Oomycete Molecular Genetics Network Meeting

Asilomar Conference Grounds, Pacific Grove CA

March 15-17, 2009

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Sunday March 15, 2009	
10 – 12:00	Registration
12-1:00	Lunch on your own
1:00	**Opening remarks: Brett Tyler**
Session 1	**Moderator: Mark Gijzen**
1:20	Thorsten Nuernberger (University of Tuebingen) A common fold of Necrosis and ethylene-inducing peptide 1-(Nep1) like proteins (NLPs) mediates microbial attack and plant defense.
1:40	Stephan Wawra (Aberdeen Oomycete Group) Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins.
2:00	Francine Govers (Wageningen University) Recognition of *Phytophthora infestans* RXLR-dEER effectors by resistance proteins is triggered by C-terminal domains comprising W motifs.
2:20	Shiv D. Kale (Virginia Bioinformatics Institute) Cell-entry motifs of effectors from three eukaryotic kingdoms bind a common receptor family.
2:40	Georgina Fabro (Sainsbury Laboratory) Analyzing the *Hyaloperonospora arabidopsidis* effector's effects.
3:00	**Break**
Session 2	**Moderator: Sophien Kamoun**
3:20	Claire Gachon (Scottish Association for Marine Science) The immune response of brown algae against the basal oomycete pathogen *Eurychasma dicksonii*.
3:40	Jens Steinbrenner (University of Warwick) Three *Hyaloperonospora arabidopsis* RXLR Effector Proteins Interact with Members of the Arabidopsis Prenylated Rab Acceptor family.
4:00	Dou Daolong (Nanjing Agricultural University) Transcriptional programming and cooperation among *Phytophthora sojae* RXLR-dEER effectors to suppress host cell death.
4:20	Yuanchao Wang (Nanjing Agricultural University) Functional Genomics of G-protein coupled receptors (GPCR) in *Phytophthora sojae*.
4:40	Qijun Xiang (University of California, Riverside) The role of MYB transcription factors in the life cycle of *Phytophthora infestans*.
5:00	**Break**
5:30-6:30	**Dinner**
7:00	Keynote Address: Richard Michelmore Lettuce downy mildew, past, present and future.

8:00-10:00	**Post Talk reception Fred Farr**
Monday March 16	
Session 3	**Moderator: Kurt Lamour**
9:00	Johanna Marcela Del Castillo Munera (LAMFU, Universidad de los Andes) Developing a taxonomic identification system based on microsatellites of *Phytophthora* species.
9:20	Daniel Gobena (University of Tennessee) Genetic diversity of *Phytophthora capsici* on Long Island, NY assessed with high resolution DNA melting analysis (DMA).
9:40	Monica Blanco (North Carolina State University) Genetic structure of populations of the tobacco blue mold pathogen, *Peronospora tabacina*, in North America, Central America and the Caribbean and Europe.
10:00	T.A.J. van der Lee (Wageningen UR) The genotypic diversity of *Phytophthora infestans* in China shows a strong correlation with the region of origin.
10:20	**Break**
Session 4	**Moderator: Manuel D. Ospina-Giraldo**
10:40	Frank Martin (USDA-ARS) Mitochondrial genome comparisons in *P. ramorum* and implications for haplotype analysis.
11:00	Erica Goss (USDA ARS) Ancient isolation and independent evolution of the three clonal lineages of *Phytophthora ramorum*.
11:20	Suomeng Dong (Nanjing Agricultural University) The *Phytophthora sojae* avirulence locus Avr3c encodes a multi-copy RXLR effector that displays sequence polymorphisms among different pathogen strains.
11:40	Rhys Farrer (Sainsbury Laboratory) Evolution of effector genes in *Phytophthora infestans* and closely related species revealed by comparative Illumina genome sequencing.
12:00-1:00	**Lunch**
Session 5	**Moderator: Nicholas Grunwald**
1:00	Marco Thines (University of Hohenheim) Phylogeny and Evolution of Oomycetes, with special reference to speciation and species concepts in downy mildews and white blister rusts.
1:20	Seogchan Kang (Penn State University) *Phytophthora* and *Pythium* Databases: A growing cyberinfrastructure supporting the identification and monitoring of the Pythiaceae.
1:40	John M. McDowell (Virginia Tech) Signatures of adaptation to obligate biotrophy in the

	Hyaloperonospora arabidopsidis genome.
2:00	Brian J. Haas (The Broad Institute) Pathogenicity correlated with genome dynamics in the *Phytophthora infestans* genome.
2:20	Rays H.Y. Jiang (The Broad Institute) Mobile elements drive dynamic evolution of the *Phytophthora infestans* genome.
2:40	**Break**
3:00-5:00	**Poster Session**
6:00-715 pm	Banquet dinner
8:00-10:00	Networking Social @ Fred Farr

Tuesday March 17	
Session 6	**Moderator: Paul Morris**
9:00	Steve Whisson (Scottish Crop Research Institute) Silencing of candidate pathogenicity factors in *Phytophthora infestans*: transient and stable silencing.
9:20	Joann Mudge (National Center for Genome Resources) *Phytophthora capsici* genome assembly: hybrid sequencing using 454 titanium and Sanger technologies.
9:40	Xiu-Guo Zhang (Shandong Agricultural University) Functional analysis of the Pectin Methylesterase gene Pcpme 1 isolated from *Phytophthora capsici*.
10:00	Harold J.G. Meijer (Wageningen University) *Phytophthora* phospholipase D genes and their role in plant cell degradation.
10:20	Liliana Cano (the Sainsbury laboratory) Transcriptome sequencing of *P. ipomoea* and *P. mirabilis* to understand effector evolution in the *Phytophthora infestans* species cluster.
10:40	Vincent Bulone (AlbaNova University Center) Cell wall polysaccharide biosynthesis in *Saprolegnia*.
11:00-11:30	**Concluding remarks, Brett Tyler**

1:20

A common fold of Necrosis and ethylene-inducing peptide 1-(Nep1) like proteins (NLPs) mediates microbial attack and plant defense

T. Nürnberger, I. Küfner, C. Ottmann, B. Luberacki, F. Brunner, M. Weyand, L. Mattinen, M. Pirhonen, G. Anderluh, H. U. Seitz, C. Oecking. Center of Plant Molecular Biology, Tübingen University, D72076 Tübingen, Germany.

Many phytopathogens secrete toxins that enhance microbial virulence by killing host cells. Typically, these toxins are produced by particular taxonomic groups of pathogens. NLPs are widely found in bacteria, fungi and oomycetes. In addition to their necrotic activities, several NLPs have been shown to trigger plant innate immunity-associated responses. However, the functional conservation among NLPs from different taxa and the relationship between the different activities of NLP proteins are unknown. We have determined the crystal structure of NLP_{Pya} from *Pythium aphanidermatum*, to 1.35Å resolution. The protein exhibits similarities to cytolytic toxins produced by marine organisms. Modelling the 3-D structure of related proteins from *Phytophthora parasitica* and *Pectobacterium carotovorum* showed a high extent of fold conservation. Expression of different NLPs in a *P. carotovorum nlp*- strain restored bacterial virulence, suggesting that NLPs from both prokaryotic and eukaryotic microorganisms are functionally conserved. NLP mutant protein analyses further revealed that the same structural properties of NLP proteins were required to cause plasma membrane disintegration and cytolysis in plant cells as well as to restore virulence in the *nlp*-deficient *P. carotovorum* strain. Our results demonstrate that NLPs are cytolytic, virulence-promoting phytotoxins exhibiting an evolutionary conserved fold that is widely distributed across taxa. We further show that the same fold is also essential for NLP-induced plant defence gene expression, suggesting that NLP-mediated interference with host cell integrity signals the activation of plant immune responses. Damage-associated activation of defenses in plants is reminiscent of microbial toxin-induced inflammasome activation in vertebrates and, hence, reveals an additional conceptual similarity in eukaryotic innate immunity.

1:40

Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins

S. Wawra[1], A.J. Phillips[1], K. Minor, S[1]. Grouffaud[1,2], J. Bain[1], E. Gilroy[2], E.J. Robertson[1], V.L. Anderson[1], C.R. Bruce[1], L.J. Grenville-Briggs[1], N.R. Horner[1], S.C. Whisson[2], P.R.J. Birch[2], A.J. Porter[1], C.J. Secombes[1], P. van West[1]. [1] University of Aberdeen, Aberdeen, Scotland, UK.
[2] Scottish Crop Research Institute, Invergowrie, Scotland, UK.

Most oomycete pathogens invade their hosts in a biotrophic manner, which means that they try to avoid host recognition and/or suppress host immune responses. Several biotrophic pathogens translocate effector-proteins into their host cells, which help to establish a successful infection. Oomycete pathogens do not possess a type III secretion machinery as bacteria have, and instead they seem to have developed a different effector translocation system. They are able to translocate proteins that contain an RxLR-EER motif located after the signal peptide. It was shown that this motif is important for effector translocation as mutating this domain stops translocation into the host cells. The mechanism by which oomycetes direct their RxLR-EER effectors into host cells is as yet unknown and is the main focus of our research. It has been postulated that endocytosis processes or protein transporters are responsible. Here we present our latest results, which give insight into the mechanism of the oomycete RxLR-EER protein translocation system.

2:00 PM

Recognition of *Phytophthora infestans* RXLR-dEER effectors by resistance proteins is triggered by C-terminal domains comprising W motifs

Francine Govers, Klaas Bouwmeester, Jun Guo, Pieter M.J.A. van Poppel, Rays H.Y. Jiang and Rob Weide Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Binnenhaven 5, NL-6709 PD, Wageningen, The Netherlands.

The *Phytophthora infestans* avirulence genes *PiAvr1* and *PiAvr4* encode RXLR-dEER effector proteins and belong to a family of oomycete avirulence homologs (*Avh*). Avh proteins are rapidly evolving but nevertheless, the majority has recognizable C-terminal motifs (Jiang et al. 2008 PNAS). *PiAvr4* was isolated by positional cloning. Loss of avirulence on *R4* potato is caused by frame shift mutations resulting in truncated PiAvr4 proteins (van Poppel et al. 2008 MPMI). The genomic region harboring *PiAvr4* shows conserved synteny with *Phytophthora sojae* and *P. ramorum* but *PiAvr4* itself is located on a 100 kb indel that breaks the conserved synteny, and is surrounded by transposons. In the C-terminus PiAvr4 has three W motifs and one Y motif. W2 in combination with either W1 or W3 triggers necrosis in potato plants carrying resistance gene *R4*. *PiAvr1* was isolated by anchoring *Avr1*-associated markers on the genome sequence. This lead to a 800 kb region with seven *Avh* genes, one of which is *PiAvr1*, the counterpart of resistance gene *R1*. Also PiAvr1 has W and Y motifs. Domain swapping revealed which motifs determine avirulence on *R1* potato. Analysis of the role of PiAvr1 and PiAvr4 in virulence is in progress.

2:20 PM

Cell-entry motifs of effectors from three eukaryotic kingdoms bind a common receptor family

Kale, S.D.*, Capelluto, D. †, Gu, B.* ‡, Dou, D.* ¶, Arredondo, F.D.*, Kang, Z.S. ‡, Lawrence, C.B.*, Shan, W.-X. ‡ and Tyler, B.M. * * Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA† Department of Biological Sciences. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA ‡ Department of Plant Pathology, Northwest Agriculture and Forestry University, 712100, China ¶ Current Address: Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China

Bacterial effectors have been shown to enter host cells via the type three secretion system. There is no evidence of anything resembling a type three secretion system in oomycetes or in any other eukaryotes.The N-terminal RXLR-dEER motif from *P. sojae* effector Avr1b can translocate proteins including GFP into soybean root cells without any pathogen encoded machinery. The N-terminal RXLR-dEER motif of Avr3a from *P. infestans* has been shown to translocate beta-glucuronidase and the C-terminus of Avr3a into host cells. Effectors from the malaria parasite, *Plasmodium* utilize an N-terminus PEXEL (RXLXE/D/Q) motif to enter host cells. The oomycete and *Plasmodium* protein translocation motifs have been shown to be interchangeable amongst the two pathogens. We have shown that fungal effectors contain variant RXLR-dEER motifs that can also translocate proteins into plant cells. We have identified a family of candidate receptors that bind to RXLR-dEER-like motifs from all three kingdoms of eukaryotic pathogens. The identity and location of the receptors suggest that all these effectors enter cells via receptor-mediated endocytosis.

2:40 PM

Analyzing the *Hyaloperonospora arabidopsidis* effector's effects

Georgina Fabro, Sophie Piquerez, David Greenshields, Jonathan D G Jones, The Sainsbury Laboratory and collaborators from the ERA-PG Effectoromics consortium*.

The oomycete *Hyaloperonospora arabidopsidis* (*Hpa*) is an obligate biotroph of *A. thaliana*. *Hpa* requires living host tissue for growth and reproduction. The pathogen uses a wide range of effectors to gain access to resources available in its host, and at the same time avoid or suppress host defence responses. These effectors are predominantly small secreted proteins containing signal peptide and RxLR motifs. Bioinformatic analysis of the *Hpa* Emoy2 race genome was carried out and ≈ 140 potential effectors were identified. In a shared effort with the ERA-PG Consortium, 102 effectors have been cloned. We analyzed the complement of *Hpa* effectors for (i) Functions in virulence /avirulence in *A. thaliana* (ii) Genetic variation in the host for their recognition. For this, we used a Heterologous (EDV) System delivering one effector at the time via the *Pseudomonas syringae* (Pst) TTSS (Sohn et al., Plant Cell 2007). Using different modified Psts (LUX and ΔCEL) we could assess if a given *Hpa* effector could increase or decrease bacterial growth "*in planta*", and suppress PAMP triggered immunity (PTI). Preliminary results indicate that 88% of the 61 effectors tested can enhance bacterial growth on at least one *A.t*. accession, and of these 55% do so in 3 to 5 more accessions. 18% of the tested effectors can restrict bacterial growth, and some of these provoke HR-like lesions on specific *A.t.* accessions. Also, 50% of the effectors can suppress callose accumulation when delivered through Pst ΔCEL, indicating PTI suppression. Mixed infections using sets of Psts carrying different effectors are also being developed and results will be presented.

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

3:20 PM

The immune response of brown algae against the basal oomycete pathogen *Eurychasma dicksonii*

C. Gachon, M. Strittmatter, D.G Mueller and F.C. Kuepper
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The oomycete pathogen *Eurychasma dicksonii* is both the most abundant eukaryotic pathogen of marine brown algae, and the most basal member of the oomycete lineage. Despite being an obligate biotroph, it has the largest reported host range among marine pathogens - infecting virtually every brown algal species tested so far. Remarkably, virtually nothing is known about many fundamental aspects of its pathogenicity, biology, epidemiology, and ecology. Due to its availability in culture and the recently-completed sequencing of the genome of one of its main brown algal hosts (*Ectocarpus siliculosus*), *Eurychasma* is a particularly attractive model to study oomycete infection strategies and algal defense mechanisms. The reaction of different algal strains against *Eurychasma* range from extreme susceptibility to complete resistance against infection, suggesting a genetic basis for disease resistance in algae. In all cases investigated, resistance is associated with the early death of the challenged algal cell, which prevents further spread of the disease. This holds true across eight species tested, suggesting that resistance-associated cell death might be a conserved immune mechanism of brown algae. We will report our progress on the molecular characterization of this response, such as the development of *in situ* labeling techniques or mining of the *Ectocarpus* genome for potential disease resistance genes.

3:40 PM

Three *Hyaloperonospora arabidopsidis* RXLR effector proteins interact with members of the *Arabidopsis* prenylated Rab acceptor family

J. Steinbrenner, M. Coates, S. Donovan, T. Payne, P. Bittner-Eddy, S. Bimanadham and Jim Beynon
Warwick HRI, Wellesbourne, University of Warwick, UK CV35 9EF

An open question is how microbial pathogens manipulate the plant immune system to establish disease. Oomycete effectors have been reported to be delivered to the host cell to alter host immunity. Despite the fact that the primary sequence of these effectors is distinct, they have in common a signal peptide for secretion from the pathogen, followed by the motif RXLR and an acid region often ending in the sequence EER, that enables entry into the host. Bioinformatic analysis of the *Hyaloperonospora arabidopsidis* genome revealed over 200 RXLR effector genes. We have used yeast two hybrid screens, using a small number of RXLRs as baits, to identify interacting proteins from Arabidopsis. Three RXLRs identified members of the PRA family as potential protein targets. PRAs are small transmembrane proteins that play a role in the regulation of vesicle trafficking. Altering plant secretory pathways would be a logical target for pathogenicity effectors and we will describe confirmation of the interaction *in planta* and consequences of the presence of the RXLR proteins to the host.

4:00 PM

Transcriptional programming and cooperation among *Phytophthora sojae* RXLR-dEER effectors to suppress host cell death Wang Qunqing[1], Han Changzhi[1], Dou Daolong[1,2], Adriana Ferreira[2], Shiv Kale[2], Wang Xiaoli[1], Yu Xiaoli[1], Liu Tiuli[1], Yao Yao[1], Wang Xinle[1], Dong Suomeng[1], Zhang Zhengguang[1], Zheng Xiaobo[1], Brett Tyler[2], Wang Yuanchao[11] Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China [2] Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

About 370 candidate effectors, sharing the RXLR-dEER motifs, have been identified from *Phytophthora sojae* genome and are believed to enter its host cells. However, the functions of the most effectors are still poorly understood. Here, we test 168 effectors and find over 60% have the capability to block cell death process triggered by the mouse Bax, which is accordance with the functions of *P. sojae* Avr1b proteins in the pathogenicity. Interestingly, we show most of the early-infection-induced effectors can suppress Bax or INF1 induced cell death, indicating the possible strategies used by *P. sojae* to benefit its biotrophic infection process. However, plant also develops the successful resistance mechanism by recognization of some early-infection-induced effector proteins, such as Avh238 and Avh241. We show that *P. sojae* can escape from the recognization by point mutations, or suppress the recognization by delivering many other effectors. Furthermore, we demonstrate Avh238 can cooperate with the other effectors, such as Avh172, to inhibit the basal resistance triggered by INF1. Taking together, our results indicate the roles of large number of predicted *P. sojae* effectors, then begin to illustrate how pathogen establish the successful infection by delivering effectors with different functions at the proper time points and how those effectors collaborately suppress the host defense.

4:20 PM

Functional Genomics of G-protein coupled receptors (GPCR) in *Phytophthora sojae*

Wei Zhao [1], Yonglin Wang [1], Xiaoli Wang [1], Chulei Hua [1], Hao Wu [1], Daolong Dou[1], Brett Tyler[2], Francine Govers[3], Yuanchao Wang [1] [1] Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China[2] Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA [3] Laboratory of Phytopathology, Plant Sciences Group Wageningen University Binnenhaven 5, NL-6709 PD Wageningen, The Netherlands

G protein coupled receptors (GPCRs) present a large family of receptors involved in a broad spectrum of cell signaling, which are responsive for transmitting the extracellular signals to intracellular response by stimuli. Despite exhibiting striking diversity in primary sequence and biology function, all GPCRs possess the conserved fundamental architecture consisting of seven transmembrane domains and share common mechanisms of signal transduction. This class of protein were primarily identified in the gnome of *Phytophthora sojae*, the destructive cause of stem and root rot in soybean. *P. sojae* have approximately 41 seven transmembrane proteins, which are classified into five classes based on sequence homology, including metabotropic glutamate receptors, rhodopsin-like superfamily, cyclic AMP receptors, one novel class phosphatidylinositol phosphate kinases with a G-protein coupled receptor, and other unclassified proteins. Subsequently, the expression profiles were evaluated during asexual lifestages, stresses treatments and infection stage. The results showed that some genes were constitutively expressed; in contrast, the other genes are induced during zoosporogenesis or stresses. Finally, genetic transformation of homology-dependent gene silencing of *P. sojae* were performed and the gene-silenced mutants of five genes were generated respectively. We found that out of five genes, three genes are probably involved in sexual reproduction and pathogenicity, while two genes are not obviously affected the phenotypes including chemotaxis to isofalvone. Further study on these mutants, and other GPCRs genes will be focused on to elucidate the functions of all GPCRs genes in *P. sojae* growth, development, stress response , chemotaxis and pathogenesis.

4:40 PM

The role of MYB transcription factors in the life cycle of *Phytophthora infestans*

Qijun Xiang and Howard Judelson. Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA

Asexual spore production in the oomycete *Phytophthora infestans* involves distinct temporal and spatial developmental switches which presumbly are promoted by groups of differentially expressed genes. This study focuses on identifying the transcription factors and cognate promoter binding sites that are responsible for sporulation-specific gene expression. Dissections of the promoters of several sporulation-specific genes have identified putative transcription factor binding sites important in their developmental regulation. For example, analyses of the pks1 gene showed that the motif CCGTTG determines its transcription at an intermediate stage of sporulation. This motif is significantly enriched in promoters highly active in sporangia and specifically binds a protein complex from nuclear extracts of sporangia. As CCGTTG is known to bind MYB transcription factors in animals and plants, such proteins are being characterized in P. infestans. MYB transcription factors are proteins with one, two or three incomplete repeats of a conserved DNA binding domain consisting of about 50 amino acids, and those regulating development typically carry two or three tandem repeats. *P. infestans* possesses multiple proteins with two or more tandem repeats, and many of them are differentially expressed during sporulation based on RT-PCR. Phylogenetic analysis is being carried out to determine how these MYBs relate to counterparts in other eukaryotes. Gene silencing experiments are also being conducted to determine whether MYB proteins regulate the spore cycle.

9:00 AM

Developing a taxonomic identification system based on microsatellites of *Phytophthora* species

J. Del Castillo, A.J. Bernal, S. Restrepo Laboratorio de Micología y Fitopatología Uniandes (LAMFU), Universidad de Los Andes. Bogotá, Colombia.

Phytophthora spp. is the most important genus of Oomycetes plant pathogens. Actually there are 80 described species, and most of these are primary invaders of plant tissues, and they are the causal agent of diseases in a wide range of crops and natural plants. In order to develop control strategies against *Phytophthora* spp., it is important to know the biology, mechanisms and evolutionary processes of this important pathogen. The aim of this study was to propose and validate a low cost identification system for *Phytophthora* species based on a set of polymorphic microsatellite (SSRs) markers. For this, 29 isolates from *P. infestans, P. andina, P. sojae, P. cryptogea, P. nicotianae, P. capsici and P. cinnamomi* were obtained, and 10 SSRs, potentially transferable markers between these species were chosen. Amplification conditions, including annealing temperature were standardized for several markers. Some of them were assayed on high resolution agarose, and they specifically amplified in all species, showing different alleles depending on the species. Although this study shows preliminary results, it is expected that, in the near future, an identification code can be created to diagnose and monitor this plant pathogen.

9:20 AM

Genetic diversity of *Phytophthora capsici* on Long Island, NY assessed with high resolution DNA melting analysis (DMA) D. Gobena[1], M. T. McGrath[2], and K. Lamour[3] [1]Genome Science and Technology Graduate Program, University of Tennessee, Knoxville TN, [2] Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Riverhead NY, [3] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville TN.

Our objectives are to develop a cost effective robust method for characterizing genetic diversity in *Phytophthora capsici* and to characterize populations over time on Long Island, New York. Approximately 550 isolates of *P. capsici* were recovered during 2004 to 2008 from pumpkin, tomato, pepper and snap beans from 21 fields. Isolates are currently being assayed for multiple population specific single nucleotide polymorphisms (SNPs) loci. The SNP's are identified by re-sequencing single copy genes in two isolates from the target populations. Once a heterozygous site has been identified, PCR is used to amplify a 45-65bpamplicon that includes the SNP in a standard reaction that includes LCGreen ^{PLUS} dye . Following amplification the amplicons are heteroduplexed and melting curves resolved using a LightScanner device (Idaho Technology, Salt Lake City, UT). This approach requires no gel apparatus and most polymorphisms were synonymous substitutions. Our preliminary data indicates three major genotypes (which could be clonal lineages) and also remarkable genotypic diversity. An overview of the marker development, application and resultant findings will be presented.

9:40 AM

Genetic structure of populations of the tobacco blue mold pathogen, *Peronospora tabacina*, in North America, Central America and the Caribbean and Europe Monica Blanco-Meneses. Box 7616, Plant Pathology Department, North Carolina State University, Raleigh NC 27695

Tobacco blue mold, caused by the oomycete pathogen *Peronospora tabacina*, causes a yearly epidemic in tobacco (*Nicotiana tabacum*). The genetic structure of 54 isolates from the U.S., Central America-Caribbean-Mexico (CCAM) and Europe and the Middle East (EULE) populations was analyzed through gene sequencing of the *cox* 2 (mitochondrial) and *Igs*2 (rDNA) and *Ypt*1 (nuclear) regions. The three gene regions showed high genetic variability across all worldwide populations with 93 haplotypes for the *Igs*2 region and *Ypt*1 regions and 82 haplotypes for the *cox*2 gene. Nucleotide diversity was higher in the EULE and the CCAM region and the average per-nucleotide expected heterozygosity (Watterson's theta) was higher in the U.S. populations. The neutrality tests were significant for all populations that were analyzed and the equilibrium model of neutral evolution was rejected, indicating an excess of recent mutations or rare alleles. Hudson's tests (S_{nn}) were performed to quantify population genetic structure within and among populations. Analysis of the U.S. and the CCAM populations together allowed pooling into two genetically subdivided populations: North and South. Migrations from the South to the North part of these regions were demonstrated using IM analysis. Within the European population the Hudson's test was significant allowing pooling of three groups: North-Central EU, Western EU and Lebanon, which were genetically subdivided. The IM analysis indicated migration from Lebanon into the North-Central EU, and from the North-central EU into the Western EU tobacco fields. Blue mold populations were highly diverse and migration plays a major role the structure of worldwide populations.

10:00 AM

The genotypic diversity of *Phytophthora infestans* in China shows a strong correlation with the region of origin. Ying Li[1], Jiehua Zhu[3], Guanghui Jin[4], Chengzhong Lan[5], Baowei Liu[6], Ruofang Zhang[7], Zhijian Zhao[8], Yanli Yang[9], Sanwen Huang[1], Evert Jacobsen[2], Theo van der Lee[2] [1]Institute of Vegetables and Flowers, China [2]Wageningen UR, the Netherlands [3]Hebei Agricultural University, China [4]Heilongjiang August First Land Reclamation University, China [5]Fujian Academy of Agricultural Sciences, China [6]Sichuan Academy of Agricultural Sciences, China [7]Inner Mongolia University, China [8]Yunnan Agricultural Academy, China [9]Yunnan Agricultural University, China;

We performed a comprehensive survey of *P.infestans* isolates in six important potato growing provinces in China. In 2006 and 2007, 119 isolates from potato and tomato were sampled and characterized using the recently established single tube SSR fingerprinting protocol and the realtime PCR for functional polymorphisms in *Avr3a* and *Avr4*. The isolates were genotyped using forteen highly informative nuclear SSR markers and two mitochondrial DNA markers. Additionally, five functional polymorphisms identified in the *Avr3a* and *Avr4* were screened using TaqMan PCR. The mating type was determined using tester isolates. Based on the genotypic analysis a selection of isolates was tested for virulence on potato R-gene differentials. In two of the six regions both the A1 and A2 mating type were found, but genetic analysis did not indicate any sexual reproduction. In the other regions only the A1 mating type was found. The occurrence of the three mitochondrial haplotypes found (Ia, IIa, IIb), strongly correlated with the origin of the isolate. With the forteen SSR markers a total of 40 genotypes was identified. Only two genotypes were present in more than one province while 38 genotypes were specific for their region of origin. The virulence of isolates on the potato differential set was identical within the same genotype, but varied strongly among isolates originating from different regions.

10:40 AM

Mitochondrial genome comparisons in *P. ramorum* and implications for haplotype analysis

Frank N. Martin USDA-ARS, Salinas, CA

Current efforts to sequence the mitochondrial genomes from a range of *Phytophthora* spp. has provided opportunities to do comparative genomics for clarification of the processes driving evolutionary divergence of the genome. It has also provided a wealth of information that can be used for development of species-specific molecular markers and mitochondrial haplotype analysis. Mitochondrial haplotype analysis can be particularly useful in population analyses of clonally reproducing pathogens such as *P. ramorum*. Comparison of a North American (NA) and European (EU) isolates of this pathogen identified 13 SNPs and a 180 bp insertion in the EU genome. Sequence analysis of the regions containing the SNPs was done on additional isolates of *P. ramorum* and a total of 28 SNPs were observed that separated into 4 distinct haplotypes. The haplotypes for the EU (haplotype I), NA (haplotype II) and the third lineage of the pathogen from Washington State (haplotype III) were distinctly different. Interestingly the NA II haplotype was separated into two haplotypes; the IIa was the predominant haplotype while IIb represented a subpopulation of isolates recovered from the Oregon forest (there was a single SNP difference between them). To simplify identification of haplotypes new amplification primers were developed to generate smaller amplicons encompassing the regions containing the SNPs and melt curve analysis was used to differentiate haplotypes. The use of this approach for haplotype analysis of additional species will be discussed.

11:00 AM

Ancient isolation and independent evolution of the three clonal lineages of *Phytophthora ramorum*

E. M. Goss, I. Carbone, N. J. Grünwald Horticultural Crops Research Laboratory, USDA ARS, Corvallis OR 97330, USA

Phytophthora ramorum is the causal agent of sudden oak death, an emerging disease in North America and Europe. In its known range, *P. ramorum* occurs as three distinct clonal lineages. We inferred the evolutionary history of *P. ramorum* from DNA sequence variation at five nuclear loci, including two avirulence gene homologs, using coalescent-based approaches. We found that the lineages have been diverged for at least 11% of their history, an evolutionarily significant amount of time roughly estimated to be on the order of 165,000 to 500,000 years. There was also strong evidence for historical recombination between the lineages, indicating that the ancestors of the *P. ramorum* lineages were members of a sexually reproducing population. Due to this recombination, the ages of the lineages varied within and between loci, but analyses suggested that the European lineage may be older than the North American lineages. The divergence of the three clonal lineages of *P. ramorum* supports a scenario in which the three lineages originated from different geographic locations that were sufficiently isolated from each other to allow independent evolution prior to introduction to North America and Europe.

11:20 AM

The *Phytophthora sojae* avirulence locus *Avr3c* encodes a multi-copy RXLR effector that displays sequence polymorphisms among different pathogen strains Suomeng Dong[1,2], Dinah Qutob[1], Jennifer Tedman-Jones[1], Kuflom Kuflu[1], Yuanchao Wang[2], Brett M. Tyler[3], and Mark Gijzen[1] [1]Agriculture and Agri-Food Canada, London, ON, N5V 4T3, Canada [2]Nanjing Agricultural University, Nanjing, 210095, China [3]Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Root and stem rot of soybean is caused by the oomycete *Phytophthora sojae*. The *Avirulence* (*Avr*) genes of *Phytophthora sojae* control race-cultivar compatibility. In this study, we identify the *P. sojae Avr3c* gene and show that it encodes a secreted RXLR effector protein of 220 amino acids. Sequence and transcriptional data were compared for predicted RXLR effectors occurring in the vicinity of *Avr4/6,* as genetic linkage of *Avr3c* and *Avr4/6* was previously suggested. Mapping of DNA markers in a F_2 population was performed to determine whether selected RXLR effector genes co-segregate with *Avr3c* phenotype. The results pointed to one RXLR candidate gene as likely to encode *Avr3c*. This was verified by testing selected genes by a co-bombardment assay on soybean plants with *Rps3c*, thus demonstrating functionality and confirming the identity of *Avr3c*. The *Avr3c* gene together with eight other predicted genes are part of a repetitive segment of 33.7 kb. Three near-identical copies of this segment occur in a tandem array. In *P. sojae* strain P6497, two identical copies of *Avr3c* occur within the repeated segments whereas the third copy of this RXLR effector has diverged in sequence. Virulent alleles of *Avr3c* that differ in amino acid sequence were identified in other strains of *P. sojae*. The *Avr3c* gene is expressed during the early stages of infection in all *P. sojae* strains examined. Overall, the results illustrate the importance of segmental duplications and RXLR effector evolution in the control of race-cultivar compatibility in the *P. sojae* and soybean interaction.

11:40 AM

Evolution of effector genes in *Phytophthora infestans* and closely related species revealed by comparative Illumina genome sequencing Rhys A. Farrer[1], Liliana M. Cano[1], Nicole Donofrio[2], Thomas Evans[2], Blake Meyers[2], Rays Jiang[3], Brian Haas[3], Mike Zody[3], Chad Nusbaum[3], David Studholme[1], Sophien Kamoun[1] [1]The Sainsbury Laboratory, Norwich, UK [2]University of Delaware, Newark, DE, USA [3]Broad Institute, Cambridge, MA,

The Irish potato famine organism *Phytophthora infestans* is an economically important specialized pathogen that causes a destructive disease on *Solanum* plants. *P. infestans* and its clade 1c sister species, *P. andina, P. ipomoeae, P. mirabilis* and *P. phaseoli*, have most likely evolved by host jumps followed by adaptation to unrelated host plant species. The availability of multiple genome sequences of *P. infestans* and its sister species will provide unprecedented opportunities to understand the basis of genome evolution and host adaptation. We initiated Illumina genome sequencing and generated >50 million 35/36nt single reads for seven genomes representing 5 species, including *P. infestans* reference strain T30-4. To investigate overall abundance of single nucleotide polymorphisms, we aligned Illumina reads to the T30-4 supercontigs containing 18155 genes. We calculated the average rates of synonymous nucleotide substitutions per synonymous site (dS), and showed that it correlates with the current clade 1c phylogeny. Families of effector genes (RXLR, Crinklers, enzyme inhibitors) evolve faster than the remainder of the genome. We also detected a higher proportion of effector genes with no reads aligning to the reference sequence. The level of selection pressure was calculated revealing the effector genes under positive selection. In conclusion, our comparative genomics analyses will help to unravel the evolutionary fate of effector genes following a host jump and will ultimately advance our interpretation of the *P. infestans* genome sequence.

1:00 pm

Phylogeny and Evolution of Oomycetes, with special reference to speciation and species concepts in downy mildews and white blister rusts

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The diversity of the Oomycota can be roughly divided into five main lineages, of which three consist of basal holocarpic, predominantly marine organisms. The crown group of oomycete radiation is defined by the Sapolegniomycetes and the Peronosporomycetes. Within the latter group, three main orders can be defined, the Rhipidiales, Albuginales, and Peronosporales. The Peronosporales contain the important, yet para- and polyphyletic genera *Lagenidium*, *Pythium*, *Halophytophthora*, and *Phytophthora*. Especially the latter genus will have to face dramatic taxonomic rearrangements. The downy mildews are the probably monophyletic crown group of *Phytophthora* and currently divided into 15 monophyletic genera. Within the downy mildews, extensive radiation and multiple host jumps can be observed, especially in the crown groups of *Bremia*, *Hyaloperonospora* and in the *Plasmopara halstedii* complex. *Bremia* is commonly believed to contain only a single species, *Bremia lactucae*, but is divided into at least a dozen highly distinct species. Radiation in *Bremia* has started from hosts in Lactuceae. In a crown group, recent host jumps have taken place to the distant Cardueae and Senecioneae. A similar situation can be observed in the *Plasmopara halstedii* species complex, as well as in the *Hyaloperonospora arabidopsidis* and *Hyaloperonospora brassicae* species clusters. Within the Albuginales, three genera are currently recognized, each harbouring diversity comparable to the downy mildews. For example, *Albugo*, parasitic to Brassicaceae, is subdivided into a generalist and several highly divergent specialist species, including a new species (*Albugo laibachii*) parasitic to *Arabidopsis thaliana*. Mechanisms of speciation and host jumping will be discussed, with focus on biotic interactions.

1:20 PM

***Phytophthora* and *Pythium* databases: a growing cyberinfrastructure supporting the identification and monitoring of the Pythiaceae** S. Kang[1], D. M. Geiser[1], S. Isard[1], M. Mansfield[1], G. Moorman[1], B. Park[1], M. Peiman[2], M. Coffey[2], K. Ivors[3], J. Park[4], Y. Lee[4], C. Garzon[5], J. Blair[6], A. de Cock[7], A. Lévesque[8], G. Abad[9], and F. Martin[10] [1]Dept. of Plant Pathology, Penn State [2]Dept. of Plant Pathology, UC-Riverside [3]Dept. of Plant Pathology, NCSU [4]Dept. of Agricultural Biotechnology, Seoul National Univ. [5]Dept. of Entomology & Plant Pathology, Oklahoma State [6]Dept. of Biology, F&M College [7]CBS; [8]Agriculture and Agri-food Canada [9]USDA-APHIS [10]USDA-ARS

The movement of non-indigenous pathogen species and exotic variants of indigenous ones will likely increase due to the rapid expansion of global commerce and human travel. However, to date efforts to study and manage this threat have been fragmented, mostly regional, and limited to coping with immediate crises. Here we will present the scope and progress of global efforts to document and catalog the genetic and phenotypic diversity of the Pythiaceae, which include many economically important pathogens. Although the Pythiaceae is one of better characterized families in the kingdom Stramenopila, its current taxonomy still lacks clarity due to the large number of described and newly recognized species/genera and the overlap in morphological features among some groups. There has been a concerted global effort to clarify the taxonomy and phylogenetic relationships in *Phytophthora* via the *Phytophthora* Database project (www.phytophthoradb.org). We are organizing an international project to construct a comprehensive multigene phylogenetic framework of the genus *Pythium*, to determine its relationship with other genera in the Pythiaceae, and to create a web-accessible global community resource termed the Pythium Database (www.pythiumdb.org) to disseminate the resulting data. Since the same genetic loci that were sequenced for *Phytophthora* species will also be sequenced in the *Pythium* project, these data will provide a solid foundation for current species classification and future descriptions of new species, facilitate cooperation in conducting and enhancing research and education on the Pythiaceae, and serve as the foundation for constructing the Stramenoplia tree of life.

1:40 PM

1:40

Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome

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

2:00 PM

Pathogenicity correlated with genome dynamics in the *Phytophthora infestans* genome

Brian J. Haas[1], Sophien Kamoun[2], Michael C. Zody[1], Rays H.Y. Jiang[1], Liliana Cano[2], Sylvain Raffaele[2], Trudy Torto-Alalibo[2], Tolga O. Bozkurt[2] and Chad Nusbaum[1] [1]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02142, USA [2]The Sainsbury Laboratory, Norwich NR4 7UK, UK

The genome sequence of *P. infestans* has substantially improved our understanding of *Phytophthora* biology and evolution. Comparisons between the sequenced *Phytophthora* genomes, *P. infestans* (240 Mb), *P. sojae* (95 Mb) and *P. ramorum* (65 Mb), indicate extensive conservation of gene order, with genome expansions largely explained by a proliferation of Gypsy family LTR retrotransposons. Conserved genes typically reside in gene-dense regions where gene order is conserved. In contrast, secreted RXLR and CRN effectors are located in gene-sparse repeat-rich regions of the genome where gene order is not conserved. The *P. infestans* genome harbors large families of these virulence effectors, including 563 RXLRs and 196 Crinklers (CRN), far more than the other sequenced genomes. Analysis of CRN protein sequences revealed a complex domain architecture involving a conserved N-terminal region followed by diverse sets of unrelated C-terminal domains. *In planta* expression of *crn2* deletions delineated its C-terminal DXZ domain as the source of cytoplasmic virulence activity. Four additional necrosis-inducing domains were identified, two with putative kinase and phosphotransferase functions, potentially capable of altering host physiology. CRN genes cluster in the genome by domain architecture, and recombination between CRN domain families may be driving CRN diversity. Manual annotation of CRN genes identified an additional 255 CRN pseudogenes and fragments, many of which cluster with functional versions. The high rate of birth and death evolution observed in effector families localized to dynamic regions of the genome correlates with the high evolutionary potential of *P. infestans* and may explain its rapid adaptability to host plants.

2:20 PM

Mobile elements drive dynamic evolution of the *Phytophthora infestans* genome

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The oomycete *Phytophthora infestans* causes late blight of potato and is notorious as the causal agent of the Irish Potato Famine. Rapid evasion of resistance is a key reason why *P. infestans* remains such a destructive pathogen. Analysis of the genome suggests that adaptation is heavily dependent on high turnover of genes encoding effector proteins. These genes typically reside in gene-sparse, repeat-rich regions of the genome showing little conservation to other *Phytophthora* genomes, a structure that may facilitate their dynamic evolution. The repeat content of the *P. infestans* genome is 74%, likely making it the most repeat rich genome yet sequenced. The genome contains a wide range of transposable element families, including representatives of all major known mechanisms of transposition. These mobile elements appear to be undergoing a burst-elimination type of rapid turnover, as recently transposed elements make up the majority of the genome. Further, expression analysis shows mobile elements are among the most abundantly expressed genetic elements. In addition, Illumina sequencing of multiple strains and related species revealed that repeat content is changing rapidly, differentiating closely related sibling species. The enrichment of repeats near effector genes suggests a model whereby the rapid insertion and deletion of mobile elements provides the means to duplicate successful effector genes and delete deleterious ones, enhancing pathogenic success.

2:40 PM

Silencing of candidate pathogenicity factors in *Phytophthora infestans*: transient and stable silencing

Stephen Whisson[1], Ramesh Vetukuri[2], Zhendong Tian[1], Christina Dixelius[2], Laura Grenville-Briggs[3], Pieter van West[3], Paul Birch[4], Anna Avrova[1].
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Phytophthora infestans causes late blight on the economically important host plants potato and tomato. Genome sequencing and expression profiling have revealed many genes up-regulated during host plant infection that may encode candidate pathogenicity factors. Since *P. infestans* is diploid, the most efficient way to assay for involvement in pathogenicity is to remove the activity of a protein using RNA mediated gene silencing of the encoding gene. We have used transient RNA silencing in a screen of over 50 *P. infestans* infection-upregulated genes to identify 40 genes that, when silenced, lead to a reduction in pathogenicity. Proteins implicated in *P. infestans* pathogenicity ranged from (for example) novel small secreted proteins, secreted and translocated proteins (RXLR effectors), nutrient transport proteins, structural proteins, and protease inhibitors. To assist in more detailed downstream analyses, we are adapting existing and novel strategies for generating *P. infestans* transformants stably silenced for genes of interest.

Tuesday March 17, 9:00 AM

***Phytophthora capsici* genome assembly: hybrid sequencing using 454 titanium and Sanger technologies**Joann Mudge[1], Kurt H. Lamour[2], Sophien Kamoun[3], Neil Miller[1], Paul M. Richardson[4], Darren Platt[4], Igor Grigoriev[4], Alan Kuo[4], Jeremy Schmutz[5], Olga Chertkov[6], Cliff S. Han[6], Chris Detter[6], Greg D. May[1], William D. Beavis[7], Jason Affourtit[8], Michael Egholm[8], Jim Knight[8], Stephen F. Kingsmore[1] [1]National Center for Genome Resources, Santa Fe, New Mexico, USA, [2]The University of Tennessee, Department of Entomology and Plant Pathology, Knoxville, Tennessee, [3]Sainsbury Laboratory, Norwich, UK, [4]USA Department of Energy Joint Genome Institute, Walnut Creek, California, USA, [5] Stanford Human Genome Center, Department of Genetics, Stanford University School of Medicine, Palo Alto, California, USA [6]Los Alamos National Laboratory, Los Alamos, New Mexico, USA, [7]Iowa State University, Ames, Iowa, USA, [8]Roche

The organism, *Phythophthora capsici,* is an oomycete and devastating pathogen of vegetable crops. Its highly repetitive, 60 Mb genome, with characteristic eukaryotic complexity, as well as the availability of closely related Sanger-sequenced genomes make *P. capsici* an excellent target for benchmarking hybrid sequencing technologies in eukaryotes. Next generation sequencing technologies have created sequencing opportunities by increasing throughput and decreasing costs. They have also introduced several challenges compared to traditional sequencing technology. In *de novo* assembly, assembly strategies must account for the large amount of sequencing data, shorter read lengths, and distinctive error profiles of each technology. Hybrid assemblies mitigate some of these issues by combining next generation sequencing with traditional Sanger sequence and have been particularly effective in *de novo* sequencing of prokaryotic genomes. We are assembling with Newbler a draft consensus of a eukaryotic genome sequenced using Sanger sequence and the 454 sequence technology using 400 bp Titanium reagents with much reduced homopolymer-induced errors. The assembly includes 15X shotgun coverage of 454 Titanium pyrosequencing sequencing averaging 338 nt, 4.5X coverage of 454 Titanium 20kb paired end sequencing averaging 155 nt, and 5X coverage of Sanger 6 or 36 kb paired end sequencing averaging 900 nt. Finally, Solexa cDNA libraries from nine life stages have been sequenced to improve gene annotation, identify SNPs, and compare gene expression levels. The successes and challenges of doing a 454-based de novo genome sequencing project in an oomycete will be discussed.

9:20 AM

Functional analysis of the Pectin Methylesterase gene *Pcpme* 1 isolated from *Phytophthora capsici*

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Abstract: *Phytophthora capsici* is an especially important pathogen that is responsible for serious disease of numerous plants. A remarkable array of CWDEs play an important role in virulence during oomycete pathogens interaction with plants. PMEs is an important CWDEs that play a subtle yet important role in the pathogenesis. In order to undersdand PMEs related to the vriulence of *P. capsici* pathogen. Here we purified the wild protein (PCPME 1) on *Pcpme* 1 expression, and further confirmed that PMEs activity trend in PCPME 1 treatment pepper leaves was consistent with increased symptoms development, and PCPME 1 was able to degradate the pepper leaves cell walls and even resulted in producing necrotic lesions on the pepper leaves. Moreover, we also demonstrated that *Pcpme* 1 was strongly expressed in the course of increasing symptoms development after *P. capsici* infection of pepper leaves, and proved that Asp residues in active site within *Pcpme* 1 could effect on the PCPME 1 activity, and the glycosylation sites had almost no effect on PCPME 1 activity and its virulence on pepper leaves. All these results suggested that *Pcpme* 1 might be an important gene in *Pcpme* genes, and it produced a pathogenesis-related protein that contribute to necrotic lesions development for virulence of *P. capsici* pathogen. Also, the functions of *pme* members within the *pme* gene family in *P. capsici* pathogen is remarkable. This study also provides more beneficial to analyze pathogenicity mechanism during oomycete pathogens interaction with hosts.

9:40 AM

***Phytophthora* phospholipase D genes and their role in plant cell degradation**

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Plant pathogens secrete a large repertoire of proteins that may have a role in pathogenicity such as modulation of host defense (e.g. effectors) or degradation of host tissue. *Phytophthora infestans*, the potato late blight pathogen, possesses genes encoding secreted proteins with a phospholipase D (PLD) catalytic domain and this suggests that the *Phytophthora* secretome comprises this class of enzymes. PLD catalyzes the hydrolysis of membrane phospholipids leading to the production of phosphatidic acid and a free headgroup. Phosphatidic acid is a key player in the arena of cellular signalling. It is involved in many processes, including G-protein regulation, protein phosphorylation, transcription, cell proliferation and growth. PLD activity and phosphatidic acid production in plants is also correlated with membrane degradation during senescence and wounding.

Phytophthora infestans, has 18 genes encoding PLDs. One PLD is a universal PLD that is present in all eukaryotes. A second one has homology to a novel class of PLD(-like) proteins. The other sixteen, divided over 4 subfamilies, are more diverged from known PLDs in eukaryotes and unique for *Phytophthora*. Two of these sub-families are small, secreted proteins. We detected PLD activity in exudates of *Phytophthora* and showed that these exudates are capable of degrading lipid vesicles. Transient *in planta* expression of the genes encoding secreted PLDs resulted in a calcium dependent induction of cell death responses. This could point to a function in host membrane modification and/or degradation. Further molecular and biochemical characterization of the various *Phytophthora* PLDs is in progress.

10:20 AM

Transcriptome sequencing of P. ipomoea and P. mirabilis to understand effector evolution in the *Phytophthora infestans* species cluster. Liliana M. Cano1, Sebastian Schornack r1, Rhys Farrer1, Joe Win1, David Studholme1, Brian Haas2, Michael Zody2, Chad Nusbaum2, and Sophien Kamoun1 1The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK 2Broad Institute 7 Cambridge Center, Cambridge, MA 02142, USA

The highlands of Central Mexico are the center of origin of the potato late blight pathogen Phytophthora infestans. They are also home to several species closely related to P. infestans, namely P. mirabilis on Mirabilis jalapa and P. ipomoeae on Ipomoea longipedunculata. These species are thought to have evolve by host-jump followed by adaptation and specialization on distinct host plants. P. infestans secretes a large repertoire of effector proteins that evolve rapidly through birth-and-death evolution and typically exhibit adaptive selection. Our aim is to to identify candidate effectors that show species-level polymorphisms that can be related to host adaptation. We applied Illumina technology to sequence the transcriptomes of P. mirabilis and P. ipomoeae represented in normalized cDNA libraries constructed from mixed developmental stages including mycelia and germinated cysts. The alignments of cDNA reads to P. infestans reference genome allowed us to detect different selection pressures among effector genes. With de novo assembly, we identified the full length sequence of a RxLR effector, annotated as a pseudogene in P. infestans T30-4. Our study highlights the value of comparing transcriptomes to identify candidate effectors in species with no prior gene sequence information. The aim now is to connect the discovered genes to effector activities to reveal a putative role in host adaptation.

10:40 AM

Cell wall polysaccharide biosynthesis in *Saprolegnia*

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One of the most important features that distinguish Oomycetes from true fungi is their specific cell wall composition. The cell wall of Oomycetes essentially consists of (1→3)-beta-glucans, (1→6)-beta-glucans and cellulose whereas chitin, a major cell wall component of fungi, occurs in minute amounts in the walls of some Oomycetes. Cell wall polysaccharides play a central role in vital processes like the morphogenesis and growth of Oomycetes. Thus, the enzymes responsible for their biosynthesis represent potential targets of drugs that can be used to control the diseases provoked by pathogenic Oomycetes. However, the proteins associated to the Oomycete carbohydrate synthase complexes and their corresponding mechanisms are not well characterized. This presentation will summarize our latest results on the biosynthesis of cellulose, (1→3)-beta-glucan and chitin in the Oomycete *Saprolegnia monoica*. In particular, the isolation and characterization of new families of genes encoding cell wall carbohydrate synthases with unique properties, and the significance of our recent discovery that (1→3)-beta-glucan and chitin synthases of Oomycetes are located in lipid rafts will be discussed.

Poster 1

Screening of four *Capsicum* spp. for resistance against *Phytophthora capsici* clonal PcPE-1 in Peru

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Phytophthora capsici is the causal agent of root rot, and crown rot in *Capsicum* crops across Peru. We previously reported that a single clonal lineage of *P. capsici* (PcPE-1) appears to be widely dispersed in the Peruvian coastal area and it survives through several years. In an effort to detect possible sources of resistance against *P. capsici*, 50 different accessions of four different species of *Capsicum* (*C. annum*, *C. baccatum*, *C. chinense*, and *C. pubescens*) from across Peru, are being screened against the major *P. capsici* clonal lineage. Two to three weeks old seedlings (30 seedlings per accession) are being inoculated with a $5x10^3$ zoospores per mL. One week after inoculation, plantlets that survive the treatment are transferred into individual pots and observations are made until plants reach maturity (2-3 months old). Our results indicate that accessions from *C. pubescens* has a moderate resistance to *P. capsici*.

Poster 2

Addressing the biological activity of *Aphanomyces euteiches* cell wall glucan-chitosaccharides in *Medicago truncatula*

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Plant immunity can be activated through the perception of signals called Microbial-Associated Molecular Patterns, such as chitin fragments originating from the fungal cell wall. Oomycetes are cellulosic microorganisms which usually lack chitin in their cell wall, but legume plants have evolved the ability to recognize instead beta-1,3-1,6-glucan cell wall fragments. The saprolegniale *Aphanomyces euteiches* is a major legume parasite, which is pathogenic on the model plant *Medicago truncatula* (Gaulin et al., in press, in: "Oomycete Genetics and Genomics: Biology, Interactions with Plants and Animals, and Toolbox", eds: Lamour and Kamoun, Wiley). Analysis of the *A. euteiches* cell wall showed the presence not only of glucose, but also of *ca.* 10% N-acetylglucosamine. Interestingly, the latter compound is not involved in crystalline chitin, but in amorphous chitosaccharides that are both structural and exposed surface polysaccharides (Badreddine et al., 2008, Eukaryotic Cell 7:1980-93). Biochemical studies showed that these chitosaccharides are strongly associated with beta-glucans, suggesting that *A. euteiches* exposes composite MAMPs at its surface. Accordingly, preliminary data showed that glucan-chitosaccharides solubilized from the *A. euteiches* cell wall are recognized by the host plant *M. truncatula*. Here we present a project aimed at elucidating their biological activity and structure-activity relationships. This study will bring insight into the recognition events involved in legume-microorganism interactions.

Poster 3

Cellular responses of *Phytophthora infestans* to cyclic lipopeptide surfactants produced by *Pseudomonas* species

Harold J.G. Meijer, Ha Tran, Judith E. van de Mortel, Peter A.C. van Gisbergen, Lia Wagemakers, Jos M. Raaijmakers and Francine Govers. Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Binnenhaven 5, NL-6709 PD, Wageningen, The Netherlands. harold.meijer@wur.nl

Oomycete pathogens cause devastating diseases on plants and animals and their control heavily depends on agrochemicals. With increasing concerns about adverse effects of agrochemicals on food safety and environment the development of novel, environmentally friendly control strategies, preferably based on natural products, is demanded. Cyclic lipopeptides (CLPs) produced by *Pseudomonas* species were discovered as a new class of natural compounds with strong activity against oomycetes including the late blight pathogen *Phytophthora infestans*. The *Pseudomonas fluorescens* CLP massetolide A (MassA), has zoosporicidal activity, induces systemic resistance and reduces late blight in tomato. To gain further insight in the modes of action of CLPs, effects on mycelial growth, sporangia formation, and zoospore behavior were investigated, as well as the involvement of G-proteins in sensitivity of *P. infestans* to MassA. In addition to zoospore lysis, MassA disturbed other developmental stages in the life cycle of *P. infestans*. G alpha gain-of-function mutants were less sensitive to MassA suggesting involvement of G-protein signaling in the response of *P. infestans* to this CLP. In order to reveal primary targets of CLPs we also monitored genome wide changes in gene expression. A distinct set of genes appeared to be up- or down-regulated after exposure to MassA, including genes encoding membrane transporters, alkaline phosphatases and pirins. Further characterization of these genes is in progress.

Poster 4

A newly discovered role for cell-cycle regulator Cdc14 in basal body function suggested by studies in *Phytophthora infestans*. A.M.V. Ah-Fong, and H.S. Judelson. Department of Plant Pathology and Microbiology, University of California, Riverside, California, USA.

Functions of Cdc14 orthologues reported to date have involved regulating various aspects of mitosis such as mitotic exit and cytokinesis. The *Phytophthora infestans* ortholog (PiCdc14) complements a yeast Cdc14 mutation, suggesting a role in mitosis. However, PiCdc14 is normally expressed only in spores. Moreover, *P. infestans* transformants overexpressing PiCdc14 exhibit normal nuclear behaviour, growth, sporulation, and germination, which suggests that it might not act in mitosis. To further explore its function, transformants expressing PiCdc14 tagged N-and C-terminally with GFP were analysed. Despite some heterogeneity amongst spores, a trend in the subcellular localization of PiCdc14 during development was discerned. PiCdc14 is distributed throughout the cytoplasm in undifferentiated sporangia. In chilled sporangia and zoospores, PiCdc14 tends to accumulate next to nuclei in an organelle which appears to be the basal body (the microtubule nucleation site from which flagella develop). This suggests a model whereby the ancestral role of Cdc14 involved the function of the centrosome, which in many species regulates aspects of mitosis. In some eukaryotes this role diversified to regulate basal bodies, which are related to centrosomes.

Poster 5

A *Phytophthora sojae* HOG1-homolog is involved in zoospore development and pathogenicity

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Mitogen-activated protein kinase (MAPK) cascades are highly conserved in the eukaryotic cells and play crucial roles in transmitting extracellular stimuli. However, MAPK signaling pathways are almost unknown in oomycete growth, development and pathogenisis. We bioinformatically identified 14 MAPKs in *Phytophthora sojae* genome. One of them encoded a homologue of yeast MAP kinase Hog1 protein and was designated as *PsHOG*1 in this study. *PsHOG*1 was a single copy gene, and was highly transcripted in sporulation stage. *PsHOG1*-silenced transformants exhibited no significant alternation on hyphal growth and sporulation but disturb the zoospore development, including zoospore encystment and cysts germination. The *PsHOG1*-silencing also decrease the virulence of *Phytophthora sojae* to the susceptible soybean cultivar. The *PsHOG*1-silencing mutants also exhibited more sensitive to the osmotice stress. Our results indicated *PsHOG*1 played important roles in stress, pathogenisis and zoospore development

Poster 6

PsCZF1* gene encoding for a C_2H_2 zinc finger protein is required for the growth, development and pathogenesis in *Phytophthora sojae

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C_2H_2 zinc finger proteins form one of the largest families of transcriptional regulators in eukaryotes. We identified a *Phytophthora sojae* C_2H_2 Zinc Finger (*PsCZF1*), which was highly conserved in the sequenced oomycete pathogens. In the transformants of *P. sojae* containing the *PsCZF1* promoter fused to the β-glucuronidase (GUS) reporter gene, GUS activity was highly induced in the *P. sojae* oospores stage and upregulated by the infection. To elucidate the function, expression of *PsCZF1* was silenced by introducing antisense constructs into *P. sojae*. *PsCZF1* silencing transformants exhibited none alternation in the cell size and morphology of sporangia and hyphae; however, the hyphal growth rate was around 50% reduced in the mutants. *PsCZF1*-deficient mutants were also impaired in the production of oospores, swimming zoospores and the germinating cysts, indicating that the gene was involved in the various stages of the life cycles. Furthermore, we found *PsCZF1*-deficient mutants completely lost the virulence on the host soybeans. Our results suggested that this oomycete specific C_2H_2-type zinc finger protein played the important roles in the growth, development and pathogenesis, therefore, PsCZF1 might be an attractive oomycete-specific target for chemical fungicides screening.

Poster 7

Characterizing Conserved Effector Proteins from *Hyaloperonospora arabidopsidis*.

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Plant pathogens utilize effector proteins that are secreted to the inside of host cells, where they interact with host targets to promote disease. Oömycete effectors carry a host targeting sequence that is needed for their translocation into the host cell. We are currently using the interaction between the model plant *Arabidopsis* and its downy mildew pathogen *Hyaloperonospora arabidopsidis* (*Ha*) to understand how oömycete effectors manipulate plant cells. A draft sequence of the *Ha* genome has recently become available. Bioinformatic analyses have revealed over 130 candidate effector genes with a secretory leader, RXLR, and dEER motifs. We are focusing on a small subset of these genes that have conserved homologs in the soybean pathogen *Phytophthora sojae*. Secreted effectors conserved between *Ha* and *Phytophthora* may have important functions in oömycete pathogenicity. We have determined *in planta* expression for each candidate effector during the course of the *Ha* interaction with *Arabidopsis*. In addition, we are examining sub-cellular localization of these effectors *in planta* and are carrying out various assays to test their potential virulence functions. Current progress towards understanding the functions of these effectors will be described.

Poster 8

The *Phytophthora sojae* avirulence locus Avr3c encodes a multi-copy RXLR effector that displays sequence polymorphisms among different pathogen strains

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Root and stem rot of soybean is caused by the oomycete Phytophthora sojae. The Avirulence (Avr) genes of Phytophthora sojae control race-cultivar compatibility. In this study, we identify the P. sojae Avr3c gene and show that it encodes a secreted RXLR effector protein of 220 amino acids. Sequence and transcriptional data were compared for predicted RXLR effectors occurring in the vicinity of Avr4/6, as genetic linkage of Avr3c and Avr4/6 was previously suggested. Mapping of DNA markers in a F2 population was performed to determine whether selected RXLR effector genes co-segregate with Avr3c phenotype. The results pointed to one RXLR candidate gene as likely to encode Avr3c. This was verified by testing selected genes by a co-bombardment assay on soybean plants with Rps3c, thus demonstrating functionality and confirming the identity of Avr3c. The Avr3c gene together with eight other predicted genes are part of a repetitive segment of 33.7 kb. Three near-identical copies of this segment occur in a tandem array. In P. sojae strain P6497, two identical copies of Avr3c occur within the repeated segments whereas the third copy of this RXLR effector has diverged in sequence. Virulent alleles of Avr3c that differ in amino acid sequence were identified in other strains of P. sojae. The Avr3c gene is expressed during the early stages of infection in all P. sojae strains examined. Overall, the results illustrate the importance of segmental duplications and RXLR effector evolution in the control of race-cultivar compatibility in the P. sojae and soybean interaction.

Poster 9

Association of soil chemical and physical properties with *Pythium* species diversity, community composition, and disease incidence. K. D. Broders, M. W. Wallhead, G. D. Austin, P. E. Lipps and P. A. Paul, and A.E. Dorrance*

This study examined the relationship of soil physical and chemical properties with *Pythium* communities and species diversity, and variability in disease incidence among communities. A high-throughput baiting and identification process identified more than 7000 isolates of *Pythium* from 88 locations in Ohio. Isolates were identified using DC-PCR followed by SSCP, and communities were assembled using the Jaccard similarity coefficient and cluster analysis. Both univariate and multivariate statistics were used to evaluate differences in soil properties between communities, and Canonical Discriminant Analysis (CDA) was used to assess the strength of the association of soil variables within communities from 83 of the locations. Twenty-one species of *Pythium* were identified, but only six were recovered from greater than 40% of the locations. Five communities were formed using the cluster analysis, and significant differences were observed in disease incidence, as well as soil pH, calcium, magnesium, and cation exchange capacity between communities. Stepwise multiple discriminant analysis and CDA identified pH, calcium, magnesium, and field capacity as contributing the most to the separation of the five *Pythium* communities. There was a strong association between abiotic soil components and the structure of *Pythium* communities, as well as diversity of *Pythium* species collected from agronomic production fields in Ohio.

Poster 10

Silencing avirulance and effector genes in Phytophthora sojae.

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Avirulance (Avr) genes in *P. sojae* interact with Rps resistance genes in the soybean plant triggering a response responsible for the containment of the spread of the pathogen in the plant. The exact functions and molecular mechanisms of most Avr genes in *P. sojae* are largely unknown. Silencing Avr genes will help us to confirm the identity of the genes and to understand the role that each Avr gene plays during infection. Silencing can also test the possible effector functions of bioinformatically identified avirulence homolog (Avh) genes. Genes of interest are introduced into P. sojae for silencing by the protoplast/PEG method. Full or partial silencing is determined by real time reverse transcription PCR and avirulance tests in soybean plants.

Poster 11

***Plasmodium falciparum* and *Hyaloperonospora parasitica* effector translocation motifs are functional in *Phytophthora infestans*.**

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Like bacteria and fungi, the potato blight pathogen *Phytophthora infestans* translocates effector proteins into host plant cells during infection. In *Phytophthora* this process depends on a short conserved amino acid sequence (RxLR) located near the signal peptide of hundreds of secreted proteins. This motif is similar to the host cell targeting-signal found in virulence proteins from the malaria parasite *Plasmodium falciparum*. A recent study showed that the RxLR motif from AVR3a was sufficient to export the green fluorescent protein (GFP) from *P. falciparum* to the erythrocyte, suggesting a conserved mechanism to deliver effector/virulence proteins into host cells. We have used the AVR3a-R3a interaction as a reporter for translocation in *P. infestans* transformants and replaced the AVR3a RxLR-EER motifs with the RxLR-EER, RxLR only, or RxLxE/D/Q from a related oomycete or *P. falciparum*, respectively. Transformation of a virulent *P. infestans* isolate with the various *Avr3a* constructs yielded avirulent transformants, implying that the alternative sequence is functionally similar to the native RxLR-EER.

Poster 12

Functional characterization of an Oospore specific gene family from the mycoparasite *Pythium oligandrum*

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The oomycete *Pythium oligandrum* is used as a biocontrol agent because of the symbioses it forms with plants, fungi, and other oomycetes. It can parasitize phytopathogenic fungi and oomycetes providing protection to crop plants.
P. oligandrum expressed sequence tags derived from vegetative mycelia and a *P. oligandrum-P. infestans* interaction were analyzed in an attempt to find sequences that may be involved in its biotic interactions. Many sequences with similarity to previously described effectors from fungi, oomycetes, and bacteria were revealed.
We also identified a family a genes in the EST libraries that encode small proteins that are tyrosine-rich (STR) with some similarity to nematode eggshell protein-encoding genes. Using a *P. oligandrum* transformation protocol, we silenced the expression of these genes by homology-dependent gene silencing. Oospores from silenced strains displayed major ultrastructural abnormalities and were sensitive to degradative enzyme treatment. The proteins were localized to the oogonial and oospore wall. We therefore suggest that these proteins are integral components of the oospore/oogonial cell wall.

Poster 13

Using PHRINGE for understanding oomycete genomes. J. L. Boore1, B. M. Tyler2, S. Tripathy2, R. Stiles1,3, and S. I. Fuerstenberg1. 1Genome Project Solutions, Hercules, CA 94547, USA, 2Virginia Bioinformatics Institute, Blacksburg, VA 24061, USA, and 3Roundtrip Networks, Hercules, CA 94547, USA. The PHRINGE (Phylogenetic Resources for Interpreting Genomes, see http://genomeprojectsolutions.com/PHRINGE_project.html) system was developed from our earlier work called "PhIGs". PHRINGE takes the complete gene sets of various organisms, clusters them into gene families using a graph-based method that considers the evolutionary relationships among the organisms, and performs maximum likelihood evolutionary trees of each. Users can view the multiple sequence alignments and evolutionary trees, compare intron-exon structures, see top blast hits, and view the relative arrangements of homologous genes. There is extensive linking to information on gene structure and function at the Virginia Microbial Database and elsewhere. We have applied this system to the complete gene sets of four oomycetes and two diatom outgroups. Results can be viewed at HYPERLINK "http://oomycetes.genomeprojectsolutions-databases.com/" http://oomycetes.genomeprojectsolutions-databases.com/.

Poster 14

Identification of plant proteins targeted by oomycete RXLR effectors using in planta co-immunoprecipitation. Joe Win*, Tolga O Bozkurt, Alex Jones, and Sophien Kamoun
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Oomycete plant pathogens secrete a class of so called RXLR effectors that are translocated inside the plant cells to establish infection. These effectors may function by targeting the plant proteins through physical interactions which may alter the functions of the targeted proteins resulting in a favourable outcome for the pathogen. We aim at identifying the plant proteins targeted by oomycete effectors and the affected physiological processes. We selected 52 validated oomycete RXLR effectors and homologs for this study. We made expression constructs based on high-expression vector pJL-TRBO, a binary plasmid containing a modified *Tobacco mosaic virus* with its coat protein gene replaced by cDNAs coding for FLAG-tagged mature oomycete effectors. Effector constructs were delivered into the leaves of *Nicotiana benthamiana* by agroinfiltration and effectors were expressed under the control of the viral coat protein promoter. The leaves were harvested 2-3 days after infiltration and total proteins were extracted. Effector proteins and their interactors from the plant were co-immunoprecipitated (co-IP) with anti-FLAG resins under non-denaturing conditions. Bound proteins were specifically eluted using 3X FLAG peptides, separated by SDS-PAGE and visualized by colloidal Coomassie blue staining. Protein bands were excised, digested with trypsin, and identified by LC-MS/MS peptide ion spectrum matching. So far we have expressed over 30 effectors to sufficient levels for co-IP and subsequent MS identification of precipitated proteins. We will report and discuss identified effector target proteins, and any alterations in plant immunity resulted from overexpression or virus-induced gene silencing of these targets. The interactions will also be confirmed by alternative approaches such as reverse Co-IP, yeast two hybrid or BiFC techniques.

Poster 15

A novel family of *cellulose synthase* genes from the Oomycete *Saprolegnia monoica*: functional characterization using cellulose synthesis inhibitors

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Cellulose biosynthesis is a vital but yet poorly characterized pathway in Oomycetes. It represents a potential target for specific inhibitors of the plant and animal pathogens from this class of microorganisms. As a first step towards the characterization of the cellulose synthase machinery in the order Saprolegniales, we have isolated a new family of cellulose synthase genes from the species *Saprolegnia monoica* (*SmCesA*). Southern blot experiments revealed the occurrence of at least three homologues in the genome of *S. monoica* and phylogenetic analyses confirmed that Oomycete *CesAs* form a clade of their own. The gene products contained the D, D , D, QXXRW signature of processive glycosyltransferases, including cellulose synthases, but their N-terminal ends exhibited domains that are not encountered in CesA proteins from other classes of organisms, namely Pleckstrin Homology domains, or zinc-finger domains together with additional putative transmembrane domains. When the mycelium was grown in the presence of the cellulose biosynthesis inhibitors 2,6-dichlorobenzonitrile or Congo Red, the growth of the mycelium was inhibited while a higher expression of all genes was observed. In addition, these higher expression levels correlated with higher *in vitro* glucan synthase activities. Altogether, the data strongly suggest a direct involvement of the isolated *SmCesA* genes in cellulose biosynthesis.

Poster 16

Towards identification of effector proteins in the lettuce downy mildew pathogen Bremia lactucae

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Bremia lactucae is an obligate biotrophic pathogen of lettuce (Lactuca sativa). Like other downy mildews and Phytophthora species it belongs to the oomycetes (kingdom Stramenopiles). During infection Bremia grows intercellular hyphae and forms haustoria in host cells. In lettuce cultivation Bremia is mainly controlled by dominant resistance genes that are rapidly overcome by new isolates. Durable resistance is more desirable than ever, as Bremia is also becoming increasingly resistant to fungicides.

The aim of this project is to identify Bremia effector proteins and to study their role in the infection process and in disease susceptibility. We have generated 5' cDNA and random-primed normalised cDNA from both Bremia spores and heavily infected lettuce leaves for 454-sequencing. An initial run yielded 46699 assemblies with an average length of 346 base pairs. To increase coverage and lengthen these assemblies additional 454-sequencing is being performed. Potential effectors will be selected from assembled EST sequences by the presence of predicted signal peptides, RXLR-motifs and other known effector characteristics. Currently we are assessing bio-assays to test for disease-promoting and defence suppressing activities of the potential effectors identified by sequencing. We will report on the preliminary analysis of the Bremia transcriptome.

The knowledge gained from this project will be used to screen for lettuce lines that are insensitive to the action of important effector proteins that can then be deployed for resistance breeding.

Poster 17

Assessing genotypic distributions of *Phytophthora spp*. isolates recovered from nursery plants and environmental samples of Tennessee

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In Tennessee, from 2004-2008, state-wide surveys of symptomatic foliage of nursery plants resulted in isolation of 113 isolates of *Phytophthora spp*.. This sample set includes several reported species as well as new *Phytophthora spp*. In 2008, a leaf baiting survey was initiated to recover *Phytophthora* from eight watersheds in East Tennessee, some of which are in close proximity to nurseries sampled previously. Healthy *Rhododendron* leaves were submerged for approximately one week and *Phytophthora spp*. mycelium was isolated from leaf lesions onto an amended agar medium. A total of six baiting periods were completed, and in total, 186 *Phytophthora* isolates were recovered. All isolates from both surveys were characterized using morphology, ITS sequences, and amplified fragment length polymorphism (AFLP) analyses. Our primary objective is to determine if *Phytophthora* species and genotypes commonly found in the nursery trade are also distributed in the sampled watersheds of Tennessee. The implications for *Phytophthora* ecology in Tennessee will be discussed.

Poster 18

Phosphoproteome analysis and the role of protein kinases during sporulation in Phytophthora infestans. Kavitha Madishetty and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside, California, 92521 USA

The asexual sporangia of P. infestans and the zoospores that they release serve as the primary inoculum for potato and tomato late blight. Expression-profiling studies using microarrays indicated that many protein kinases, as well as protein phosphatases, are differentially expressed during sporulation and zoosporogenesis; of these many are spore-specific. The role of protein phosphorylation in sporangial development is being addressed through two approaches. The first involves gene silencing of five spore-specific protein kinases, either using stable transformants expressing hairpin constructs and or by dsRNA-mediated transient silencing. The second entails a global analysis of phosphorylated proteins during sporulation. Proteins from non-sporulating mycelia, sporulating mycelia, and sporangia have been analyzed by two-dimensional electrophoresis. Phosphorylated proteins are detected using Pro-Q Diamond and total proteins using Sypro Ruby. Comparisons are then made to identify phosphoproteins that are newly synthesized during each developmental stage as well as proteins that have been modified by kinases or phosphatases. Excised spots are then subjected to mass spectroscopy and compared to Phytophthora sequence databases.

Poster 19

Comparative genomics: *Phytophthora phaseoli* causing downy mildew of lima bean

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The phylogenetic relationship between different *Phytophthora* species has shown that *Phytophthora phaseoli*, causal agent of downy mildew of lima bean is closely related to *Phytophthora infestans*. Currently, there is little molecular information available about the host pathogen interaction between lima bean and *P. phaseoli*. In an effort to determine whether *P. phaseoli* contains and utilizes effectors similar to *P. infestans,* we used a degenerate primer set for *INF1* and a gene specific primer set for *Avr3a*, two well-known *Phytophthora* effector genes, and were able to amplify several candidates from our pathogen. This finding prompted us to explore global gene expression in *P. phaseoli*, for which we created two RNA pools: the first is from *P. phaseoli* in culture, and the second from *P. phaseoli* during infection. The data has been generated using SBS (sequencing by synthesis) technology at the NCGR (National Center for Genomic Research) under the direction of Greg May. With this *P. phaseoli* data, we will study the pathogen gene expression patterns during infection and determine if there are any genes that are common to or different from other *Phytophthora* species, in particular *P. infestans*. We will be comparing the millions of short reads that were generated from *P. phaseoli* during infection against the available expressed sequence tag (EST) information from *P. sojae, P. ramorum, P. infestans* and *P. brassicae*. A subset of the candidates will be validated by biological assays, to see if they are involved in effector functions. These studies will help us to understand which genes are evolving rapidly or slowly, while also providing a rich data resource for future molecular studies of *P. phaseoli*.

Poster 20

Transcriptome sequencing to understand effector evolution in the Phytophthora infestans species cluster. Liliana M. Cano1, Sebastian Schornack1, Rhys Farrer1, Joe Win1, David Studholme1, Chad Nusbaum2, and Sophien Kamoun1. 1The Sainsbury Laboratory, Norwich, UK, 2Broad Institute, Cambridge, MA, USA

The highlands of Central Mexico are the center of origin of the potato late blight pathogen Phytophthora infestans. They are also home to several species closely related to P. infestans, namely P. mirabilis on Mirabilis jalapa and P. ipomoeae on Ipomoea longipedunculata. These species are thought to have evolve by host-jump followed by adaptation and specialization on distinct host plants. P. infestans secretes a large repertoire of effector proteins that evolve rapidly through birth-and-death evolution and typically exhibit adaptive selection. Our aim is to to identify candidate effectors that show species-level polymorphisms that can be related to host adaptation. We applied Illumina technology to sequence the transcriptomes of P. mirabilis and P. ipomoeae represented in normalized cDNA libraries constructed from mixed developmental stages including mycelia and germinated cysts. De novo sequence assembly revealed a novel RXLR effector present in both P. ipomoeae and P. mirabilis species but annotated as a pseudogene in the P. infestans T30-4 reference genome due to a 5-bp deletion. We found that transient expression of functional alleles resulted in suppression of plant immunity and we are currently expanding these experiments to the various hosts. Our study highlights the value of comparing transcriptomes from closely related species to identify candidate effectors in species with no prior gene sequence information. We now aim to connect the discovered genes to effector activities to reveal a putative role in host adaptation.

Poster 21

Production, viability and germination of oospores from A1 X A2 mating types crosses of *Phytophthora infestans* in Colombia. M.C. Cespedes*, M. Cardenas, and S. Restrepo. Laboratorio de MicologÌa y FitopatologÌa, Universidad de los Andes, Bogota, Colombia

Phytophthora infestans is one of the most important plant pathogens in Colombia. It is the causal agent of late blight, which attacks in this country important crops as *Physalis peruviana, Solanum tuberosum, Solanum phureja, Solanum betaceum* and *Solanum lycopersicum*. A recent research identified for the first time in Colombia an individual of A2 mating type from *Physalis peruviana* plants, suggesting the possibility of sexual reproduction. Despite the presence of the A2 mating type, the Colombian population of *P. infestans* showed a low genetic variability. In order to verify the capability of the pathogen to reproduce sexually, the production, viability and germination of oospores from A1 X A2 crosses were investigated. Mating between A1 from *Physalis peruviana*, *S. betaceum, S. phureja, S. lycopersicum, S. quitoense* and *S. tuberosum* and the A2 isolate of *P. infestans* produced oospores *in vitro* in most of the cases examined. Viability of oospores from the crosses was tested using the plasmolysis test in NaCl solution. Germination of oospores was also assessed by presence/absence of germ sporangia. This study allowed us to understand the population dynamics of this pathogen.

Poster 22

Evaluation of the genetic diversity of *Phytophthora infestans* in the Northern Andean region. **M. Cárdenas*** (3), R. Sierra (3), A. Grajales (3), A. Rojas (3), A. González (3), A. Vargas (3), C. Salazar (3), M. Marín (1), G. Fermín Muñoz (4), L. E. Lagos (2), A. Bernal (3), S. Restrepo (3). (1) Universidad Nacional, Medellin, Antioquia, Colombia; (2) Universidad de Nariño, Pasto, Nariño, Colombia; (3) Universidad de los Andes, Bogotá D.C., Colombia; (4) Universidad de los Andes, La Hechicera, Merida, Venezuela.

Phytophthora infestans, the causative agent of the late blight disease in potato (*Solanum tuberosum*) and other members of the solanaceae family (*S. phureja*, *S. lycopersicum*, *S. betaceum*, *S. melongena*, *S. quitoense* and *Physalis peruviana*), has not been studied from a population perspective in the northern Andean region. Particularly, the structure of this pathogen population is unknown, with no information available about the relatedness among isolates present in different locations and hosts. In order to make some inferences about the relationships among the *P. infestans* populations, 5 genic regions including 4 nuclear (Internal Transcribed Spacer, Ras Intron-Ras, ß-tubulin and Avr3a) and 1 mitochondrial (Cox I) were analyzed on 92 isolates of *P. infestans* from different hosts in Colombia (including 4 regions: Boyacá, Cundinamarca, Antioquia and Nariño), Ecuador, and Venezuela. Surprisingly, a low genetic diversity was found in the Andean region where potato has its center of origin. No genetic structure could be established regarding the host or geographic origin, suggesting the dispersal of a clonal population of the pathogen in this region. Nonetheless, a new allele was found in the Avr3a gene restricted to some isolates from the southern location of the region (Nariño and Ecuador) and shared with *P. andina*. We are exploring the *P. infestans* genome for new molecular markers needed to understand the population dynamics of *P. infestans* in order to successfully design disease control strategies.

Poster 23

Species boundaries of *Phytophthora capsici* and related taxa inferred from eleven nuclear and mitochondrial loci Mansfield, M. A (1)., Coffey, M. D. (2), Martin, F. N.(3), Sunkavally, B. (2), Blair, J. E. (4), Abad, G. Z. (5), Kang, S. (1), and Geiser, D. M. (1). (1) Dept. Plant Pathology, The Pennsylvania State University, University Park, PA (2) Dept. Plant Pathology and Microbiology, University of California Riverside, Riverside, CA (3) USDA-ARS, Salinas, CA (4) Franklin and Marshall College, Dept. of Biology, Lancaster, PA (5) USDA-APHIS-PPQ, Molecular Diagnostics Lab, Beltsville, MD.

The oomycete plant pathogen *Phytophthora capsici* causes extensive damage worldwide to a number of important agricultural plants, including solanaceous, curcubit, and tropical species. Localized field populations of *P. capsici* are reported to have considerable morphological and physiological diversity, including resistance to chemical controls. Previous studies provided evidence for subgroups within *P. capsici*, leading to descriptions of *P. mexicana* and *P. tropicalis*. Because of the agricultural importance of this destructive pathogen, we selected 75 isolates of *capsici* and related species from the World Phytophthora Collection to investigate species boundaries among *P. capsici* and related *Phytophthora*. Using seven nuclear and four mitochondrial loci, we performed a phylogenetic analysis of isolates previously identified as *P. capsici* and *P. mexicana*, as well as *P. sp. glovera*, *P. tropicalis* and *P. siskiyouensis*. *P. capsici* and *P. mexicana* isolates together form a distinct monophyletic group, but there was no support in the multilocus phylogeny for the status of *P. mexicana*. A novel, highly supported clade of isolates from cacao in Brazil was also resolved, suggesting that these isolates constitute a new species of *Phytopthora*. There was very strong support for the monophyly of *P. sp. glovera*, *P. tropicalis* and *P. siskiyouensis*. Two isolates showed distant relationships to all other taxa, suggesting that they are single members of currently uncharacterized species. The results of analyses testing for historical recombination within the *P. mexicana/capsici* clade will be presented

Poster 24

An optimized protocol for chromatin immunoprecipitation in *Phytophthora sojae*

Phuntumart[1], V., Morohashi[2], K. and Grotewold[2], E.

[1]*Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403* **[2]***Dept. of Plant Cellular and Molecular Biology,Plant Biotechnology Center, The Ohio State University, Ohio, 43210* Chromatin structure plays an important role in complex gene regulation, and modifications of histones tails contribute to the activation or repression of transcription. Chromatin immunoprecipitation (ChIP) provides a powerful tool for the characterization of protein–DNA interactions and to establish the genomic location of histone modification in vivo. Although ChIP has been applied to several organisms, the establishment of the technique to *Phytophthora sojae,* an important soybean pathogen, remains relatively challenging. The protocol presented here provides the first instance of ChIP being applied to study histone modifications in this economically important oomycete.

Poster 25

Multiple horizontal gene transfer events and domain fusions have created novel regulatory and metabolic networks in the oomycete genome. Paul F Morris, Laura Rose Schlosser, Katherine Onasch, Tom Wittenschlaeger, Ryan Austin, Nicholas Provart. PFM, LRS, KDO, TW: Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio, 43403; RA and NJPUniversity of Toronto, Toronto, Ontario, Canada. M5S 3G5. pmorris@BGSU.edu

Analysis of the *Phytophthora sojae* genome revealed 273 novel multifunctional proteins that are largely conserved across *Phytophthora and Pythium* genomes. Each of these proteins contains combinations of protein motifs that are not present in bacterial, plant, animal, or fungal genomes. Only 11% of these proteins models are also found in the diatom genome and thus the majority of these proteins have formed after the split between diatoms and oomycetes. We postulated that the novel multifunctional proteins of oomycetes might have value as potential Rosetta Stones to identify interacting proteins of conserved metabolic and regulatory networks in eukaryotic genomes. However ortholog analysis of each domain within the multifunctional proteins using the reciprocal smallest distance algorithm against 39 sequenced bacterial and eukaryotic genomes identified only 25 candidate Rosetta Stone proteins. Since the majority of proteins are not Rosetta Stones, they may instead serve to identify novel metabolic and regulatory networks in oomycetes. Since multifunctional proteins in metabolic pathways must act cooperatively with other components of the pathway, we looked in detail at the phylogenetic origins of enzymes in the sulfate assimilation, lysine and serine biosynthetic pathways. Each of these pathways had one or more bifunctional enzymes. Phylogenetic analysis of the proteins in these pathways revealed multiple examples of horizontal transfer from both bacterial genomes and the photosynthetic endosymbiont in the ancestral genome of Stramenopila. The complexity of the phylogenetic origins of these metabolic pathways and the paucity of Rosetta Stones relative to the total number of multifunctional proteins, suggests that the protein regulome of oomycetes has few features in common with other Kingdoms.

Poster 26

Transcriptional programming and cooperation among *Phytophthora sojae* RXLR-dEER effectors to suppress host cell death Wang Qunqing[1], Han Changzhi[1], Dou Daolong[1,2], Adriana Ferreira[2], Shiv Kale[2], Wang Xiaoli[1], Yu Xiaoli[1], Liu Tiuli[1], Yao Yao[1], Wang Xinle[1], Dong Suomeng[1], Zhang Zhengguang[1], Zheng Xiaobo[1], Brett Tyler[2], Wang Yuanchao[1]1, Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China 2, Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

About 370 candidate effectors, sharing the RXLR-dEER motifs, have been identified from *Phytophthora sojae* genome and are believed to enter its host cells. However, the functions of the most effectors are still poorly understood. Here, we test 168 effectors and find over 60% have the capability to block cell death process triggered by the mouse Bax, which is accordance with the functions of *P. sojae* Avr1b proteins in the pathogenicity. Interestingly, we show most of the early-infection-induced effectors can suppress Bax or INF1 induced cell death, indicating the possible strategies used by *P. sojae* to benefit its biotrophic infection process. However, plant also develops the successful resistance mechanism by recognization of some early-infection-induced effector proteins, such as Avh238 and Avh241. We show that *P. sojae* can escape from the recognization by point mutations, or suppress the recognization by delivering many other effectors. Furthermore, we demonstrate Avh238 can cooperate with the other effectors, such as Avh172, to inhibit the basal resistance triggered by INF1. Taking together, our results indicate the roles of large number of predicted *P. sojae* effectors, then begin to illustrate how pathogen establish the successful

infection by delivering effectors with different functions at the proper time points and how those effectors collaborately suppress the host defense.

Poster 27

A novel approach for discovering motifs regulating spore-specific transcription in the phytopathogen Phytophthora infestans. S.Roy and H. S. Judelson. Genetics, Genomics and Bioinformatics Program and Department of Plant Pathology & Microbiology, University of California, Riverside, California, USA.

The oomycete *Phytophthora infestans* is one of the most devastating phytopathogens, causing late blight in potato and tomato. Its pathogenic success depends on forming different asexual spores such as sporangia and zoospores. Our goal is to identify what regulates transitions between such stages, by understanding what determines stage-specific transcription. To help accomplish this, transcription factor binding sites (TFBSs) acting during development are being identified by integrating bioinformatics and traditional molecular biology techniques. Microarray data was employed to help assemble sets of stage-specific and coexpressed promoters. These were searched for over-represented motifs (putative TFBSs) using Gibbs sampling, enumerative search, and expectation maximization algorithms. Phylogenetic footprinting involving eight Phytophthora genomes, and tests for positional bias, are being used to provide robust TFBS predictions. So far, data has been obtained from genes co-expressed in sporangia and cleaving sporangia. For each, multiple putative TFBSs were identified. Known motifs, such as the "cold box" which regulates some zoosporogenesis-induced genes, were in the output; this supports the validity of the computational approach. In addition, many motifs show evolutionary conservation and positional bias, suggesting that they are biologically relevant. Functional analyses of selected motifs are showing encouraging results, confirming that integrating bioinformatics with traditional promoter analysis methods reduces the time needed to identify TFBSs. This should lead to a better understanding of signaling pathways regulating spore development and provide insight into new disease control strategies.

Poster 28

Comparative genomics: *Phytophthora phaseoli* causing downy mildew of lima bean

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The phylogenetic relationship between different *Phytophthora* species has shown that *Phytophthora phaseoli*, causal agent of downy mildew of lima bean is closely related to *Phytophthora infestans*. Currently, there is little molecular information available about the host pathogen interaction between lima bean and *P. phaseoli*. In an effort to determine whether *P. phaseoli* contains and utilizes effectors similar to *P. infestans,* we used a degenerate primer set for *INF1* and a gene specific primer set for *Avr3a*, two well-known *Phytophthora* effector genes, and were able to amplify several candidates from our pathogen. This finding prompted us to explore global gene expression in *P. phaseoli*, for which we created two RNA pools: the first is from *P. phaseoli* in culture, and the second from *P. phaseoli* during infection. The data has been generated using SBS (sequencing by synthesis) technology at the NCGR (National Center for Genomic Research) under the direction of Greg May. With this *P. phaseoli* data, we will study the pathogen gene expression patterns during infection and determine if there are any genes that are common to or different from other *Phytophthora* species, in particular *P. infestans*. We will be comparing the millions of short reads that were generated from *P. phaseoli* during infection against the

available expressed sequence tag (EST) information from *P. sojae, P. ramorum, P. infestans* and *P. brassicae*. A subset of the candidates will be validated by biological assays, to see if they are involved in effector functions. These studies will help us to understand which genes are evolving rapidly or slowly, while also providing a rich data resource for future molecular studies of *P. phaseoli*.

Poster 29

Small RNA Pathways in the Oomycetes Phytophthora sojae, Phytophthora ramorum, and Phytophthora infestans. N. Fahlgren[1], K. Kasschau[1], S. Bollmann[2]*, C. Press[2], C. Sullivan[1], N. Grunwald[2], and J. Carrington[1]. [1]Center for Genome Research and Biocomputing, and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331. [2]Horticultural Crops Research Laboratory, USDA Agricultural Research Service, Corvallis, OR 97330.

The oomycetes are a distinct lineage of eukaryotes that contain many important pathogens of plants, including *Hyaloperonospora parasitica* and several *Phytophthora* species. Oomycetes are closely related to photosynthetic algae and share some pathogenic features with members of the apicomplexans, such as *Plasmodium falciparum*, the causal agent of malaria. Plant pathogens of the genus *Phytophthora* are among the most well-studied oomycetes and include *P. infestans*, a pathogen of potato that caused the Irish potato famine, *P. sojae*, a pathogen of soybean, *P. ramorum*, the cause of sudden oak death, and *P. capsici*, a pathogen of pepper. Recently the genomes of these four *Phytophthora* species and *H. parasitica* were sequenced. Most eukaryotes have RNA silencing systems that use small RNAs to suppress a wide range of genes, genetic elements, and viruses. One important silencing pathway is the microRNA pathway. miRNAs (usually 21-22 nucleotides) are derived from processing of self-complementary foldback RNAs derived from *MIRNA* genes. miRNAs associate with protein effector complexes containing ARGONAUTE proteins, and the miRNA serves to guide cleavage, translational repression or redirection of the target transcript within the cell. Here, we identified the core small RNA biogenesis components and effectors in three *Phytophthora* species, *P. sojae*, *P. ramorum*, and *P. infestans*. We used high-throughput pyrosequencing (454 Life Sciences) and sequencing-by-synthesis (Illumina) to profile small RNA. Analysis of these data revealed several candidate *MIRNA* genes from one gene family that are conserved in all three species. In addition, large numbers of siRNA-generating loci were identified throughout the *Phytophthora* genomes.

Poster 30

The brown algal pathogen Eurychasma dicksonii: A model oomycete to study the evolution of pathogenicity M. Strittmatter*, C. Gachon, and F.C. Kuepper Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, PA37 1QA Oban, UK. HYPERLINK "mailto:claire.gachon@sams.ac.uk" claire.gachon@sams.ac.uk

The intracellular, obligate-biotrophic pathogen Eurychasma dicksonii is the most widespread eukaryotic pathogen of marine brown algae, and also the most basal member of the oomycete lineage. This algal parasite has the broadest host range described so far for marine pathogens and occurs worldwide in cold and temperate waters. Currently, nothing is known about the molecular mechanisms that determine the pathogenicity of this generalist parasite. We have developed a Real-Time PCR assay that reliably quantifies Eurychasma infection in brown algae and found that various clonal Ectocarpus strains show differential susceptibility towards the oomycete pathogen. Established on laboratory cultures this assay is also applicable for the detection of the pathogen in natural brown algal populations. Due to its availability in culture, its phylogenetic position and the recently completed sequencing of the genome of one of its brown algal hosts, Ectocarpus siliculosus, we are currently using Eurychasma dickosnii as a model organism to study the evolution of pathogenicity among oomycetes and chromealveolates.

Poster 31

The *Phytophthora sojae* avirulence locus Avr3c encodes a multi-copy RXLR effector that displays sequence polymorphisms among different pathogen strains

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Root and stem rot of soybean is caused by the oomycete Phytophthora sojae. The Avirulence (Avr) genes of Phytophthora sojae control race-cultivar compatibility. In this study, we identify the *P. sojae* Avr3c gene and show that it encodes a secreted RXLR effector protein of 220 amino acids. Sequence and transcriptional data were compared for predicted RXLR effectors occurring in the vicinity of Avr4/6, as genetic linkage of Avr3c and Avr4/6 was previously suggested. Mapping of DNA markers in a F2 population was performed to determine whether selected RXLR effector genes co-segregate with Avr3c phenotype. The results pointed to one RXLR candidate gene as likely to encode Avr3c. This was verified by testing selected genes by a co-bombardment assay on soybean plants with Rps3c, thus demonstrating functionality and confirming the identity of Avr3c. The Avr3c gene together with eight other predicted genes are part of a repetitive segment of 33.7 kb. Three near-identical copies of this segment occur in a tandem array. In *P. sojae* strain P6497, two identical copies of Avr3c occur within the repeated segments whereas the third copy of this RXLR effector has diverged in sequence. Virulent alleles of Avr3c that differ in amino acid sequence were identified in other strains of *P. sojae*. The Avr3c gene is expressed during the early stages of infection in all *P. sojae* strains examined. Overall, the results illustrate the importance of segmental duplications and RXLR effector evolution in the control of race-cultivar compatibility in the *P. sojae* and soybean interaction.

Poster 32

Shared genetic mechanisms between oomycetes and fungi.

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Phytophthora is a member of a class oomycetes, which belongs to a distinct kingdom Stramenopila. The Stramenopila lineage diverged from major kingdoms of eukaryote, i.e., Animals, Fungi, and Plants in the remote past. Because of this reason, a large part of Phytophthora genome is distinct from genomes of model organisms such as Arabidopsis, Drosophila and Saccharomyces. Nevertheless, oomycetes are composed of mycelia and they feed on decaying matter, just like fungi. It is not clear if this resemblance of fungi and oomycetes is a consequence of a convergent evolution or alternatively, resulted from homologous genetic mechanisms. Taking the advantage of abundant Eukaryotic genome data, we systematically conducted comparative genomics and examined phylogenetic distribution of gene homologs. We are specifically interested in genes which are conserved between oomycetes and fungi but are diverged or undetectable in the genomes of animals, plants or diatoms (Stramenopila algae). It was found that 116 genes are shared exclusively between oomycetes and fungi. In addition, functional category analysis revealed that genes for "polysaccharide metabolism" were overrepresented in this subset. We will discuss gene regulation and possible functions of the Fungal/Oomycetes specific genes.

Poster 33

Finishing and resequencing of the *Phytophthora sojae* genome

Brett M. Tyler[1], Sucheta Tripathy[1], Felipe Arredondo[1], Jeff L. Boore[2], Robert Stiles[2,3], Susan I. Fuerstenberg[2], Hongbin Zhang[4], Igor Grigoriev[5], Clive Evans[1], Richard M. Myers[6], Jane Grimwood[6] and Jeremy Schmutz[61] Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24060; [2] Genome Project Solutions, Hercules, CA 94547, USA, [3] Roundtrip Networks, Hercules, CA 94547, USA; [4] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474; [5] DOE Joint Genome Institute; [6] Stanford University Human Genome Center and HudsonAlpha Institute for Biotechnology, Huntsville, Alabama 35806.

In order to create a high quality reference sequence for the oomycetes, we are advancing the quality of the genome sequence of *Phytophthora sojae* strain P6497 towards finished quality. To date, a new BAC library with >150kb inserts derived from MboI digestion, with 12x coverage has been created, to complement the exiting HindIII and BamHI libraries. These BAC libraries, jointly giving 25x coverage, have been fully end-sequenced, and the data used to create a new assembly (version 4) which is currently being annotated. Automated gap closure has been completed, and individual BACs with complex regions are being targeted, with a focus on those containing arrays of effector genes. 98.9% of the sequence is now in 29 scaffolds of 100 kb in length or better. Within each scaffold, only 1.5% of the sequence is represented by gaps. In addition, we have Sanger sequenced 5,000 short subclones from *P. sojae* strain P7064 to identify polymorphisms, which will be used for genetic mapping of the sequence scaffolds. Illumina bead polymorphism assays will be used to map into the scaffolds onto genetic maps created by crosses P6497 x P7064 (from Virginia Tech) and UQ1200 x US7 (from the University of Queensland, Australia). In addition to placing phenotypic traits such as Avr genes onto the sequence, the genetic mapping will help identify adjacent scaffolds and mis-assemblies. Synteny analysis also is being used to suggest adjacent scaffolds and mis-assemblies. Finally, we are using 454 Titanium sequencing (500 bp

reads) to carry out 5x resequencing of *P. sojae* strains P7064, P7076 and P7074, representing the three other major *P. sojae* genotypes. These sequences will provide a genome wide map of nearly all ancestral polymorphisms in the species.

Poster 34

Functional Genomics of G-protein coupled receptors (GPCR) in *Phytophthora sojae*

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G protein coupled receptors (GPCRs) present a large family of receptors involved in a broad spectrum of cell signaling, which are responsive for transmitting the extracellular signals to intracellular response by stimuli. Despite exhibiting striking diversity in primary sequence and biology function, all GPCRs possess the conserved fundamental architecture consisting of seven transmembrane domains and share common mechanisms of signal transduction. This class of protein were primarily identified in the gnome of *Phytophthora sojae*, the destructive cause of stem and root rot in soybean. *P. sojae* have approximately 41 seven transmembrane proteins, which are classified into five classes based on sequence homology, including metabotropic glutamate receptors, rhodopsin-like superfamily, cyclic AMP receptors, one novel class phosphatidylinositol phosphate kinases with a G-protein coupled receptor, and other unclassified proteins. Subsequently, the expression profiles were evaluated during asexual lifestages, stresses treatments and infection stage. The results showed that some genes were constitutively expressed; in contrast, the other genes are induced during zoosporogenesis or stresses. Finally, genetic transformation of homology-dependent gene silencing of *P. sojae* were performed and the gene-silenced mutants of five genes were generated respectively. We found that out of five genes, three genes are probably involved in sexual reproduction and pathogenicity, while two genes are not obviously affected the phenotypes including chemotaxis to isofalvone. Further study on these mutants, and other GPCRs genes will be focused on to elucidate the functions of all GPCRs genes in *P. sojae* growth, development, stress response , chemotaxis and pathogenesis.

Poster 35

Unraveling transcriptional regulatory mechanisms involved in sexual reproduction of *Phytophthora infestans*. Xiaofan Niu and Howard Judelson. Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA

Phytophthora infestans, the causal agent of potato and tomato late blight, is one of the most devastating plant pathogens in the world. Its sexual reproduction cycle serves an important role in disease since the sexual spores (oospores) are important for survival over the long-term and through unfavorable conditions. To understand the transcriptional regulatory machinery involved in sexual development, ten genes that based on microarray studies are expressed specifically during mating, or up-regulated more than 100-fold, were selected for detailed analysis by fusing their promoters with the GUS reporter gene, and transformed into *P. infestans* to examine their temporal and spatial expression patterns. The promoter of gene Pi 000192, which is predicted to encode an elicitin-like protein, has shown specific activity in oogonia and antheridia. Deletions, site-directed mutagenesis, and electrophoresis mobility gel shift assays are now being used to define the regulatory elements in this promoter. To acquire further information on the sexual stage, a global proteomics comparison of the vegetative (nonsporulating mycelium) and sexual (oospore) life stages of *P. infestans* is being conducted. A preliminary study indicated that oospores contain mitochondrial proteins, ATPase, crinkler family proteins, and others. In addition, the functions of selected mating-induced proteins are being tested, such as a RNA-binding protein belonging to the Puf family. Tandem affinity purification tags were attached to the Puf RNA binding domain to use affinity approach to identify mRNAs that bind and are presumably regulated by Puf during sexual development.

Poster 36

PsCZF1* gene encoding for a C_2H_2 zinc finger protein is required for the growth, development and pathogenesis in *Phytophthora sojae Yonglin Wang, Daolong Dou, Xiaoli Wang, Aining Li, Chenlei Hua, Yuting Shen, Binyan Cheng, Xiaobo Zheng, Yuanchao Wang Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China

C_2H_2 zinc finger proteins form one of the largest families of transcriptional regulators in eukaryotes. We identified a *Phytophthora sojae* C_2H_2 Zinc Finger (*PsCZF1*), which was highly conserved in the sequenced oomycete pathogens. In the transformants of *P. sojae* containing the *PsCZF1* promoter fused to the β-glucuronidase (GUS) reporter gene, GUS activity was highly induced in the *P. sojae* oospores stage and upregulated by the infection. To elucidate the function, expression of *PsCZF1* was silenced by introducing antisense constructs into *P. sojae*. *PsCZF1* silencing transformants exhibited none alternation in the cell size and morphology of sporangia and hyphae; however, the hyphal growth rate was around 50% reduced in the mutants. *PsCZF1*-deficient mutants were also impaired in the production of oospores, swimming zoospores and the germinating cysts, indicating that the gene was involved in the various stages of the life cycles. Furthermore, we found *PsCZF1*-deficient mutants completely lost the virulence on the host soybeans. Our results suggested that this oomycete specific C_2H_2-type zinc finger protein played the important roles in the growth, development and pathogenesistherefore, PsCZF1 might be an attractive oomycete-specific target for chemical fungicides screening.

Poster 37

The potential host range of the sister species *Pseudoperonospora cubensis* and Ps. *humuli* is extending across host orders F.Runge, M. Thines. University of Hohenheim, Institute of Botany 210, D-70593 Stuttgart, Germany. <rungefab@uni-hohenheim.de>

Pseudoperonospora cubensis (*Pc*) is a serious threat for cucurbitaceous crops (Cucurbitaceae), while *Pseudoperonospora humuli* (*Ph*) is an important pathogen of hop (Cannabaceae). Although parasitic to different Angiosperm orders, these pathogens are highly similar in morphology, ITS and *cox2* sequence. Thus, it can be concluded that the host jump from Urticales (Rosales s.l.) to Cucurbitales has occurred in evolutionary recent times. Infection experiments were done to evaluate the host range of these pathogens. The focus of these experiments was to clarify, whether *Ph* is potentially infective to Cucurbitaceae and whether *Pc* may infect hop. If perennial hop could be infected by *Pc*, this would be a possible retreat for this pathogen to overwinter. Also the perennial Cucurbitaceae *Bryonia dioica* was tested as a possible overwintering host. In our experiments under controlled conditions, we could achieve infection of two Ps. cubensis strains on several annual Cucurbitaceae, but also on the perennial *Bryonia dioica*. *Pseudoperonospora humuli* was also able to reproduce on *Bryonia dioca*. For both pathogens back infections to their respective original hosts were successful. Interestingly, limited infectivity of *Pc* to hop was also observed, and *Ph* was also able to infect *Cucumis sativus*. These findings raise questions regarding species delimitation in *Pseudoperonospora* and suggest that there could be a population continuum between *Pc* and *Ph*. However, an analysis of more than 40 *Ph* strains revealed complete identity in ITS. Further studies are on the way to determine the variability of *Pc* in Europe and the worldwide phylogenetic patterns of these pathogens

Poster 38

Genetic structure of populations of *Phytophthora infestans* from China. Liyun Guo[1], Xiao-qiong Zhu[1], Chia-Hui Hu[2] and Jean Beagle Ristaino[2].[1]Department of Plant Pathology, China Agricultural University, Beijing, China, 100094, China and [2]Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695*Phytophthora infestans* causes late blight and is the most devastating disease of potato in China. China grew more than 72 million tons of potato in 2007 in four major potato growing areas in the north and south. One hundred modern isolates of *P. infestans* collected from ten provinces including Beijing, Chongqing, Fuijan, Gansu, Hebei, Heilongjiang, Jilin, Inner Mongolia, Sichuan, and Yunnan between 1999-2004 were analyzed for mating type, metalaxyl resistance, mitochondrial DNA (mtDNA) haplotype, and a subset of 21 isolates were analyzed for allozyme genotype with *Glucose-6-phosphate isomerase* (*Gpi*), and *Peptidase* (*Pep*), restriction fragment length polymorphism fingerprint (RFLP) with the RG-57 probe, and DNA sequence variability from two nuclear (Ras, and Intron Ras) and two mitochondrial (P3 and P4) gene regions. All isolates tested were the A1 mating type except three isolates that were A2 and self fertile. Four allozyme genotypes including *Gpi* 86/100, *Pep* 92/100; *Gpi* 86/100, *Pep* 100/100; *Gpi* 100/100, *Pep* 100/100; and *Gpi* 100/111, *Pep* 100/100 were found. Three isolates from tomato and one isolate from potato were the US-1 genotype (*Gpi* 86/100, *Pep* 92 or 100/100, Ib mt DNA haplotype). A previously described genotype from Siberia called SIB-1 (*Gpi* 100/100, *Pep* 100/100, IIa mtDNA haplotype) was identified among isolates and was widely

distributed in China in Gansu, Hebei, Heilongjiang, Inner Mongolia, and Jilin provinces in the north and Sichuan and Yunnan province in the south. A new genotype named CN-9 (*Gpi* 100/111, *Pep* 100/100, IIb mtDNA haplotype) was found only in the south of China. The remaining genotypes CN10-CN12 (*Gpi* 100/100, *Pep* 100/100, Ia mtDNA haplotype) had unique RFLP fingerprints and were named CN-9-CN12. We have examined potato leaves infected with *P. infestans* from herbarium collections (1901-1981) from China, Southeast Asia, India, Russia, and Australia. Twelve samples from China containing late blight lesions collected between 1938 and 1982 from Beijing, Hebei, Sichuan, Shanxi, Chongqing, and Yunnan were examined. The Ia mtDNA haplotype of *P. infestans* was found earlier in China than other mtDNA haplotypes and was likely the initial haplotype introduced. Samples collected by different researchers in China from tomato in 1938 in Kunming and from potato in 1940 in Chengjiang and Chongqing were the Ia mtDNA haplotype. In contrast, the earliest record of the Ib haplotype (US-1 genotype) of *P. infestans* in China was in 1952 on potato in the Sichuan region, in 1954 on potato in Hebei and in 1956 on tomato in Beijing. The Ib haplotype still occurs in the Beijing area on tomato, but the IIa haplotype is now dominant in modern populations. The predominant genotype of *P infestans* in China was SIB-1 (IIa haplotype) and identical to those from Siberia suggesting a Russian ancestry.

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