Zdarec (Zdr-1) were assembled with combined Sanger and Illumina sequencing of fosmid clones. Functional members from each *RPP1* locus, RPP1-EsA and RPP1-ZdA, contain large leucine-rich-repeat insertions relative to previously characterized alleles. We employed a site-directed mutagenesis approach, informed by variation across ATR1 alleles, to identify surfaces on the ATR1 protein specifically recognized by RPP1-EsA and RPP1-ZdA. Surprisingly, ATR1 residues on the second tandem WY-domain condition recognition by RPP1-EsA, contrasting with previously characterized residues on the head or first WY-domain of the protein. Recognition of ATR1 on multiple WY-domains suggests that domain count in a given effector could expose a pathogen to recognition, possibly constraining the evolution of oomycete effectors.

Embracing discovery of effectors in the hop downy mildew plant pathogen

Liliana M. Cano[1], Saunia Withers[1], David H. Gent[2], Lina M. Quesada-Ocampo[1]
[1]Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695 USA; [2]Dept. of Botany and Plant Pathology, Oregon State University, OR 97331 USA

Oomycete plant pathogens secrete effector molecules that manipulate host physiology to achieve colonization. Effector proteins have been identified in various oomycetes species but little is known about the sequence, function, and evolution of effectors of downy mildew plant pathogens. *Pseudoperonospora humuli* is the causal agent of downy mildew of hop, an economically important disease that occurs worldwide and has gained importance in recent years in the United States due to an increase of hop production. The main goal of our study was to identify and make available to the oomycete community a set of candidate pathogen effectors from *P. humuli* using transcriptomics. Identified effector candidates can be employed in high throughput functional genomics screens, commonly referred to as effectoromics, to test hop germplasm for specific pathogen recognition by resistance *R* genes. Here we report the identification of various effector families that are expressed during *P. humuli* – hop

interaction and discuss their potential roles in disease. Our findings are a first step toward effector-assisted breeding in hop for resistance to downy mildew.

Phytophthora infestans GPCR-PIPK GK4 is a membrane localized PI4P5-kinase and is required for virulence

Johan van den Hoogen, Chenlei Hua, Harold JG Meijer, Francine Govers. *Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*

Signaling networks involving heterotrimeric G-proteins and phospholipids lie at the base of many cellular processes in eukaryotes. Oomycetes possess a family of novel proteins called GPCR-PIPKs (GKs) that are composed of a G-protein-coupled-receptor (GPCR) domain fused to a phosphatidylinositol phosphate kinase (PIPK) domain. Based on this domain structure GKs are anticipated to link G-protein and phospholipid signaling but their functions and biochemical activities are currently unknown. Previously we analyzed the function of one of the twelve GKs in the potato late blight pathogen *Phytophthora infestans* by gene silencing and overexpression and showed that PiGK4 is involved in spore development, sporangial cleavage, hyphal elongation and virulence (Hua, Meijer et al. 2013, Mol. Microbiology). Overexpression of subdomains of PiGK4 fused to a fluorescent protein revealed that the GPCR domain targets PiGK4 to membranes surrounding certain cellular compartments. With GPCRs as the main target of active agents, and their biological importance, GKs pose potential as oomicide drug targets. To determine the enzymatic activity of the PIPK domain in PiGK4 we make use of the temperature sensitive yeast mutant mss4ts that can be complemented with the full-length PiGK4 gene. Here we will present a more detailed analysis of the function of the various conserved domains in PiGK4 by complementation essays in yeast mss4ts using modified versions of PiGK4 generated by deleting and swapping domains.

Deciphering nuclear localization signals in *Phytophthora sojae*

Yufeng Fang, Brett M. Tyler. *Interdisciplinary Ph.D. program in Genetics, Bioinformatics & Computational Biology, Virginia Tech, Blacksburg, VA 24061 and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331 USA*

Nuclear localization signals (NLSs) are amino acid sequences that direct proteins from the cytoplasm into the nucleus in eukaryotic cells. To date, sequences that determine nuclear localization have not been defined in oomycetes. In this study we have investigated NLS functioning in *Phytophthora sojae*, a model species of oomycetes that infects soybeans. Using confocal microscopy, we found that two well-studied NLS types, classical NLS and Proline-Tyrosine NLS, cannot direct fluorescent proteins into the nuclei of *P. sojae* transformants. Surprisingly, we also found that in highly conserved nuclear-localized proteins, such as ribosomal proteins and core histones, NLSs defined in human or yeast, did not function in the case of their *P. sojae* orthologs. Those results suggested that *P. sojae* may use a non-canonical mechanism for nuclear cargo import. To identify functional NLSs in *P. sojae*, we experimentally examined 20 *P. sojae* nuclear-localized proteins, and defined in detail the NLS in three of those proteins. We

found *P. sojae* NLSs have several unique characteristics: (1) *P. sojae* NLSs are composed of several sub-sequences dispersed across the protein sequence. (2) Weak NLSs can either work individually when present in multiple copies or work collectively to direct proteins into the nucleus. (3) One class of *P. sojae* NLS could be defined as three clusters of four positively charged amino acids with a minimum spacing. When the non-canonical *P. sojae* NLSs were tested in *Arabidopsis* and mammalian cells, they directed nucleolar localization, suggesting that the *P. sojae* NLSs may have evolved from nucleolar localization signals.

Co-regulation of gene expression in *Phytophthora*

Jasmine Pham[1,2], Remco Stam[1,2], Julie Squires[2,3], Christian Cole[4], Pieta Schofield[4], Peter Cock[2,3], Leighton Pritchard[2,3], Paul Birch[1,2,3], Michael Csukai[5], Steve Whisson[2,3], Edgar Huitema[1,2.] *[1]The University of Dundee at The James Hutton Institute, Invergowrie, Dundee UK; [2]Dundee Effector Consortium; [3]The James Hutton Institute, Invergowrie, Dundee, UK; [4]Division of Computational Biology, College of Life Sciences, University of Dundee, Dundee, United Kingdom; [5]Syngenta, Jealott's Hill International Research Centre, Bracknell, UK.*

Species of *Phytophthora* represent economically important plant pathogens which are notoriously difficult to control. Progression through the infection process requires the development of infection structures and production of proteins required for virulence, such as effector proteins. These changes are driven by co-ordinated regulation of gene expression, which in turn, suggests the involvement of promoter motifs and transcription factors in *Phytophthora*. The identification and characterisation of such regulatory elements may thus help understand and disrupt basic processes required for infection.
Here, we describe our efforts towards the identification of promoter motifs and transcriptional regulators in *P. capsici* and *P. infestans*. Using transcriptomics data from microarray and RNA-sequencing timecourse experiments of *P. capsici* infection on tomato, we identified clusters of co-regulated genes using both supervised and unsupervised clustering methods. Alignment and analysis of the promoter regions of clustered genes identified potential promoter motifs driving co-regulation of gene expression. We have also identified a number of transcription factors that appear to be regulated during biotrophy. Amongst those, a potential transcriptional regulator with similarity to the transcriptional repressor protein NmrA of *Aspegillus nidulans* was identified in both *P. capsici* and *P. infestans*. Over-expression of this NmrA-like protein in *P. capsici* resulted in reduced lesion formation and altered expression of the *PcHmp1* biotrophy marker gene *in planta*. Further work to determine how both transcription factors, as well as their putative target motifs, control *Phytophthora* gene expression during biotrophy is currently underway. We anticipate that a thorough understanding of transcriptional regulatory networks will help devise new and more targeted strategies to control *Phytophthora* epidemics in the field.

Nitrate assimilation pathway contributes to pathogenicity of *Phytophthora infestans*

Melania Abrahamian, Howard Judelson. *Dept. of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA*

Phytophthora infestans is a devastating potato and tomato pathogen that triggered the Irish potato famine in 1845. Since the 1840s efforts have been made towards understanding its pathogenicity. Our lab has generated RNA-seq data for *P. infestans* cultured on media or isolated from infected potato tubers and tomato leaves. Our RNA-seq data analysis identified a group of genes in *P. infestans* that are up-regulated highly when the pathogen is feeding on its host. This group includes genes for the uptake of amino acids, and the transport and assimilation of nitrate. We are interested in understanding the transcriptional regulation and importance of these genes in the fitness of *P. infestans*. For this purpose, we have generated knockdown strains for nitrate assimilation-related genes by homology-based silencing, and changes in growth, development, and pathogenicity were identified. According to our analyses there is no significant difference between wild type and knockdown strains when they are cultured on media. The knockdown strains however have reduced growth on leaves from nitrate-fertilized tomato plants. These results suggest that nitrate assimilation pathway contributes to the survival and pathogenicity of *P. infestans* on the host.

***Phytophthora* Suppressor of RNA silencing 1 (PSR1) targets a novel component of small RNA biogenesis in plants to promote infection**

Yongli Qiao[1,2,3], Jinxia Shi[1,2], Yingnan Hou[1,2], Yi Zhai[1,2], Wenbo Ma[1,2]
[1]Dept. of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA; [2]Center for Plant Cell Biology, University of California, Riverside, CA 92521 USA; [3]Current address: Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

A broad range of parasites rely on the functions of effector proteins to subvert host immune response and facilitate disease development. The notorious *Phytophthora* pathogens evolved effectors with RNA silencing suppression activity to promote infection in plant hosts. Here we report that the *Phytophthora* Suppressor of RNA Silencing 1 (PSR1) directly targets an evolutionarily conserved nuclear protein containing the DEAH-box RNA helicase domain in *Arabidopsis thaliana*. This protein, designated PSR1-Interacting Protein 1 (PINP1), regulates the biogenesis of both microRNAs and endogenous small interfering RNAs in Arabidopsis. A null mutation of *PINP1* causes embryonic lethality; and silencing of *PINP1* leads to developmental defects and hypersusceptibility to *Phytophthora* infection. These phenotypes are reminiscent of transgenic plants expressing *PSR1*, supporting PINP1 as a positive regulator of plant immunity and a direct virulence target of PSR1. We further demonstrate that the localization of the Dicer-like 1 protein is impaired in the nucleus of *PINP1*-silenced or *PSR1*-expressing plants, indicating that PINP1 may facilitate small RNA processing by regulating the function of Dicer-like proteins. A similar function of *PINP1* homologous genes in development and immunity was also observed in *Nicotiana benthamiana*. These findings highlight a conserved RNA helicase as a novel component

of RNA silencing and a general regulator of distinct classes of small RNAs in plants. Importantly, *Phytophthora* has evolved effectors to target PINP1 in order to promote infection.

CRN13 from *Aphanomyces euteiches* trigger host DNA damage

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

An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor

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Autophagy is a multifaceted membrane trafficking pathway involved in adaptation to cellular stress conditions such as starvation and pathogen infection. Activation of autophagy leads to formation of special vesicular structures called autophagasomes, which carry autophagic cargo to lysosomes or vacuoles for degradation. A form of autophagy, known as selective autophagy, can specifically degrade toxic substances such as invading pathogens. Selective autophagy functions through autophagy cargo receptors that confine the cargo within a special set of autophagasomes. Although the

role of autophagy in antibacterial defense responses has been documented in animals, the role of autophagy in plant-microbe interactions is unclear and somewhat controversial. Here, we discovered that a secreted RXLR-WY type effector of *Phytophthora infestans*, named PexRD54, binds to the autophagy marker protein ATG8. We identified an **A**TG8 **I**nteracting **M**otif (AIM) in PexRD54. Mutations in the AIM prevented both *in vivo* and *in vitro* PexRD54-ATG8 interactions. Consistently, overexpression of PexRD54 increased the number of GFP:ATG8 labeled autophagasomes and enhanced stability of ATG8 protein. To investigate the biological function of PexRD54, we studied the autophagy cargo receptor Joka2, which also interacts with ATG8. Overexpression of Joka2 *in planta* limited *P. infestans* infection, suggesting a role for Joka2/ATG8 selective autophagy in response to oomycete infection. Remarkably PexRD54, but not the AIM mutant of PexRD54, was able to out-compete Joka2 for binding to ATG8 and restore full pathogen virulence. Our findings point to a model in which an RXLR-WY effector from *P. infestans* antagonizes a selective autophagy cargo receptor to enhance pathogen virulence.

Effector Directed Breeding to Improve Soybean Resistance against *Phytopthora sojae*

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Phytopthora sojae is the causal agent of soybean root and stem rot, which is estimated to cause $1-3 billion in crop losses per year worldwide. We are using *P. sojae* RxLR effector genes to screen for novel resistance genes in accessions of cultivated soybean. The screening system uses *Pseudomonas fluorescens* (EtHAN) to transiently deliver a *P. sojae* effector into soybean cells by Type III secretion. If the effector is recognized by an *R* gene, a macroscopic hypersensitive response (HR) appears on the leaf. To improve signal intensity, we co-infiltrate with *Pseudomonas syringe pv. glycinea* race 4 which increases the growth of EtHAN *in planta*. Three known *P. sojae* avirulence effectors were used to validate this system. We then used this system to screen for novel *R* genes against *P. sojae* effectors *Avh*16, *Avh*180, and *Avh*240. These effectors are conserved, highly expressed, and important for *P. sojae* virulence. We screened 31 *G. max* lines that were selected for resistance against three resistance-breaking isolates of *P. sojae*. 19 lines responded to one or more of these three effectors. Resistant line 32 responded to both *Avh*16 and *Avh*240 and was selected for locus mapping. Line 32 (R) was crossed with Williams (S). A screen of segregating F3 populations suggests that line 32 contains at least 2 dominant *R* genes. We are using the data from this screen to map the genetic locus for each *R* gene. Our goal is to produce breeding lines with novel and durable *R* genes against *P. sojae*.

Clotrimazole as a potent Agent for Treating the Oomycete Fish Pathogen *Saprolegnia parasitica* through Inhibition of Sterol 14α-Demethylase (CYP51)

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A candidate CYP51 gene encoding sterol 14α-demethylase from the fish oomycete pathogen Saprolegnia parasitica (SpCYP51) was identified based on conserved CYP51 residues among CYPs in the genome. It was heterologously expressed in Escherichia coli, purified and characterized. Lanosterol, eburicol and obtusifoliol bound to purified SpCYP51 with similar binding affinities (K_s 3 to 5 μM). Eight pharmaceutical and six agricultural azole antifungal agents bound tightly to SpCYP51 with posaconazole displaying the highest apparent affinity (K_d ≤3 nM) and prothioconazole-desthio the lowest (K_d ~51 nM). The efficaciousness of azole antifungals as SpCYP51 inhibitors was confirmed by IC_{50} values of 0.17 to 2.27 μM using CYP51 reconstitution assays. However, most azole antifungal agents were less effective at inhibiting S. parasitica, S. diclina and S. ferax growth. Epoxiconazole, fluconazole, itraconazole and posaconazole failed to inhibit Saprolegnia growth (MIC >256 μg ml^{-1}). The remaining azoles only inhibited Saprolegnia growth at elevated concentrations (MIC 16 to 64 μg ml^{-1}) with the exception of clotrimazole, which was equally potent as malachite green (MIC ~1 μg ml^{-1}). Sterol profiles of azole treated Saprolegnia species confirmed that endogenous CYP51 enzymes were being inhibited with the accumulation of lanosterol in the sterol fraction. The effectiveness of clotrimazole against SpCYP51 activity (IC_{50} = ~1 μM) and inhibiting the growth of Saprolegnia species in vitro (MIC ~1 to 2 μg ml^{-1}) suggests that clotrimazole could be used against Saprolegnia infections including as a preventative measure by pretreatment of salmonid and other fish eggs and for fresh water farmed fish as well as in leisure activities.

Novel *Aphanomyces* sp. isolates from Lake Tahoe (USA) signal crayfish (*Pacifastacus leniusculus*)

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The causative agent of crayfish plague, *Aphanomyces astaci,* originates from North America. Regardless of that, the isolation and characterization of strains have mainly been conducted using samples from European crayfish populations. In Europe the disease has caused dramatic collapses of native crayfish populations. In this study, live specimens (n=20) of the signal crayfish (*Pacifastacus leniusculus*) from Lake Tahoe were imported to Finland after previous Asilomar OMGN-meeting 2013. The individuals were transferred in quarantine conditions and kept in separate tanks at +10 °C

containing sterilized lake water. Dead individuals were freshly mounted on peptone-glucose agar containing antibiotics. Several *A. astaci* isolates and two undescribed *Aphanomyces* sp. were isolated from the dead individuals. Based on the sequence analysis, the obtained *A. astaci* isolates were 100 % identical with the isolates of *A. astaci* PsI-genotype found in Europe. Furthermore, the sequence comparisons indicated that the *Aphanomyces* sp. isolates differed from the previously described *Aphanomyces* species. The first isolate showed closest similarity to the *Aphanomyces* sp. strain previously isolated from Daphnia in Europe. The second *Aphanomyces* sp. showed the highest similarity against *Aphanomyces* sp. strain from Japanese ice fish, being closest related to *A. frigidophilus*. An infection experiment was conducted to test if these strains have any effect on native European crayfish, but neither symptoms nor mortality were observed. In this presentation, we will describe these new isolates and discuss about their relevance as signal crayfish parasites.

Mitochondrial marker systems for studying *Phytophthora* and *Pythium* for community analysis

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Multiple species of *Phytophthora* and *Pythium* can often be present in a single environmental sample (e.g. soil, riverstreams, or plant tissue) and many have the potential to cause significant damage to agriculture and native ecosystems. Currently the markers that are available either have extensively long amplicons (>1kb) or do not fully discriminate individual species. New marker systems based on mitochondrial gene order differences were developed to overcome these challenges. While these marker systems are incredibly versatile tools for diagnostics they also have potential to facilitate community analysis in complex environmental samples. For *Phytophthora,* independent databases have been developed for two loci (*trnM-trnP-trnM* and *atp9-nad9*), which contain approximately 250 and 900 sequences, respectively. Furthermore, genus and species-specific TaqMan probes have been tested and validated for both loci and over 30 validated probes in addition to the 14 reported by Bilodeau *et al*., (2014) are currently available. Additionally, an isothermal detection method for rapid field diagnostics has been developed using recombinase polymerase amplification for *Phytophthora* at a genus specific and species-specific level for select species. For *Pythium* a database for another mitochondrial locus has been developed and currently consists of over 40 species. These tools represent a comprehensive hierarchal approach to understand *Phytophthora* and *Pythium* communities in environmental samples by using loci that have shorter amplicons (< 400 bp) with a high degree of polymorphisms between species. Taken together these markers should provide a useful infrastructure to metagenomic analyses for *Phytophthora* and *Pythium* species.

Lectin receptor kinase; sentinels in defense against plant pathogens.
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Plant breeders continuously face the challenge to obtain new cultivars with adequate levels of resistance to a variety of pathogens. In recent years, we identified a novel type of immune receptors that could be employed as disease resistance components towards both *Phytophthora* and bacterial pathogens. This resistance is mediated by cell surface receptors belonging to the family of L-type lectin receptor kinases (LecRKs). LecRKs are wide-spread in plants, and justifies exploitation of LecRKs as novel sources of crop resistance. The LecRK multi-gene family in Arabidopsis consists of 45 members, and their individual role in defense was determined in a genome-wide phenotypic analysis of T-DNA insertion mutants in infection assays. We found that multiple LecRKs play a role in resistance to a variety of plant pathogens, and that overexpression of various LecRKs enhances disease resistance. In addition, we screened for LecRK-Interacting-Proteins (LIPs) using mass spectrometry. LecRK interaction was confirmed for one of the candidates, LIP1, in co-immunoprecipitation assays. Further functional analysis showed that Arabidopsis Lip1 mutants are compromised in Phytophthora resistance in a similar fashion as LecRK mutant lines. Understanding how LecRK-mediated resistance is functioning is crucial to design novel resistance in crops against *Phytophthora* and bacterial plant pathogens.

Development of Downy-Mildew Resistant Cucumbers for the Eastern U.S.
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Cucurbit downy mildew, a disease caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov., is a serious threat to cucumber (*Cucumis sativus* L.) production worldwide and can result in 100% yield losses in affected environments. In the last decade, strains of the pathogen have overcome the resistance of commercial cultivars in the United States, and currently no cultivar has robust resistance to the disease. This lack of resistance has been especially problematic for cucumber growers seeking to capture the late-season market, when downy mildew is ubiquitous throughout Eastern and Great Lakes production environments. To improve resistance, resistance genes from the moderately-resistant cucumbers 'Ivory Queen' and 'Marketmore 97' were pyramided together into a new highly-resistant, late-maturing breeding line: DMR-NY264. In 2012 and 2013 field trials that included 27-35 entries of Cornell breeding material and the most resistant cultivars and USDA accessions identified in previous studies, DMR-NY264 had downy mildew resistance equal to or exceeding the most resistant wild accessions, and higher resistance and yields under disease pressure than all cultivars trialed. DMR-NY264 was subsequently crossed with early-maturing elite cucumbers in the slicing, pickling, and beit alpha backgrounds in order to develop highly-resistant, early, high-yielding

cucumber inbreds of diverse market types. The resulting populations are being leveraged to elucidate the genic regions contributing to this multigenic resistance.

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

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Internalization of oomycete and fungal pathogen effectors into host plant cells has been reported to be blocked by proteins that bind to the effectors' cell entry receptor, phosphatidylinositol-3-phosphate (PI3P). This finding suggests a novel strategy for disease control by engineering plants to secrete PI3P-binding proteins. We tested this strategy using the chocolate tree, *Theobroma cacao*, as a proof-of-concept crop system. Both transient and stable expression of secreted, functional PI3P-binding proteins in detached leaves of *T. cacao* greatly reduced infection by two oomycete pathogens, *Phytophthora capsici* and *Phytophthora palmivora* and the fungal pathogen *Colletotrichum theobromicola*. Microarray and quantitative reverse transcriptase PCR analyses revealed that cacao leaves transiently expressing a functional, secreted PI3P-binding protein showed a highly similar profile of differentially-regulated genes to control leaves infected with *P. capsici*. Many of the up-regulated genes were related to production of reactive oxygen intermediates, suggesting a form of priming response may be present. Staining with 3,3-diaminobenzidine (DAB) showed an increased basal level of hydrogen peroxide in cacao leaves with both stable and transient expression of functional PI3P-binding proteins. These results suggest that secretion of PI3P-binding proteins enhances resistance to oomycete and fungal pathogens, potentially by activating defense signaling in addition to the possibility of inhibiting effector entry.

Manipulation of host proteins to generate resistance to *Phytophthora infestans* in solanaceous crops.

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The *Phytophthora infestans* RxLR effector PexRD2 is secreted into the host cell cytoplasm where it interacts with a host Mitogen Activated Protein Kinase Kinase Kinase (MAPKKKε). MAPKKKε forms part of a signalling cascade which initiates a localised cell death response upon recognition of an.unidentified MAMP. PexRD2 forms

a homodimer *in planta* and directly interacts with MAPKKK$_\varepsilon$ in order to suppress cell death. The present study used random mutagenesis PCR to generate a library of mutant variants of MAPKKK$_\varepsilon$ to be screened for insensitivity to effector activity. >560 variants were screened in transient assays for their ability to initiate cell death when co-expressed with PexRD2. Currently, two mutant sequences, carrying two amino acid mutations each, have been demonstrated to reproducibly exhibit insensitivity to the effector. Each point mutation is now being examined for its contribution to insensitivity MAPKKK$_\varepsilon^{Asp241Asn}$ appears to be sufficient for insensitivity whilst MAPKKK$_\varepsilon^{Ser200Thr}$ appears to have no effect when examined individually, as opposed to together. MAPKKK$_\varepsilon^{Phe10Leu}$ and MAPKKK$_\varepsilon^{Pro49Arg}$ appear on the same variant sequence and seem to be additive, as incidence of cell death appears to be higher when the mutations are combined. Future work will aim to assess the potential of these kinase mutants to generate resistance *in planta* and to elucidate the biochemical mechanisms of suppression.

Displacements and evolutionary origins of US lineages of *Phytophthora infestans*

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Phytophthora infestans, causal agent of the Irish potato famine, is a threat to food security globally and an important pathogen in the southeastern US on both tomato and potato. In 2009, potato and tomato late blight epidemics in the eastern US were the worst in modern history due to widespread inoculum distribution from infected tomato transplants and conducive rainy weather events. USABlight (www.usablight.org) was launched in 2011, and recent lineages in the US have been monitored. The mefenoxam sensitive US-22, more common on tomato in 2009, has been displaced by US-23, which infects both hosts. The genetic relationships and origins of 18 of the 24 clonal lineages found in the US since the 1970s were examined using 12 simple sequence repeat (SSR) markers. The US-1 lineage formed a cluster distinct from most modern US lineages in PCA and STRUCTURE analyses of both RFLP and SSR datasets. Five clusters of lineages were identified, and many US lineages showed similarity to Mexican lineages with the exception of US-1 and US-23. US-1 was not the first lineage introduced into the US or Europe in the 19th century; mitochondrial haplotype data and NGS data from herbarium specimens indicate that the Herb-1 (Ia) lineage was introduced first. The US-1 (Ib) lineage was introduced later and found multiple times in the US and globally in early 20th-century specimens – some of which originated from South American sources. The evolutionary relationship of recent US lineages will be discussed in the context of possible pathogen migration routes of the pathogen in the Americas.

Population genomics of New World *Phytophthora infestans*.

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The appearance of the plant pathogen *Phytophthora infestans* in Europe in 1845 triggered the Irish potato famine and massive crop losses that continued until effective fungicides were widely deployed in the 20th century. The pathogen is now ubiquitous in global potato and tomato fields, and more aggressive and virulent strains have surfaced within the last two decades. Recent studies used herbarium specimens of infected potatoes to investigate genomic differences between early European *P. infestans* and those strains circulating in modern fields. Phylogenetic analysis of complete mitochondrial genome sequences derived from modern and 19th-century specimens showed that historical samples of *P. infestans* belonged to a mitogenomic lineage distinct from other known lineages and now likely rare or extinct in modern times. We report 44 additional *P. infestans* mitogenome sequences, including seven from historical Europe and many from across modern populations in the New World. Through maximum-likelihood and Bayesian phylogenetic analyses, we show that the HERB-1 lineage is still maintained in Mexican and South American populations. We also argue that multiple mitochondrial haplotypes were present in early European potato fields, and that this lineage began to diverge an estimated 75 years before the first reports of potato late blight in Europe. Ongoing population genetic analyses of the entire set of available *P. infestans* whole nuclear genomes, along with those of several outgroup species, provide an early view of the complex demographic history pre-dating migrations of this New World species to Europe and the rest of the world.

Genomic signatures of host jumping onto raspberry and strawberry in two *Phytophthora* sister taxa

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Contrasting the genomes of sister taxa with narrow but different host ranges provides an ideal system for studying host adaptation. Selection pressures imposed by different hosts are expected to lead to adaptation by gradual divergence of populations and eventual speciation due to reproductive isolation. This process of host-mediated speciation is expected to leave signatures (such as mutations, recombination, indels, or duplications) in the corresponding pathogen genomes. Using paired-end approaches on the Illumina HiSeq2000 platform we sequenced and annotated the genomes of two sister species in the plant pathogen genus *Phytophthora*, *P. rubi* and *P. fragariae*, to

study which genomic signatures are involved in host adaptation (*P. rubi* and *P. fragariae* exclusively infects the genus *Rubus* or *Fragariae*, respectively). Genomes were assembled using SOAPdenovo version 1.05. Gene calling was performed using MAKER, AUGUSTUS, and SNAP. The genomes of *P. rubi* and *P. fragariae* yielded 9,434 scaffolds and an estimated 18,268 genes for *P. rubi* and 8,511 scaffolds and 17,832 genes for *P. fragariae*. Functional annotation showed a similar number of genes involved in different biochemical pathways, such as sugar processing, metabolism, and amino acid synthesis between the two species. Positive selection (dN/dS) was detected for three pairs of orthologous effector proteins in the RxLR class. Work is ongoing to identify other candidate genes under selection and genomic signatures that may have been involved in the process of speciation via host jumping.

De novo assembly and analysis of transcriptome of _Peronospora belbahrii_

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Basil is an important herb crop widely used in Mediterranean cuisine, traditional medicine and cosmetics. Basil downy mildew, a disease caused by the obligate biotrophic oomycete pathogen *Peronospora belbahrii*, is threating basil production worldwide. This foliar disease is very damaging due to its fast disease development and rapid spread. Understanding its pathogenesis mechanisms is key to developing novel effective and environmental friendly disease control strategies. To this end, we performed multiplexing mRNA-seq analysis with total RNA isolated from purified *P. belbahrii* sporangia, non-infected basil leaves, and infected basil leaves during the infection time course. The reads generated from *P. belbahrii* sporangia were assembled into transcripts through *de novo* assembly using Trinity. A local BLAST search against *Phytophthora infestans* protein database showed that the amino acid sequences of many conserved proteins from both species were well aligned, suggesting that the quality of the Illumina sequencing and assembly is acceptable. The secretome, putative effectors and their expression profiling during infection are being analyzed. In addition, the expression patterns of basil genes during infection are being analyzed to gain insight into the molecular basis of plant responses. Detailed results will be presented in the meeting.

Dynamics of the actin cytoskeleton in _Phytophthora infestans_ hyphae and infection structures.

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The actin cytoskeleton is a dynamic but well organized intracellular framework that is indispensable for the viability of eukaryotic cells. Its functions range from intracellular transport, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. We studied the actin cytoskeleton dynamics in the filamentous oomycete plant pathogen *Phytophthora infestans* in transgenic lines expressing the

actin binding peptide Lifeact-eGFP by fluorescence microscopy. This showed that in hyphae actin filament cables and plaques are cortically localized. The distance between the hyphal tip and the first actin filament plaque correlated strongly with growth velocity. Upon growth termination, actin filament plaques appeared in the hyphal tip. The plaques were nearly immobile with average lifetimes exceeding one hour; much longer (over 500-fold) than the lifetimes of actin patches in fungi. Plaque assembly required ~30 seconds while disassembly took only ~10 seconds. In contrast to actin patches in yeast, plaque disassembly was not accompanied with formation and internalization of endocytic vesicles (Meijer et al. 2014, Cell. Microbiol.). We also investigated the *in vivo* actin dynamics during early stages of pathogenesis. At the site of contact with the plant cell a condensed transient actin structure was observed that resembles aster-like actin structures formed upon encountering hard surfaces. Our results suggest that the actin cytoskeleton has distinct functions during the *P. infestans* lifecycle. Future efforts will focus at identifying interactors and key regulators of the actin cytoskeleton and pinpoint features in the actin network that are unique for oomycetes.

Displacement of US-1 clonal lineage by a new lineage of *Phytophthora infestans* on potato in Kenya and Uganda

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Phytophthora infestans, the causal agent of potato late blight, was formerly present as an old clonal lineage (US-1) in much of sub-Saharan Africa. A collection from South Africa, Mozambique, Malawi, Tanzania, Burundi, Rwanda, Uganda, and Kenya, made in 2007 consisted primarily of the US-1 clonal lineage (Ib mitochondrial haplotype), although one lineage, designated KE-1 (Ia mitochondrial haplotype), was found in two fields in Kenya. Analysis using mtDNA and microsatellite markers of samples collected in 2011 / 2012 showed that US-1 lineage has been completely displaced in Kenya on potato by KE-1. In Uganda, the eastern part had the 1a haplotype while western Uganda had mostly the 1b haplotype with the 1a haplotype being found in 8 fields, which is consistent with an on-going displacement process. All tomato isolates were the US-1 lineage. KE-1 lineage has not displaced US-1 on tomato implying differences between potato and tomato US-1 genotypes. Further analyses on Ipi0 allelic diveristy of the two lineages revealed presence of diverse virulent isolates within US-1 genotype that could break RB resistance gene. KE-1 lineage is supposedly more aggressive than the US-1 lineage since it has displaced it on potato. Presence of KE-1 lineage will undoubtedly increase difficulties in controlling potato late blight in east Africa.

Population Structure of Downy Mildew on Spinach

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Spinach production in the United States has increased exponentially with the popularity of baby spinach. The increase in demand has changed production practices such as denser plantings and decreased rotation. Downy mildew on Spinach is caused by the oomycete *Peranospora farinosa* f. sp. s*piniacae.* This diploid organism is an obligate, heterothallic pathogen. *P. farinosa* is race typed using a panel of commercial cultivars. This process uses a significant amount of resources and time. The rate of race identification has increased in recent years making it difficult for breeders to keep up. We sequenced two isolates representing two races in order to find single nucleotide polymorphisms (SNPs) between the isolates. The SNPs chosen are silent, genic, and ranging across multiple contigs. Population structure of isolates on a variety of spinach cultivars from Arizona and California is assessed using targeted sequencing of SNPs. The results and implications will be discussed.

Developing species-specific molecular diagnostics for cucurbit downy mildew

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Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is the number one disease limiting cucurbit production in the United States. Growers experience significant losses every year due to severe defoliation of plants that reduce fruit yield and quality. Currently, the disease can only be controlled through aggressive and expensive fungicide programs that are initiated when an outbreak has occurred in the state or surrounding counties. Outbreaks are typically reported when disease symptoms are severe enough in the plant to be detected through visual inspection. Early detection of *P. cubensis* prior to infection by using spore traps or in the initial stages of infection by using molecular tools could help reduce fungicide applications and production costs for growers. To address this need, we used Illumina next generation sequencing and comparative genomics between *P. cubensis* and its sister species *P. humuli*, the causal agent of hop downy mildew, to identify candidate genomic regions for species-specific molecular detection of *P. cubensis*. We compared the transcriptomes of diverse *P. cubensis* and *P. humuli* isolates, selected candidate genomic regions unique to *P. cubensis*, and tested these candidates against a diverse *P. cubensis* and *P. humuli* panel to confirm specificity. In addition to delivering a molecular diagnostic tool for *P. cubensis*, through this project we have generated genomic data for two economically important downy mildew pathogens that can increase our understanding of evolutionary relationships and host specificity in these sister species.

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

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Phytophthora sojae is the causal agent of soybean root and stem rot. Attempts to control this pathogen have been focused on the use of resistance genes (*R* genes) that recognize pathogen effector proteins. Our aim is to identify *R* genes against core effectors in *P. sojae*-resistant cultivars. We hypothesize that a resistance gene against a core effector will provide durable resistance against a broad range of *P. sojae* isolates. We further hypothesize that *Glycine soja*, a sexually compatible, wild relative of cultivated soybean, can be exploited as a source of new *R* genes against *P. sojae*. We are using an effectoromics approach to identify *R* genes against *P. sojae* effectors. Using essential effectors as probes we have found several *G. soja* cultivars that produce effector-specific hypersensitive responses, suggestive of *R* genes that recognize these pathogens. We are currently breeding selected lines that contain putative *R* genes to validate their functions and map the resistance loci. We are screening F3 populations descended from crosses of resistant, effector-responsive *G. soja* or *G. max* lines to the susceptible soybean (*Glycine max*) cultivar Williams. We will present data from a preliminary screen of progeny from Line 326 X Williams. Line 326 responds to the core effector Avh180 and is resistant to three isolates of *P. sojae* that have collectively overcome all *R* genes currently deployed in cultivated *G. max*. We hope to use these data to map the resistance gene and develop breeding lines with a new *R* gene against this destructive pathogen.

The oomycete phytobiome, a case study of soybean roots

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The root and surrounding rhizosphere ecosystem is dynamic and subject to microbial communities and their interactions, which has a profound impact on host physiology which in turn affects the associated microbiota. Oomycetes are part of this ecosystem and are some of the most aggressive and important plant pathogens, many of which are soilborne. Yet, few studies have examined oomycetes from a community perspective, including the role of pathogen complexes. In the United States soybeans are produced across 76 million acres of highly productive land, but can be severely impacted by diseases caused by oomycetes. We initially utilized a two-year culture based survey to study oomycetes associated with soybean seedlings from across 11 states in the Midwest, characterizing the communities and profiling phenotypic traits such as pathogenicity and aggressiveness. With this approach a total of 3,500 isolates were collected. Within this isolate collection a total of 83 different oomycete species were identified and characterized. To further investigate and characterize oomycete

communities associated with agricultural systems we are developing and utilizing markers for amplicon-based community analysis. Through the use of amplicon-based community analysis together with our oomycete culture phenotype data (i.e. pathogenicity) and the role of climatic, edaphic and biotic factors (such as host and plant growth stage), we will improve our understanding of the phytobiome and the factors that community and disease dynamics. This will enable improved disease management and will assist with maximizing yield and sustainability of production.

Mining of a plant pathogen genome to identify candidate WY-domain containing pathogenicity proteins

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The most destructive plant pathogens use secreted proteins known as effectors to hijack host metabolism and suppress the immune system, often by direct protein-protein interaction with their host targets. Effector-target interactions are not well understood on a biochemical level; however, crystal structures of several oomycete effectors have recently revealed a structurally conserved WY-domain that may play a role in these protein-protein interactions. The genome of *Bremia lactucae*, the causative agent of lettuce downy mildew, has been sequenced by the Michelmore lab and comparative and functional genomic analysis of the draft genome is underway. We have mined the *B. lactucae* genome for genes encoding WY-domain candidate effectors. We identified a family of expressed proteins with variable numbers of WY-domains per protein. Several candidates have no known homologs in the oomycetes and those with homologs were only distantly related, suggesting lineage specific divergence. Many of these WY-proteins are predicted to be secreted and a subset of them also contains the RXLR motif that is characteristic of secreted oomycete effectors. These candidate effectors will be functionally tested *in planta* for suppression of cell death and effector targets will be identified using high- throughput yeast two-hybrid assays and co-immunoprecipitation. Effector and host target identification will provide the basis for future research into the molecular mechanisms of pathogenesis.

POSTER ABSTRACTS

(listed alphabetically by name of submitting author)

Session 1: odd numbers
Session 2: even numbers

1. Tools for Functional Analysis of Effector Genes in *Phytophthora sojae*

Felipe Arredondo[1], Shiv D. Kale[2], Asula Megan[1], Tian Zhou[2], Hua Wise[1], Brett Tyler[1].
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Plants produce a molecular defense response when they are challenged by a plant pathogen. *Phytophthora sojae*, a soybean pathogen, produces effector proteins to promote infection. Effectors include Avirulence (Avr) genes products that can interact with plant resistance proteins (Rps), if present, resulting in effector-trigger immunity. There are possibly hundreds of effector genes in *Phytophthora* species that are involved during infection of a plant host. Understanding the function of these genes during infection is still not clear and more studies of this complex system are needed. Our lab uses several molecular tools to assess the role of these genes in pathogenicity. Some of these tools and methodologies could be used in other *Phytophthora* species to confirm and support evidence of effector genes. An effective and reliable method is the protoplast/PEG transformation with DNA or dsRNA. These methods can introduce fluorescent proteins as cytological markers or over express or silence a target gene. Silencing genes via DNA or dsRNA can produce stable or transiently silenced transformants respectively. Silencing effector genes in *P. sojae* has been used to test the role of many effector genes during infection. Another molecular tool is the double-barrel particle bombardment, which enables transient expression of *P. sojae* genes in soybean tissue and accurate measurement of the effects of that expression.

2. Quorum sensing of the zoospores in *Phytophthora capsici*

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Phytophthora capsici is a soil-borne oomycete pathogen, it can infect multiple hosts, and cause considerable economic loss to agriculture. As the secondary infection in disease cycle, zoospores are vital to pathogenicity of *P. capsici* in disease epidemic. In this study, we investigated the quorum sensing effect in zoospores of *P. capsici*. It was observed that the zoospores would gather together in a certain density, and the lowest concentration for aggregation was 0.5×10^5 ml^{-1}. Pathogenicity tests showed that the infection ability of the zoospores would be weakened through reduced inoculum concentration, and a single zoospore can't infect successfully. Furthermore, we found that zoospore free fluid (ZFF) can promote the zoospores aggregation, and also can enhance the infection ability of a single zoospore. The effect of ZFF on different growth stages of *P. capsici* was investigated. The result showed that ZFF can promote cyst germination and germ tube elongation. Interestingly, ZFF also can promote infection ability of zoospores in *phytophthora infestans*. It is presumed that there are some signal

molecules in ZFF to regulate the zoospores aggregation and infection, and these signal molecules may be active across species boundaries. Further research is currently in progress to clarify the regulatory effect of signal molecules on the quorum sensing in zoospores of *P. capsici.*

3. Before war: transcriptional reprogramming of *Phytophthora parasitica* prior to infection in citrus

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Brazil and USA are the top orange producers in the world, however, there is a constant threat from diseases, such as root rot caused by *Phytophthora parasitica*. It is well known that *Phytophthora* species secrete hundreds of effectors that enable infection. Manipulating the effectors can lead to pathogen loss of virulence. The mechanistic molecular functioning of effectors remains poorly understood for phytophthora-citrus diseases. We were particularly interested in the moments before infection, i.e. how *P. parasitica* prepares itself facing signals that plants are around, also how many genes are differentially expressed and which one are putative effectors? Are there differences in the transcription reprogramming of *P. parasitica* facing signals from a susceptible or a resistant host? To answer these questions we cultivated *P. parasitica* in liquid culture with or without root extracts of a susceptible citrus variety (Sunki) and a resistant one (*Poncirus trifoliata*). We harvested the mycelia and extracted RNA to perform a RNAseq through Illumina sequencing. Surprisingly, there are much more down-regulated genes than up-regulated ones in both treatments with the susceptible or resistant plant extracts in comparison with control treatment. However, among the up-regulated genes, we have found several RxLR and Crinkler effectors (also plotted in the genome architecture of *P. parasitica*). There were much more up-regulated effectors in the mycelia treated with the sunki (susceptible variety) extract in comparison with the ones treated with *P. trifoliata* extracts. The RxLR and CRN effectors found in this analysis will be target for functional characterization during infection in citrus plants.

4. Identifying mechanisms at the intersection of plant nutrient transport and oomycete virulence

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Successful plant pathogens must execute two tasks. The first task, suppression of plant immunity, has been studied intensively and is increasingly well understood. The second task, equally important but much less understood, is to acquire nutrients from the plant host. Emerging evidence suggests that plant pathogens reprogram host pathways for nutrient biosynthesis and transport. However, little is known about the mechanisms through which oomycetes extract nutrients from susceptible host plants. We have initiated a project to define how oomycetes accomplish this task, with emphasis on

identifying host susceptibility genes. In particular, we intend to identify plant pathways for nutrient acquisition and transport that are co-opted by pathogens. We will describe new experimental tools that are under development to support this project, along with preliminary functional genomic experiments to identify plant nutrient acquisition/transport genes that are necessary for pathogen colonization.

5. Cold spring rains brought perfect conditions for *Pythium* in Ohio and a few more surprises

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The 2014 season brought widespread seedling damping-off to most of Ohio. Cool temperatures (40-50°F) and excess rainfall (over 2 inches) within a week of planting provided environmental conditions that were especially conducive to soil-borne pathogens. Samples of symptomatic seedlings or soil for baiting were collected from more than 20 fields in Ohio. Both *Pythium spp.* and *Fusarium spp.* were recovered from symptomatic seedlings. Nine *Pythium* species were identified from 15 of these fields including: *P. conidiophorum, P. dissotocum, P. heterothallicum, P. inflatum, P. perplexum, P. sylvaticum, P. torulosum, P. ultimum var. ultimum*, and *P. vexans*. Twenty of twenty six isolates were insensitive to metalaxyl in a preliminary test. Therefore, seed treatments with this single active ingredient will not provide protection from the wide diversity of pathogens contributing to the seedling disease complex. In order to improve stand count and decrease the rate of damping-off, seed treatments should include a combination of active ingredients.

6. Variability of *Phytophthora infestans* effectors that determine the outcome of R gene based resistance to potato late blight

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International potato center (CIP) is working on a transgenic approach to obtain a late blight (LB) resistant potato variety for Africa. The potato cultivars Desiree and Victoria were transformed with three LB resistance genes *RB* (*Rpi-blb1*), *Rpi-blb2* from *Solanum bulbocastanum*, and *Rpi-vnt1* from *S. venturii*, with the hope of obtaining durable resistance. These three genes recognize pathogen proteins (effectors) that act as avirulence genes (*Avr* genes) leading to the resistance reaction. Functionality and durability of the resistance of potato against late blight is determined by the effector allele composition and their expression in the *P. infestans* strains present in the field. Here we describe the level of allelic and expression variation of the effectors Avr-blb1, Avr-vnt1 and Avr-blb2 in the *P. infestans* isolates collected from Peru and Uganda.

7. Functional characterization of putative extracellular cystatins in *Phytophthora palmivora* pathogenicity on papaya

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Phytophthora palmivora is an economically important oomycete pathogen that causes root rot, stem canker, fruit rot, and damping off of papaya. Fruit lesions produce milky latex, which contains large amounts of cysteine proteases, including papain. In addition, latex from papaya stems and leaves is also rich in papain. Proteolysis of non-self proteins has been established as an important aspect of host defense. Due to the high abundance of papain in papaya latex and its broad specificity in protein degradation, it is likely that *P. palmivora* has developed ways to interact with and inhibit papain to facilitate infection. Four putative cystatin-like extracellular protease inhibitors (PpmEPICs) were identified from *P. palmivora* raw sequence data. *PpmEPICs* transcripts were detected during infection of papaya fruit tissue, though no significant upregulation was observed. One of these proteins, PpmEPIC-G4, inhibited papain hydrolysis of casein substrate in a dose dependent manner. These results demonstrate that *PpmEPICs* are expressed during infection of papaya fruit and that PpmEPIC-G4 is a functional cystatin capable of arresting papain enzyme activity. We are currently testing whether PpmEPIC-G4 plays a role in virulence by gene silencing and overexpression. Detailed results will be presented in the meeting.

8. Identification of the *DNA methyltransferase* 1-*associated* protein in the Oomcyete

Alexander Howard, Vipaporn Phuntumart. Bowling Green State University, Ohio, USA

Oomycetes are fungus-like eukaryotic organisms that are mostly known as pathogens to various forms of cash crops and animals. These complex organisms require various mechanisms for gene regulation to maintain expression of their genes. However, DNA methylation has not yet been characterized for these organisms. Given that, a bioinformatics approach was used to identify the *DNA methyltransferase* 1-*associated* protein, DMAP-1 in *P. sojae*. Protein-protein BLAST was used to perform search against the non redundant protein database (nr) via FungiDB and National Center for Biotechnology Information (NCBI) to find oomycete homologues of DMAP-1. The conservation of this gene in the genome of oomycetes indicates that DMAP-1 may play a vital role in these organisms. A phylogenetic tree was constructed in order to gain a better understanding the evolutionary route and, in turn, hint at the origins of the gene. Expression data of the DMAP-1 gene will also be presented.

9. Biochemical Characterization of the *Phytophthora sojae* effector protein Avr1b expressed in *Pichia pastoris*

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The plant pathogenic oomycete *Phytopthora sojae* is the cause of soybean root rot and results in significant loses each year to global soybean production. Races of *P. sojae* expressing *Avr1b* result in an avirulent phenotype on *Rps1b* soybean cultivars. Avr1b is one of several known oomycete RxLR-dEER effector proteins capable of translocating into host cells both in the presence and absence of the pathogen. Using the methylotrophic yeast *Pichia pastoris* we have established a platform to express and purify milligram quantities of secreted Avr1b as well as other oomycete and fungal effectors. Using differential scanning fluorimetry and circular dichroism spectroscopy we show the protein is well folded. Using surface plasmon resonance and liposome binding assays we show the protein has strong affinity (~250 nM) to PtdIns-3-P.

10. The *Phytophthora* genus sequencing project

Brent Kronmiller, Danyu Shen, Javier Tabima, Stephanie Bollmann, Felipe Arredondo, Niklaus Grunwald, Brett M. Tyler, Beijing Genome Institute (BGI), Phytophthora Genus Sequencing Consortium

Phytophthora is a genus of Oomycetes containing over 120 species that cause a huge variety of plant diseases. While some *Phytophthora* species infect only one plant host, some have a broad host range and can cause diseases on thousands of plants. Six species of *Phytophthora* have been previously sequenced. These six species are relatively diverse; they are found on four distinct clades of the ten-clade *Phytophthora* genus phylogenetic tree. Two sequenced species, *P. sojae* and *P. infestans*, show very different genomes. *P. sojae* has an assembled genome length of 83Mb and is 40% repetitive, while *P. infestans*' length is 229Mb with 74% repeats. In order to better understand the differences observed within the *Phytophthora* genus, the Phytophthora Genus Sequencing Consortium has generated sequencing data for 29 additional species. Here we report on genomes from eleven *Phytophthora* species from clades 7 and 8c. On average 56M Illumina 90 base pairs (bp) paired end reads were sequenced per genome by the BGI. This resulted in approximately 5 Gb of sequence per genome - for a 100 Mb *Phytophthora* genome this equaled 50X coverage. Two RNA samples from each species were also sequenced to produce an average of 26M 90bp paired end transcript reads per genome. Genomes were assembled by the BGI. RNAseq reads were de-novo assembled to create transcriptomes for each species. Genes were annotated using the genomic and RNAseq data sets with MAKER, and functional annotations were assigned.

11. Functional characterization of a conserved *Phytophthora* RxLR effector with RNA silencing suppression activity

Ariel Tung Kuan[1,2], Shuyi Duan[1,3], Wenwu Ye[3], Yi Zhai[1,2], Qin Xiong[1,3], Duseok Choi[1,2], Yuanchao Wang[3], Wenbo Ma[1,2]. *[1]Dept. of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA; [2]Center for Plant Cell Biology, University of California, Riverside, CA 92521 USA; [3]Dept. of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China*

Phytophthora are a group of oomycete plant pathogens that devastate economically important crops and wood trees worldwide. Sequence analysis of *Phytophthora* genomes revealed hundreds of virulence proteins, also known as cytoplasmic effectors, that function inside the plant cells to promote infection/disease. Recently, we have identified two RxLR effectors functioning as silencing suppressors from *Phytophthora sojae*, and named them ***P****hytophthora* **S**uppressors of **R**NA silencing **1** and **2** (PSR1 and PSR2). Importantly, transient silencing of *PSR2* in *P. sojae* (*PsPSR2*) led to significant reduction of disease symptoms on soybean seedlings, suggesting that PSR2 is an essential virulence factor in *P. sojae*, probably by inhibiting plant RNA silencing pathways. Here we analyzed the prevalence of *PSR2* homologs in *Phytophthora* by using the genomes of several *Phytophthora* species. Phylogenetic analysis suggested that *PSR2* family is highly conserved in *Phytophthora*; furthermore, each *Phytophthora* species produces approximately 20-30 *PSR*2 homologs with diversified sequences. Characterization of a *PsPSR2* homolog in *P. infestans* (*PiPSR2*) confirmed that it also possesses the RNA silencing suppression activity, suggesting that PSR2 homologs may have a conserved function in manipulating host RNA silencing activity. Interestingly, the PSR2 homologs exhibit a highly specific pattern containing multiple repeat units and each repeat unit is consisted of the conserved W, Y and L motifs. Ongoing investigation on specific repeat(s) that is indispensable for the RNAi suppression activity of PSR2 and potential targets of PSR2 in plants will be discussed.

12. Diversity of determinants of mefenoxam insensitivity in *Phytophthora infestans*

Michael Matson and Howard Judelson. *Dept. of Plant Pathology and Microbiology, Univ. of California, Riverside, CA USA*

Recently, a mutation determining sensitivity to the fungicide mefenoxam was identified at nt 1145 of the gene encoding the largest subunit of RNA Polymerase I (RPA190) of *Phytophthora infestans* (Randall et al, 2014). We have developed a High Resolution Melt (HRM) assay that can predict SNP T1145A at this position and presumably the sensitivity phenotype of isolates. Our screening of diverse isolates has revealed many insensitive strains lacking the resistance SNP as well as isolates showing a diversity of response phenotypes independent of the dosage of T1145A alleles. We sought to investigate resistance in these isolates, and generated crosses between resistant isolates and other sensitive isolates to follow segregation of the SNP. Sequencing of the progeny of two such crosses showed no linkage of resistance to RPA190. However, RAPD markers previously associated with resistance in *P. infestans* were found to be genetically linked to resistance in these crosses. The fragmented nature of

the *P. infestans* reference genome makes walking along the sequence to search for candidate genes difficult, so a Bulked Segregant Analysis sequencing approach is being used to identify genes conferring resistance in these crosses.

13. Marker development for the identification of field isolates of *Phytophthora phaseoli*

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Lima bean is the cornerstone of the Delaware vegetable processing industry. However, this specialty crop is susceptible to the yield-reducing oomycete *Phytophthora phaseoli*, causal agent of downy mildew on lima bean. Genetic inference on geo-tagged samples of isolates from lima bean production farms could facilitate pathotype determination and suitable cultivar choices for a given growing season. Furthermore, the ability to monitor regionally defined – Mid-Atlantic region (MAR) – race evolution may enable more informed breeding approaches aimed at the development of resistant varieties. Performing genotyping-by-sequencing on a collection of physiologically validated isolates of *P. phaseoli* belonging to racial classes C, D, E and F, this study aims to identify genotypic profiles predictive of racial structure. No reference genome sequence exists for *P. phaseoli*. Therefore, two approaches for SNP discovery were employed: 1) using a related species (*P. infestans*) as a reference genome, ~32,000 SNP loci were identified; 2) using a *de novo* cluster-based method, ~1,500 SNP loci were identified. Markers selected from these loci will be tested on populations of *P. phaseoli* that have been collected from the MAR, with 68 isolates having been collected over the 2013-2014 seasons. Physiological race testing is also being conducted on these isolates.

14. Functional and phylogenetic analyses of exo-1,3-beta glucanase gene (*PinsEXO1*) from the pathogenic oomycete *Pythium insidiosum*

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Pythium insidiosum is an animal pathogenic oomycete. It is the causative agent of pythiosis, which is a disease affecting mammals, including humans Recently, an exo-1,3-beta glucanase gene (*PinsEXO1*), encoding a 74-kDa immunoreactive protein recognized by serum samples from patents with pythiosis, was identified in all strains of *P. insidiosum* tested. Glucanases are hydrolytic enzymes responsible for the hydrolysis of glucans, polymers of glucose found abundantly in plant, fungi and oomycete cell walls. *PinsEXO1* is highly expressed during infection, and it is hypothesized to be involved in cell wall remodeling of the oomycete. The gene for *PinsEXO1* was subsequently cloned into the pRSET-B plasmid vector and expressed in *E. coli*. Here, a functional analysis of *PinsEXO1* has been developed to determine substrate specificity. In addition, a phylogenetic tree has been constructed to investigate the evolutionary relationships of *PinsEXO1* with glucanase enzymes in other oomycetes. This has allowed for the examination of the homology between glucanases from both plant and

animal pathogens. The significance of these results as they pertain to furthering our understanding of the role *PinsEXO1* plays in infection will be discussed.

15. A screen for *S. cerevisiae* mutants hypersensitive to the *P. sojae* effector PsAvh172

Matthew Sydor and William Morgan. *Dept. of Biology, College of Wooster, Wooster, OH, USA 44691*

Phytophthora sojae encodes hundreds of host-translocated RxLR effector proteins, many of which are expressed while establishing stem and root rot in soybean. Functional genomics analysis in the yeast *Saccharomyces cerevisiae*, a model eukaryote, has previously been used to identify conserved biological pathways targeted by bacterial effectors. The ability of *P. sojae* RxLR effectors to inhibit yeast growth suggests that each targets a biological pathway conserved among eukaryotes. To explore the function of individual oomycete effectors, we have begun to screen for yeast knock-out mutants that are hypersensitive to a *P. sojae* RXLR effector. Using the complete collection of nearly 5000 viable yeast knockout mutants, Kramer *et al.* (2007) initially demonstrated that the host target of a pathogen effector could be predicted based on which mutants are hypersensitive to effector expression. Bosis *et al.* (2011) subsequently demonstrated that a carefully selected subset of less than 100 yeast knockout mutants was nearly as powerful for identifying probable effector targets. The reduced number of mutant strains required for this modified procedure has allowed us to now pursue this approach in our undergraduate research laboratory. Preliminary results indicate that the growth of several yeast knockout strains is compromised by expression of the *P. sojae* effector PsAvh172. Bioinformatics analysis of verified hypersensitive mutants promises to suggest likely targets of this and other oomycete effectors.

16. High throughput assay to detect novel inhibition of *Phytophthora sojae* by environmental bacterial isolates

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We have developed a high throughput *in vivo* assay to determine the protective qualities of natural environmental microbes against significant soybean pathogens, such as *Phytophthora sojae,* in order to ultimately identify mechanisms of oomycete antagonism. We use a high-throughput approach to identify culturable environmental microbes that are able to inhibit the growth of *P. sojae* when grown together on a V8 agar media. We have identified microbes with a strong inhibitory phenotype against *P. sojae* and developed a soybean seed assay to determine if the inhibitory activity against the oomycete on media translates to protection of a germinating soy plant from active root rot infection. The results from repeated seed assays has shown that germinating soybean seeds co-grown with specific environmental isolates are remarkably protected from infection by *P. sojae*. We are developing a full plant assay to expand upon our seed assays and determine if we can fully protect a seed through its germination period

into maturity when grown in the presence of *Phytophthora sojae.* Those environmental microbes that offer the greatest protection against *P. sojae* will be characterized to determine the mechanism of protection. To address this question, we have successfully optimized genetic tools, including transposon mutagenesis and fosmid library expression, which can be used to identify the genetic basis for the protective phenotype. These methods provide a foundation for the continuing collaborative research with the overall goal to develop a systematic approach for the identification of novel inhibitory compounds that can be used to combat plant pathogens.

17. Mechanisms of qualitative and quantitative resistance to *Aphanomyces* root rot in alfalfa

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Aphanomyces root rot (ARR), caused by *Aphanomyces euteiches*, is one of the most important diseases of alfalfa (*Medicago sativa*) in the United States. Two races of the pathogen are currently recognized. Most modern alfalfa cultivars have high levels of resistance to race 1 but few cultivars have resistance to both races. Surveys in the eastern and Midwestern US found that race 2 is predominant and new pathotypes are emerging that overcome resistance in current cultivars. Broad race-nonspecific resistance is needed to avoid the boom and bust cycles of disease resistance breeding. The aim of this project is to identify alfalfa plants with quantitative race-nonspecific resistance, understand the mechanisms of race-specific and race-nonspecific resistance, and identify DNA markers associated with resistance. Analysis of 98 alfalfa accessions from the USDA National Genetic Resources Program identified three accessions with novel quantitative resistance to *A. euteiches*. In these lines, the pathogen initially colonizes hypocotyl cortical cells but does not cause decay, and is excluded from the stele. Colonized cells ablate as the vascular system enlarges and the seedling develops. In contrast, race-specific resistance is characterized by a hypersensitive response of individual epidermal or cortical cells upon pathogen attack. In both types of resistance, suberized cells surround the stele and strong autofluorescence occurs in cortical cells after inoculation, indicating the presence of phenolic compounds. In susceptible plants extensive cortical decay occurs, the stele is colonized, and little suberization develops. These accessions are promising source materials for developing cultivars with durable resistance to ARR.

18. RNA-Seq for identifying novel transcripts, alternate splicing and improving current gene annotations in plant pathogen Phytophthora infestans

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Phytophthora infestans is responsible for the late blight disease of potato and tomato plants. Several other *Phytophthora* species infect potato and tomato. Disease management strategies require correct identification of the pathogen as different species call for different controlling strategies. Correct gene models and annotations are key to successfully study any organism. In the present study we used RNA-Seq data to modify the gene annotations of *P. infestans.* We have used Trinity and PASA to update the existing gene models, identify novel genes and alternative splicing events. Alt-splicing has been not been previously studied in *P. infestans*. We have used genome-guided Trinity to first align the reads to the genome and then PASA was used to align the transcripts obtained by Trinity back to the T30-4 genome. Out of 18,179 genes currently annotated in the genome, we have modified ~8,000 genes with additions of untranslated regions, changes in CDS boundaries and gene merging. We have also identified ~800 genes with alternative splicing which were initially not known. Apart from the current genome annotations, we have identified ~8,000 overlapping transcripts that were not having any corresponding genes in the current annotation. Out of these 8,000 transcripts we have identified ~400 *P. infestans* genes which were previously not annotated. This study will help researchers in many ways: correctly identifying any signal peptides present in the gene affecting their localization, identification of transcription factor binding sites, any additional exons affecting the enzymatic activity of certain genes. To conclude we find that RNA-Seq is a great tool to improve gene models and identify novel transcripts.

19. Analysis of Soybean Promoters Involved in Partial Resistance to *Phytophthora sojae*

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Phytophthora root and stem rot is a major disease of soybean in Ohio, especially when production occurs in poorly drained soils. The disease is primarily controlled through the use of host resistance, which is both qualitative and quantitative or partial. Partial resistance is more durable but is poorly understood. Previous studies identified sequence, expression and/or functional annotations of genes underlying QTL in the resistant cultivar Conrad that confer this type of resistance in soybean. In the present study, the promoters of some of these genes were tested for sequence differences and for their ability to drive expression of a GUS reporter gene during inoculation with *P. sojae.* The promoter of a putative WRKY had expression differences between Conrad and the susceptible cultivar Sloan during the early stages of infection. The promoter of a

putative GH3 had sequence differences between the cultivars but did not have a clear expression pattern.

20. Relationship between PI3P, lipid rafts and plasmodesmata during invasion by *Phytophthora* pathogens

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Oomycete and fungal pathogens invade plants by using secreted effectors. Entry into host cells is thought to involve binding to phosphatidylinositol-3-phosphate (PI3P) or other phosphoinositide monophosphates, possibly located in lipid rafts in the plasma membrane. However, details of this process are still unclear and especially there is a lack of detailed cytological evidences. Furthermore, the possibility of symplastic trafficking of effectors from cell to cell has not been examined. Symplastic trafficking involves membranous structures known as plasmodesmata (PD). Many viral pathogens take advantage of the PD for movement. Since PD are a continuous extension of the cell membrane, they are a potential trafficking channel for movement of lipid rafts and effectors to neighboring cells. We are developing fluorescent protein probes for lipid rafts (Remorin from *Solanum tuberosum*), and PDs (TMV-MP from *Tomato mosaic virus*) along with effectors and the specific PI3P-binding protein Vam7P from *Saccharomyces cerevisiae.* We are using these probes together with cytological stains to examine the spatial distribution of lipid rafts, PDs, PI3P and effectors before and during plant infection, by live-image confocal microscopy. These experiments aim to clarify how *Phytophthora* pathogens manipulate PI3P in lipid rafts on host membranes for cell entry and systemic translocation.

21. The narrative of *Bremia lactucae* in California over three decades

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Downy mildew, caused by *Bremia lactucae*, is the number one lettuce disease in California and worldwide. This oomycete pathogen is highly variable and can rapidly change to overcome resistant cultivars. We are characterizing the pathogen population in order to determine which sources of wild germplasm will provide effective resistance in California that minimizes dependency on chemical protectants and aids organic farmers. We have been monitoring *B. lactucae* in California and storing isolates since 1982, mostly on an opportunistic basis. In December 2013, we began monthly surveys to provide a more comprehensive data set on pathogen variability. We will report the patterns in virulence phenotypes observed in the *B. lactucae* population over the past 30 years and the appearance of a second mating type, thus the potential for sexual reproduction. This may explain the recent rise in novel phenotypes and may require a change in strategy for durable disease resistance. We will also report on genomic

sequence analysis of representative isolates collected throughout the three decades as a window to understanding variation of *B. lactucae* in California.

22. FungiDB: An integrated functional genomics database for oomycetes (and fungi).

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FungiDB (http://FungiDB.org) is a free online database that enables data mining and analysis of the pan-fungal and oomycete genomic sequences and functional data. This resource was developed in partnership with the Eukaryotic Pathogen Bioinformatics Resource Center (http://EuPathDB.org). Using the same infrastructure and user interface as EuPathDB, FungiDB allows for sophisticated and integrated searches to be performed over an intuitive graphical system. Release 3.2 of FungiDB contains sequence and annotation for 20 Oomycete genomes including members of *Albugo* (2), *Aphanomyces* (2) *Hyaloperonospora* (1), *Phytophthora* (6), *Pythium* (7), and *Saprolegnia* (2). In addition to the genomic sequence data and annotation, FungiDB includes transcriptomic data based on RNA sequence and microarray experiments and all expressed sequence tag data from GenBank. All genomes in FungiDB are run through a standard analysis pipeline that generates additional data such as signal peptide and transmembrane domain predictions, GO term and EC number associations and orthology profiles. The graphical user interface in FungiDB allows users to conduct *in* silico experiments that leverage the available data and analyses. For example, a search in FungiDB can identify all genes in *Phytophthora sojae* that do not have orthologs in mammals, have a predicted signal peptide, are annotated as a kinase and are expressed under conditions of high oxygen stress. FungiDB is supported in part by the Burroughs Wellcome Fund, the Alfred P. Sloan Foundation, USDA NIFA and NIH HHSN272201400030C.

23. The inositol polyphosphate phosphatase family in oomycetes.

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Phosphoinositides (PIs) play essential roles in intracellular transport and communication. They interact with a plethora of proteins, often modulating their activity. The level of individual PIs is governed by PI kinases [PI(P)Ks], and PI phosphatases (INPPs). Genome wide inventories in plant pathogenic *Phytophthora* spp. previously revealed that they encode an unique variety of PI(P)Ks. Among them GPCR-PIPKs

(GKs), composed of a G-protein-coupled-receptor (GPCR) domain fused to a PIPK domain. So far nothing is known about PI(P)K counterparts in phosphoinositide signalling, the INPPs. We performed a genome-wide inventory for INPP genes in *Phytophthora* spp. and other oomycetes. Most genomes enclose around 22 INPPs genes in their genome, based on domain and motif conservation. Oomycete INPPs are well conserved in their catalytic domains and correlate to all known INPPs from other organisms. However, a subset of INPP are fusion products of INPP4 and INPP5 catalytic domains with an N-terminal GPCR moiety. This structure mimics that of GKs. As anticipated counterparts and the involvement of GKs in developmental transitions (Hua et al., 2013) this suggest that together these might determine specific spatiotemporal distribution of PIs. With the ongoing research on putative phospholipid based transport of proteins essential in pathogenicity processes it is worth to deduce their *in vivo* role. Also, with GPCRs as the main target of active agents, the GPCR-PI metabolizing enzymes might be valuable oomicide targets.

24. Is stress in farmed salmon (*Salmo salar*) an important factor for saprolegniosis?

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Saprolegniosis is a major concern to fish farms leading to heavy losses due to infection by this filamentous oomycete pathogen. These infections are often observed after procedures likely to induce significant stress to the fish such as vaccination. In this study we have analysed the expression of a number of genes as putative stress markers, from fish at different stages of the vaccination process. These include steroidogenic acute regulatory protein (StAR) and cytochrome P450side-chain-cleavage, responsible for the regulation of steroidogenesis, the glucocorticoid receptor (GR) and heat shock proteins 70 and 90. First data shows significant activation of these stress markers as well as raised blood cortisol and glucose concentrations likely to affect susceptibility of salmon to *Saprolegnia* after vaccination. We aim to link stress marker gene expression to immune gene expression in future studies, with major changes in the latter seen during infection of salmon with *Saprolegnia*.

25. Taxonomic characterisation of Oomycetes from the Falkland Islands

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Oomycetes are a class of well-known economically and ecologically important pathogenic organism affecting a broad variety of plant and animal species. The Falkland Islands are an unexplored area and thus little is known of the diversity of oomycete species on this archipelago. Using molecular taxonomic techniques we characterised oomycete biodiversity and present evidence of novel oomycete species isolated from

the islands. Isolates were collected from different aqueous sites during sampling campaigns in 2011-2013, using sterile baits and novel sampling techniques. The internal transcribed spacer (ITS) was sequenced and phylogenetic analysis was performed and confirmed the presence of several different species of oomycete covering four genera *Saprolegnia, Achlya, Leptolegnia* and *Pythium.* Phylogenetic analysis of the sequenced data with closely related species sequences revealed unique species within these genera. Here we present molecular and morphological characterisations and infection studies of several of these novel species.

26. *Phytophthora sojae* effector Avr1b can be delivered into soybean cells by heterologous PI3P-binding proteins during infection.

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Oomycete and fungal pathogens secrete effector proteins that can enter plant cells to modify the physiology of their hosts. A major class of effectors produced by oomycetes contains RXLR motifs that mediate entry of these effectors into plant cells. We previously showed that RXLR effectors can enter host cells in the absence of any pathogen. Furthermore, these effectors can bind to specific lipids including phosphatidylinositol-3-phosphate (PI3P). PI3P-binding requires the RXLR motif, plus in some cases, C-terminal regions of the protein. Previously we showed that PI3P binding is required for the effectors to enter into host cells when the purified proteins are introduced into root or leaf tissue. Here we show that the RXLR motif of Avr1b is sufficient for cell entry *in vivo*, independent of the C-terminal PI3P-binding residues. In order to validate that PI3P-binding mediates host cell entry in planta, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RXLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. The Avr1b and various derivative mutant proteins can be specifically detected in culture supernatants after de-glycosylation, indicated that Avr1b is post-translationally modified.

27. **Folding Based Molecular Dynamics Simulations of *Phytophthora sojae* Avh5 Effector**

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Using previously solved X-ray structures of C-terminal domains from *Phytophthora* avirulence genes, a model for Avh5 from *P. sojae* was generated. We utilized the server, Swiss-model to generate models based on best fit amino acid sequence homology. Of these, a model comprising the terminal 65 amino acids was chosen as a starting point, which included 4 α-helices, with the first 58 amino acids of the N-terminus, comprising the RxLR domain, having no tertiary structure. The C-terminal

portion of this was then used as a docking receptor for the suspected ligand Inositol-1,3-Di-phosphate (IP3P), with the software AutoDock Vina, showing a site in a single loop. This initial model was then embedded into a solvent system of water and and ions. Using the forcefield 54a7 and the software Gromacs, the entire model was allowed to run in an unrestrained molecular dynamics (MD) simulation, allowing the N-terminal region to adopt a rudimentary fold. After removing the IP3P from the model, a series of further refolding simulations were conducted, to test effects of putative theories regarding tertiary structure. These included extended simulations of 200 nanoseconds in the same solvent makeup, addition of a second Avh5 protein in the same state, and addition of a a lipid bilayer composed DPPC, and cholesterol. This later simulation mimics a standard lipid raft system for small scale MD simulations. From this entire process, a starting model was generated which would allow further experimental research into the various process involved in Avh5 interactions with membranes and specifically IP3P.

28. 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 to 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 causal 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. These essential effectors will make excellent candidates for screening for new, durable resistance to *P. sojae*, as these genes presumably 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. sojae*, 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. Silencing of 13 of these effectors produced reduced virulence. Among these effectors, PsAvh16, PsAvh180 and PsAvh240 showed substantially reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. These three effectors are being used as candidates in a high throughput screen system utilizing Pseudomonas Type III secretion system to screen for new resistance genes against *P. sojae*.

29. Improving *Phytophthora ramorum* draft genome using Pacbio-illumina hybrid assembly

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Phytophthora ramorum is an invasive plant pathogen and causal agent of Sudden Oak Death in California. In 2006, the *P. ramorum* draft genome was published using strain Pr-102 from coast live oak (*Quercus agrifolia*). Whole genome shotgun Sanger sequencing at 7x coverage was used to assemble the genome with an estimated size of 65 Mb, 2,576 scaffolds and 7,589 contigs. Since the first *P. ramorum* genome has been published, there have been many advances in high throughput sequencing and bioinformatics tools. We are currently working to improve *P. ramorum* (Pr-102) genome assembly. PacBio single molecule real time sequencing produced ~435,399 reads at 12x genome coverage. The PBJelly tool from PBSuite Software was used to align PacBio reads to the 2006 *P. ramorum* draft genome. Our preliminary results have 1,595 contig-gaps filled and 669 gaps overfilled (contigs adjacent to a gap are extended but do not overlap and the sum of the new sequences that cover the gap are larger than the predicted gap size). From contig extension perspective, there were 1,089 single end extensions, 174 double end extensions. About 7% (30,958) of the PacBio reads were unplaced. The genome size is now estimated to be 66.6 Mb with 2,475 scaffolds. We are currently optimizing a hybrid assembly of paired-end illumina short reads and PacBio sequences using ECTools. By improving the draft assembly of *P. ramorum*, we will be able to more effectively detect changes in *P. ramorum*'s genome in response to environmental factors and conduct population and evolutionary genomic studies.

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