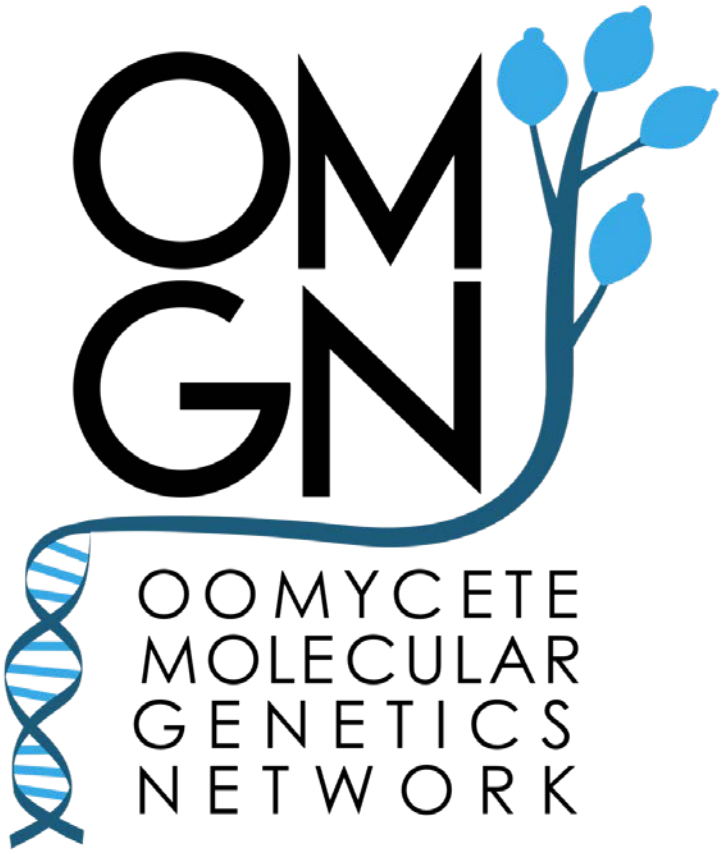
OMGN 2017

March 11 - 14, Asilomar Conference Grounds



Oomycete Molecular Genetics Network Meeting 2017

The Oomycete Molecular Genetics Research Network (OMGN) was initially funded by an NSF Research Coordination Network grant in 2001 and continued to receive funding from the NSF for many years. More recently, the Network has received funding from various USDA AFRI programs. The purpose of our annual meeting is to promote communication and collaboration, and minimize the duplication of effort within the oomycete molecular genetics community. Our community now numbers well in excess of 100 laboratories from around the world, and research on oomycetes attracts considerable attention from outside the community as well as within. The OMGN annual meeting alternates between Asilomar, CA, and another venue, usually outside of the USA. This year, the meeting returns to California, and we are delighted to welcome you- or welcome you back- to the Asilomar Conference Grounds. The 2017 meeting will cover some of the latest research on Oomycete Genomics, Evolution, Population Biology, Host Interactions, and Effector Biology. We look forward to an engaging and dynamic meeting!

ORGANIZERS

Scientific Program:

- Lina Maria Quesada-Ocampo (Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.)
- Aurelien Tartar (Department of Biological Sciences, Nova Southeastern University, Fort Lauderdale, FL, U.S.A.)

Meeting Logistics:

- Joel Shuman (Virginia Tech)
- Paul Morris (Bowling Green State University)

ACKNOWLEDGEMENTS

This meeting is supported in part by a coordinated agricultural project and a conference grant provided by the USDA's National Institute of Food and Agriculture. The project "Integrated Management of Oomycete Diseases of Soybean and Other Crop Plants" was competitive grant 2011-68004-30104 in Agriculture and Food Research Initiative program area A5121 "Oomycete Pathosystems in Crop Plants to Minimize Disease" with Dr. Ann Lichens-Park as program leader. The conference grant "18th Annual Oomycete Molecular Genetics Meeting" is a competitive grant in AFRI program area A1112 "Pests and Beneficial Species in Agricultural Production Systems" with Dr. Mary Purcell-Miramontes as program leader.

OMGN logo design: Kelsey Wood, University of California, Davis

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AT-A-GLANCE PROGRAM

Oral presentations are in Fred Farr Forum. Reception/Poster sessions are in Kiln Hall. All meals are in Crocker Dining Hall, except on Monday evening (Seascape banquet room).

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8:30 - 9:00	Registration	
9:00 - 9:05	Welcome	
9:05 - 10:25	**Genomics I**	**Chair: Alamgir Rahman**
9:05 - 9:20	Prediction and characterization of WY-domain effectors in downy mildews	Kelsey Wood
9:25 - 9:40	CRISPR/Cas9, a powerful new tool for genetic studies of oomycete pathogens	Yufeng 'Francis' Fang
9:45 - 10:00	Systematic approach for development of markers for plant pathogenic oomycetes	Frank Martin
10:05 - 10:20	Identification of effectors and structural variants in the improved genome assemblies of *Phytophthora ramorum* isolates using novel bioinformatics pipelines	Takao Kasuga
10:25 - 10:50	Break	
10:50 - 12:00	**Keynote Speech**	**Chair: Aurelien Tartar and Lina Quesada**
	Comparative genome evolution in fungi, from populations to phyla	Jason Staijich
12:00 - 2:00	Lunch	
2:00 - 3:20	**Host interactions and resistance I**	**Chair: Patricia Manosalva**
2:00 - 2:15	*Phytophthora* suppressor of RNA silencing 2 (PSR2) suppresses small RNA accumulation by interacting with Double-stranded RNA Binding protein 4 (DRB4) in *Arabidopsis*	Yi Zhai
2:20 - 2:35	Management of downy mildew in lima bean	Terence Mhora

2:40 - 2:55	*Phytophthora infestans* produces small phospholipase D-like proteins that elicit plant cell death and promote virulence	Francine Govers
3:00 - 3:15	Plant recognition of a novel *Phytophthora* PAMP XEG1	Yan Wang
3:20 - 3:50	Break	
3:50 - 5:10	**Effectors I**	**Chair: Joël Klein**
3:50 - 4:05	A *Phytophthora palmivora* cystatin-like protease inhibitor targets papain to contribute to virulence on papaya	Miaoying Tian
4:10 - 4:25	An effector-targeted susceptibility factor mediates degradation of an immunity-associated vesicle trafficking regulator SWAP70	Qin He
4:30 - 4:45	Downy mildew conserved RXLR effectors drive the discovery of sunflower broad-spectrum resistance	Laurence Godiard
4:50 - 5:05	*Phytophthora* effectors suppress plant immunity by interfering with the Ca2+-signaling pathway	Gul Shad Ali
5:10 - 5:25	FungiDB: new, cool, and under-utilized features for the oomycete communiy	Omar Harb
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7:30 - 10:00	Reception/Poster session (odd numbers)	

Monday, March 13th		
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9:20 - 9:35	Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*	Alamgir Rahman
9:40 - 9:55	Target enrichment sequencing of *Phytophthora* species enables massively parallel diagnostic analysis of known avirulence genes and revised genome annotation of *Phytophthora infestans* RXLR effectors	Ingo Hein
10:00 - 10:15	Genomic investigations of heterokaryosis in the oomycete pathogen of lettuce, *Bremia lactucae*	Kyle Fletcher
10:20 - 10:50	Break	
10:50 - 11:50	**Effectors II**	**Chair: Miaoying Tian**
10:50 - 11:05	A *Phytophthora capsici* virulent RXLR effector targets plant PP2a isoforms that confer Phytophthora blight resistance	Xiao-Ren Chen
11:10 - 11:25	The contribution of effectors to nonhost resistance	Hazel McLellan
11:30 - 11:45	Identification and monitoring of effector proteins in the spinach downy mildew pathogen *Peronospora farinosa*	Joël Klein
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2:00 - 3:20	**Host interactions and resistance II**	**Chair: Yi Zhai**
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2:20 - 2:35	Secreted Nep1-like proteins of oomycetes, fungi, and bacteria trigger immunity in Arabidopsis; genetic dissection of NLP-induced plant defense	Guido Van den Ackerveken
2:40 - 2:55	Understanding plant immunity to *Phytophthora* spp.: from model plants to crops	Patricia Manosalva
3:00 - 3:15	Utilizing host resistance for the management of the oomycete pathogen *Phytophthora sojae*	Colin Davis
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3:50 - 5:10	**Oomycete biology, populations, and evolution I**	**Chair: Javier F. Tabima**
3:50 - 4:05	Phylogeography of the tropical oomycete *Phytophthora palmivora*	Erica M. Goss
4:10 - 4:25	Diversity of *Phytophthora* species from natural and semi-natural ecosystems in Portugal, Chile and Vietnam	Marília Horta Jung
4:30 - 4:45	Live cell imaging of the cytoskeleton in *Phytophthora* pathogens reveals unique actin and microtubule configurations	Kiki Kots
4:50 - 5:05	Morphological and molecular identification of *Phytophthora palmivora* Butler as causal agent of black pod rot of cocoa (*Theobroma cacao* L.) from coastal Ecuador	Miriam Escos
5:10 - 5:55	Committee meeting	
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7:30 - 10:00	Refreshments/Poster session (even numbers)	

Tuesday, March 14th		
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9:00 - 9:15	Genome biology, evolution and recognition of *Albugo candida* CCG effectors	Oliver J. Furzer
9:20 - 9:35	Oomycete evolution reconstructed with an automated phylogenomics pipeline using publicly available genome data from 39 taxa and three additional taxa not previously sequenced	Hai D.T. Nguyen
9:40 - 9:55	Updated view of genome structure, variation, and transcriptional dynamics in *Phytophthora infestans* based on a PacBio assembly and revised gene models	Howard Judelson
10:00 - 10:15	Transcriptional programming of *Phytophthora sojae* for organ-specific infection	Wenwu Ye
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10:50 - 11:50	**Oomycete biology, populations, and evolution II**	**Chair: Erica M. Goss**
10:50 - 11:05	Population dynamics of *Phytophthora rubi* indicate high rates of migration between states and nurseries in the Pacific Northwestern United States	Javier F. Tabima
11:10 - 11:25	*Nothophytophthora* prov. nom., a new sister genus of *Phytophthora*	Marília Horta Jung
11:30 - 11:45	Hidden diversity in the oomycete genus Olpidiopsis is a global threat to red algal cultivation	Yacine Badis
11:50 - 12:00	Closing remarks	
12:20 - 2:00	Lunch/Adjourn	

OMGN
OOMYCETE MOLECULAR GENETICS NETWORK

TRAVEL FELLOWSHIP AWARDEES

This meeting is supported in part by a coordinated agricultural project and a conference grant provided by the USDA's National Institute of Food and Agriculture. The project "Integrated Management of Oomycete Diseases of Soybean and Other Crop Plants" was competitive grant 2011-68004-30104 in Agriculture and Food Research Initiative program area A5121 "Oomycete Pathosystems in Crop Plants to Minimize Disease" with Dr. Ann Lichens-Park as program leader. The conference grant "18th Annual Oomycete Molecular Genetics Meeting" is a competitive grant in AFRI program area A1112 "Pests and Beneficial Species in Agricultural Production Systems" with Dr. Mary Purcell-Miramontes as program leader.

United States Department of Agriculture

National Institute of Food and Agriculture

Both the project and conference grant support local meeting costs, invited speaker costs, and 27 travel awards to students, postdoctoral associates, and new or junior faculty to the oomycete research community. The awardees are listed in the table below.

Awardee Name, Affiliation, Mentor(s)	Abstract/Thesis Topic
Rodger Belisle University of California-Riverside Patricia Manosalva	*Elucidating the molecular basis of plant immunity against P. cinnamomi*
Xiao-Ren Chen University of California-Riverside Wenbo Ma	*A Phytophthora capsici virulent RXLR effector targets plant PP2a isoforms that confer Phytophthora blight resistance*
Ronaldo Dalio Agronomic Institute of Campinas, Brazil Wolfgang Osswald	*Screening and functional characterization of candidate RxLR and CRN effector proteins on the interaction Phytophthora parasitica-citrus: epistasis and immunity manipulation*
Colin Davis Virginia Tech Saghai Maroof	*Identification and mapping of disease resistance genes in soybean*
Kasia Dinkeloo Virginia Tech Guillaume Pilot, John McDowell	*New tools to identify mechanisms of nutrient transport from plants to biotrophic pathogens*
Gregory Edwards Nova Southeastern University Aurelien Tartar	*Detection of Lagenidium giganteum in phytotelmata microbiomes*

Yufeng 'Francis' Fang
Virginia Tech, Oregon State University
Brett Tyler

Nuclear localization of proteins and genome editing in the oomycete Phytophthora sojae

Kyle Fletcher
University of California-Davis
Richard Michelmore

Genomic investigations of possible heterokaryosis in the oomycete pathogen of lettuce, Bremia lactucae

Paula Leoro Garzon
Nova Southeastern University
Aurelien Tartar

Detection of Lagenidium giganteum in phytotelmata microbiomes

Andrew Gonedes
Nova Southeastern University
Aurelien Tartar

Detection of Lagenidium giganteum in phytotelmata microbiomes

John Herlihy
Virginia Tech
John McDowell

Assessing the impact of low iron availability on oomycete pathogenicity

Yingnan Hou
University of California-Riverside
Wenbo Ma

Phased siRNAs derived from specific gene loci regulate plant immunity

Natasha Jackson
University of California-Riverside
Patricia Manosalva

Elucidating the role of MORC1 during plant immunity against Phytophthora spp.

Miriam Escos Martinez
University Zaragosa-Spain
Juan Barriuso Vargas, Karina Solis Hidalgo

Biological control of Phytophthora palmivora by Trichoderma spp.

Terence Mhora
University of Delaware
Nicole Donofrio, Tom Evans

Management of downy mildew in lima bean

Jiangqiang Miao
Oregon State University, China Agricultural University
Brett Tyler, Xili Liu

Oomycete resistance mechanisms to fungicides and the function of oxysterol binding protein

Natasha Navet
University Hawaii-Manoa
Miaoying Tian

Functional characterization of a cytoplasmic effector gene highly conserved in plant pathogenic oomycetes

Alamgir Rahman
North Carolina State University
Lina Quesada-Ocampo

Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen Pseudoperonospora cubensis

Maria Ratti
University of Florida-Gainesville
Erica Goss

Determining oomycete species diversity and disease risk

Camilo Humberto Parada Rojas
North Carolina State University
Lina Quesada-Ocampo

Development of microsatellites and population analyses of Phytopthora capsici infecting vegetable crops

Mauricio Serrano-Porras
Iowa State University
Alison Robertson

Evaluating soybean seed treatments for control of seed and root rot caused by Pythium spp.

Unnati Sonawala
Virginia Tech
Guillaume Pilot, John McDowell

Understanding how biotrophic pathogens manipulate plant amino acid transporters to acquire nutrients using the Arabidopsis thaliana and Hyaloperonospora arabidopsidis pathosystem

Javier Felipe Tabima
Oregon State University
Nik Grünewald

Comparative and population genomics of Phytophthora rubi and P. fragariae

Anna Thomas
North Carolina State University
Peter Ojiambo, Ignazio Carbone

Population genetic structure of Pseudoperonospora cubensis as influenced by cucurbit host types and geography in the United States

Avery Wilson
College of Wooster
William Morgan

Investigating the effect of the PsAvh110 effector on genome-wide yeast expression levels to identify putative targets

Kelsey Wood
University of California-Davis
Richard Michelmore

Prediction and characterization of WY domain effectors in downy mildews

Yi Zhai
University of California-Riverside
Wenbo Ma

Elucidating the molecular mechanisms of Phytophthora effector-mediated RNA silencing suppression.

ABSTRACTS OF ORAL PRESENTATIONS

(in order of appearance)

Sunday, March 12th		
9:05 - 10:25	**Genomics I**	**Chair: Alamgir Rahman**
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9:45 - 10:00	Systematic approach for development of markers for plant pathogenic oomycetes	Frank Martin
10:05 - 10:20	Identification of effectors and structural variants in the improved genome assemblies of *Phytophthora ramorum* isolates using novel bioinformatics pipelines	Takao Kasuga

Prediction and characterization of WY-domain effectors in downy mildews

Kelsey Wood[1], Lida Derevnina[1,2], Juliana Gil[1], Sebastian Reyes Chin Wo[1], and Richard Michelmore[1]
([1]Genome Center and Department of Plant Sciences, University of California, Davis, CA; [2]The Sainsbury Lab, Norwich, UK)

Identification of effectors in oomycete genomes is of major interest for understanding the mechanisms of pathogenesis, for monitoring field pathogen populations, and for breeding pathogen resistant plants. Using comparative genomics and bioinformatics, we have identified candidate effectors from several economically important downy mildew species by searching for the WY domain, a conserved structural element found in *Phytophthora* effectors that has been implicated in their immune-suppressing function. Searching for the WY-domain uncovered additional effector candidates that were missed by searching for the RXLR domain alone. There is significant variation among the WY effector candidates in both sequence and domain architecture. The candidate effectors show several characteristics of pathogen effectors, including an N-terminal secretion signal, lineage specificity, and evidence of gene duplication and gene family expansion. Unexpectedly, only a minority of WY effectors contained the canonical N-terminal RXLR motif, which is a conserved feature in *Phytophthora* effectors. Functional characterization of nine of the WY domain effectors revealed three effectors that elicited an immune response on lettuce containing introgressions from wild lettuce species. None of the immune eliciting effectors contained an RXLR motif. These results suggest that there has been an evolutionary divergence in sequence motifs between genera that has important implications for effector prediction in the oomycetes.

CRISPR/Cas9, a powerful new tool for genetic studies of oomycete pathogens

Yufeng 'Francis' Fang[1,2,4], Meng Cai[2,3], Biao Gu[2], Linkai Cui[2], Dulani P. Wellappili[2], Xili Liu[3], Brett M. Tyler[1,2]*
([1.] Interdisciplinary Ph.D. Program in Genetics, Bioinformatics & Computational Biology, Virginia Tech, VA 24061; [2.] Center for Genome Research & Biocomputing and Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR, 97331; [3.] Department of Plant Protection, China Agricultural University, Beijing, the People's Republic of China, 100193; [4.] Current address: Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC)

Despite the availability of several oomycete genome sequences, the lack of efficient techniques for genome editing has long hampered genetic studies of this organism. In our recent study, we successfully accomplished targeted mutagenesis and gene replacement using an oomycete-optimized CRISPR/Cas9 in the soybean pathogen *Phytophthora sojae*. Using the RXLR effector gene *Avr4/6* as a target, we observed that in the absence of a homologous template, the repair of Cas9-induced double-strand

breaks (DSBs) in *P. sojae* was mediated by non-homologous end joining (NHEJ), primarily resulting in short indels. Most mutants were homozygous, presumably due to gene conversion triggered by Cas9-mediated cleavage of non-mutant alleles. When donor DNA was present, homology-directed repair (HDR) was observed, which resulted in the replacement of the target gene with the donor DNA. Our results establish a powerful tool for studying functional genomics in *Phytophthora*, which provides new avenues for better control of this pathogen. Here, we will present our latest progress on optimization of the oomycete CRISPR/Cas9 system, and report studies of two fungicide resistance genes and effectors using the system.

Systematic approach for development of markers for plant pathogenic Oomycetes
Frank Martin

In an effort to simplify the identification of loci for phylogenetic analysis, mitochondrial haplotype analysis and development of diagnostic assays a comparative mitochondrial genomics approach has been used. Close to 500 oomycete mitochondrial genomes for 87 taxa have been assembled for a range of oomycetes. Comparison of genome assemblies for single sporangia and oospore isolates has provided insight to mechanisms associated with genome evolution. Annotated genes have been useful for conducting phylogenetic analysis of a wide range of taxa. The assembly of mitochondrial genomes from multiple isolates of the same species has provided resources for identification of mitochondrial haplotype markers for population studies. Comparative genomics has been used to identify gene order differences among genera to enhance specificity of detection and regions of high sequence divergence that are useful for design of molecular detection assays. Thus far highly specific diagnostic assays have been developed for *Phytophthora* (multiplexed genus and species specific TaqMan real time PCR and isothermal RPA assays), *Pythium* (genus specific TaqMan and RPA assays validated, species specific under evaluation), *Aphanomyces* (genus and species specific detection work done with collaborators) and several downy mildew taxa (*Bremia*, *Peronospora* and *Pseudoperonospora* sp.). The ability to use comparative genomics with assembled mitochondrial genomes has been particularly useful for design of amplification primers specific for obligate downy mildew pathogens.

Identification of Effectors and Structural Variants in the improved genome assemblies of *Phytophthora ramorum* isolates using novel Bioinformatics Pipelines
Mathu Malar C, Takao Kasuga

We report the improved effector repertoire of two newly assembled *P. ramorum* genomes, Pr102 and ND886. The *P. ramorum* isolate ND886 was derived from foliage of ornamental Camellia and Pr102is a well-studied isolate from a stem canker on oak, both from Marin County, California. PacBio long reads and Illumina reads were generated for these two isolates and we combined both short and long reads with the original draft assembly of Pr102 to assemble the genomes. We used a combination of error correction and hybrid assembly methods to assemble the genomes. The latest genome assembly size is 67.9 Mb and 60.2 Mb for *P. ramorum* Pr102 and ND886 respectively. The N50 of the assemblies were 78830, 687183 respectively. Only 2422 and 214 bases of gaps are present in Pr102 and ND886 assemblies, which is a great improvement from 12,227,423 gaps occurred in the earlier assembly. Falcon assembler proved to be the best option for the hybrid assembly. As most of the gaps have been closed, we wanted to improve the effector repertoire using a variety of approaches. A pipeline that encompassed ORF prediction, HHM search, signalP,TargetP, TMHMM, BigPI predictions resulted in best output. We predicted about 435 and 506 effectors in the genome assemblies. These effectors contained the earlier effectors (V1 of Pr102) and many additional effectors. We are working on the syntenic relationship between other *Phytophthora* spp. having effector rich region.

KEYNOTE ADDRESS

Sunday, March 12th		
10:50 - 12:00	**Keynote Address**	**Chair: Aurelien Tartar and Lina Quesada**
	Comparative genome evolution in fungi, from populations to phyla	Jason Staijich

Comparative genome evolution in fungi, from populations to phyla

Jason Stajich
University of California-Riverside, Riverside, CA USA

Understanding the evolutionary relationships of organisms, how gene sequences change so that gene products are incorporated into new pathways, or how populations of individuals within a species change is important to study for understanding the emergence of pathogens and for the overall study of evolution. Inexpensive DNA and RNA sequencing has simplified how we inventory a species or population for the genetic material. Comparative biology of genes and genomes can now be performed on the scale of hundreds of eukaryotic species. The Fungal kingdom has been a useful proving ground for large-scale genome sequencing with relatively small (10-100Mb) genomes, which has lead to the production of more than 500 genomes of fungi. I will present progress we have made on understanding fungal phylogeny and evolution using the 300+ fungal genomes and phylogenomic methods for resolving species relationships as part of the 1000 Fungal Genomes project. This work has lead us to revise some of the relationships of phyla, especially in the zygomycete and zoosporic fungal lineages. I will show results detailing the pivotal emergence of multicellular fruiting bodies in the Ascomycete fungi (Nguyen et al. Nature Comm 2017. DOI: 10.1038/ncomms14444) by utilizing genetic and comparative genomics. Finally, I will present work on population genomics of pathogenic fungi and illustrate the power of comparing recent evolutionary changes at a population scale in populations of plant and animal pathogens. Together these studies will highlight some of the conclusions we can draw from broad genome sequencing across a kingdom with diverse morphologies and ecological associations.

Sunday, March 12th		
2:00 - 3:20	**Host interactions and resistance I**	**Chair: Patricia Manosalva**
2:00 - 2:15	*Phytophthora* suppressor of RNA silencing 2 (PSR2) suppresses small RNA accumulation by interacting with Double-stranded RNA Binding protein 4 (DRB4) in *Arabidopsis*	Yi Zhai
2:20 - 2:35	Management of downy mildew in lima bean	Terence Mhora
2:40 - 2:55	*Phytophthora infestans* produces small phospholipase D-like proteins that elicit plant cell death and promote virulence	Francine Govers
3:00 - 3:15	Plant recognition of a novel *Phytophthora* PAMP XEG1	Yan Wang

Phytophthora suppressor of RNA silencing 2 (PSR2) suppresses small RNA accumulation by interacting with Double-stranded RNA Binding protein 4 (DRB4) in _Arabidopsis_

Yi Zhai[1,2], Jinqiu He[3], Duseok Choi[1,2], Yingnan Hou[1,2], Ariel Kuan[1,2], Wenwu Ye[4], Jinbiao Ma[3], Wenbo Ma[1,2]

([1] Department of Plant Pathology and Microbiology, University of California, Riverside, CA; [2] Center for Plant Cell Biology, University of California, Riverside, CA; [3] Department of Biochemistry, School of Life Sciences, Fudan University, Shanghai, China; [4] Department of Plant Pathology, Nanjing Agriculture University, Nanjing, China)

Phytophthora contains important plant pathogens that cause devastating diseases of crops. Genome sequences of *Phytophthora* pathogens revealed a large number of secreted virulence proteins; some of these so-called effectors function inside the host cells to facilitate colonization and infection. Previously, we discovered that *Phytophthora* produce effectors with RNA silencing suppression activity to promote infection. *Phytophthora* Suppressor of RNA silencing 2 (PSR2) belongs to a conserved RxLR effector family with tandem repeats of L-W-Y motifs. Using transgenic *Arabidopsis* plants, PSR2 was found to specifically affect the abundance of phased small interfering RNAs (phasiRNAs). In order to understand the virulence mechanism of PSR2, we characterized PSR2-associating protein(s) in plants. Our results show that PSR2 interacts with Double-stranded RNA-Binding protein 4 (DRB4) in the nucleus. DRB4 has a known function in phasiRNA biogenesis by partnering with the endonuclease Dicer-like protein 4 (DCL4), which processes long double-stranded RNA precursors into siRNAs. Genes involved in the phasiRNA pathway positively regulate plant defense against *Phytophthora*, indicating that PSR2 promotes infection by targeting phasiRNA production through its interaction with DRB4.

Management of downy mildew in lima bean

Terence Mhora[1], Colin Scanlan[1], Alden Duckett[1], Andrew Kness[1], Nancy Gregory[1], Emmalea Ernest[1], Randall Wisser[1], Thomas Evans[1], Nicole Donofrio[1]

([1]Department of Plant and Soil Sciences, University of Delaware, Newark, DE)

The oomycete pathogen *Phytophthora phaseoli* (Thaxter) causes significant yield losses in lima bean, the cornerstone crop of Delaware's vegetable processing industry. An integrated pest management (IPM) approach using chemical control and host resistance is being improved upon to reduce related yield losses due to this pathogen. A collection of 101 pathogen isolates from the Mid-Atlantic Region was assessed for mefenoxam sensitivity due to growing concerns over fungicide resistance. We identified five mefenoxam insensitive isolates originating from two of the fields from which the isolates were sampled. Morphological differences between field isolates and their derived single sporangial isolates suggested the presence of mixed populations in grower fields. Host resistance is useful to augment chemical control strategies. However, there are currently no horticulturally acceptable cultivars containing resistance to race F, the predominant physiological race of this pathogen in the field. To facilitate the breeding process, molecular markers for detecting race F resistance in lima bean were developed from

germplasm containing resistance to race F using bulked segregant analysis via genotyping-by-sequencing. These markers accurately predicted race F resistance in a collection of 256 genetically and geographically diverse lima bean accessions (diversity panel). To implement resistance gene pyramiding as part of our IPM strategy, phenotyping of the diversity panel was conducted through dew chamber and field experiments to find alternate sources of resistance. Phenotyping resulted in the discovery of an additional 12 race F resistant accessions. Here, we will report our current progress on improving our IPM strategy.

Phytophthora infestans produces small phospholipase D-like proteins that elicit plant cell death and promote virulence

Harold Meijer[1,2*], Charikleia Schoina[1*], Shu-tong Wang[1], Klaas Bouwmeester[1], Chen-lei Hua[1], Francine Govers[1]
[1] Laboratory of Phytopathology and [2] Wageningen Plant Research, Wageningen University and Research, Wageningen, The Netherlands

Successful invasion of host tissue by biotrophic plant pathogens is dependent on modifications of the host plasma membrane to facilitate two-way transfer of proteins and other compounds. Haustorium formation and establishment of extrahaustorial membranes is likely dependent on a variety of enzymes that modify membranes in a coordinated fashion. Phospholipases, enzymes that hydrolyze phospholipids, have been implicated as virulence factors in several pathogens. The oomycete *Phytophthora infestans* that causes late blight of potato, is a biotrophic pathogen that possesses different classes of phospholipase D (PLD) proteins including small PLD-like proteins with and without signal peptide (sPLD-likes and PLD-likes, respectively). Here we studied the role of sPLD-like-1, sPLD-like-12 and PLD-like-1 in the infection process. The three encoding genes are expressed in expanding lesions on potato leaves and during growth *in vitro* with the highest expression levels in germinating cysts. When expressed *in planta* in the presence of the silencing suppressor P19, all three (s)PLD-likes elicited local cell death, a response that was visible at the microscopic level and was strongly boosted by the presence of calcium. Moreover, inoculation of leaves expressing the (s)PLD-like genes resulted in increased lesion growth and more sporangia. However, when the PLD catalytic motifs (HDK) were mutated there was neither cell death elicitation nor increased lesion growth thus pointing to the necessity for enzymatic PLD activity in these processes. These results suggest that the *P. infestans* (s)PLD-likes are catalytically active and function by executing membrane modifications to support growth of this pathogen in the host.

Plant recognition of a novel _Phytophthora_ PAMP XEG1

Yan Wang[1], Yuanpeng Xu[1], Jiaming Qi[1], Huibin Wang[1], Yujing Sun[1], Yuanyuan Shao[1], Bowen Wan[1], Wenwu Ye[1], Suomeng Dong[1], Yuanchao Wang[1]
([1.] Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China)

Phytophthora species are notorious plant pathogens that cause great damage on crops. Soybean root rot caused by *Phytophthora sojae* is destructive to soybean and often leads to an annual loss up to tens of billions dollars. Recently, we identified XEG1, a novel pathogen-associated molecular pattern (PAMP) in *P. sojae*. XEG1 belongs to the glycoside hydrolase family 12 and is widely distributed across microbial taxa. XEG1, on the one hand, is essential for *Phytophthora* virulence, and on the other hand can be recognized by plants and triggers cell death in various plant species, indicating that plants have a conserved system to recognize XEG1. In this study, we employed *Nicotiana benthamiana* as a model plant and characterized the function of membrane-localized receptors in XEG1 recognition. In this way, we successful identified the recognition receptor that responses to XEG1. This receptor mediates plant responses to multiple XEG1 homologues. In addition, infection assays demonstrated that the XEG1 recognition receptor mediates resistance to different *Phytophthora* pathogens in *N. benthamiana*. This

study provides novel insights on understanding plant innate immunity against *Phytophthora* pathogens and will facilitate the development of durable disease resistance resources.

Sunday, March 12th		
3:50 - 5:2	**Effectors I**	**Chair: Joël Klein**
3:50 - 4:05	A *Phytophthora palmivora* cystatin-like protease inhibitor targets papain to contribute to virulence on papaya	Miaoying Tian
4:10 - 4:25	An effector-targeted susceptibility factor mediates degradation of an immunity-associated vesicle trafficking regulator SWAP70	Qin He
4:30 - 4:45	Downy mildew conserved RXLR effectors drive the discovery of sunflower broad-spectrum resistance	Laurence Godiard
4:50 - 5:05	*Phytophthora* effectors suppress plant immunity by interfering with the Ca2+-signaling pathway	Gul Shad Ali
5:10 - 5:25	*FungiDB: new, cool, and under-utilized features for the oomycete community*	Omar Harb

A *Phytophthora palmivora* cystatin-like protease inhibitor targets papain to contribute to virulence on papaya

Rebecca Gumtow[1], Dongliang Wu[1], Sebastian Schornack[2], Janice Uchida[1], and Miaoying Tian[1] ([1.]Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI; [2.] Sainsbury Laboratory, University of Cambridge, Cambridge, United Kingdom)

Latex from papaya fruit, stems and leaves is very rich in papain, a cysteine protease that has been shown to mediate plant resistance against various pathogens and insects. Yet the oomycete *Phytophthora palmivora* is a destructive pathogen of papaya, causing root rot, stem canker, fruit rot, and damping off of seedlings, suggesting that *P. palmivora* have evolved cysteine protease inhibitors to inhibit papain to enable successful infection. Out of four putative cystatin-like extracellular protease inhibitors (PpmEPICs) from *P. palmivora* transcriptomic sequence data, one named PpmEPIC8 was highly induced during infection. Purified recombinant PpmEPIC8 protein strongly inhibited papain enzyme activity, suggesting that it is a functional cystatin. *P. palmivora* PpmEPIC8 mutant strains were generated via *Agrobacterium*-mediated transformation and CRISPR/Cas9 gene editing. Mutants with 4bp, 6bp or 8bp-deletion in the PpmEPIC8 protein coding region were obtained. Increased papain sensitivity of *in vitro* growth and reduced pathogenicity during infection of papaya fruits were observed for PpmEPIC8 mutants compared with the wild-type strain, suggesting that PpmEPIC8 indeed plays a significant role in *P. palmivora* pathogenicity by inhibiting papain. Although cystatin-like extracellular protease inhibitors were previously characterized as important virulence factors of oomycete pathogens using biochemical and other approaches, genetic evidence was lacking. This study provides first genetic evidence demonstrating that oomycete pathogens secrete cystatins as important weapons to invade plants.

An Effector-targeted susceptibility factor mediates degradation of an immunity-associated vesicle trafficking regulator SWAP70

Qin He[1], Shaista Naqvi[1], Hazel McLellan[1], Petra Boevink[2], Eleanor Gilroy[2], Ingo Hein[2], Paul Birch[1,2]
[1]Division of Plant Science, University of Dundee; and [2]Cell and Molecular Sciences; both located at James Hutton Institute, Invergowrie, Dundee, UK

Plant pathogens deliver effectors into plant cells to manipulate host processes. Much attention has been focused on identifying their host targets. Recently, we reported that *Phytophthora infestans* RXLR effector Pi02860 targets NRL1, a host non-phototrophic hypocotyl 3/root phototropism 2 (NPH3/RPT2)-like protein, in the host cytoplasm and at the cell plasma membrane. NRL1 is susceptibility factor that suppresses INF1-triggered cell death. A dimerization-dead NRL1 mutant attenuates infection and loses its ability to suppress INF1-triggered cell death. Moreover, expression of NRL1-mut reduces the ability of Pi02860 to attenuate INF1-mediated HR, demonstrating that host NRL1 activity is required for Pi02860 to

promote disease. NRL1 interacts with a guanine nucleotide exchange factor (GEF), SWAP70, which localises to endosomes. Virus-induced gene silencing of SWAP70 in *N. benthamiana* resulted in enhanced *P. infestans* colonization and compromised INF1-triggered cell death. Overexpression of SWAP70 showed reduced *P.infestans* infection and accelerated INF1-triggered cell death, indicating that this host protein acts as a positive regulator of immunity. Moreover, suppression of INF1-triggered cell death by Pi02860 was significantly attenuated by co-expression with SWAP70. Importantly, Pi02860 enhances the interaction between NRL1 and SWAP70, promoting destabilisation of SWAP70. We argue that Pi02860 uses host protein NRL1 to target SWAP70, potentially blocking host vesicle trafficking to suppress immunity.

Downy mildew conserved RXLR effectors drive the discovery of sunflower broad-spectrum resistance

Yann Pecrix[1], Luis Buendia[1], Charlotte Penouilh-Suzette[1], Felicity Vear[2], Stéphane Muños[1], Ludovic Legrand[1], Jérôme Gouzy[1], Laurence Godiard[1].
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Plasmopara halstedii is an obligate biotroph oomycete causing downy mildew disease on sunflower, *Helianthus annuus*, an economically important oil crop. *P. halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different *Pl* resistance genes, not yet cloned. During the last decades, *P. halstedii* pathotypes showing new virulent patterns appeared, concomitantly with the breakdown of *Pl* resistance loci used in fields. Finding broad-spectrum and more durable resistance against downy mildew disease is therefore an agronomic issue. We focused on *P. halstedii* RXLR effectors conserved among pathotypes to drive the discovery of sunflower broad range resistance. High-throughput sequencing and *in silico* methods led us to identify potential RXLR effectors, 58% of which were conserved at the protein level in 17 *P. halstedii* pathotypes. Thirty of them expressed in pathogen infecting leaves were cloned for transient expression experiments. Seventeen cloned effectors suppressed Pathogen Triggered Immunity induced by *Phytophthora infestans* INFESTIN1 in *N. benthamiana,* suggesting they were true effectors. Subcellular localization of the effectors was performed in sunflower cells and their ability to trigger hypersensitive responses in sunflower lines was tested. Four cloned RXLR effectors including C1 induced HR in resistant lines carrying different *Pl* loci. Testing the C1 effector on F3 populations segregating for resistance indicated co-segregation of C1 induced-cell death activity with the *Pl* resistance locus. This *Pl* locus was finely mapped in a region of 3 Mbp of the sunflower genome thanks to AXIOM SNP arrays.

***Phytophthora* effectors suppress plant immunity by interfering with the Ca2+-signaling pathway**

Shaheen Bibi, Zunaira Afzal, Aftab Khan, David Norman, Mary Brennan, and Gul Shad Ali

Phytophthora spp. express several hundred virulence effectors, which, they actively translocate inside the host cells. Most translocated effectors contain an N-terminal RXLR motif (Arg, any amino acid, Leu, Arg) and a C-terminal domain with effector activity. Most RXLR effectors share little sequence homology to characterized proteins thus hindering our ability to assign homology-based function to effectors. To assign function to RXLR effectors, one of the most commonly employed strategies is to identify their host targets using protein-protein interaction-based screening. Several studies have reported on a number of host targets of *Phytophthora* effectors. These limited but significant studies suggest that the RXLR effectors target diverse host targets. However, compared to the 100s of *Phytophthora* effectors, these few examples are just the tip of an iceberg. Our understanding of effector biology remains incomplete and a lot more remains to be discovered and investigated to fully understand how effectors manipulate host cellular processes. In a Y2H screening, we have identified several putative host targets for several RXLR effectors. Using biochemical, cell biological and computer modelling combined with in vivo

functional analyses, we show that one of these effectors targets a calcium (Ca2+) signaling proteins. Our findings are consistent with numerous reports showing a central role for Ca2+ homeostasis in plant-microbe interactions. Here we will report on our progress on how RXLR effectors modulate the Ca2+-signaling pathway to suppress plant immunity. We will also discuss mechanistic insights into how knowledge obtained in our studies could be utilized for rationally designing disease resistant plants.

Monday, March 13th		
9:00 - 10:20	**Genomics II**	**Chair: Kelsey Wood**
9:00 - 9:15	Comparative genomics to assess mycoparasitism and pathogenicity in the oomycetes	Laura Grenville-Briggs
9:20 - 9:35	Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*	Alamgir Rahman
9:40 - 9:55	Target enrichment sequencing of *Phytophthora* species enables massively parallel diagnostic analysis of known avirulence genes and revised genome annotation of *Phytophthora infestans* RXLR effectors	Ingo Hein
10:00 - 10:15	Genomic investigations of heterokaryosis in the oomycete pathogen of lettuce, *Bremia lactucae*	Kyle Fletcher

Comparative genomics to assess mycoparasitism and pathogenicity in the oomycetes
Ramesh Vetukuri, Kurt Lamour, and Laura Grenville-Briggs

Emerging oomycetes, such as *Phytophthora colocasiae* are serious threats to global food security, whilst others have potential as biological control agents, such as the mycoparasites *Pythium oligandrum* and *Pythium periplocum* and the entomopathogen *Lagenidium giganteum*. We have carried out de novo genome sequencing and genome-wide transcriptomics (RNA-seq) of these four understudied species using paired end and mate pair Illumina HiSeq sequencing. We are now investigating the genetic and molecular determinants of host specificity and pathogenicity in these four understudied oomycetes. Genome sizes range from 36 Mb to 100 Mb. RxLR class effectors were found in *P. colocasiae* but not in the other three species. Crinkler (CRN), and elicitin proteins were detected in all the four oomycetes sequenced. Novel effector families are present in the insect pathogen. RNA-seq data from *P. oligandrum* myco-parasitising the potato late blight oomycete *Phytophthora infestans* specific up-regulation of 1940 *P. oligandrum* genes 12 hours after infection and 300 genes at 24hrs. Some of these genes are involved in secondary metabolism and terpenoid biosynthesis, transport and cell wall degradation. Novel transcripts from *P. infestans* reveal how this oomycete defends itself against mycoparasitic attack and may provide vital clues for durable control of potato late blight. Comparative analyses of the hyper aggressive mycoparasite *P. oligandrum* versus the weaker mycoparasite *P. periplocum* provide clues to the success of *P. oligandrum*. Identification of common and specific pathogenicity determinants and defence pathways opens the way for more durable oomycete control measures and better use of oomycetes as biological control agents.

Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*
Alamgir Rahman, Frank Martin, Aidan Shands, Timothy Miles, and Lina M. Quesada-Ocampo

Pseudoperonospora cubensis, an obligate oomycete pathogen, causes cucurbit downy mildew (CDM) on a variety of cucurbit host plants including cucumber, cantaloupe, watermelon, pumpkin, and squash. In 2004 the disease re-emerged in the United States after overcoming host resistance in cucumber and fungicides used for disease control in other cucurbits. CDM is now the major threat to cucurbit production and control can only be achieved via expensive and frequent fungicide applications, which show limited efficacy. Since airborne sporangia of *P. cubensis* initiate epidemics every year after being dispersed from overwintering sites, detection prior to infection could warn of potential disease outbreaks. In addition, *P. cubensis* displays host adaptation and fungicide resistance, thus, incorporating host preference and fungicide resistance markers into biosurveillance efforts would allow reduction of unnecessary fungicide

applications. Using Next Generation Sequencing and bioinformatics, candidate genomic regions were identified as diagnostic markers for *P. cubensis* presence, host-preference, and resistance to carboxylic acid amide and quinone outside inhibitor fungicides. These diagnostic markers were used to develop specific primers and/or LNA probe-based qPCR assays, and tested for specificity and sensitivity when monitoring *P. cubensis*. The detection level of *P. cubensis* DNA extracted from sporangia and infected plant tissue was determined. Pathogen trap plots and spore traps were deployed in North Carolina to test diagnostic markers in the field. We will discuss the utility of comparative genomics in identifying unique genomic DNA regions that can be used for biosurveillance of *P. cubensis*.

Target enrichment sequencing of *Phytophthora* species enables massively parallel diagnostic analysis of known avirulence genes and revised genome annotation of *Phytophthora infestans* RXLR effectors

Gaetan Thilliez, Joanne Tze-Yin Lim, Katie Baker, Miles Armstrong, Edgar Huitema, Paul Birch, and Ingo Hein

The oomycete pathogens *Phytophthora infestans* and *P. capsici* cause significant crop losses worldwide. Key components required for their host infections are effectors, which for both pathogens include RXLR containing proteins. A consequence of RXLR effector recognition by cognate immune receptors in planta is resistance. The purpose of this study was to develop target enrichment sequencing for *P. infestans* and *P. capsici* secreted proteins including RXLR effectors. Target enrichment sequencing of 579 *P. infestans* and 574 *P. capsici* genes was conducted in parallel for 12 isolates comprising six individually barcoded isolates of each species. Included in the experiments were the reference isolates T30-4 for P. infestans and LT1534 for P. capsici. Under high-stringent mapping conditions, enriched sequencing reads displayed a higher than 50% ontarget rate and more than 87% of all target genes from the reference genomes were fully represented. The enriched sequence representation achieved for the individual *P. infestans* isolates revealed a combination of presence/absence variations as well as sequence polymorphisms for known Avr genes across different pathogen genotypes. In addition, we report on cross-hybridization between *P. capsici*-derived baits and the T30-4 *P. infestans* genome, which enabled the identification of RXLR effectors in *P. infestans* that were previously thought to be unique to *P. capsici*. A de novo annotation of RXLR effectors in *P. infestans* was subsequently conducted and identified more than 1200 putative RXLR-containing effectors in the T30-4 reference genome.

Genomic investigations of heterokaryosis in the oomycete pathogen of lettuce, *Bremia lactucae*.

Kyle Fletcher[1], Juliana Gil[1], Sebastian Reyes Chin-Wo[1], Aubrey Kenefick[1], Lien Bertier[1], Lin Zhang[1], Keri Cavanaugh[1], Cayla Tsuchida[1], Joan Wong[1,2], Richard Michelmore[1]
([1.] The Genome Center, University of California, Davis, CA, 95616; [2.] Bayer Vegetable Seeds, Nunhem, the Netherlands)

Bremia lactucae is a highly specialized obligate biotrophic oomycete pathogen that causes downy mildew of lettuce. Genome assembly has been challenging due to high levels of heterozygosity; high quality draft assemblies have been produced for isolates SF5 (Illumina, Moleculo & PacBio) and C82P24 (Illumina), with the aid of several *in silico* approaches. The current reference genome of *B. lactucae*, SF5 contains 885 scaffolds spanning 116 Mb, with an N_{50} of 283 Kb. Mapping Illumina reads of multiple modern and historical field isolates to the SF5 reference revealed multiple distributions of allele frequencies. However, total genome size estimates of all isolates measured by flow cytometry were consistently ~304 Mb, providing no evidence for polyploidy in *B. lactucae*. Additionally, allele frequencies in eight F_1 progeny derived from SF5 (1:1 allele frequencies) x C82P24 (1:3 allele frequencies) were 1:1. Kinship analysis identified two sets of progeny from this cross, indicating that they were derived from three different parental nuclei. Phenotyping and sequencing 20 single spore isolates of C82P24 showed no evidence for multiple isolates being present, consistent with heterokaryosis being the most

parsimonious explanation for these observations. Field surveys suggests that this phenomenon may be common in isolates of *B. lactucae* and single spore isolation of additional, potentially heterokaryotic isolates is underway to investigate its prevalence.

Monday, March 13th		
10:50 - 11:50	**Effectors II**	**Chair: Miaoying Tian**
10:50 - 11:05	A *Phytophthora capsici* virulent RXLR effector targets plant PP2a isoforms that confer Phytophthora blight resistance	Xiao-Ren Chen
11:10 - 11:25	The contribution of effectors to nonhost resistance	Hazel McLellan
11:30 - 11:45	Identification and monitoring of effector proteins in the spinach downy mildew pathogen *Peronospora farinosa*	Joël Klein

A *Phytophthora capsici* virulent RXLR effector targets plant PP2a isoforms that confer *Phytophthora* blight resistance

Xiao-Ren Chen[1,2,3], Gui-Lin Sheng[1], Yan-Peng Li[1], Yu-Ping Xing[1], Yi Zhai[2,3], Wen-bo Ma[2,3]
([1.] Department of Plant Protection, Yangzhou University, Yangzhou, China 225009; [2.] Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521; [3.]Center for Plant Cell Biology, University of California, Riverside, CA 92521)

Plant pathogens deliver an array of effectors to alter host physiology and defense responses. To understand the molecular basis underlying these, it is important to identify and characterize the target proteins of pathogen effectors in plants. Here we show that the RXLR effector PcAvh1 of *Phytophthora capsici* is highly conserved across *Phytophthora* genus. The encoding gene *PcAvh1* is upregulated during the plant infection stages versus barely expressed during the developmental stages. The effector is important for the pathogen virulence on pepper and *Nicotiana benthamiana* via activity in the host nucleus and cytoplasm and of RNA silencing suppression. PcAvh1 interacts with two host protein phosphatase 2a (PP2a) structural isoforms. Silencing of the *PP2a* isoforms facilitates pathogen infection. Furthermore, silencing of the isoforms together severely compromises the growth of plants. These results demonstrate that host PP2a activity is required for the plant resistance against the pathogen. Taken together, we conclude that PcAvh1 is an important virulence determinant that targets host PP2a isoforms to attenuate plant resistance.

The Contribution of Effectors to Nonhost Resistance

Hazel McLellan[1], Sarah Harvey[2], Jens Steinbrenner[3], Petra Boevink[4], Miles Armstrong[4], Ingo Hein[4], Jim Beynon[5], Katherine Denby[2] and Paul Birch[1,4]
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The majority of plants are resistant to most pathogens and this is thought to be due to nonhost resistance (NHR), which is both broad spectrum and durable. Yet the molecular mechanisms underlying this type of resistance are not fully understood. Using a system employing the oomycete pathogens *Phytophthora infestans* and *Hyaloperonospora arabidopsidis* and their respective hosts Potato, *Nicotiana benthamiana* and *Arabidopsis thaliana* we show that effector-host protein target interactions may contribute to nonhost resistance. Through a large scale matrix Y2H screen we explore the extent of effector: target interactions conserved in nonhost plants, identifying several different categories of interaction. We demonstrate the differences in efficacy of effectors to contribute to virulence in host versus nonhost systems, showing that the majority of effectors from a pathogen will not function in distantly related nonhost plants. Moreover, we then assess the potential of swapping non-targeted proteins from nonhost to host systems to provide increased resistance.

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

Joël Klein[1], Marcel Van Verk[1,2] and Guido van den Ackerveken[1]
([1]Plant-Microbe Interactions, and [2]Bioinformatics, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands)

Peronospora farinosa f. sp. *spinaciae* (*Pfs*) is an obligate biotrophic oomycete pathogen of spinach, on which it causes downy mildew disease. *Pfs* employs secreted effector proteins to modulate plant innate immunity and enable infection. Resistance genes in the spinach host provide protection against *Pfs*, by recognizing the pathogen, through its effectors. Although newly bred resistant cultivars are initially protected, new *Pfs* races rapidly break the employed resistance genes by adaptation of their recognized effector proteins. A reference genome of *P. farinosa* race 1 (*Pfs1*) of 32 Mbp was generated by sequencing using PacBio, and Illumina technology, followed by a hybrid assembly. Sequencing of *Pfs1* mRNA of 7 infection stages before and during infection contributed significantly to generate accurate gene models. The corresponding protein models have been used to identify more than 60 *Pfs1* effectors, by selecting proteins with signal peptides followed by conserved translocation motifs. To study adaptation of effectors we sequenced 14 other *Pfs* races using Illumina. The genomes of these races were *de novo* assembled, and the effector repertoires determined. Polymorphisms in effector sequences of these races are under study to provide insight into effector evolution. Furthermore, we assembled the mitochondrial genomes of *Pfs1* and the 14 other races to infer their phylogenetic relatedness. Combined with the data on effector evolution this will give us insight into mechanisms by which this downy mildew breaks resistance in spinach cultivars.

Monday, March 13th		
2:00 - 3:20	**Host interactions and resistance II**	**Chair: Yi Zhai**
2:00 - 2:15	Autophagy is a central cellular process in the interaction between the kelp *Macrocystis pyrifera* and *Anisolpidium ectocarpii* (Oomycota)	Pedro Murúa
2:20 - 2:35	Secreted Nep1-like proteins of oomycetes, fungi, and bacteria trigger immunity in Arabidopsis; genetic dissection of NLP-induced plant defense	Guido Van den Ackerveken
2:40 - 2:55	Understanding plant immunity to *Phytophthora* spp.: from model plants to crops	Patricia Manosalva
3:00 - 3:15	Utilizing host resistance for the management of the oomycete pathogen *Phytophthora sojae*	Colin Davis

Autophagy is a central cellular process in the interaction between the kelp *Macrocystis pyrifera* and *Anisolpidium ectocarpii* (Oomycota)

Pedro Murúa[1,2,3], Dieter G. Müller[4], Pieter van West[2], Claire M. M. Gachon[3]
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The Anisolpidiales were recently identified as a group of uniflagellate, marine oomycete pathogens of brown algae. A pathosystem was established between *Anisolpidium ectocarpii* and *Macrocystis* female gametophyte, and subjected to ultrastructural investigations. Electron microscopy revealed that successful penetration by *Anisolpidium* spores leads to the development of a walled syncytium. In optimal growth conditions, this syncytium fills the entire volume of the cell wall. Sometimes however, and likely as a result of starvation, *Anisolpidium* syncytium shrinks inside the space delimited by the cell wall, plasmalemma invaginations appear in the periplasmic space, and some spore initials undergo autophagy, trying to rescue the development of a limited number of pathogen spores. Sometimes, entirely abortive *Anisolpidium* syncytia showed evidence of uncontrolled autophagy despite the presence of lipid reserves. Infected algal cells exhibit many digestive vacuoles, some of them with *Anisolpidium* structures. Autophagy, therefore, may be a key cellular process used by both pathogen and host. Our working hypothesising is that 1) autophagy is used by the host as a response to mobilise energy and/or kill the intruder; 2) autophagy is normally used by the pathogen to adjust its sporulation to the level of resources it can retrieve from its host; 3) both the host and the pathogen may hijack their counterpart's autophagic process for their own benefit.

Secreted Nep1-like proteins of oomycetes, fungi, and bacteria trigger immunity in *Arabidopsis*; genetic dissection of NLP-induced plant defense.

Tom M. Raaymakers, Ruben Hijne, Tijmen van Butselaar, Guido van den Ackerveken
Plant-Microbe Interactions, Department of Biology, Institute of Environmental Biology, Utrecht University, Utrecht, The Netherlands

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are secreted by a wide range of plant-associated microorganisms, in particular by many phytopathogenic oomycetes and fungi. Both cytotoxic and non-cytotoxic NLPs act as a microbe-associated molecular pattern (MAMP) in *Arabidopsis thaliana*. Specifically, a 24 amino acid fragment, nlp24, derived from a conserved region of oomycete, fungal, and bacterial type 1 NLPs triggers defense. The widespread distribution of NLPs makes this family the first proteinaceous MAMP identified in three different kingdoms of life. A receptor complex consisting of RLP23–SOBIR1–BAK1 mediates NLP-triggered immunity (NTI) in *Arabidopsis*. Here, we

present our data on the genetic dissection of NTI, using a transgenic *Arabidopsis* line expressing an estradiol-inducible version of *HaNLP3* of the Arabidopsis downy mildew pathogen *Hyaloperonospora arabidopsidis*. When treated with estradiol plants become severely stunted and plant immunity is strongly activated. M2 seeds of an EMS-mutagenized population were screened for loss of NLP-triggered growth inhibition. We selected ~40 mutants showing normal growth after estradiol treatment and insensitivity to the nlp24 peptide. The obtained *decreased NTI* (*dni*) mutants are analysed for defects in responsiveness to other MAMPs and their susceptibility to downy mildew infection. We will report on our progress on *dni* mutant analysis and cloning of the corresponding *DNI* genes.

Understanding plant immunity to *Phytophthora spp.*: from model plants to crops

Jackson N., Belisle, R., Xu, G., McKee, B., Ceballos V., and Manosalva P.
Department of Plant Pathology and Microbiology, University of California Riverside, Riverside, CA, USA

Oomycetes are destructive filamentous microorganisms capable of causing enormous economical losses on crops worldwide as well as environmental damage by destroying natural ecosystems. Species such as *Phytophthora infestans* (*Pi*) infects a relatively narrow host range limited to the Solanaceae family whereas others, such as *P. cinnamomi* (*Pc*), are able to infect over 1000 plant species. *P. cinnamomi* is called the “biological bulldozer” for its capacity to destroy natural communities and is the causal agent of Phytophthora root rot (PRR), the major constraint for avocado production worldwide. This disease affects approximately 60-75% of California (CA) growers causing losses of $40 millions annually. In addition, CA growers are threatened by the introduction of more aggressive *Pc* isolates. Our data indicates that there is significant phenotypic variation among CA *Pc* isolates regarding: i) vegetative growth, ii) virulence, and iii) fungicide sensitivity. The molecular and genetic basis of plant immunity to *P. cinnamomi* is largely unknown. Here, we describe the role of the Microrchidia (MORC) proteins, new epigenetic factors, in regulating plant immunity to *P. infestans* and *P. cinnamomi*. We will also report our recent findings regarding the molecular mechanisms for which MORC1 regulates plant immunity to these oomycete pathogens. Our results suggest that MORC1 regulates resistance by interacting with specific host proteins and regulating microRNAs associated with different plant immune processes. Finally, we will discuss our strategies to dissect the molecular and genetic basis of plant - *P. cinnamomi* interactions using model plants and avocados.

Utilizing Host Resistance for the Management of the Oomycete Pathogen Phytophthora sojae

Colin Davis, John McDowell, M.A. Saghai Maroof, Brett Tyler, Michael Fedkenheuer , Kevin Fedkenheuer, Rachelle Mathieson, Alison Robertson

Phytophthora sojae (*P. sojae*), the causal agent of soybean root and stem rot disease, is an oomycete pathogen responsible for over 400 million dollars of soybean crop damage annually in the US, and over one billion dollars worldwide. For successful P. sojae infection, the pathogen must secrete effector proteins into the host, which function to suppress the host’s immune system. Rps genes are able to recognize specific pathogen effectors inside the host and activate the defense response in turn. However, the effectiveness of current R genes is decaying as certain pathotypes of P. sojae evolve to overcome the resistance through mutation or silencing of the cognate effectors, which are typically non-essential for virulence. This project seeks to identify novel R genes conferring durable resistance to P. sojae. Resistance gene screening targeting core P. sojae effectors is expected to provide more durable R-genes, because the loss of the cognate effector would compromise virulence. We have developed and utilized an effector-based screening assay using Pseudomonas fluorescens to deliver core RXLR effectors from P. sojae to screen for new Rps genes in Glycine germplasm and to conduct segregation analysis and genetic mapping in segregating populations. Additionally, we have developed a trifoliate assay to screen for R-genes using virulent P. sojae. In F2:3 populations,effector recognition is dominant and for each R-gene that we have screened. We are currently using our pathogen-based assay in

conjunction with our effector-based assay on RIL populations to map the positions of R-genes that recognize core P. sojae effectors.

Monday, March 13th		
3:50 - 5:10	**Oomycete biology, populations, and evolution I**	**Chair: Javier F. Tabima**
3:50 - 4:05	Phylogeography of the tropical oomycete *Phytophthora palmivora*	Erica M. Goss
4:10 - 4:25	Diversity of *Phytophthora* species from natural and semi-natural ecosystems in Portugal, Chile and Vietnam	Marília Horta Jung
4:30 - 4:45	Live cell imaging of the cytoskeleton in *Phytophthora* pathogens reveals unique actin and microtubule configurations	Kiki Kots
4:50 - 5:05	Morphological and molecular identification of *Phytophthora palmivora* Butler as causal agent of black pod rot of cocoa (*Theobroma cacao* L.) from coastal Ecuador	Miriam Escos

Phylogeography of the tropical oomycete *Phytophthora palmivora*

Jianan Wang[1*], Michael D. Coffey[2], Erica M. Goss[1,3]
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The genetic structure and diversity of oomycetes are shaped by their evolutionary history, including coevolutionary interactions with hosts, geographic spread, and long-distance migration events, often mediated by humans. In turn, diversity and dispersal of present populations impact the risk of disease epidemics and strategies for controlling disease. *Phytophthora palmivora* is a globally distributed oomycete that infects a broad range of cash crops and fruit trees in the tropics and subtropics, including cocoa and palms. The center of diversity of *P. palmivora* is in Southeast Asia, but it is an important and widespread pathogen in South America. Our multilocus sequence analysis showed that the centers of origin of the pathogen are located in the Philippines and Indonesia. We then tested alternative models for the emergence of *P. palmivora*. We found genetic variation consistent with historical movement among Pacific Islands, likely associated with coconut. The population structure of *P. palmivora* is consistent with a bridgehead effect, such that the colonization of South America and host shift to cocoa led to further global dispersal, including gene flow back to Southeast Asia. We propose that the extensive genetic diversity in *P. palmivora* in Southeast Asia is the result of a complex history, including long-term co-evolution with native hosts, geographic isolation with migration, and re-introduction of genotypes from South America.

Diversity of *Phytophthora* species from natural and semi-natural ecosystems in Portugal, Chile and Vietnam

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Surveys of *Phytophthora* diversity were performed using baiting assays and direct plating of necrotic plant tissues. Isolates were identified using both classical identification and sequence analysis of ITS and *cox1*. In Portugal, 68 forest stands, 12 forest nurseries, 38 river systems and 4 lagoon ecosystems were surveyed. The isolates obtained belonged to 29 known species, 3 informally designated taxa, and 1 previously unknown taxon of *Phytophthora*, nine new *Phytophthora* hybrid taxa from Clades 6 and 9, *Nothophytophthora homothallica* nom. prov., 1 known and 10 new *Halophytophthora* species, 7 known species and one new taxon of *Phytopythium*, and multiple *Pythium* species were isolated. In Chile, the survey was performed in 13 natural forest stands and 20 forest streams located in two protected areas near Valdivia and in a temperate mountain forest in the Concepción area, and in each one planted stand of the introduced tree species *Castanea sativa* and *Fagus sylvatica*. Eight described species (including *P. kernoviae*) and 2 previously unknown taxa of *Phytophthora* were isolated. In addition, a diverse array of Clade 6 hybrids, and *Nothophytophthora caduca* nom. prov., *Nothophytophthora chlamydospora* nom. prov. and *Nothophytophthora valdiviana* nom. prov., were obtained. In Vietnam the survey was performed in 23 natural forest stands and 10 forest streams and rivers in temperate montane and tropical lowland regions, and in 14 rubber plantations. Sixteen described species (including *P. ramorum*), 3 designated taxa and 23 previously unknown taxa of *Phytophthora*, amongst them 9 Clade 9 hybrid taxa, were isolated. In addition, *Nothophytophthora vietnamensis* nom. prov. and a diverse array of known and new taxa of *Phytopythium*, *Pythium* and *Elongisporangium* were recovered. The implications of these findings for plant biosecurity and the development of a deeper understanding of the evolution and adaptability of the genus *Phytophthora* will be discussed.

Live cell imaging of the cytoskeleton in *Phytophthora* pathogens reveals unique actin and microtubule configurations

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The cytoskeleton is a dynamic but well organized intracellular network that is essential for proper functioning of eukaryotic cells. We study the cytoskeleton in *Phytophthora* species, oomycete plant pathogens that cause devastating diseases worldwide. We use Lifeact-eGFP expressing *Phytophthora infestans* for live cell imaging of the actin cytoskeleton in various developmental stages. Previously we identified actin plaques as highly immobile, long-lived structures that are unique for oomycetes. Here we present two other unique actin configurations; one associated with plug deposition in germ tubes and the other with appressoria, infection structures formed prior to host cell penetration. Plugs are composed of cell wall material that is deposited in hyphae emerging from cysts to seal off the cytoplasm-depleted base after cytoplasm retraction towards the growing tip. Preceding plug formation a typical local actin accumulation was observed that remained associated with the leading edge during plug deposition. In appressoria we observed an aster-like actin configuration at the contact point with the underlying surface. These findings strongly suggest a role for the actin cytoskeleton in plug formation and plant cell penetration. For live cell imaging of the microtubule cytoskeleton we have generated a *Phytophthora palmivora* transformant expressing GFP-α-tubulin allowing us to visualize the dynamics of microtubules in oomycetes for the first time. The data presented here provide a better understanding of the structure and functioning of the *Phytophthora* cytoskeleton. The long term goal is to uncover oomycete or *Phytophthora* specific features in the cytoskeleton that might be instrumental for drug design.

Morphological and molecular identification of *Phytophthora palmivora* Butler as causal agent of black pod rot of cocoa (*Theobroma cacao* L.) from coastal Ecuador.

Carmita Suarez-Capello[1], Miriam Escos[2], Karina Solis[1,2,3], Ana Garces-Claver[2,4], Denny Carriel[1], Juan J. Barriuso[4].
([1.] Quevedo State Technical University, Quevedo, Ecuador; [2.] Plant Food Research Laboratory, Agrifood Reseach and Technology Centre of Aragon (CITA), Zaragoza, Spain; [3.] National Institute of Agricultural Research (INIAP), Ecuador; [4.] Agrifood Institute of Aragon, Zaragoza University (IA2), Zaragoza, Spain).

By the end of last century, black pod disease and losses of productive cocoa (*Theobroma cacao*) trees in Ecuador were below 1% of damage and it was therefore considered a minor problem for the cocoa industry. Since then, incidence and aggressiveness of the pathogen have increased coinciding with the propagation of plantings from a single high yield clone. This is especially true in new planting areas that now report black pod disease as the main sanitary problem on cocoa. The aim of the study was to determine the *Phytophthora* species associated with black pod disease of cocoa in the coastal region of Ecuador. Isolation of *Phytophthora* species was done from naturally infected pods in different localities throughout the main cocoa region. One week-growth colonies in PDA plates were morphologically described and identified molecularly by sequencing the ITS regions. For the first time, results of a systematic study started by Quevedo State Technical University in Ecuador and Zaragoza University in Spain demonstrate that *Phytophthora palmivora* Butler acts as the causal agent of pod rot of cocoa in Ecuador.

Tuesday, March 14th		
9:00 - 10:20	**Genomics III**	**Chair: Laura Grenville-Briggs**
9:00 - 9:15	Genome biology, evolution and recognition of *Albugo candida* CCG effectors	Oliver J. Furzer
9:20 - 9:35	Oomycete evolution reconstructed with an automated phylogenomics pipeline using publicly available genome data from 39 taxa and three additional taxa not previously sequenced	Hai D.T. Nguyen
9:40 - 9:55	Updated view of genome structure, variation, and transcriptional dynamics in *Phytophthora infestans* based on a PacBio assembly and revised gene models	Howard Judelson
10:00 - 10:15	Transcriptional programming of *Phytophthora sojae* for organ-specific infection	Wenwu Ye

Genome biology, evolution and recognition of *Albugo candida* CCG effectors

Oliver J. Furzer[1], Volkan Çevik[1,2], Amey Redkar[1], Agathe Jouet[1], Kate Bailey[1], Eric B. Holub[3], Jonathan D.G. Jones[1]
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We study the obligate biotroph oomycete species *Albugo candida,* races of which infect plants of the *Brassicaceae,* including the model *Arabidopsis thaliana*. We aim to understand how races of this pathogen are recognised by resistant lines, and the basis for evasion of recognition. Previous studies on *Albugo laibachii* revealed a new class of secreted effector candidates with the N-terminal, post signal peptide, motif CxxCxxxxxG (CCG). Multiple CCG proteins are recognised by the Arabidopsis TIR-NB-LRR (TNL) class Resistance protein WRR4A. To understand host resistance to *Albugo* we have mined the "non-host" immunity found in Arabidopsis to crop-infecting *A. candida* races, and identified a further four functional Resistance proteins, all TNLs. Among these, WRR4B, a WRR4A paralog, also recognises several CCG effectors. To understand better the *Albugo* effector complement and its evolution, we have performed PacBio genome and extensive Illumina cDNA sequencing on *A. candida* race Ac2V. Assembly of PacBio reads in conjunction with previous Illumina reads has produced a greatly improved genome assembly. *A. candida* race Ac2V has ~100 CCG effectors, mostly in gene-sparse regions of the genome. I will present a synthesis of our work on resistance to *Albugo* and an analysis of the new *Albugo* PacBio genome with particular emphasis on the evolution of the CCG effectors.

Oomycete evolution reconstructed with an automated phylogenomics pipeline using publicly available genome data from 39 taxa and three additional taxa not previously sequenced.

Hai D.T. Nguyen[1], Hannah E. Morrison[1], Kasia Dadej[1], Tara L. Rintoul[1], Tahera Sultana[1], Christoffel F. J. Spies[1], C. André Lévesque[1]
([1.] Agriculture and Agri-Food Canada, Ottawa, Canada)

The elucidation of oomycete evolutionary history has been limited by phylogenetic analyses with conflicting hypotheses about relationships at higher taxonomic levels within the group. To provide more information about these relationships, whole genome data from 39 publicly available oomycete taxa were downloaded and re-annotated. Three additional oomycete taxa; *Pythium helicandrum*, *Pythium undulatum* and *Halophytophthora epistomium* were sequenced, assembled and annotated. We developed an automated phylogenomics pipeline (APP) for performing quick and routine phylogenetic reconstruction with genome data. We will report our progress on the utilization of the latest oomycete genomic dataset for resolving phylogeny and compare our findings with previous research.

Updated view of genome structure, variation, and transcriptional dynamics in *Phytophthora infestans* based on a PacBio assembly and revised gene models

Howard Judelson, Michael Matson, Jolly Shrivastava, Audrey Ah Fong
(Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA)

Most published analyses of the *P. infestans* genome and transcriptome are based on a Sanger assembly, gene models generated with limited transcript support, and microarray expression data. To enable more accurate analyses, a new assembly was developed using Pacific Biosciences technology (N50 of 22 kb) and 250-nt Illumina reads of isolate 1306. The new assembly has 220 Mb of DNA within 550 contigs having an N50 of 1.5 Mb, compared to 190 Mb in 18,288 contigs (4921 scaffolds) in the Sanger assembly. The new assembly has been validated and improved with optical maps and long-range linking libraries. The N50 value is diminished by the presence of many short contigs, which may represent haplotype fragments that did not coalesce during assembly. The new assembly was used to confirm the occurrence of copy number and structural variation within haplotypes of 1306, and between isolates of *P. infestans.* Non-Mendelian events arising from crosses were also identified. QTL mapping of several traits confirmed that the PacBio assembly is more useful for genetic analyses than the Sanger assembly. RNA-seq data from 1306 was used to update the *P. infestans* gene models, identify new genes, and characterize transcription during the life cycle. Parallel studies of two isolates generated robust expression calls for about 16K of the 18K predicted genes in hyphae, sporangia, chilled sporangia, zoospores, and germinated cysts. This more than doubles the number of genes that we estimate were measured in the published Affymetrix microarray studies, providing more insight into metabolism, signaling, and pathogenesis.

Transcriptional programming of *Phytophthora sojae* for organ-specific infection

Wenwu Ye, Yang Wang, Long Lin, Jiawei Wu, Yachun Lin, Yuanchao Wang
(Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China 210095)

Effector proteins are secreted by many plant pathogens to promote infection. The genome of the soybean root rot pathogen *Phytophthora sojae* contains at least 380 genes encoding candidate RxLR family effectors. Previously, we found that different RxLR effectors could be transcriptionally programmed following infection, and the mechanism facilitated the effectors target different functional branches of the plant defense response. To further learn effector functions and the associated transcriptional regulation mechanism for pathogenicity, we compared the transcriptomes of *P. sojae* during the early stage of infection in soybean roots and leaves. We analyzed the differentially expressed genes and the enriched pathogenicity-related gene families. Functions of several candidate genes were further studied based on CRISPR/CAS9-mediaed gene knock out. A model on how the *Phytophthora* pathogens regulating effectors to facilitate its organ-specific infection, as well as the associated evolutional mechanism, were proposed.

Tuesday, March 14th		
10:50 - 11:30	**Oomycete biology, populations, and evolution II**	**Chair: Erica M. Goss**
10:50 - 11:05	Population dynamics of *Phytophthora rubi* indicate high rates of migration between states and nurseries in the Pacific Northwestern United States	Javier F. Tabima
11:10 - 11:25	*Nothophytophthora* prov. nom., a new sister genus of *Phytophthora*	Marília Horta Jung
11:30 - 11:45	Hidden diversity in the oomycete genus Olpidiopsis is a global threat to red algal cultivation	Yacine Badis

Population dynamics of *Phytophthora rubi* indicate high rates of migration between states and nurseries in the Pacific Northwestern United States

Javier F. Tabima, Inga A. Zasada, and Niklaus J. Grünwald

The Pacific Northwestern US (PNW) is the main producer of red raspberry (*Rubus idaeus*), with the states of Washington, California and Oregon producing the most berries in the country. The most common plant pathogen causing disease in raspberry fields in the PNW is *Phytophthora rubi*, an oomycete plant pathogen known to cause red root disease in red raspberry. *P. rubi* is found in 90% of the red raspberry crops in the state of Washington, resulting in millions of dollars in losses to the berry industry of the United States. Recent studies have shown an absence of population structure in *P. rubi* across the PNW, indicating that constant migration is taking place in this region. To study the population dynamics of *P. rubi*, we used genotyping-by-sequencing (GBS) to characterize genetic diversity and study population structure and migration between states and nurseries of the American PNW. Our results show that *P. rubi* forms a single population in the PNW, and has high rates of migration between geographic locations. Analysis of directed migration show that nurseries in the state of Washington are sinks of disease from nurseries in California and Oregon, most likely due to the transport of contaminated soil and plant material. These findings pose a concern for the nursery industry, indicating that the pathogen is moving towards regions with high production of red raspberry, thus exposing a necessity to improve mechanisms of control and detection of *P. rubi* to avoid cross-contamination via human-mediated migration across states in the PNW.

Nothophytophthora* prov. nom., a new sister genus of *Phytophthora

ThomasJung[1,2,3], Bruno Scanu[4], József Bakonyi [5], Diána Seress [5], Alvaro Durán [6], Eugenio Sanfuentes von Stowasser [7], Leonardo Schena[8], Saveria Mosca [8], Pham Quang Thu[9], Chi Nguyen Minh[9], Sebastian Fajardo[7], Mariela González [7], Ana Pérez-Sierra [10], Helen Rees [10], Cristiana Maia[1], Marília Horta Jung[1,2] ([1]Laboratory of Molecular Biotechnology and Phytopathology, Center for Mediterranean Bioresources and Food (MeditBio), University of Algarve, 8005-130 Faro, Portugal; [2]Phytophthora Research Centre, Mendel University, 613 00 Brno, Czech Republic; [3]Phytophthora Research and Consultancy, 83131 Nußdorf, Germany; [4]Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), Università degli Studi di Sassari, 07100 Sassari, Italy; [5]Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest, Hungary; [6]Ontario Forest Research Institute, P6A 2E5 Sault Ste. Marie, Canada; [7]Laboratorio de Patología Forestal, Facultad Ciencias Forestales y Centro de Biotecnología, Universidad de Concepción, Concepción, Chile; [8]Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea di Reggio Calabria, 89124 Reggio Calabria, Italy; [9]Forest Protection Research Centre, Vietnamese Academy of Forest Sciences, Duc Thang Ward, Northern Tu Liem District, Hanoi, Vietnam; [10]Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom.)

During various surveys of *Phytophthora* diversity in Europe, Chile, and Vietnam slow growing oomycete isolates were obtained from soil samples and small streams in natural and planted forest stands.

Phylogenetic analyses of ITS, β-tubulin, LSU and *cox1* sequences placed them into six new species belonging to a new genus, officially described as *Nothophytophthora* gen. nov., which clustered sister to *Phytophthora*. *Nothophytophthora* species share numerous morphological characters with *Phytophthora*: persistent (all *Nothophytophthora* spp.) and caducous (*N. caduca*, *N. chlamydospora*, *N. valdiviana*, *N. vietnamensis*) sporangia with variable shapes, internal differentiation of zoospores and internal, nested, extended (*N. chlamydospora*, *N. caduca*) and external (all *Nothophytophthora* spp.) sporangial proliferation; smooth-walled oogonia with amphigynous (*N. amphigynosa*) and paragynous (*N. amphigynosa*, *N. intricata*, *N. vietnamensis*) insertion of the antheridia; chlamydospores (*N. chlamydospora*) and hyphal swellings. Comparisons of morphological structures of both genera provide clues about the morphology and ecology of their common ancestor which are discussed. Including *Nothophytophthora* in coalescence analyses will give new insights into the evolutionary history of *Phytophthora*. Production of caducous sporangia by *N. caduca*, *N. valdiviana* and *N. chlamydospora* from Valdivian rainforests and *N. vietnamensis* from a wet mountain forest in Vietnam indicates a partially aerial lifestyle as adaptation to these humid habitats. Presence of tree dieback in all forests from which *Nothophytophthora* spp. were recovered and partial sporangial caducity of several *Nothophytophthora* species suggests they may be facultative pathogens. Pathogenicity tests are urgently required to clarify whether the individual *Nothophytophthora* species have a pathogenic or saprophytic lifestyle.

Hidden diversity in the oomycete genus *Olpidiopsis* is a global threat to red algal cultivation
Yacine Badis, Tatyana Klochkova, Martina Strittmatter, Andrea Garvetto, Pedro Murùa, Craig Sanderson, Gwang Hoon Kim, Claire Gachon

Seaweed cultivation is the fastest-growing of all aquaculture sectors, with an annual growth rate of 8 % and a value in excess of $5bn. Emerging diseases are a threat to its sustainable development. The oomycete genus *Olpidiopsis* notably encompasses pathogens of red seaweeds, including the most economically damaging disease in *Pyropia* (ex-Porphyra) farms in Asia. Here we identified three new european *Olpidiopsis* species, and a Scottish variety of *O. porphyrae*, a devastating pathogen only reported in Japanese seaweed farms. Importantly, two of the new species infected *Porphyra* and *Palmaria* sp., which are subject extensive farming trials in Europe and North America. To further assess the extent of undescribed *Olpidiopsis* diversity and the threat it might pose to aquaculture in different regions, we screened targeted *Porphyra* metagenomes as well as global metagenomic barcoding campaigns. Over 700 new sequences attributable to *Olpidiopsis* were detected with a worldwide distribution. Close relatives of the Korean *O. pyropiae* are also reported for the first time in Europe and the United States. In the light of our restricted sampling, our results highlight the diversity and abundance of *Olpidiopsis* worldwide. In the context of worsening impact of *Olpidiopsis* pathogens in Asia, this worldwide distribution should be treated as a serious threat to the global seaweed industry and wild red algal populations. Our data calls for more efforts towards the documentation of these pathogens, and for adequate biosecurity measures to be developed.

ABSTRACTS OF POSTER PRESENTATIONS

(odd number presented on Sunday, even number on Monday)

1. Screening and functional characterization of candidate RxLR and CRN effector proteins on the interaction *Phytophthora parasitica*-citrus: epistasis and immunity manipulation?

Ronaldo J. D. Dalio, Heros J. Maximo, Tiago S. Oliveira, Marcos A. Machado.
(Centro de Citricultura Sylvio Moreira, Instituto Agronômico de Campinas, Cordeirópolis, SP, CEP13490-970)

Phytophthora parasitica is a very destructive root pathogen of citrus plants. It is known to secrete several effectors proteins to establish infection. The mechanistic molecular functioning of effectors remains elusive in Phytophthora-citrus interactions. We took advantage of *P. parasitica* genome sequences to screen for citoplasmatic candidate effectors. Through a bioinformatics pipeline, we have identified 171 candidate RxLR and 66 CRN effectors. Subsequently we have searched those effectors among the up-regulated genes of an RNAseq database of *P. parasitica*. Five RxLR and 11 CRN were suppressed/repressesd when already in contact with root extracts of citrus plants, before infection. These effectors have predicted citoplasmatic, nuclear or chlroroplastic sub-celular localizations. In infection experiments, all these candidate effectors were consistently up-regulated at the beginning of the infection or at later time-points. We have then proceeded to transient expression in *Nicotiana benthamiana* plants via agroinfiltration, in parallel with *INF1*. Preliminary results show that PpRxLR2 effector might have an immunity suppression function, deactivating INF1 necrosis induction. On the other hand, we have found that *PpCRN13* might be related to have an epistasis function together with *INF1*, maximizing hypersensitive response and necrosis. Further studies will validate these results. We will discuss which experiments can be designed to deepen our understanding on the effectors deployment of *P. parasitica* infecting citrus and how to target these effectors to break pathogen virulence.

2. (Student Poster) Investigating the effect of the PsAvh110 effector on genome-wide yeast expression levels to identify putative targets

Avery Wilson[1], William Morgan[1]
Department of Biology, College of Wooster, Wooster, OH

There are over 350 predicted effectors in the *Phythopthora sojae* genome, but the functions of the vast majority of these remain uncharacterized. Here, we use yeast as a eukaryotic model to investigate the activity of one *P. sojae* effector, PsAvh110. Using yeast as a model is based off of the observation that heterologous expression of PsAvh110 causes significant growth inhibition of yeast, suggesting that the effector targets a conserved eukaryotic pathway. To investigate this pathway, we heterologously expressed PsAvh110 in yeast and used RNA-sequencing to characterize how the effector affects genome-wide expression levels. We found that the expression of PsAvh110 in yeast caused the expression of 25 genes to be induced at least two-fold, and that it caused the expression of 13 genes to be repressed at least two-fold. We then performed gene set enrichment analysis on the set of genes significantly up- or down-regulated to identify statistically overrepresented Gene Ontology (GO) terms. Within the set of genes induced two-fold, we found the overrepresentation of GO terms relating to fructose and mannose transport, as well as serine family amino acid catabolism. For the set of genes repressed two-fold, we found the overrepresentation of GO terms relating to iron homeostasis. Further analysis of these results is needed to identify direct putative targets of the effector.

3. The evolution of RxLR genes in the oomycete pathogens *Phytophthora infestans* and *Phytophthora andina*.

Amirhossein Bahramisharif, Ian Beddows, Thorsten Klösges, Laura Rose

(Institute of Population Genetics, Heinrich Heine University, Düsseldorf, Germany)

The plant pathogen *Phytophthora infestans* causes serious yield losses on economically important crops. Although most losses in the 1990s were caused by a new genotype of *P. infestans,* the origin of the Irish famine strain still appears complex. A recent study on the evolutionary history of *P. infestans* and *Phytophthora andina* provided evidence that *P. infestans* might have originated from South America. These oomycete pathogens secrete effector proteins that modulate host responses and have been implicated in pathogenesis. One prominent class of effector proteins is characterized by a conserved RxLR motif that is required for translocation inside host cell. Therefore, to gain a better insight into the mechanisms involved in plant-pathogen interactions, the aim of this study was to evaluate the molecular evolution of 36 RxLR genes from *P. infestans* and *P. andina*. Next-generation based read libraries from 27 pathogen isolates were mapped on to the *Phytophthora* T30-4 reference genome. Genotype calling and haplotype imputation were conducted with ANGSD and BEAGLE software. Next, isolate-specific genomes were reconstructed using the SNP data. The coding sequences for 36 RxLR genes were extracted from the reconstructed genomes and used for population genetic statistical analyses. In total, five genes showed evidence of recent diversifying selection. These genes have been prioritized for functional studies of infections on a panel of wild and cultivated tomato genotypes.

4. An AGC family protein kinase, associated with PsGPA1, contribute to virulence and sexual/asexual reproduction in *Phytophthora sojae*.

Min Qiu[1], Baiyu Zhang[1], Yaling Li[1], Xin Zhang[1], Brett M. Tyler[2], Yuanchao Wang[1]
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Sporangia reproduction and zoospore motility are essential in the early stages of oomycete pathogen *Phytophthora sojae* infection. Meanwhile, oospore reproduction during sexual stages makes great significance in poor surrounding. G proteins are essential for growth, asexual and sexual development, and virulence in both animal and plant pathogens. Previously, we have identified a G-protein α subunit encoded by *PsGPA1* which regulates the chemotaxis and pathogenicity of *Phytophthora sojae*. In the present study, we used affinity purification to identify PsGPA1-interacting proteins, including PsYPK2, a ser/thr protein kinase belongs to AGC family protein kinase. PsYPK2 can interact with PsGPA1 in vitro and an analysis of the CRISPR-knockout mutants reveals that PsYPK2 contributes to virulence during interaction with a susceptible soybean cultivar. Further assay shows the mutants have abnormal germ tubes that were highly branched and exhibited swelling. In addition, production of sporangia is evidently decreased and the mutants lose the ability to produce oospore. These results suggest that PsYPK2 regulates the process of sexual and asexual reproduction. However, PsYPK2 mutants have normal zoospore chemotaxis. It probably due to G protein pathway can response to numerous stimuli, which simultaneously activate multiple downstream pathways that may potentially cross talk and affect the biological net outcome.

5. (Student Poster) Functional characterization of a cytoplasmic effector gene highly conserved in plant pathogenic oomycetes.

Navet Natasha[1], Wu Dongliang[1], Shao Dandan[1] and Tian Miaoying[1]
([1]Department of Plant and Environmental Protection Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, HI-96822)

Plant pathogenic oomycetes secrete a diverse array of RxLR cytoplasmic effectors potentially involved in infection of host plants. In identification of RxLR effectors from two plant pathogenic oomycetes, including basil downy mildew pathogen *Peronospora belbahrii* and the broad host range pathogen *Phytophthora palmivora*, we identified an RxLR effector protein that is highly conserved and broadly present in all plant pathogenic oomycetes with genomic sequences available; we designated this as Oomycete RxLR Conserved Effector 1 (ORCE1). ORCE1 homologs exhibit remarkable degree of amino acid sequence conservation and are under purifying selection suggesting that they play fundamental role in parasitizing their host plants. Functional validation of the signal peptide of *Phytophthora palmivora* ORCE1 using yeast invertase secretion assay revealed that ORCE1 likely represent a *bona fide* effector. Overexpression of ORCE1 in *Phytophthora palmivora* increased pathogen virulence on papaya plants. ORCE1 is expressed during different development stages of *Phytophthora palmivora* but high levels of transcripts were present throughout infection stages, suggesting that this gene is induced during contact with host tissue. CRISPR-Cas9 is being used to edit ORCE1 to further determine its role in development and pathogenicity. Identification of host targets of ORCE1 is underway using yeast-two-hybrid assay. This study is attempting to dissect the molecular mechanisms that enable oomycete pathogens to successfully infect plants.

6. (Student Poster) Protein coding genes in *Phytophthora* genomes exhibit GC bias at their terminals.

Chandra Sarkar and Paul F. Morris.
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While designing primers to amplify *Phytophthora sojae,* genes, we noted that the T_m of the 5'-terminals of primers were significantly higher than those for the 3'-end. To test the hypothesis that there was a general N-terminal GC bias across all coding sequences, we examined the codon bias of a curated dataset of 13,106 coding sequences obtained from FungiDb. The GC bias was estimated using a sliding window of 6 codons along the N- and C-terminals. The mean GC content of this gene set was 58.69% with average gene length of 1752bp. The N-terminal regions had an elevated GC content of 62.5% (±3%) from bases 4 to 70. Around base position 50, it reached a maximum of 64.9%, and remained slightly above the mean (59.8%) at 450 bp. In contrast, the C-terminal at bases 4-25 had a GC content of 56.5% and increased to reach the mean GC content around 60 bp. An analysis of 2511 predicted signal peptides indicated that these proteins had a slightly higher GC content of 59.5%. A more pronounced N-terminal GC bias was observed with a maximum of 67.3% around bases 45-50, and the mean GC content of 61% at 450 bp was significantly higher than for other proteins. The C-terminal regions for predicted signal peptide proteins was also slightly higher than other proteins. This pattern of positional GC bias is also observed in *P. ramorum, P. infestans, P. parasitica,* and *P. cinnamoni* . Other expression patterns will also be reported.

7. (Student Poster) Lessons learned from massive amplicon sequencing of cytochrome oxidase II for determination of Oomycete species diversity

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Studies of soil microbial communities are valuable to find links between taxa and environmental characteristics. When applied to particular plant pathogens, these studies provide insights about their abundance and survival in different environments. Our aim was to describe Oomycete species composition in soil samples from cacao farms and contrast them with neighboring crops and uncultivated zones by using high-throughput multiplexed sequencing of the cytochrome oxidase II gene (cox2). Total DNA was extracted from collected soil samples and used for library preparation. Amplicons of ~580bp were sequenced by Illumina MiSeq 2x300. Resulting reads were quality filtered before conducting OTU picking with usearch method against a custom Oomycete sequence database. The identified species belonged to *Phytophthora* and *Pythium* genera. The main advantage of using cox2 comes from the use of a custom database, which reduces analysis time and returns better resolution than default databases. On the other hand, PCR bias and significant heterogeneity in read counts per sample undermines downstream analysis. Most known pathogens are present both in cacao crops and in the more diverse plant communities adjacent to farms; suggesting contribution of neighboring zones to the ecology of Oomycete pathogens affecting cacao.

8. (Student Poster) Elucidating the role of MORC1 during plant immunity against *Phytophthora* spp.

Jackson N., Xu G., Ceballos V., Nam J. C., Kang H. G., Fei Z., Klessig D. F., Manosalva P.

Microrchidia (MORC) proteins are a subset of the GHKL ATPase superfamily, containing GHKL andS5 domains that form a catalytically active ATPase module. Proteins containing this GHKL ATPase motif play roles in chromatin remodeling, heat shock responses, signal transduction, and DNA mismatch repair. MORC proteins have been recently described as components involved in the RNA directed DNA methylation (RdDM) pathway and heterochromatin silencing. Previously, we reported that MORC1 regulates plant immunity against *P. infestans* and cell death in a species-specific manner behaving as a positive regulator in *Arabidopsis* and potato and as a negative regulator in tomato and tobacco. This antagonistic phenotype has been mapped to the C-terminal region of these proteins suggesting that the MORC1 species-specific effects are mainly due to how and to whom these proteins interacts at their C-terminal regions. In this study, we found that MORC1 is also required for plant immunity against the wide-host-range oomycete, *P. cinnamomi*. We have identified two nuclear proteins that differentially interact with the C-terminal region of potato and tomatoMORC1 using yeast two-hybrid system. In addition, we shown that these two proteins are associated with defense responses against *Phytophthora* spp. MicroRNA Illumina sequencing in tomatoMORC1-silenced plants suggest that micro RNAs targeting hormone signaling, epigenetic factors, and resistance components are regulated by MORC1 in tomato. Our results suggest that MORC1 is involved in resistance against different oomycete pathogens by interacting with specific host proteins and regulating microRNAs associated with different plant immune processes.

9. (Student Poster) Phased siRNAs derived from specific gene loci regulate plant immunity

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Small RNAs are key players controlling gene expression by mediating transcriptional or posttranscriptional silencing. In plants, recognition of microbe-associated molecular patterns activates immune response, which requires transcriptional reprogramming. Small RNAs have been shown to regulate plant immunity. In particular, microRNAs that regulate phytohormone signaling indirectly influences the expression of defense-related genes. Recently, we identified a *Phytophthora* RxLR effector PSR2 that promotes infection by affecting the accumulation of secondary siRNAs derived from coding or non-coding loci with specific microRNAs as the triggers. Here, we investigated the function of

the secondary siRNAs pathway in plant defense using the *Arabidopsis thaliana-Phytophthora capsici* plant-pathosystem.
We show that mutants defective in secondary siRNAs production were also compromised in defense, indicating that they play a role in plant immunity. To identify specific secondary siRNAs species involved in regulating immune response, we analyzed differential accumulation of small RNAs during *P.capsici* infection in wild-type Arabidopsis and the PSR2 transgenic lines using Illumina sequencing. Our results showed that miR161 was induced in wild type plants during *P. capsici* infection. miR161 triggers the production of phased small interfering RNAs (phasiRNAs) specifically from gene transcripts encoding pentatricopeptide repeat (PPR) proteins. As a result, these *PPR*-derived phasiRNAs were also induced by *P. capsici* infection. Over-expression of *MIR161* led to enhanced resistance; furthermore, knock-out mutants of several *PPR* genes exhibited hypersusceptibility. These results suggest that phasiRNAs derived from *PPR* loci are positive regulators of plant immunity. Importantly, induction of miR161 or *PPR*-derived phasiRNAs were not observed in *PSR2*-expressing plants, indicating that they are the primary small RNA targets of PSR2 in Arabidopsis to promote infection.

10. A nucleus-localized effector from *Phytophthora sojae* modulates histone acetylation to suppress plant immunity

Haiyang Li, Haonan Wang, Maofeng Jing, Jinyi Zhu, Baodian Guo, Yang Wang , Yachun Lin, Han Chen, Jiawei Wu, Zhenchuan Ma, Yan Wang, Wenwu Ye, Suomeng Dong, Yuanchao Wang
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Filamentous fungi and oomycete pathogens secrete many intracellular effectors to manipulate host immunity during infection. Identification of plant targets of these effectors will help uncover the mechanisms on how effectors suppress PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) in plants. Previously we have determined that PsAvh52, an effector secreted from soybean root rot pathogen *Phytophthora sojae*, can suppress the cell death induced by both the effectors and PAMPs in *Nicotiana benthamiana*. Here, we demonstrated that PsAvh52 interacts with a soybean histone acetyltransferase (GmHAT1), a key factor manipulating epigenetic modifications. GmHAT1 localizes in plant cell cytoplasm, but it was translocalized from the cytoplasm to nucleus when co-expressed with PsAvh52. The nucleus-localized GmHAT1 regulates the expression of plant defense-related genes and attenuates plant resistance by increasing the level of histone acetylation. Taken together, these results indicate that PsAvh52 manipulates epigenetic modifications to enhance *Phytophthora sojae* colonization in soybean.

11. (Student Poster) Towards understanding the role of plant amino acid transporters in oomycete nutrient acquisition

Unnati Sonawala, Kevin Fedkenheuer, Guillaume Pilot, John McDowell
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Hyaloperonospora arabidopsidis (*Hpa*) is an oomycete that naturally causes downy mildew disease of *Arabidopsis thaliana.* Downy mildew pathogens are obligate biotrophs that extract nutrients exclusively from living plant cells. Genome data suggest that *Hpa* has lost the ability to assimilate inorganic nitrogen and sulfur; therefore, *Hpa* is likely to acquire these nutrients in organic forms, such as amino acids. Analysis of publicly available Arabidopsis transcriptome data in response to *Hpa* in immune vs. susceptible genotypes revealed upregulation of many host amino acid transporters in the susceptible accession. T-DNA knockout lines for some of these transporters display reduced growth of *Hpa* on the host; suggesting that the pathogen is directly or indirectly benefitting from plant amino acid transporters for nutrient acquisition. Moreover, double combinations of some of these mutants show additive reduction in pathogen growth. Here we will report our recent progress on exploring the underlying mechanism of interaction of *Hpa* with plant amino acid transporters.

12. (Student Poster) Cold stress at different times after planting increases damping-off of soybean caused by *Pythium sylvaticum*

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Pythium sylvaticum is an Oomycete that causes damping-off of soybeans in Iowa resulting in stand loss particularly when cool weather occurs soon after planting. The objective of this experiment was to evaluate the effect of cold stress at different times after planting on damping-off caused by *P. sylvaticum*. Soybeans were planted in 237 mL cups that were inoculated with the pathogen, placed at 18 ºC, and subjected to 96 hours of cold stress (4 ºC or 10 ºC) at different times after planting (0, 24, 48, 96, 144 and 192 hours after planting). Non-inoculated cups served as controls. We detected a significant effect of the timing of cold stress at 4 ºC and 10 ºC on emergence (P=0.0013 and P=0.0090, respectively). Emergence was lowest when cold stress at either temperature occurred 48 or 96 hours after planting. Emergence was further delayed when *P. sylvaticum* was present. These results indicate that periods of cold stress at or soon after planting increase the susceptibility of soybean to damping-off caused by *P. sylvaticum* which could contribute to reduced plant stands and decreased yields.

13. (Student Poster) New tools to identify mechanisms of nutrient transport from plants to biotrophic pathogens

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Successful plant pathogens must execute two tasks. The first task, suppression of plant immunity, has been studied intensively and is increasingly well understood. The second task, equally important but much less understood, is to acquire nutrients from the plant host. Emerging evidence suggests that plant pathogens reprogram host pathways for nutrient biosynthesis and transport. However, little is known about the mechanisms through which oomycetes extract nutrients from susceptible hosts. We have initiated a project to define how oomycetes accomplish this task. We hypothesize that the expression and localization of nutrient transporters are manipulated by pathogens to export nutrients across the plant plasma membrane to the pathogen's feeding structure. We are developing a method to identify these transporters and potential regulatory proteins. This method utilizes translating ribosome immunopurification technology (TRAP), regulated by pathogen-responsive and tissue-specific promoters, to isolate RNA. By collecting currently translating RNA from specific cell sets interacting with the pathogen, this method will enable the discovery of plant transporter genes that are manipulated by the pathogen to extract nutrients. The long-term goal of this project is to engineer these transporter genes in crops so that they can no longer be repurposed by the pathogen, effectively cutting the pathogen's supply lines and retarding disease development. In principle, this approach will provide resistance against a wide range of pathogens and would be very difficult for pathogens to overcome by co-evolution.

14. β-Rubromycin inhibits the growths of *Phytophthora infestans* and *Pythium aphanidermatum* on host plants

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To help understanding of the relevant molecular mechanisms of the cold-induced sporangium development in *Phytophthora infestans*, we screened for compounds from actinomycetes that exhibit stage specific inhibition of the sporangium development. Testing samples were prepared by mixing equal

parts of acetone and actinomycetes culture. Sporangia with or without samples were kept at 10˚C for 16 hours and observed morphological change under the microscopy. Among 700 samples tested, a strain was found to produce consistently the compound inhibiting cyst germination, named *Streptomyces* sp. no. 750. *Streptomyces* sp. no. 750 was cultivated in liquid medium at 30˚C with shaking to yield 76.4 liter culture broth. Equal volume of EtOAc was added to the culture broth to extract the active compounds, and which were applied for series of ODS column chromatography. We finally obtained 2.9 mg of the purified compound. The purified molecule was identified as β-rubromycin from the ESI-MS, ^{1}H-NMR, and ^{13}C-NMR data. Biological activities of the purified molecule and β-rubromycin on the *P. infestans* sporangium development were also identical. When sporangia were treated with β-rubromycin, only cyst germination was inhibited at low temperatures. Further analyses revealed that β-rubromycin inhibited hyphal elongation but not germination from sporangia at 25˚C in *P. infestans*. β-Rubromycin also inhibited cyst germination and oospore germination in *Pythium aphanidermatum*.

16. (Student Poster) Phenotypic diversity among representative *Phytophthora cinnamomi* isolates from California

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Phytophthora cinnamomi (*Pc*) is the causal agent of Phytophthora root rot (PRR), the most serious disease of avocado worldwide. Pagliaccia *et al*. (2013) first assessed the genetic diversity of this pathogen in California and identified 16 genotypes corresponding to two distinct clades of A2 mating type isolates. Additionally, differences in virulence were observed between these two clades after infecting avocado rootstocks with different levels of resistance to *Pc*. Additional phenotypic differences have been discovered between these specific genotypes by analyzing representative isolates from both A2 clades. These isolates were characterized for: i) mycelial growth rate, ii) optimal temperature for growth, and iii) fungicide sensitivity. More virulent *Pc* isolates of clade II had a slower radial growth and a higher optimal temperature for growth *in vitro*. The more virulent group was also significantly more sensitive to the new fungicide oxathiapiprolin (Orondis®) when tested *in vitro*. Variation in sensitivities between the individual isolates was confirmed with another new chemistry, fluopicolide (Presidio®), as well as the traditionally used mefenoxam (Ridomil Gold® SL). Fungicide efficacy among these isolates was tested under greenhouse conditions using avocado seedlings. Sixteen weeks after inoculation with the most virulent isolates, the seedlings treated with oxathiapiprolin (Orondis®) showed significantly less symptoms of PRR compared to the untreated control and all other fungicides tested. Our data suggests that there is significant phenotypic variation among the isolates of *P. cinnamomi* collected from California avocado orchards for all the traits assessed in this study.

15. A bZIP transcription factor of *Phytophthora sojae* plays a key role in organ-specific infection

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Transcriptional regulation is required for precise gene expression during infection of pathogens. The basic leucine zipper transcription factors (bZIP TFs) are widely found in eukaryotes and control gene expression during life cycle through binding the cis-element in promoters. We have previously identified an expanded family of bZIP TFs with novel variants of DNA-binding domain in *Phytophthora sojae* genome. To further learn the biological functions of the bZIP TFs and screen those essential for virulence, we knocked out several genes in *P. sojae* based on CRISPR/Cas9 system. Interestingly, one bZIP TF gene exhibited a high expression level during soybean root infection, but a low expression level during leaf infection. The gene knock-out mutants showed defective virulence to hypocotyls but normal virulence to leaves. Based on RNA-seq analysis for the wild type and mutants, we identified candidate genes that might be affected due to the knock-out of the bZIP gene. We further found that many of these

candidate genes showed gene expression patterns that were associated with the organ-specific infection in wild type *P. sojae* strains. In summary, these data reveal a transcriptional regulation mechanism in *P. sojae* for its organ-specific infection, and the bZIP TF is one of the key regulators.

17. Distinctive nuclear localization signals in the oomycete *Phytophthora sojae*

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Nuclear localization signals (NLSs) are stretches of residues that direct proteins from the cytoplasm into the nucleus in eukaryotic cells. Despite their critical roles in protein regulation, NLSs in oomycetes have not been defined. Here, we use the soybean pathogen *Phytophthora sojae* as a model to investigate these sequences in oomycetes. By establishing a reliable *in vivo* NLS assay based on confocal microscopy, we found that *P. sojae* uses NLS for translocation of proteins into the nucleus that differ from conventional well-characterized NLSs. Most tested classical NLSs and proline-tyrosine NLSs (PY-NLSs) from model organisms showed poor nuclear import activities in *P. sojae*. In comparison, functional NLS from *P. sojae* nuclear proteins resembled conventional ones but required additional basic residues. In addition, multiple weak NLSs could also work collaboratively for the efficient nuclear import of proteins. To learn more about the features of NLS-mediated transport of nuclear proteins in *P. sojae*, we further characterized in depth the nuclear import mechanism of a *P. sojae* basic leucine zipper transcription factor, PsbZIP1. We found that the nuclear translocation of PsbZIP1 was determined by a central conserved region which consists of by four distinct motifs, and was independent of conserved DNA binding residues. Three motifs showed autonomous NLS activity and the fourth motif served as a nuclear localization enhancer. Sequence comparison and mutational analysis of these nuclear localization motifs revealed a new form of bipartite NLS consisting of a triplet of basic residues followed by a tail of scattered basic amino acids.

18. A chitin synthase gene PcCHS is involved in sporangial development, zoospore production, and plant infection of *Phytophthora capsici*

Can Zhang, Weizhen Wang, Zhaolin Xue, Yongzhao Diao, Jianqiang Miao, Li Liu, Xili Liu

Phytophthora species are destructive plant pathogens that attack a wide range of ornamentally and agriculturally important plants. As opposed to fungi which contains chitin as a cell wall scaffold, oomycetes are classically described as containing no or very small amounts (< 1%) of this carbohydrate. Interestingly, putative chitin synthase genes are presented in *Phytophthora* species. In this study, we identified a chitin synthase gene PcCHS in *P. capsici*. PcCHS was strongly expressed in three asexual developmental states (sporulating hyphae, zoospores and cysts), as well as in infection stages. Silencing of PcCHS did not affect cell wall integrity but affected the sporangiophores, sporangial development, and zoospore production in *P. capsici*. Scanning electron microscopy observation revealed that PcCHS was required for the production of inclusions (spore specific components) in sporangia of *P. capsici*. The silenced transformants showed severely diminished pathogenicity, which was not only caused by an impaired ability to penetrate but also related to the decreased number of germinating cysts and the shorter length of germ tubes. Thus, our results showed that CHS gene-silenced *Phytophthora* transformants display abnormal growth during sporangial development, zoospore production, and the loss of pathogenicity in the hosts.

19. (Student Poster) Detection of fungicide-resistant *Pseudoperonospora cubensis* isolates using novel molecular tools
Aidan Shands, Emma Wallace, Timothy Miles, Lina Quesada

Pseudoperonospora cubensis, the causal agent of cucurbit downy mildew (CDM), is the most destructive pathogen to members of Cucurbitacae. Since the 2004 CDM resurgence, the preferred method of control is the application of single-site fungicides. Fungicide applications are expensive and sometimes ineffective due to pathogen-acquired resistance, often leading to crop loss. The resistance mechanisms of *P. cubensis* isolates to carboxylic acid amide (CAA) and quinone outside inhibitor (QoI) fungicides were associated with different single nucleotide polymorphisms (SNPs). The SNPs conferring CAA resistance is located in the cellulose synthase 3 gene (CesA3) at amino acid positions 1105 and 1109. The SNPs conferring QoI resistance is located in the cytochrome b gene (cyt b) at amino acid position 143. Molecular assays were developed to identify the 1105, 1109 and 143 SNPs to determine the occurrence of CAA and QoI resistance in a variety of *P. cubensis* isolates. Isolates (n=68) were collected across North Carolina from diverse hosts including cucumber, melon, squash, gourd, pumpkin and watermelon, and evaluated for fungicide resistance with the molecular assay. Isolates of *Pseudoperonospora humuli*, the causal agent of hop downy mildew and a sister species to *P. cubensis*, were also evaluated in these assays. Results from the CesA3 and cyt b sequence alignments and corresponding TaqMan® assays showed that resistance was most prevalent in cucumber *P. cubensis* isolates. This research will aid cucurbit farmers in the development of targeted fungicide programs to maximize the efficacy of fungicides, while reducing the occurrence of fungicide-resistance.

20. (Student Poster) Assessing the Impact of Low Iron Availability on Oomycete Pathogenicity
John Herlihy, Guillaume Pilot, Terri Long, John McDowell

Oomycete pathogens represent a significant threat to global food production and natural ecosystems. Novel modes of disease control could provide increases in crop yield, and reduce dangerous chemical application to food crops. Susceptibility genes (S-genes) are plant genes required by pathogens for successful colonization. Researchers have identified S-genes such as nutrient transporters or transcription factors that the pathogen utilizes or repurposes to achieve its ends. Discovery of new S-genes and their subsequent genetic manipulation may provide novel, durable disease resistance. We are particularly interested in the micronutrient iron, and the mechanisms by which oomycetes acquire it from their hosts. Low iron environments, such as calcareous soils, negatively impact plant growth, but little has been done to understand how oomycete pathogens respond to these conditions. Iron's scarcity and necessity make it a target of all pathogens, and the plant immune system has evolved to monitor iron status. We are investigating the role of plant iron metabolism and iron starvation on this early stage of oomycete pathogenesis. We hope to uncover if growth of biotrophic oomycetes requires plant S-genes. Growing plants hydroponically allows for nutrient control and cytological observation of the root pathogen *Phytophthora capsici* in iron limiting conditions. Screening T-DNA insertion mutants of known iron related genes and conducting transcriptome profiling is a first step toward understanding how oomycetes acquire iron and how host iron metabolism affects disease outcomes. We hope to uncover new S-genes, related to iron, that when removed, starve pathogens of this important nutrient.

21. (Student Poster) Validation of predicted miRNAs in *Phytophthora sojae* and *Phytophthora infestans*
Cassidy M. Madison, Manuel Ospina-Giraldo

The post-transcriptional regulatory environment in oomycetes, especially in the economically important *Phytophthora infestans* and *P. sojae*, is very poorly understood. In particular, the expression of

microRNAs (miRNAs), and their potential roles in infection, is a topic of increasing interest. In this study, we attempted to experimentally verify the existence of several miRNAs from both *P. infestans* and *P. sojae*, which had been predicted using in silico approaches. In addition, we investigated the possibility of these miRNAs playing a role in *Phytophthora* pathogenicity. The presence of two of the five miRNAs, namely psj-miR8788 (predicted based on its stem-loop structure and sequence conservation between *P. sojae*, *P. ramorum*, and *P. infestans*) and PimiRNA3, predicted in *P. infestans*, was experimentally confirmed *in vivo* by amplification using specific stemloop primers and subsequent cloning and sequencing. The predicted targets of psj-miR8788 include members of the amino acid/auxin permease family, which serve important amino acid transport functions, while the targets for PimiRNA3 include a diverse group of proteins, some of them with enzymatic activity. We also measured the transcriptional activity of psj-miR8788 during infection of soybean plants and determined that psj-miR8788 is upregulated mostly at the earlier stages of infection, peaking at 24 hours post inoculation. This suggests that psj-miR8788 is important during the biotrophic stage of infection, and perhaps plays a role in suppressing plant defense systems by modulating amino acid transport systems in the plant.

22. **(Student Poster)** ***Trichoderma harzianum*** **(Rifai) and** ***T. gamsii*** **(Samuels & Druzhinina) as biological control agents against** ***Phytophthora palmivora*** **Butler, causal agent of black pod rot of cocoa (*Theobroma cacao* L.) in coastal Ecuador**

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Trichoderma is one of the most widely used biological control agent, its efficacy is associated with secondary metabolites production and mycoparasitism due to hydrolytic enzymes secretion and its wall-degrading ability. Antagonism assays against *Phytophthora palmivora*, causal agent of black pod rot of cocoa in coastal Ecuador, were carried out using two isolates from *Trichoderma* genus (*T. harzianum* and *T. gamsii*). Confrontation assays on PDA plates between *T. harzianum*/*T. gamsii* and *Phytophthora palmivora* showed growth inhibition of the pathogen growth. *Trichoderma* antifungal *in vitro* activity was also evaluated on membranes in order to quantify the ability of the *Trichoderma* to produce metabolites with inhibitory action against *P. palmivora*. *Trichoderma* species were cultivated on PDA plates covered with a cellophane membrane and then removed to inoculate with *P. palmivora* plugs. Both secondary metabolites produced by *T. gamsii* and *T. harzianum* showed 100% of inhibition growth of the cocoa black pod rot causal agent *P. palmivora*.

23. (Student Poster) Detection of ***Lagenidium giganteum*** **in phytotelmata microbiomes**

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae, but phylogenetic analyses have consistently demonstrated that it is a close relative to plant pathogens (*Phytophthora* and *Pythium* spp.). In addition, a recent transcriptome analysis showed that *L. giganteum* expresses oomycete genes that have been associated with plant infection. These observations suggest that *L. giganteum* might have evolved from a plant pathogen to an invertebrate pathogen, and have retained the ability to establish symbiotic or pathogenic interactions with plant tissues. To test this hypothesis, a metagenomic survey of plant material was initiated. Specifically, phytotelmata collected from plant axils (Bromeliaceae) were processed for metagenomic DNA extraction, and Polymerase Chain Reactions (PCR) were performed in an effort to (i) detect *L. giganteum*, and (ii) estimate the relative abundance of *L. giganteum* compared to other oomycetes. First, the presence of oomycetes in

all sampled phytotelmata was confirmed by PCR reactions, using oomycete-specific *cox1* primers that were previously published and tested in a barcoding study. Next, the use of a *L. giganteum*-specific primer set demonstrated that the *L. giganteum* cox1 barcode can be amplified and sequenced from phytotelmata metagenomic DNA, suggesting that this oomycete is able to colonize environments that are consistent with a close relationship to both plant tissues and mosquito hosts. Finally, the phytotelmata oomycete community was profiled by high throughput sequencing (PacBio platform) of the amplified cox1 barcodes (800bp), in order to determine the relative abundance of *L. giganteum* among all oomycete species.

24. (Student Poster) Development of microsatellites and population analyses of *Phytophthora capsici* infecting vegetable crops

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Phytophthora capsici is a devastating oomycete that affects solanaceous, cucurbitaceous, fabaceous, and other crops in the United States (US) and worldwide. The release of the *P. capsici* genome allows for design of robust markers for genetic studies. We surveyed and characterized simple sequence repeats (SSRs) in the *P. capsici* transcriptome using an *in silico* approach. A total of 1,855 SSRs were identified, representing 9.36% of the total number of sequences examined. A subset of 50 SSR primers was evaluated in a diverse set of *P. capsici* isolates using agarose gels. Confirmation of polymorphism by fragment analysis revealed variations in the repeats of 11 SSR primers in 30 *P. capsici* isolates from different states, hosts, mating types, and fungicide sensitivities. *P. capsici* isolates seemed to cluster geographically by state. Preliminary phenotypic evaluations of *P. capsici* isolates from North Carolina indicates variation on fungicide sensitivity *in vitro* to mefenoxam, fluopicolide, and dimethomorph. North Carolina *P. capsici* populations contain both mating types A1 and A2, with the A2 mating type being the most predominant. The identified SSRs will facilitate the genetic characterization and complement phenotypic characterization of *P. capsici* populations, which may assist in deployment of disease management strategies.

25. (Student Poster) Population genetic structure of *Pseudoperonospora cubensis* as influenced by cucurbit host types and geography in the United States

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A global resurgence of cucurbit downy mildew (CDM) caused by the obligate oomycete, *Pseudoperonospora cubensis* was observed in the last two decades. In the United States, resurgence of CDM occurred in 2004 and caused extensive yield losses and CDM remains as one of the most important yield-limiting factor for cucurbit production to date. The population genetic structure of *P. cubensis* was examined using 93 isolates collected on diverse host types from different locations in eastern United States based on the double digest Restriction Associated DNA Sequencing (ddRADSeq) technology. Results obtained from phylogenetic and STRUCTURE analyses showed that the pathogen population in the United States is composed of two host specialized lineages and a significant subdivision of the population was detected between lineage I and lineage II isolates (F_{ST} = 0.72). Lineage II was found to be specialized on *Cucumis sativus* and lineage I was specialized on *Cucurbita* spp. and *Citrullus lanatus*. In addition, the presence of a distinct genetic cluster within lineage I, associated primarily with *C. lanatus*, was detected. The two lineages were also associated with mating types of *P. cubensis* with lineage II and I being associated with the A1 and A2 mating type, respectively. However, STRUCTURE analysis did not detect a significant subdivision of the pathogen population based on

geography. Information generated from this study may further aid in the development of diagnostic tools and more precise management strategies for CDM on different cucurbit host types.

26. The role of *Phytophthora infestans* effector *Avr1-like* in modulating plant defense

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Phytophthora infestans secretes many RXLR effectors potentially involved in infection of host plants. *Avr1-like* is one such RXLR effector that acts as a virulence factor, promoting colonization. Avr1-like is closely related to Avr1 but does not trigger R1-mediated resistance. Unlike Avr1, Avr1-like is present in all the modern European isolates tested in our study. High numbers of transcripts encoding *Avr1-like* were present throughout infection stages, suggesting that this gene is specifically induced in contact with host tissue to modulate plant defences, as seen with other known avirulence effectors such as *Avr3a* and *Avr2*. In this study we demonstrate that *Avr1-like* acts as a suppressor of RNA silencing. Using mutagenesis analysis, we found that a motif in Avr1-like that plays a major role in its suppression activity. Subcellular localization of Avr1-like in *Nicotiana benthamiana* by transient expression revealed that this RXLR effector was localized to both the nucleus and the cytoplasm.

27. Hidden diversity in the oomycete genus *Olpidiopsis* is a global threat to red algal cultivation

Yacine Badis, Tatyana Klochkova, Martina Strittmatter, Andrea Garvetto, Pedro Murùa, Craig Sanderson, Gwang Hoon Kim, Claire Gachon

Seaweed cultivation is the fastest-growing of all aquaculture sectors, with an annual growth rate of 8 % and a value in excess of $5bn. Emerging diseases are a threat to its sustainable development. The oomycete genus *Olpidiopsis* notably encompasses pathogens of red seaweeds, including the most economically damaging disease in *Pyropia* (ex-Porphyra) farms in Asia. Here we identified three new european *Olpidiopsis* species, and a Scottish variety of *O. porphyrae*, a devastating pathogen only reported in Japanese seaweed farms. Importantly, two of the new species infected *Porphyra* and *Palmaria* sp., which are subject extensive farming trials in Europe and North America. To further assess the extent of undescribed *Olpidiopsis* diversity and the threat it might pose to aquaculture in different regions, we screened targeted *Porphyra* metagenomes as well as global metagenomic barcoding campaigns. Over 700 new sequences attributable to *Olpidiopsis* were detected with a worldwide distribution. Close relatives of the Korean *O. pyropiae* are also reported for the first time in Europe and the United States. In the light of our restricted sampling, our results highlight the diversity and abundance of *Olpidiopsis* worldwide. In the context of worsening impact of *Olpidiopsis* pathogens in Asia, this worldwide distribution should be treated as a serious threat to the global seaweed industry and wild red algal populations. Our data calls for more efforts towards the documentation of these pathogens, and for adequate biosecurity measures to be developed.

28. (Student Poster) Prediction and characterization of WY-domain effectors in downy mildews

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Identification of effectors in oomycete genomes is of major interest for understanding the mechanisms of pathogenesis, for monitoring field pathogen populations, and for breeding pathogen resistant plants. Using comparative genomics and bioinformatics, we have identified candidate effectors from several economically important downy mildew species by searching for the WY domain, a conserved structural element found in *Phytophthora* effectors that has been implicated in their immune-suppressing function. Searching for the WY-domain uncovered additional effector candidates that were missed by searching for the RXLR domain alone. There is significant variation among the WY effector candidates in both sequence and domain architecture. The candidate effectors show several characteristics of pathogen effectors, including an N-terminal secretion signal, lineage specificity, and evidence of gene duplication and gene family expansion. Unexpectedly, only a minority of WY effectors contained the canonical N-terminal RXLR motif, which is a conserved feature in *Phytophthora* effectors. Functional characterization of nine of the WY domain effectors revealed three effectors that elicited an immune response on lettuce containing introgressions from wild lettuce species. None of the immune eliciting effectors contained an RXLR motif. These results suggest that there has been an evolutionary divergence in sequence motifs between genera that has important implications for effector prediction in the oomycetes.

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