Phytophthora
Molecular Genetics Network Workshop

(Harvard Square photo courtesy of Jean Ristaino)

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PROGRAM

Note: Presenters are listed in bold print

Sunday, March 13

3:00-10:00	Registration
6:00	Dinner
7:00	Keynote Lecture **John Taylor:** Molecular Natural History Department of Plant Biology and Microbiology University of California, Berkeley

Monday, March 14

7:30	Breakfast
8:45	Welcome and announcements
Session I	**Populations and Evolution**
9:00	Generation of a large set of microsatellites markers for *Phytophthora infestans* by mining sequence data. **Theo van der Lee**, Odette Mendes, Hannneke van der Schoot, Caroline Ruyter-Spira, Bas te Lintel Hekkert, Francine Govers and Gert H. J. Kema
9:20	Population History of *Phytophthora infestans* inferred from nuclear and mitochondrial DNA sequences. **J. B. Ristaino,** L. Gomez, J. Thorne, and I. Carbone
9:40	Mitochondrial genome sequencing of the haplotypes of the Irish Potato famine pathogen, *Phytophthora infestans*. Cruz Avila-Adame, Luis Gómez-Alpizar, Robin C. Buell, and **Jean B. Ristaino**
10:00	Coffee
10:20	Mitochondrial genome organization in *Phytophthora*; what *P. infestans, P. ramorum* and *P. sojae* tell us. **F.N. Martin,** USDA-ARS, Salinas, CA
10:40	Genetic structure of *Phytophthora infestans sensu lato* attacking *Solanum quitoense* in Ecuador. **R.F. Oliva**, M.G. Chacon, M.V. Galarza, J. Ochoa, W. Flier, C. Gessler, and G.A. Frobes

11:00 Microsatellite and AFLP analyses reveal *Phytophthora ramorum* to be an exotic pathogen in North America and Europe and detect a novel lineage in a US nursery. **M. Garbelotto**, K. Ivors, and P. Bonants

Session II Virulence, Avirulence, and Resistance

11:20 A gene amplification associated with the *Avr3-Avr10-Avr11* locus in *Phytophthora infestans*. **Rays H.Y. Jiang**, Jun Guo, Rob Weide, and Francine Govers

11:40 An ancestral oomycete locus contains late blight avirulence gene *avr3a*, encoding a protein that is recognised in the host cell cytoplasm. **Miles R Armstrong,** Stephen C Whisson, Leighton Pritchard, Jorunn I B Bos, Eduard Venter, Anna O Avrova, Anne P Rehmany, Sanwen Huang, Sophien Kamoun, Jim L Beynon and Paul R J Birch

12:00 Lunch

1:40 Genetic Evidence for a Role of *Phytophthora infestans* protease inhibitors in disease. **Jing Song**, Nicolas Champouret, Joe Win, Miaoying Tian, and Sophien Kamoun

2:00 Avirulence and pathogenicity genes in *Hyaloperonospora parasitica*. **Jim Beynon**, Rebecca Allen, Laura Baxter, Peter Bittner-Eddy, Mary Coates, Sharon Hall, Julia Meitz, Anne Rehmany and Laura Rose

2:20 Identification of cyst surface proteins from the fish pathogen *Saprolegnia parasitica*. **Emma Robertson** and Pieter van West

Session III Expression, Signaling, and Function

2:40 A proteomic approach to identify extracellular and cell wall proteins involved in the *Phytophthora infestans* – plant interaction. **Catherine R Bruce**, Shuang Li, Neil AR Gow and Pieter van West

3:00 Coffee

3:20 Functional screening of the tomato late blight proteome for secreted proteins reveals a novel pheromone-like protein in *Phytophthora infestans*. **B.S. Kelley**, S.-J. Lee, C.M.B. Damasceno, and J.K.C. Rose

3:40 Comparative genomics and synteny studies revealing the reservoir of secreted proteins in *Phytophthora*. **Rays H.Y. Jiang**, Brett Tyler and Francine Govers

4:00 Promoter motifs needed for inducing *Phytophthora infestans* genes during zoosporogenesis in response to cold and increased membrane rigidity. **Shuji Tani** and Howard S. Judelson

4:20 Distinct signaling pathways regulate plant cell death induced by INF1, CRN2 and PiNPP1.1 of *Phytophthora infestan*. **Edgar Huitema**, Cahid Cakir, Thirumala-Devi Kanneganti, Natalia Norero and Sophien Kamoun

4:40 Discussion: Gene Naming Conventions

6:00 Dinner

7:30 Posters/Mixer

Tuesday, March 15

7:30 Breakfast

8:45 Nominations for Network Steering Committee

9:20 Characterization of *P. infestans* Glucanase Inhibitor Proteins (GIPs) and their Interaction with Tomato Endo-beta-1,3-glucanases. **Cynthia M. B. Damasceno**, Thirumala-Devi Kanneganti, Joe Win, Sophien Kamoun, and Jocelyn K. C. Rose

9:40 Functional genomics of *Phytophthora infestans* effectors of plant disease. Thirumala-Devi Kanneganti, **Carolyn Young**, Jorunn Bos, Joe Win and Sophien Kamoun

Session IV Genomics, Data Mining, and Application

10:00 Development of an Expressed Sequence Tags resource for the pathogenic oomycete *Aphanomyces euteiches*. **Arnaud Bottin**, Christophe Jacquet, Hélène San-Clemente, Bernard Dumas, and Elodie Gaulin

10:20	Coffee
10:40	Phytophthora database: A global resource for detecting, identifying, and monitoring *Phytophthora*. **Seogchan Kang**, David. M. Geiser, Izabella Makalowska, Michael D. Coffey, Kelly L. Ivors, Frank N. Martin, and Kerry L. O'Donnell
11:00	Sequencing the *Phytophthora infestans* Genome: Preliminary Studies. Michael C. Zody, Keith O'Neill, Bob Handsaker, Elinor Karlsson, Francine Govers, Peter van de Vondervoort, Rob Weide, Stephen Whisson, Paul Birch, LiJun Ma, Bruce Birren, Jean Ristaino, William Fry, Howard Judelson, Sophien Kamoun and **Chad Nusbaum**
11:20	Network discussion: The Future
12:30	Lunch

Adjourn

Presentation Abstracts

Session I Populations and Evolution

Generation of a large set of microsatellites markers for Phytophthora infestans by mining sequence data

Theo van der Lee[1], Odette Mendes[1], Hannneke van der Schoot[1], Caroline Ruyter-Spira[1], Bas te Lintel Hekkert[1], Francine Govers[2] and Gert H. J. Kema[1]

Plant Sciences Group, [1]Plant Research International and [2]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

Microsatellite or simple-sequence repeat (SSR) markers are extremely useful in population studies particularly in diploid species. So far, however, only a limited number of informative microsatellite loci have been identified in the potato late blight pathogen *Phytophthora infestans* and none have been mapped. To identify additional polymorphic SSR loci, genomic and EST sequences were scanned for the presence of di- and trinucleotide units, repeated six or more times. We identified 333 unique SSR loci using an automated software pipeline. Primers flanking these SSRs were developed and tested on a set of ten previously characterized *P. infestans* field isolates. Of the 300 primers pairs tested, 203 pairs generated a clear fragment of the expected length. More than half of these amplified microsatellites (110) showed length differences among the different isolates. Some microsatellites seemed to be very variable in the *P. infestans* population with up to 9 different alleles detected in the ten genotypes. The most informative microsatellites are currently being mapped on the genetic linkage map of cross 71 (80029 x 88133). The genomic and EST sequences (xgi.ncgr.org/spc/; Randall et al. 2005 MPMI in press) proved to be an excellent source for new microsatellites. We anticipate that these SSR markers will be instrumental for efficient analyses of *P. infestans* populations and will facilitate integration of the various genetic datasets available for this important plant pathogen.

Population History of *Phytophthora infestans* inferred from nuclear and mitochondrial DNA sequences

J. B. Ristaino[1], L. Gomez[1], J. Thorne[2], and I. Carbone[1]

Box 7616, Dept. of Plant Pathology[1], and Dept. of Statistical Genetics[2], North Carolina State University, Raleigh, NC 27695, USA

Nuclear and mitochondrial DNA variability was used to examine the population history of *P. infestans*. DNA sequence data from three nuclear regions (Intron Ras, Ras, and β-tubulin) and two mitochondrial regions (P3 and P4) were obtained from ninety isolates from various locations including Brazil, Bolivia, Ecuador, Peru, Costa Rica, Mexico (Toluca Valley), the United States (USA) and Ireland. Population summary statistics show that the Mexican population from the presumed center of origin of *P. infestans*, harbored less nucleotide and haplotype diversity than South American populations, and was genetically differentiated from other populations, particularly at the mitochondrial loci. Coalescent-based genealogies of mitochondrial loci (*rpl14, rpl5, tRNAs, cox1*) and nuclear locus (Intron Ras+Ras) were congruent and demonstrated the existence of two lineages leading to the present day haplotypes of *P. infestans* associated with potatoes. A third lineage, associated with a group of isolates from *Solanum tetrapetalum* collected in the Andean Highlands of Ecuador was also found. In the mitochondrial genealogy, the two potato lineages corresponded to the mitochrondrial haplotypes Type I and Type II described elsewhere. The same mitochondrial haplotype was associated with different nuclear backgrounds. Haplotypes found in the Toluca Valley population were derived from only one of the two lineages in both mitochondrial and nuclear genealogies, whereas haplotypes found in South American populations (Peru and Ecuador) were derived from both lineages. Haplotypes found in USA and Ireland populations were also derived from both lineages and these populations were not genetically differentiated from Peruvian population, suggesting a common ancestry among these populations. Evidence for recombination was found for Mexican and the USA populations. *Solanum tetrapetalum* isolates were highly polymorphic within the regions analyzed and may be a new species. The results support a South American origin of *P. infestans* and will be discussed in relation of previous hypotheses regarding the geographic origin of this plant pathogen.

Mitochondrial genome sequencing of the haplotypes of the Irish Potato famine pathogen, *Phytophthora infestans*

Cruz Avila-Adame[1], Luis Gómez-Alpizar[1], Robin C. Buell[2], and Jean B. Ristaino[1]

[1]Box 7616, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA and [2]The Institute for Genomics Research, Rockville, MD, USA

The mitochondrial genome of three of the four haplotypes of the Irish Potato famine pathogen, *Phytophthora infestans*, were sequenced. The genome sizes were 37,922, 39.870 and 39,840 bp for the type Ia, IIa and IIb mitochondrial DNA (mtDNA) haplotypes, respectively. The mitochondrial genome size for the type Ib haplotype, previously sequenced was 37,957 bp. More than 90% of the genome contained coding regions and the GC content was 22.3%. Genes involved in electron transport (18), RNA-encoding genes (2), ribosomal protein genes (16) and transfer RNA genes (25) were coded in both strands and their arrangement was conserved among the haplotypes. The type I haplotypes contained 5 unique open reading frames of unknown function (ORF), and the type II haplotypes contained 12 ORF's. Polymorphisms were observed in both coding and noncoding regions but the highest variation was found in noncoding regions. The type I haplotypes (Ia and Ib) differed by only 14 polymorphic sites, whereas, the type II haplotypes (IIa and IIb) differed by 50 polymorphic sites. The largest number of polymorphic sites were found between the type IIb and Ia haplotypes (152). A large spacer flanking the genes coding for tRNA-Tyr and the small subunit RNA (rns) contained the largest number of polymorphic sites and corresponds to the region where a large indel that differentiates type II from type I haplotypes is located. The size of this region was 785 bp, 2666 and 2670 bp in type Ia, IIa and IIb haplotypes respectively. Phylogenetic and coalescent analysis revealed two lineages that corresponded to the type I and type II haplotypes. The four haplotypes were distinguished by 44 mutations. The type II haplotypes are more ancestral and evolved earlier than the type I haplotypes. More mutations were observed in the type IIb haplotypes than the other haplotypes. The Ia haplotype diverged from the Ib haplotype more recently and the mutations associated with this divergence were identified.

Mitochondrial genome organization in *Phytophthora*; what *P. infestans, P. ramorum* and *P. sojae* tell us

F.N. Martin

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Sequence analysis of the mitochondrial DNA of *P. ramorum* and *P. sojae* are byproducts of the recent effort to sequence the nuclear genomes of these species and provide an opportunity for evaluation of genome evolution by comparison with the previously published mDNA sequences of *P. infestans*. The genomes reflect molecules with a circular orientation with sizes ranging from 37,957 bp for *P. infestans* to 42,975 bp for *P. sojae*. A total of 37 genes, 19 tRNAs, and a variable number of ORFs are encoded in these genomes. While the gene order in *P. sojae* and *P. ramorum* are the same, *P. sojae* has 5 additional ORFs. Relative to these two species the gene order in *P. infestans* differs in two locations due to inversions. Unlike the other two species, there is an inverted repeat of 1,137 bp present in *P. ramorum* that includes an unidentified ORF, adjacent spacer regions, and flanking regions of specific genes. Sequence alignments of the three genomes reveals that specific regions are more variable than others. Differences in genome organization will be discussed relative to phylogeneic relationships among the species.

Genetic structure of *Phytophthora infestans sensu lato* attacking *Solanum quitoense* in Ecuador

[1]R.F. Oliva, [1]M.G. Chacon, [2]M.V. Galarza, [2]J. Ochoa, [3]W. Flier, [4]C. Gessler and [5]G.A. Frobes

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The aim of this study is to clarify the genetic structure of a novel *Phytophthora* population attacking *Solanum quitoense* in the highland tropics of Ecuador and to gain new insight into the dynamics and complexity of this important plant pathogen. Twenty-one isolates of *Phytophthora* collected from the commercial crop *Solanum quitoense* (Section *Lasiocarpa*) were characterized for metalaxyl sensitivity, mating type, *Gpi*, *Pep*, mitochondrial haplotype, RFLP (RG-57) fingerprint, and SSRs. Based on their multilocus genotype at least two groups, A and B, could be distinguished. The allele combination of each group is novel. Group A had some similarities with the US-1 lineage reported in Ecuador: *Gpi* 86/100, Ib haplotype, US-1 RFLP fingerprint, and common SSR alleles. This group has a *Pep* genotype (76/100) not present in the US-1 lineage but observed in other *Phytophthora* groups in Ecuador. The second group, B, has similarities with the multilocus genotype reported for the EC-3 lineage (*Gpi* 86/100, *Pep* 76/100 and mtDNA Ia), however, the RFLP fingerprint is different from that of any pathogen group described previously in Ecuador. One of the SSR primers did not amplify B isolates, suggesting that this group might belong to a species distinct to *P. infestans*. Genetic analysis revealed that at least, one *Phytophthora* group attacking *S. quitoense* is quite distinct from *P. infestans* populations from potato and tomato. Further analysis with Internal Transcribed Spacer (ITS) sequences should provide a better understanding of the phylogenetic relationships among *S. quitoese* groups A and B, and other closely related *Phytophthora* groups.

Microsatellite and AFLP analyses reveal *Phytophthora ramorum* to be an exotic pathogen in North America and Europe and detect a novel lineage in a US nursery

M. Garbelotto[1], K. Ivors[1], & P. Bonants[2]

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Phytophthora ramorum is one of the most destructive forest pathogens of recent discovery and unknown origin. It is thought to have been introduced both in Europe and the USA. The sequence of the entire genome of *P. ramorum* was used to identify potential microsatellite loci. A total of 14 microsatllites were analysed on approximately 100 individuals, considered representative of the geographic and host provenance for the US and the EU. Results indicated that: a)- only one genotype is present in North America, b)- only five genotypes were present among the EU isolates, , and c US and EU alleles are often alternatively fixed. By performing AFLP analyses on mitotic progeny, we were able to prove that AFLP variation will detect mitotic recombination, confirming our early hypothesis that most of the variation observed in AFLP patterns in the US was due to mitotic recombination. In light of these results, we strongly support the exotic nature of this pathogen in the West Coast of the United States, and possibly a multiple introduction or the presence of cryptic sex in the EU. Plant nurseries in the Pacific Northwest of the USA are known to have harboured both mating types of the pathogen. Here we show the discovery of an isolate carrying alleles from both US and EU lineages: multiple sequence analyses indicate this isolates is likely to be a representative of the original *P. ramorum* population.

SESSION II VIRULENCE, AVIRULENCE, AND RESISTANCE

A gene amplification associated with the *Avr3-Avr10-Avr11* locus in *Phytophthora infestans*

Rays H.Y. Jiang[1], Jun Guo[1,2], Rob Weide[1] and Francine Govers[1]

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Phytophthora infestans is a destructive oomycete pathogen causing potato late blight worldwide. Genetic analyses of potato and *P. infestans* have demonstrated that in this pathosystem, monogenic resistance mediated by resistance (*R*) genes, is based on a gene-for-gene interaction. Our aim is to clone and characterise avirulence (*Avr*) genes in *P. infestans*. We constructed high density linkage maps of two regions carrying *Avr* genes (van der Lee *et al.* 2001 Genetics 157: 949-956) and performed transcriptional profiling on F1 progeny of the mapping population. By marker landing and genome walking using cloned cDNA-AFLP fragments we could identify a candidate avirulence gene at the *Avr3-Avr10-Avr11* locus. cDNA-AFLP and RT-PCR confirmed the differential expression of the candidate gene in the different races. Southern blot analysis revealed that the gene is specifically amplified in avirulent strains. Detailed analysis is in progress.

An ancestral oomycete locus contains late blight avirulence gene *avr3a*, encoding a protein that is recognised in the host cell cytoplasm

Miles R Armstrong [1], Stephen C Whisson1, Leighton Pritchard[1], Jorunn I B Bos[2], Eduard Venter[1], Anna O Avrova[1], Anne P Rehmany[3], Sanwen Huang[4], Sophien Kamoun[2], Jim L Beynon[3] and Paul R J Birch[1]

[1]Scottish Crop Research Institute; [2] University of Ohio; [3]Horticulture Research International; [4] Wageningen University

The oomycete *Phytophthora infestans* causes late blight, the potato disease that precipitated the Irish famines in 1846 and 1847. It represents a re-emerging threat to potato production and is one of over 70 species which are arguably the most devastating pathogens of dicotyledonous plants. Nevertheless, little is known about the molecular bases of oomycete pathogenicity, or of avirulence molecules in these algae-like organisms that are perceived by host defenses. Disease resistance alleles, products of which recognise corresponding avirulence molecules in the pathogen, have been introgressed into the cultivated potato from a wild species, *Solanum demissum*, and *R1* and *R3a* have been identified. We used association genetics to identify *Avr3a*, and show that it encodes a protein that is recognised in the host cytoplasm, where it triggers *R3a*-dependent cell death. *Avr3a* resides in a region of the *P. infestans* genome that is co-linear with the locus containing avirulence gene *ATR1*^{NdWsB} in *Hyaloperonospora parasitica,* the distantly-related oomycete pathogen of Arabidopsis. Remarkably, distances between conserved genes in these avirulence loci were often similar, despite intervening genomic variation. We suggest that *Avr3a* has undergone gene duplication and an allele evading recognition by *R3a* arose under positive selection. A lack of allelic diversity in *Avr3a* provides evidence that host factors may have contributed to genetic bottlenecks in the panglobal distribution of *P. infestans*.

Genetic Evidence for a Role of *Phytophthora infestans* protease inhibitors in disease

Jing Song, Nicolas Champouret, Joe Win, Miaoying Tian, and Sophien Kamoun

Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691

The oomycete *Phytophthora infestans* causes late blight, a reemerging and ravaging disease of potato and tomato. *Phytophthora infestans* has evolved 18 extracellular protease inhibitor genes belonging to two major structural classes: (i) Kazal-like serine protease inhibitors (EPI1-14) and (ii) cystatin-like cysteine protease inhibitors (EPIC1-4). Biochemical evidence suggests that some of the protease inhibitors target apoplastic proteases from the host plant tomato. EPI1 and EPI10 inhibit and interact with the subtilisin-like protease p69B, whereas EPIC2 interacts with the papain-like cysteine protease PIP1. To complement the biochemical studies, we first carried out stable DNA transformation of protoplasts of *Phytophthora infestans* using lipofectin, polyethylene glycol and CaCl2. We used the Gateway technology to generate transformation constructs in which the gene of interest is bordered by the oomycete *ham34* promoter and terminator, followed by the bacterial resistance gene G418 (Geneticin) fused to *hsp70* promoter and terminator. Both sense and antisense constructs of *epi* genes were used. We also used RNA interference (RNAi) triggered by dsRNA to silence *epi* genes. Preliminary evidence suggests that alteration of *epi1* expression resulted in altered virulence on tomato. These studies will lead to a better understanding of the role of protease inhibitors in disease progression.

Avirulence and pathogenicity genes in *Hyaloperonospora parasitica*

Jim Beynon[1], Rebecca Allen[1], Laura Baxter[1], Peter Bittner-Eddy[1], Mary Coates[1], Sharon Hall[1], Julia Meitz[1], Anne Rehmany[1] and Laura Rose[2]

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We are working on the interaction between *H. parasitica* (downy mildew) and Arabidopsis. We have used the SSH technique to identify pathogen genes expressed *in planta* and we have identified a range of novel gene products that show no homology to current databases. We are developing the necessary genetic and genomic resources to identify pathogen gene products that interact directly with the host plant (an update on the *H. parasitica* sequencing project will be presented). We have cloned the avirulence genes *ATR1* and *ATR13*, the products of which are recognised by the plant resistance genes *RPP1* and *RPP13*, respectively. *ATR13* is a highly variable gene matching the levels of diversity seen at the *RPP13* locus. Not all diversity in *RPP13* can be explained by the interaction with *ATR13* suggesting that alternative selective pressures are in play. The ATR1/RPP1 interaction reveals a complex story where different *RPP1* genes have different *ATR1* allele recognition capabilities, different domains of ATR1 are shown to be under different evolutionary pressures and alleles of *ATR1* can be recognised by *RPP1* in transient assays but not when expressed in the pathogen.

Arabidopsis

Identification of cyst surface proteins from the fish pathogen *Saprolegnia parasitica*

Emma Robertson and Pieter van West

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Fish pathogens, such as the oomycete *Saprolegnia parasitica*, cause devastating damage and loss of profit in the fish farming industry worldwide. These pathogens cause Saprolegniosis, a disease where filamentous mycelium grows into the fins and body of freshwater fish. The disease results in slow and sluggish movement of infected fish. Severe infection may result in death of the host. Very little is known of the molecular biology of *S. parasitica*, and pathogenicity of the oomycete is undetermined. It has been proposed that spines present on the surface of secondary zoospores hook onto the scales of the fish, and aid in the initial host-pathogen interaction. This would then permit an opportunity for further invasion and colonisation. Here we present a proteomic approach to identify hook proteins and other surface proteins that may play a role in pathogenicity. Furthermore, we are optimising an RNA interference (RNAi) protocol for *S. parasitica*. Using RNAi, it should be possible to perform functional analysis of genes in *S. parasitica*. By having a better understanding of the proteins involved in the pathogenicity of *S. parasitica*, it may be possible to identify potential drug targets and develop a new route of controlling this devastating, and economically important disease.

Saprolegnias

Malachite green as a prophylactic

mosquito larvae ? guanine

cDNA

25% Est's cyst

Salt, H_2O_2 Formalin

Chitosan, Histones

SESSION III EXPRESSION, SIGNALING, AND FUNCTION

A proteomic approach to identify extracellular and cell wall proteins involved in the *Phytophthora infestans* – plant interaction

Catherine R Bruce, Shuang Li, Neil AR Gow and Pieter van West

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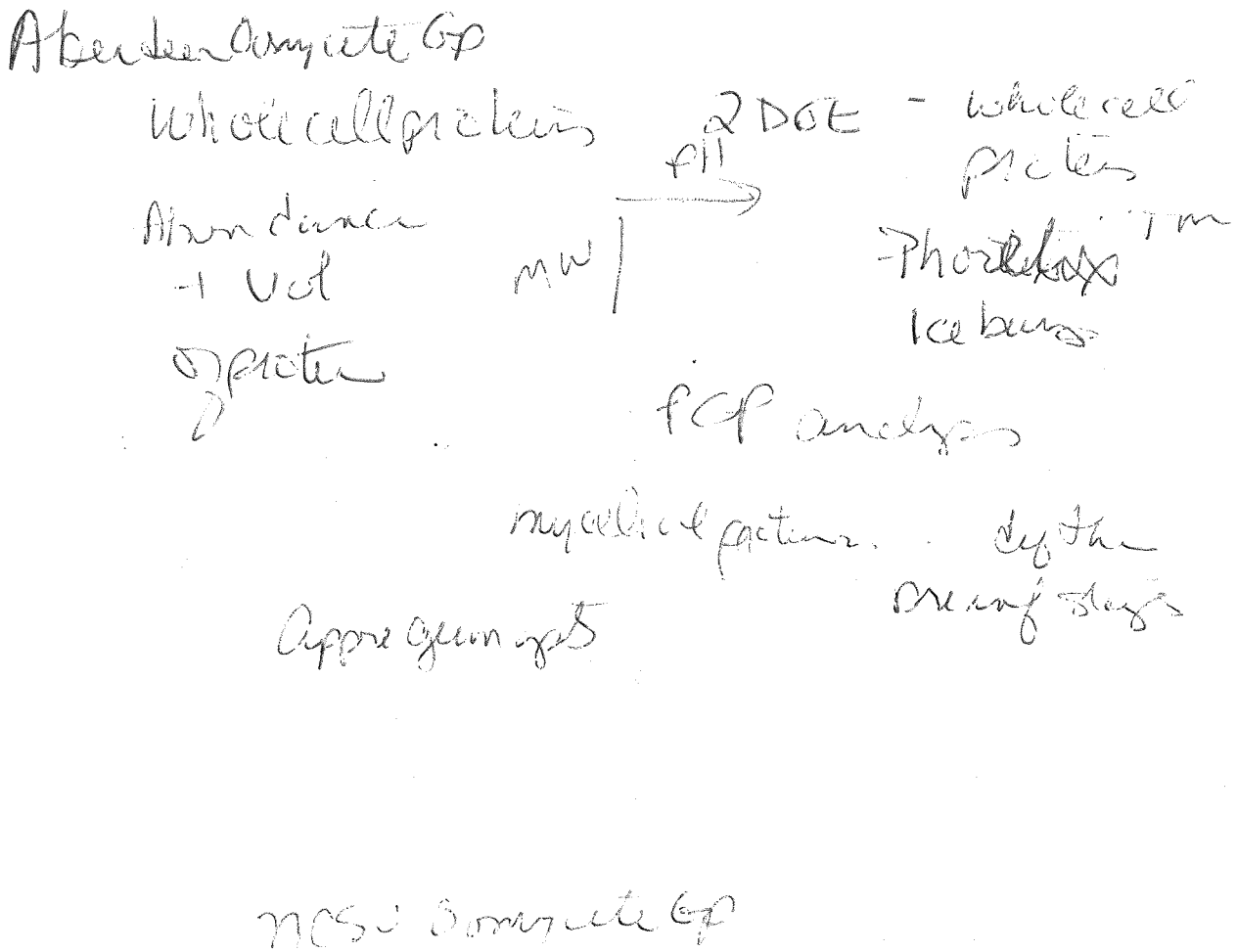
A thorough understanding of the molecular events taking place during interactions between *P. infestans* and host and non-host plants is crucial for developing new control strategies. At the plant-pathogen interface, an exchange of molecular signals is thought to determine the outcome of the interaction. We anticipate that secreted and cell wall proteins will be rich in important signalling molecules involved in disease resistance or establishing successful infection. A proteomic approach is employed to identify novel extracellular and cell wall proteins from mycelia cultured *in vitro* and from plant intercellular fluid during *P. infestans* – tomato interaction. At present we have identified over 40 protein spots. Several of these may represent effector molecules and these are characterised further. Here we present our latest results.



Functional screening of the tomato late blight proteome for secreted proteins reveals a novel pheromone-like protein in *Phytophthora infestans*

B.S. Kelley, S.-J. Lee, C.M.B. Damasceno, and J.K.C. Rose

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The plant cell wall represents the interface between plants and their pathogens. Accordingly, the expression of proteins that are secreted into the plant wall by pathogens to facilitate invasion or by the plant host for defense have a major influence on whether a plant-pathogen interaction results in resistance or susceptibility to the pathogen. A better understanding of the full complement, or proteome, of extracellular proteins that is involved in the interaction between plant pathogens and their hosts would provide important insights into critical molecular mechanisms of attack, defense and counterdefense. We used the interaction between *P. infestans* and a compatible tomato host as a model system and performed a signal sequence trap functional screen for both host- and oomycete-derived proteins that are secreted in infected leaf tissue. Genes were identified that encoded proteins with both canonical and non-canonical signal peptides for extracellular trafficking. The benefit of this screen over a computational approach was demonstrated by the identification of extracellular proteins that lack predicted signal peptides. The screen yielded twenty-four tomato genes, two of which were previously unknown. In addition, twenty-four *P. infestans* genes were identified, twenty of which have not previously been reported. The previously annotated genes included a cutinase and metallopeptidase, as well as a gene encoding a protein with similarity to a sea slug pheromone. The full-length cDNA of the pheromone-like PiE17 protein has been cloned and sequenced and orthologs have been identified in *P. sojae* and *P. ramorum*. Current efforts are directed at heterologous expression and purification of PiE17 to determine its effect on zoospore behavior or host responses as a putative extracellular signaling molecule.

Comparative genomics and synteny studies revealing the reservoir of secreted proteins in *Phytophthora*

Rays H.Y. Jiang, Brett Tyler* and Francine Govers

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Pathogenic fungi and oomycetes possess a wide range of molecules to interact with their hosts. Proteins secreted by plant pathogens are of ultimate interest because these proteins might be effector molecules that play important roles in pathogenesis. The presence of signal peptides and transmembrane domains was analyzed on all annotated genes in two sequenced *Phytophthora* genomes revealing the whole reservoir of secreted proteins. A total of 1570 and 1256 putative secreted protein genes from *P. sojae* and *P. ramorum,* respectively, were investigated for their sequence diversity, expansion of family members and genome organization. More than 80% of the secreted protein genes form gene families, and many of the families are clustered in the genome. Differences in expansion of gene families in different *Phytophthora* spp. were observed, and these expansion patterns may explain the difference in their pathogenicity. Some genes are located in genomic regions having many re-arrangements and insertions/deletions and these "hotspots are particular interesting to explore.

Promoter motifs needed for inducing *Phytophthora infestans* genes during zoosporogenesis in response to cold and increased membrane rigidity

Shuji Tani and Howard S. Judelson

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USA

Zoospores, important components of the late blight disease caused by *Phytophthora infestans*, are released from sporangia in cold water (usually <14°C). In contrast, at higher temperatures the direct emergence of germ tubes from sporangia predominates. To better understand the cold-induced signaling pathways that trigger zoosporogenesis, we are analyzing the regulation of a family of NIF (nuclear LIM-interacting interactor) genes. The three *PinifC* genes are regulated by a cold-induced inositol trisphosphate-mediated calcium signaling pathway. Using GUS reporter fusions, analyses of truncated, chimeric, and mutated *PinifC3* promoters revealed that a 7-bp sequence between positions -139 and -133 was sufficient for cold-induced transcription. This "cold box" was also detected in promoters of *PinifC1*, *PinifC2*, and orthologs from *P. sojae*. Protein(s) binding the cold box were detected by EMSA, and are being purified from nuclear extracts for sequence analysis. Furthermore, zoospore release and cold box-regulated transcription were induced by the membrane rigidifizer DMSO (mimicking a cold treatment), but inhibited by the membrane fluidizer benzyl alcohol. Our data delineate a cold signaling pathway in which sporangia perceive reduced temperatures via increased membrane rigidity, which triggers oscillations in cytosolic calcium, zoosporogenesis, and the induction of genes containing the cold box.

2003 Euk Cell 2.1376

2004 MPMI 17-330-337

DMSO - membrane rigidizer

Benzl Alcoh - membrane Fluidizer

Distinct signaling pathways regulate plant cell death induced by INF1, CRN2 and PiNPP1.1 of *Phytophthora infestans*

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Phytophthora infestans, a plant pathogenic oomycete, causes late blight on potato and tomato. Most plant species display active defense responses upon *P. infestans* infection and are fully resistant (nonhost resistance). The mechanism that underlies nonhost resistance is hypothesized to involve recognition of *P. infestans* effectors. Perception of these proteins initiates a series of discrete signaling steps, resulting in cell death and defense responses. Previously, *P. infestans* INF1, CRN2 and PiNPP1.1 were identified as necrosis inducing proteins. To expand our understanding of resistance, we investigated various aspects of INF1, CRN2 and PiNPP1.1- induced cell death. First, we used virus induced gene silencing (VIGS) to silence 35 signaling genes in *N. benthamiana* and measure their impact on elicitor induced necrosis. Second, we tested whether AVRPTOB, a suppressor of AVRPTO-induced cell death, suppresses the activity of INF1, CRN2 and PiNPP1.1. Third, we applied combinations of INF1, CRN2 and PiNPP1.1 in agroinfiltration assays to test whether cross-talk occurs between signaling pathways. Our results point to at least two distinct cell death pathways. This work is helping us dissect nonhost resistance to this economically important pathogen.

INF1 necrosis inducer, prot. - [illegible]
CRN2 - cytoplasm
PiNPP1 - apoplasm - [illegible]

Characterization of *P. infestans* Glucanase Inhibitor Proteins (GIPs) and their Interaction with Tomato Endo-beta-1,3-glucanases

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The outcomes of many plant-pathogen interactions are determined in the cell wall/apoplast at an early stage upon pathogen contact with the plant. As a defense response, plants produce hydrolytic enzymes, such as endo-beta-1,3-glucanases (EGases) and chitinases, which degrade pathogen cell wall polysaccharides and in the process generate active oligosaccharide elicitors that induce a range of general defense responses in the plant. As a counterdefense, *Phytophthora* species secrete glucanase inhibitor proteins (GIPs) into the plant apoplast, which specifically bind and inhibit the activity of plant extracellular EGases. GIPs were first reported in *P. sojae* and the cloning of the corresponding gene family demonstrated that GIPs have homology to the chymotrypsin class of serine proteases, but they do not have proteolytic activity because they lack an intact "catalytic triad". It is hypothesized that the major role of GIPs during pathogenesis is the suppression of the release of glucan elicitors from the pathogen cell wall. Despite their potential importance as suppressors of EGase-mediated defense responses, many questions remain unanswered. We are investigating: ***(1)*** the identity of the interacting GIP-EGase pairs from among their respective family members; ***(2)*** the role of GIP-EGase pairs in elicitor release and contribution of GIPs to *Phytophthora* virulence; ***(3)*** the molecular basis of GIP action and specificity. We have identified a 4-member *GIP* family (*PiGIP1-4*) in *P. infestans* by library screening and EST database search in addition to 3 potential homologs in P. *ramorum* using BLAST analysis against the *P. ramorum* genome database. This has provided a valuable phylogenetic perspective and will enable us to generate hypotheses about evolutionary relationships between GIPs and functional serine proteases. Within the *P. infestans* GIP family only *PiGIP1* encodes a protein with an intact catalytic triad. One possibility is that this represents an ancestral GIP with an unrelated function, or alternatively it may also be involved in binding EGases. We have expressed and purified a Histagged PiGIP1 from *E. coli* and are now determining whether this protein has proteolytic activity. Western blot analysis has shown that PiGIPs are present in the tomato leaf apoplast during infection. In order to identify the interacting tomato extracellular EGases, we have individually expressed PiGIPs *in planta* using an *Agrobacterium*-PVX based method (Agroinfection) and collected the apoplastic fluid for use in co-immunoprecipitation. This approach will allow us to identify individual pairs of PiGIPs-EGases that interact *in planta*, which in turn will provide insight into the specificity of these interactions.

Functional genomics of *Phytophthora infestans* effectors of plant disease

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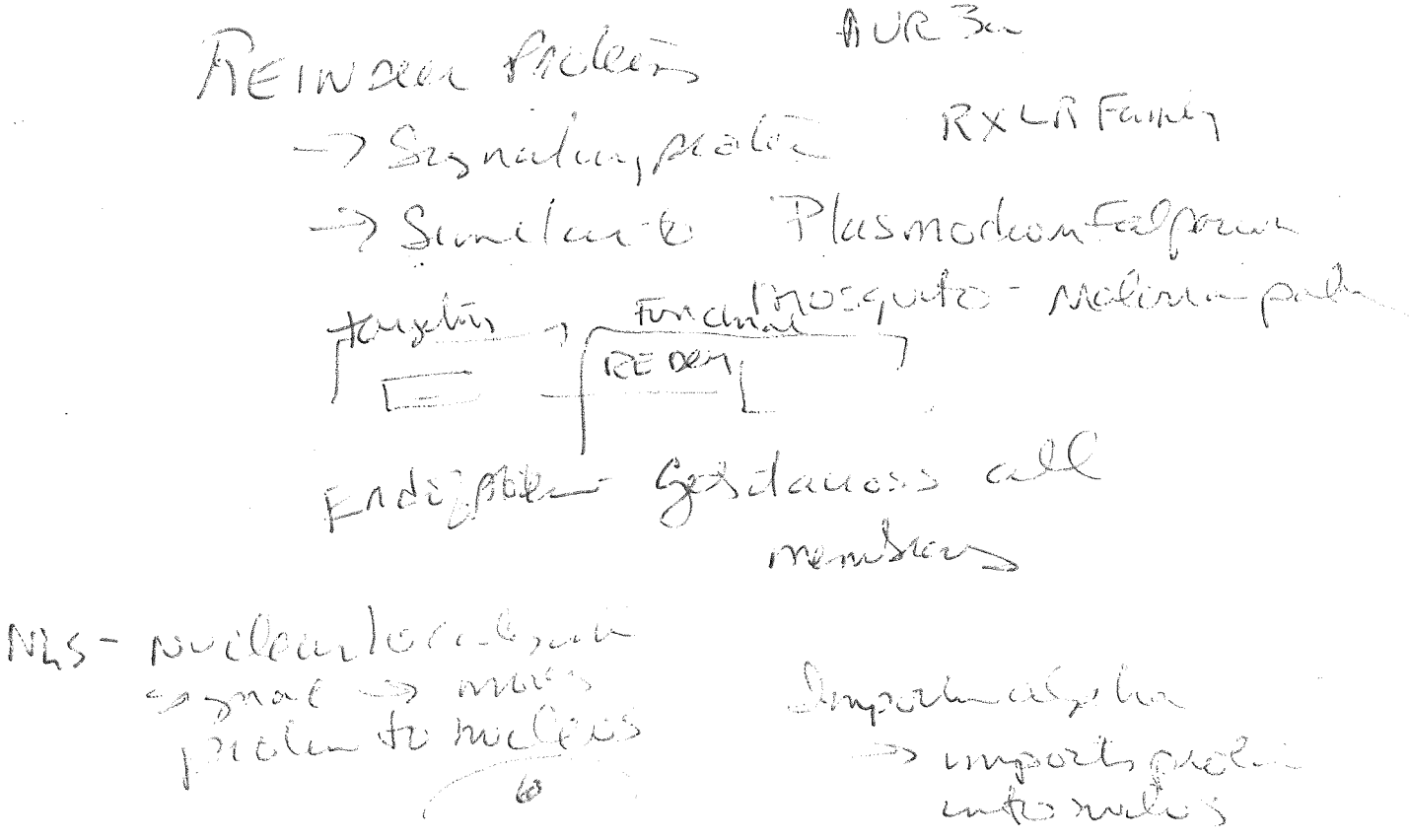
The oomycete *Phytophthora infestans* is a devastating pathogen of potato and tomato. During infection, *P. infestans* secretes a diverse array of effector proteins that manipulate host processes, leading to virulence. We hypothesize that *P. infestans* secretes two classes of effectors, one that is secreted into the plant apoplast and another that is translocated into the plant cell. Our goal is to understand the molecular mechanisms underlying *P. infestans*-plant interactions. For this purpose, computational data mining tools and robust high throughput functional assays were combined to identify candidate effector genes. We have selected ~200 full length cDNAs encoding extracellular proteins that fulfilled at least two of the following criteria: (1) up-regulated during infection; (2) conserved between *Phytophthora* and saprophytic/plant pathogenic fungi; (3) contain a nuclear localization signal; (4) possess R-dEER, a highly conserved novel amino acid motif present in virulence/avirulence proteins from three different oomycetes; (5) polymorphic between *P. infestans* strains. Single candidate genes were expressed *in planta* using virus vectors to identify genes that trigger cellular and molecular responses in plant cells. We aim to support our functional data with expression profiling and subcellular localization experiments. This research will provide the basis towards understanding *Phytophthora* effector gene function and will establish functional connections between *P. Infestans* effectors and plant processes.



SESSION IV GENOMICS, DATA MINING, AND APPLICATION

Development of an Expressed Sequence Tags resource for the pathogenic oomycete *Aphanomyces euteiches*

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The genus *Aphanomyces* from the *Saprolegniaceae* family contains parasitic species affecting either plants or animal hosts. Comparative genomics between *Aphanomyces* species, as well as between *Aphanomyces* and other Oomycetes (e.g. *Phytophthora*), should help to define the minimal gene requirements for pathogenicity on plants or animals. *Aphanomyces euteiches* is a major root pathogen of Pea and Alfalfa in Northern America, and of Pea in Europe. This homothallic microorganism forms oospores within infected tissues. After root decay, the spores can survive several years in the absence of the host. Neither effective chemicals nor resistant cultivars are available to control the disease in Pea. *A. euteiches* is also able to infect *Medicago truncatula*, a model plant in which various genomic tools will facilitate the characterization of defence and resistance mechanisms against this parasite. In order to study the molecular basis of pathogenicity in *A. euteiches*, we are developping an EST sequencing project supported by the Genoscope d'Evry (France). Starting from mycelium grown in a standard medium or from starved mycelium placed in contact with host roots, two cDNA libraries are under construction and 10.000 ESTs are going to be generated from each library. The sequences will be annotated and released on a publicly accessible web site hosted at our laboratory. Analysis of the sequences and of gene expression arrays will lead to a collection of putative pathogenicity genes. The functional characterization of these genes will involve their silencing after *Agrobacterium*-mediated transformation, which is under development at our laboratory. Progress of the project and preliminary results will be presented.

Phytophthora database: A global resource for detecting, identifying, and monitoring *Phytophthora*

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Because plant pathogens can easily migrate from one region to another, understanding pathogen diversity and dynamics in the context of global agroecosystems is crucial for developing effective regulatory and disease management strategies, and should also facilitate the monitoring of changes in pathogen communities as they respond to agricultural practices and environmental changes. The ability to accurately identify the causal agent of a disease is essential for such an understanding. However, because of the vast diversity of plant pathogens, accurate identification of pathogens is a challenge, even to experts. Morphological traits have limited resolving power in plant pathogens because such traits are not always variable between closely related species. Given these challenges, genotyping is a highly effective means for detecting and identifying new and emerging pathogens. To enhance our ability to detect, identify, monitor, and manage *Phytophthora* diseases, this newly initiated collaborative project aims to catalog genetic and phenotypic diversity of *Phytophthora* spp. in a format that can be easily accessed, utilized, and compared by the global *Phytophthora* community. This project will establish a baseline for monitoring the emergence of new/foreign pathogens and help us track the movement of *Phytophthora* via agricultural trades. Specifically, this project will accomplish the following objectives: (i) To establish a comprehensive phylogenetic framework for *Phytophthora* mainly based on the World Phytophthora Collection at UC-Riverside (ii) To develop and optimize molecular diagnostic tools based on data from the phylogenetic analysis, and (iii) To build a comprehensive genotype, phenotype, and specimen database for *Phytophthora* and supporting computational tools.

Sequencing the *Phytophthora infestans* Genome: Preliminary Studies

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Phytophthora infestans, which causes late blight of potato, is still a devastating agricultural pathogen. We have completed a pilot project to explore the *P. infestans* genome and develop an optimal strategy, in terms of both cost and quality, for generating a genome sequence. The following datasets were generated to support analysis of the genome: (1) Paired end sequences of the entire *P. infestans* BAC library (27,648 clones) (2) 0.1X sequence coverage from a plasmid library, yielding more than 40Mb of genome sequence (3) Deep shotgun coverage of five pairs of overlapping BACs from alternate haplotypes. We also included the data generated by Syngenta in our analyses. We have characterized the genome with respect to repeat content and polymorphism. Analysis of alignments of whole genome shotgun data suggests that between 50 and 70% of the genome consists of repeats. We have identified several discrete mobile elements within the sequenced BACs, accounting for over 30% of their length and ranging in size from 100 to over 14,000 bp and in frequency from ~10x to over 3000x. We find that the polymorphism rate is relatively low, with a single nucleotide polymorphism (SNP) frequency of 1/2000 and a single base insertion/deletion frequency of less than 1/7700. By alignment to the sequences of *P. sojae* and *P. ramorum* and the distantly related diatom *T. pseudonana*, we have identified several putative genes in our BAC sequences and calculated rates of divergence and both rooted and unrooted phylogenetic trees for the three *phytophthora* species. We observe an average coding nucleotide divergence greater than 15% between *P. infestans* and the other *phytophthora* species, making them somewhat more distantly related than humans and rodents.

We are working to build a dense SNP-based genetic map (to be integrated with the existing AFLP map) to anchor the genome sequence and to provide a mapping resource to the community. SNP detection is confounded by the low rate of polymorphism and extent of highly similar repeats, making the discovery of assayable markers challenging. We will present the genome sequencing strategy developed in the pilot, along with the status of our genome analysis and map construction.

POSTER ABSTRACTS

A Genomic Assessment Of Novel Multi-functional Protein Sequences In The Soybean Pathogen *Phytophthora sojae*

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The close arrangement of genes in clusters in the *Phytophthora sojae* genome http://genome.jgi-psf.org/index.html may have facilitated a trend towards greater numbers of multifunctional proteins than in other eukaryotic organisms. For example, in the shikimate pathway leading to the synthesis of aromatic amino acids, we have identified four novel multifunctional proteins in addition to the pentafunctional enzyme (ARO1) that is also present in fungi. Multifunctional proteins in the oomycetes are not limited to metabolic pathways. They also include proteins involved in nuclear trafficking, membrane proteins, and novel protein families predicted to function in signal transduction pathways. Novel multifunctional proteins are of interest for several reasons. A higher proportion of these genes may yield identifiable phenotypes by gene silencing. Multifuctional enzymes in key biosynthetic pathways are also a genetic resource that could be tapped to increase the nutritional quality of our foods. To identify the complete set of multifunctional proteins in the P. sojae genome, the predicted transcripts were downloaded from the DOE-JGI website. The sequences were subjected to BLASTx analysis against the NR database and protein motifs were identified by InterProScan software. The output was parsed into an SQL database on the Botany Beowolf Cluster. Candidate genes with multiple motifs, where the best BLASTx hit aligns to a fraction of the total length of the proteins are then selected for visual inspection on the DOE-JGI browser. Visual inspection is being used to evaluate models for inclusion in this data set.

Optimizing gene silencing and overexpression in *Phytophthora infestans* using the *PiBzp1* transcription factor, a gene required for appressorium formation and zoospore motility

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Two priorities in studies of *Phytophthora* are identifying genes important in the disease cycles of these destructive pathogens, and improving methods for manipulating genes in transformants. For example, gene silencing or overexpression provides ways to characterize the role of genes in pathogenesis and validate their utility as targets for chemical inhibitors (i.e. fungicides). To help achieve these goals, we have been studying interactors of protein kinases induced during the spore cycle of *P. infestans,* the cause of late blight of potato and tomato. An interactor of a zoosporogenesis-induced kinase was found to encode a bZIP transcription factor, and experiments to misregulate the bZIP gene *(PiBzp1)* were initiated to test its role. Silenced transformants exhibited abnormal swimming behavior, failed to make appressoria, and were nonpathogenic. Using the bZIP gene as a model, optimal methods for both gene silencing and overexpression are now being identified. Constructs containing the bZIP coding region in sense or antisense directions between the *ham34* promotor and terminator, or the entire native gene, are being evaluated. Moreover, four different transformation techniques are being tested, involving protoplasts, electroporation, particle bombardment, and *Agrobacterium*. Preliminary results show that protoplast transformation results in more efficient silencing than electroporation.

Sequence analysis of genes involved in *Phytophthora* purine biosynthetic pathway

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Purine pathway is one of the central metabolic processes in most of the organisms because its products, GMP and AMP, are important for several physiological functions: the components of DNA, RNA and some, the precursors of the energy molecule ATP and signal molecule cAMP. Some earlier studies indicated that this pathway had the unknown function in *Phytophthora sojae* virulence and sporangium, and *P. infestans* zoospores germination. Based on the genome-wide analysis, several preliminary results have been obtained: 1) All the necessary enzymes involved in this pathway have been identified in the *P. sojae, P.ramorum* and *P. infestans*, and all the enzymes are highly conserved in the *Phytophthora* species; 2) PurE, the gene coding for the NCAIR mutase, is an individual enzyme in the Prokaryote system while being a subunit of NCAIR synthetase in the Eukaryote. Surprisingly, PurE-like gene has been identified in the *Phytophthora* species and it is not supposed as a pseudogene by the EST analysis; 3) We also found a gene coding for a trifunctional protein in the *Phytophthora* species, which only appears in the insects and vertebrates. In those animals, 3 enzymes activities fall into the order of GARS-AIRS-GART or GARS-AIRS-AIRS-GART, and a monofunction GARS protein is also expressed due to the alternative splicing of the same gene. In the contrary, the order in *Phytophthora* is GARS-GART-AIRS and no alternative splicing is shown.

Now, the expression patterns of those genes are being studied and their intriguing functions are investigated by gene silencing and functional complementation strategies. Its molecular evolution is also compared. Further results will be provided in the meeting.

Abbreviations: NCAIR, Phosphoribosylcarboxyaminoimidazole; GARS, Glycinamide ribonucleotide; synthetase; AIRS, Aminoimidazole ribonucleotide synthetase; GART, Glycinamide ribonucleotide formyltransferase

Genetic characterization of Phytophthora isolates recovered during surveys for Sudden Oak Death in Tennessee

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Survey for Sudden Oak Death at nurseries (both production and retail) in Tennessee during 2004 netted more than 80 isolates of Phytophthora from a variety of hosts. *Phytophthora ramorum* was recovered from two locations. Genetic characterization is underway for the remaining isolates. Initially a field isolates genomic DNA is fingerprinted using a standard set of AFLP primers that amplify well across the genus (E+12/M+21). Isolates with identical or similar fingerprints are likely to be of the same species and representatives of a specific fingerprint type are analyzed further by sequencing the ITS region and comparing this to sequences deposited in GenBank. Of the 18 isolates analyzed thus far, four species of Phytophthora have been identified including *P. citricola*, *P. cactorum*, *P. citrophthora*, and *P. cambivora*. In addition, an unknown isolate with ITS sequence most similar (but not identical) to *P. ramorum* has been recovered. Results for all isolates will be overviewed and the implications for the nursery industry discussed.

Phytophthora sojae and *Phytophthora ramorum* contain plant-like *Mariner* transposons sequences

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Rapid genetic changes are well documented in oomycete plant pathogens including, *Phytopthora infestans* and *Phytopthora sojae*. Only recently have studies explored the presence of transposons in oomycete genomes and their role in contributing to this genetic change. *Mariner*/*Tc1* and *hAT*-like transposons have been identified in the late blight pathogen, *Phytophthora infestans*. These DNA elements transpose through a "cut and paste" mechanism catalyzed by a transposase. Mariner-like transposable elements are grouped according to spacing of the conserved DDE(D) triad of the transposase catalytic domain. Using the 43 amino acid DD39D motif from the soybean mariner element, *soymar1*, we performed *tblastn* searches to identify homologous sequences in the genome assemblies of *P. sojae* and *P. ramorum*. We identified 78 sequences, 34 in *P. ramorum* and 44 in *P. sojae*, aligned them using ClustalX and constructed a phylogenetic tree from these and other similar sequences from rice, soybean and Arabidopsis. All the sequences that we identified had 39 residues between the second D in the triad and the third conserved residue, either D or E. Thirty-three *P. ramorum* and 42 *P. sojae* sequences belong to the DD39D class. One *P. sojae* sequence belongs to the DD39E class. One from *P. ramorum* and one from *P. sojae*, were atypical and contained other residues at position 39. A phylogenetic tree generated from the transposase catalytic domain sequences of *P. sojae, P. ramorum*, rice, soybean and Arabidopsis, indicated that the Phytophthora and plant sequences fall into separate groups, but it is noteworthy that all mariner elements in these Phytophthora species are plant-like. To determine if oomycete relatives contain similar transposons, we searched the recently sequenced genome of the diatom, *Thalassiosira pseudonana*, a fellow stramenopile, for DNA elements to determine their relationship to elements from *P. sojae* and *P. ramorum*. No *P. sojae* or *P. ramorum* queries identified diatom DNA elements. Two diatom gene models were annotated as DNA elements, and these bore greater sequence identity to a Drosophila element than to plant or oomycete elements based on amino acid sequence alignment. Either the diatom lost all mariner-like elements or the oomycetes acquired them after diverging from diatoms. One possibility is that oomycetes acquired these elements via horizontal gene transfer from host plants. This analysis lays the groundwork for further study and raises interesting questions on the role of transposable elements in oomycete genome plasticity.

Analysis of microsatellite abundance and distribution in *Phytophthora sojae* and *P. ramorum*

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The first whole genome assembly of the two oomycete plant pathogens *Phytophthora sojae* (95 Mb) and *P. ramorum* (65 Mb) were examined to determine types, abundance and distribution of different simple sequence repeats (SSRs) ranging between 2 to 6 bp in motif length. We found 2,128 and 1,000 SSRs in *Ps* and *Pr*>, respectively. In general, the density of SSRs (bp per Mb) in *P. sojae* is about 1.5 times that of *Pr*. Whereas AC dinucleotide repeats appear at a higher density in *Pr*, AG and AT repeats appear at a higher density in *Ps*. Interestingly, density of most trinucleotide repeats was higher in *PS* than in *Pr*. Although *Ps* has a larger genome, the percentage of SSR loci located in coding regions is higher at 17.4% compared to *Pr* at 14.8%. Compared to other species, including *Saccharomyces cerevisiae*, repeats of length 4, 5, and 6 bp are considerably underrepresented in both *Phytophthora* genomes. Whereas in most genomes studied to date dinucleotide repeat stretches tended to be longer than other repeats, in the case of *Phytophthora* only tetranucleotide repeats were occasionally considerably longer (ACAG, ACAT, and AGAT). As expected frequency of trinucleotide repeats in exons was considerably higher when compared to di, tetra- or pentanucleotide repeats.

Cost effective, high throughput DNA isolation from Phytophthora

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The cost of isolating high quality, high molecular weight DNA can be a limiting factor for Phytophthora research. Costs include time, exposure to toxic chemicals, potentially injurious repetitive movements, pipette error and expensive kits We have developed a strategy that significantly lessens all of the above cost factors and provides consistently high quality genomic DNA from sets of 96 samples of Phytophthora mycelium. The strategy hinges upon optimization of three key factors to prepare the biological material for DNA extraction; (i) amount of starting material, (ii) lyophilization regime, and (iii) disruption regime. Once these factors have been optimized DNA is extracted from the resulting powder using homemade buffers and a 96-well glass fiber spin column plate. This system is attractive to users because there is little repetitive pipetting lessening the chance for error and injury and no toxic organic solvents such as chloroform or phenol. Financially it is attractive in that many of the liquid handling 'consumables' are re-useable and samples can be processed in a timely fashion.

Oospore isolation and germination for *Phytophthora sojae*

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Sexual recombination is a key step in the generation of functional mutants for *Phytophthora sojae* reverse genetics. Although formation of sexual oospores occurs readily on rich media such as V8 juice agar the separation of oospores from asexual material, stimulation of oospores to begin germination, and isolation of individual oospore progeny can be challenging. This is especially true when multiple individual mutants need to be taken through the sexual stage and an array of oospore progeny analyzed for each mutant. A moderately high throughput strategy has been devised that relies on a commercial hand blender and a commercially available enzyme mixture to separate oospores from asexual material and to stimulate oospore germination. An overview of this strategy will be presented.

Cloning and characterisation of the centrin gene in *Phytophthora nicotianae*

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The Ca^{2+}-sensitive, contractile protein, centrin, is associated with a number of components of the flagellar apparatus in biflagellate *Phytophthora* zoospores. Centrin aligns with microtubules to form the anterior flagellar root, is concentrated in a connecting fibre between the two basal bodies, and also occurs in the flagella. Roles in regulation of flagellar orientation and detachment have been proposed for basal body-associated centrin in other protists. In the present study, we have conducted an immunocytochemical and molecular analysis of centrin in *P. nicotianae* with the aim of increasing our understanding of its function in *Phytophthora* hyphae and spores. *P. nicotianae* was found to contain a single centrin gene which is highly expressed in sporulating hyphae, preparatory to zoospore formation. The gene is also expressed in vegetative hyphae, down-regulated in zoospores and not expressed during the early growth of germinated cysts. One and two dimensional electrophoresis and immunoblotting showed the presence of and changes in the relative abundance of two or more isoforms of the centrin protein during the asexual life cycle of *P. nicotianae*. Centrin phosphorylation and dephosphorylation is likely to be a major contributor to these post-translational modifications.

The cutinase gene family in *Phytophthora ramorum* and *P. sojae*

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The genomes of *Phytophthora ramorum* and *P. sojae* are characterized by the presence of multiple copies of a cutinase-coding gene. Four and 16 paralogs of this gene were found in *P. ramorum* and *P. sojae*, respectively. These genes encode proteins between 187 and 226 amino acids long. Protein sequence analysis from all cutinase gene copies identified in both genomes revealed a high percentage of identical amino acid residues, with sequence variation mostly occurring at the 5' end of the molecule. One sequence from *P. sojae* (PSCUT4) contains a 21-residue gap. Phylogenetic analysis conducted with Clustal W (alignment) and the Neighbor-joining method (for phylogenetic inference) indicated that *P. ramorum* and *P. sojae* cutinase genes could be classified in three major clusters (Figure 1). Three of the *P. ramorum* sequences clustered together in a group that also contains two sequences from *P. sojae*. The fourth *P. ramorum* sequence, however, is placed in a very different cluster along with three sequences from *P. sojae*. PSCUT4, the shortest and most divergent of all cutinase sequences, is also included in this cluster.

Haplotype comparison of an ultra gene dense island containing a G-protein Coupled Receptor gene in *Phytophthora infestans*

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Phytophthora infestans is a notorious oomycete pathogen causing potato late blight world wide. An ultra gene dense region of 8.9 kb containing the G-protein Coupled Receptor gene *Pigcr1* is identified as a gene island in a BAC sequence comprising 107 kb. Haplotype comparison of the surrounding 40 kb region, using the sequence of a pair of allelic BACs, revealed very little polymorphism in this part of the genome. In addition to the high gene density (on average one gene per 1,8 kb) other remarkable features are found such as alternative splicing, shared core-promoter regions and overlapping 3' UTR's. To estimate the frequency of overlapping 3' UTRs in the *P. infestans* genome a bioinformatics tool was developed and a large *P. infestans* EST contig set was analysed. Synteny studies with the *Phytophthora sojae* and *Phytophthora ramorum* genomes demonstrated that the gene island is present in all three species and that the genes in this ultra gene dense region show a highly conserved order and orientation.

A proteomic analysis of two life stages of *Phytophthora sojae* and *Phytophthora ramorum* by multidimensional protein identification technology

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The recent completion of *Phythophthora sojae* and *Phythophthora ramorum* genome sequencing allows comparative proteomic studies to be conducted. Multidimensional protein identification technology (MudPIT) was applied to two life stages (mycelium and cysts) of *P. sojae* and *P. ramorum*. Five to eight hundred proteins were confidently identified for each life stage of each of the two organisms. A significant overlap in identified proteins was observed between (i) the different life stages of each species and (ii) the same life stage of the two species. Further study of the overlapping and non-overlapping proteins may aid in understanding the differential biology of these plant pathogens. An overview of our methodology and findings will be presented.

Screening and Characterization of *Phytophthora sojae* genes differentially expressed during the early stage of soybean infection

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Suppression subtractive hybridization (SSH) was utilized to identify the *Phytophthora sojae* genes differentially expressed in the early stage of *P. sojae* and soybean compatible interaction. In this study, mycelium of *P. sojae* isolate Pm-2 was harvested for RNA extraction 0, 6, 12 and 24h after contacted with the leaves of soybean. Two populations of cDNA were created from RNA extracted from mixed mycelium that contacted with the soybean for 6, 12, and 24h and from uninoculated one. Total 507 cDNA clones exhibited differentially expression between inoculated and uninoculated mycelium was selected for sequencing and 130 unigenes were obtained. RT-PCR and virtual northern were utilized to characterize the expression of these genes. Forty-six *P. sojae* genes strongly up-regulated during infection including the genes encoding Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), NAPDH dehydrogenase, annexin, creatine kinase and lots of proteins related to energy metabolism and protein synthesis. Eighty-four down-regulated unigenes including the genes involved in metabolism (23), cellular process and signal (11), information storage and processing (9) and 41 unclassified genes. Candidate genes identified in this screening were subjected to functional analysis by yeast genetic complementation and *P. sojae* antisense inhibition. Amongst the up-regulated genes, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis, was strongly induced by H2O2, and after transferred to yeast GAPDH deleted mutant, *P. sojae* GAPDH could complement the yeast cell H2O2 tolerance. In addition, the role of two down-regulated genes, vacuolar H+-ATPase (V-ATPase) and plasma membrane H+-ATPase (P-ATPase) in *P. sojae* nutrient absorption were elucidated intensively.

Dissection of the transcriptome of *Phytophthora sojae* under oxidative stress: Source of pathogenicity factors?

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One of the hallmarks of plant defense responses against pathogens is the generation of reactive oxygen species (ROS) in a process referred to as oxidative burst. Extensive studies have been conducted to investigate the role of ROS in plant defense; however, little has been done to determine the role of the pathogen under oxidative stress. Recent studies have shown that the production of ROS in *Botrytis cinerea,* a necrotroph, leads to increased levels of ROS production in the plant, which causes death of plant tissue. Subsequently, increase in fungal growth is observed. Alternatively, the biotrophic fungus, *Claviceps purpurea*, produces scavenging enzymes to protect itself from oxidative stress. Studies have shown that some key genes such as catalase and the bZIP transcription factor identified when pathogens are under oxidative stress contribute to pathogenicity. *Phytophthora sojae* is a hemibiotroph, which causes root rot to soybean resulting in billions of dollars in losses to farmers annually. Responses of either the pathogen or the plant at the point of interaction (interactome) are not well studied. Genes differentially expressed under oxidative stress by *P. sojae* may have a role in the modulation of host defense reactions. We used the technology of microarray, which helps to look at a global expression of genes under a particular condition to study the transcriptome of *P. sojae* under oxidative stress. *P. sojae* mycelium was grown *in vitro* and subjected to 0.3mM cumene hydroperoxide. Samples were taken at six time points viz. 0, 5 mins , 15 mins, 30 mins, 1hr and 2hrs. Results from the data generated from the microarray will be presented.

Analysis of calcium- and NAPDH oxidase-mediated signal pathway in tobacco cell apoptosis induced by PB90 from *Phytophthora boehmeriae*

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The hypersensitive response (HR) is a form of cell death associated with plant resistance to pathogen infection. We previously demonstrated that PB90, a novel protein elicitor secreted by *Phytophthora boehmeriae*, an important plant pathogen that causes serious boll rot of cotton in China, induces hypersensitive cell death (HCD) and systemic acquired resistance in non-host plants. However, elucidation of the biochemical mechanism in the PB-induced HR signaling pathway is still unknown. In this report, we elucidate that PB90-induced HCD shares some features of the apoptotic cell death process in animals and investigate the signaling events that mediate hypersensitive cell death. We observed that suspension-cultured cell of *Nicotiana tabacum* BY-2 treated by PB90 results in rapid cell death, extracelluar medium alkalization and changes in ascrobate (ASC) and glutathione (GSH) metabolisms. The cell death was shown to an apoptotic cell death characterized by DNA fragmentation and 180-200 bp laddering of tobacco DNA and positive reaction of nuclei by TUNEL assay, which suggested that PB90-induced HCD is an apoptosis. The apoptotic cell death is tightly regulated by a signaling cascade involving Ca2+ and NADPH oxidase events. We observed that a Ca2+ specific chelator, EGTA and an inhibitor of mammalian neutrophil plasma membrane NADPH oxidase, DPI could block PB90-nduced tobacco apoptotic cell death and inhibit PB90-induced changes in ASC and GSH metabolisms, moreover, cycloheximide, a protein synthesis inhibitor, could also suppress cell death and ROS production induced by PB90. The results indicated that Ca2+, NADPH oxidase and protein synthesis are intimately involved in the signal transduction processes leading to PB90-induced apoptosis. In addition, involvement of apoptosis inhibitor, NtBI-1 in apoptosis results from increasing of expression of NtBI-1 induced by PB90.

Construction of BAC-based Physical Map of *Phytophthora sojae* with Multiplexed Fluorescence-labeled Fingerprinting

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Clones from 2 BAC libraries of *Phytophthora sojae* were digested with 6 enzymes and labeled simultaneously with 4 fluorescent dyes. Contigs were assembled using FPC software. A total of 8,681 clones were assembled into 257 contigs, 2,822 clones are singletons. Of these contigs, 11 contigs contain 100-200 clones; 46 contain 50-99 clones; 61 contain 35-49 clones; 84 contain 10-24 clones and 55 contain 3-9 clones. The largest contig contains 112 clones. The total unique bands of all the contigs is 34,742, with the average bands per clone being 49.3 and the total length of the contigs being 98,667 kb.