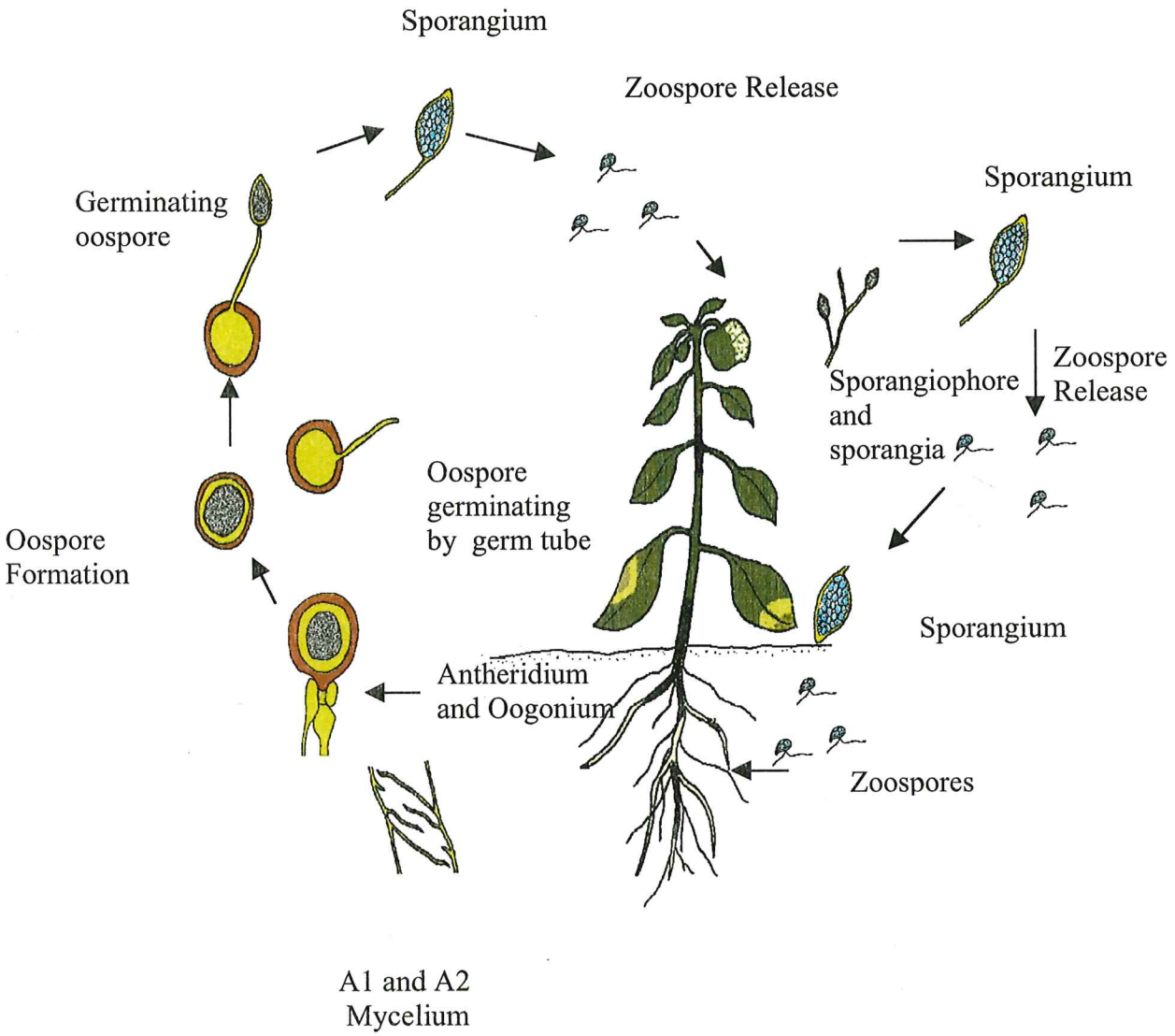
Francine

Phytophthora Molecular Genetics Workshop
Milwaukee 2002



Life History of *Phytophthora infestans*

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Phytophthora **Molecular Genetics Workshop Aug.1-2, 2002**
Hyatt Regency Milwaukee
333 West Kilbourn Ave.
Milwaukee, Wi 53203,USA
414 276 1234

Thursday August 1, 2002

7: 30 Breakfast and Registration: Gilpatrick Room

8: 40 Introductory remarks: Paul Morris.

8:45 ***The Phytophthora*** **Molecular Genetics Network**
Brett Tyler. Virginia Bioinformatics Institute. Blacksburg, VA24060.

9:00 Regulators of Spore Pathways in ***Phytophthora infestans***
Howard Judelson UC Riverside, Riverside, Ca.

9:30 ***Phytophthora*** **research at Wageningen University**
Wilco Ligterink, Laboratory of Phytopathology, Wageningen University, The Netherlands

10:00 Coffee break

10:20 Functional Genomics of Extracellular Proteins of ***Phytophthora infestans*** Trudy Torto, OSU-OARDC, Wooster

10:50 ***Phytophthora*** **Genome Update**
Brett Tyler. Virginia Bioinformatics Institute. Blacksburg, VA24060

11:20 Moderated discussion: Future directions of ***P. infestans*** **and** ***P. sojae*** **genomics**. Mark Gijzen, Agriculture Canada, London.

12:00 Buffet lunch: Regency C
Put up Posters

Session 2: Population Genetics.
Moderator: Sophien Kamoun

1:00 **A Window to the Past: Tracking Historic Migrations of the Irish Potato Famine Pathogen using herbarium specimens.** Jean Ristaino, NC State University, Raleigh, NC 27695.

1:30 **Rapid discovery of Simple Nucleotide Polymorphisms in *Phytophthora capsici* using Expressed Sequence Tags from mixed isolates.** Lamour, K. H., Michigan State University, East Lansing, MI

1:50 **Molecular Detection of *Phytophthora ramorum*, the causal agent of Sudden Oak Death.** Kelly Ivors, Department of ESPM-ES, University of California, Berkeley, CA 94720.

2:10 ***Phytophthora infestans* genome contains transposon elements of both *SINE* and *TC1/* mariner families.** Svetlana Bagirova, Moscow State University, Moscow, Russia.

2:30 **Characterization of *Phytophthora capsici* populations in Massachusetts and Development of PCR-based protocols for rapid identification of Isolates.** Bess Dicklow Department of Plant Pathology, Univ. of Massachusetts, Amherst, MA

2:45 **Genomic resources : The World *Phytophthora* Collection.** Mike Coffey, UC Riverside, Riverside, CA.

3:00 **Coffee Break**

3:20 **Molecular characterization of *pimar1*, a *mariner*-like element in the *P. infestans* genome .** William Morgan, College of Wooster, Wooster, OH.

3:40 **Moderated discussion: How can community resources foster new research in population genetics.** Sophien Kamoun, OSU –OARDC, Wooster, OH

4:00-600 Poster Session and Social: Regency C.

7:00 **Dinner: Regency C**

8:00 **Potato Structural and Functional Genomics: From ESTs to Microarrays** Robin Buell, The Institute for Genetic Research, Rockville, MD.

Friday 1 August, 2002

Session 3: Resistance and Avirulence Genes:
Moderator: Brett Tyler

8:00 **Continental Breakfast : Gilpatrick Room**

9:00 ***Rps1*-k is comprised of multiple functional *Phytophthora* resistance genes.** Madan Bhattacharyya, Agronomy Department, Iowa State University.

9:30 **Mapping of the Avr1a gene in *Phytophtora sojae,*** Mark Gijzen, Agriculture Canada.

10:00 **Nonhost resistance of Arabidopsis to the oomycete pathogen *Phytophthora infestans*.** Edgar Huitema, OSU-OARDC, Wooster, OH.

10:20 **Coffee Break**

Session 4: Functional Genomics.
Moderator: James English

10:40 **Use of Combinatorial Phage Display for Selection of Peptides that Affect Receptor-Mediated Development in *Phytophthora*.** James English Univ. of Missouri, Columbia, MO

11:10 **Necrosis inducing peptides of *P. infestans*.** Dinah Qutob, Agriculture Canada, London ON.

11:30 ***Phytophthora infestans* 1,3-β-glucanases: The genes and their regulation** Adelle McLeod, Cornell, University, Ithaca, NY

11:50 **Extracellular protease inhibitors: possible weapons of *Phytophthora infestans* involved in overcoming plant defenses.** Miaoying Tian, OSU_OARDC, Wooster, OH

1210 **Moderated Discussion: James English.**

12:30 **Wrap up**

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Key Note Address

Potato Structural and Functional Genomics: From ESTs to Microarrays

Robin C. Buell, The Institute for Genomic Research , 9712 Medical Center Drive Rockville, MD 20850 rbuell@tigr.org

The culivated potato, Solanum tuberosum, shares similar biology with other members of the Solanaceae family yet also has unique features such asmodified stems termed stolons that develop into edible tubers. Potato is also susceptible to Phytophthora infestans, an oomycete pathogen and the causal agent of potato late blight, which is a devastating disease for potato growers. To better understand the potato genome, we have undertaken a survey of the potato transcriptome using Expressed Sequence Tags (ESTs) from an array of tissue sources. A total of 61,940 ESTs were generated from aerial tissues, below-ground tissues, and tissues challenged with the late blight pathogen. Clustering and assembly of these ESTs resulted in a total of 19,892 unique sequences with 8,741 tentative consensus sequences and 11,151 singleton ESTs. We were able to correlate changes in gene expression with two major physiological events in potato biology: resistance to the late blight pathogen and tuberization. As a complement to the generation of potato ESTs, we have fabricated a 10,000-clone potato microarray. We have established that more than ample cross-hybridization is present among eight Solanaceae species and potato such that these arrays can be utilized for gene expression profiling in heterologous systems.

Session 1 : Genomics.
Gilpatrick Room
Moderator : Mark Gijzen

The Phytophthora Molecular Genetics Network

Brett Tyler. Virginia Bioinformatics Institute. Blacksburg, VA24060 bmtyler@vt.edu.

In December 2001 I received a $500,000 grant from NSF on behalf of the *Phytophthora* community to fund a Research Collaboration Network in *Phytophthora* molecular genetics. The grant funds a variety of activities, including an annual workshop such as this one, a web site, collection and distribution of research resources such as strains, plasmids and microarrays, and short traineeships. The grant does not fund any research activities directly. The annual workshop will be held in conjunction with a larger meeting such as the American Phytopathological Society meeting or the Asilomar Fungal Genetics meeting. Funding for the workshop includes 16 travel awards. The web site will include a large variety of material such as address lists, protocols, lists of resources available from the *Phytophthora* Resources Center, links of interest, news and a technical help page. The *Phytophthora* Resources Center (PRC) will collect and distribute libraries, clones, vectors and commonly *Phytophthora* strains at a subsidized cost. The PRC will also organize the creation of glass slide microarrays containing a selection of genes of interest to the community, and the distribution of those slides at a subsidized cost. My goal is to have the web site and PRC substantially in place by the end of 2002. The traineeships (8 weeks) are intended to provide practical experience in new techniques, as well establishment of new personal contacts, for all members of the community, but particularly those new to the field or who wish to expanded their activities in *Phytophthora* molecular genetics. 4-7 traineeships will be available each year. The operation of the Network will be guided by a steering committee of six *Phytophthora* researchers broadly representing the community. Subcommittees of the steering committee will select the network trainees and the travel awards.

Regulators of Spore Pathways in *Phytophthora infestans*

Howard S. Judelson*, Audrey Ah Fong, Flavio Blanco, Cristina Cvitanich, Anna-Liisa Fabritius, Kyoung Su Kim, and Shuji Tani University of California, Riverside, CA 92521 USA.
judelson@ucrac1.ucr.edu

The sexual and asexual spores made by *Phytophthora* spp. are important elements of their life and disease cycles. To better understand these pathways, relevant genes were identified using approaches such as subtraction cloning, differential hybridization to cDNA arrays, high-throughput complementation of yeast mutants, and the mining of expressed sequence tag (EST) databases. About 100 genes that show strong induction during sexual sporulation, asexual sporulation, or indirect germination were identified. Current work focuses on proteins that participate in signal transduction and transcriptional or translational regulation. Some of these include a protein kinase specific to zoosporogenesis, a phosphatase specific to asexual sporulation, and a translational regulator specific to sexual and asexual sporulation. Two general questions are being addressed: what is the function of each protein, and what regulates the transcription of each gene? Function is being tested using genetic and biochemical approaches, such as yeast two-hybrid studies. To understand what regulates transcription, the promoters of several genes were fused to the GUS reporter gene, yielding tissue-specific patterns of expression in transformants; using these promoter, deletion analysis and binding assays are identifying the binding sites of development-specific transcription factors. Also, signal transduction pathways participating in the regulation of genes have been dissected using biochemical inhibitors.

Phytophthora research at Wageningen University

Wilco Ligterink, Wubei Dong, Rays H.Y. Jiang, Guo Jun, Maita Latijnhouwers, André van 't Slot, Peter van de Vondervoort, Rob Weide, Francine Govers
Laboratory of Phytopathology, Wageningen University, The Netherlands. wilco.ligterink@wur.nl

The oomycete *Phytophthora infestans* is the causal agent of the notorious potato late blight disease. Insight in the biology and pathology of oomycetes is rather limited and therefore, pinpointing effective and specific targets for control in *P. infestans* is not yet possible. To get to know the secrets of *P. infestans* requires a major input in unraveling the signal transduction pathways and the genetic networks that underlie the synthesis of pathogenicity factors and avirulence components. In our research group, we focus on (i) G-protein mediated signal transduction in *P. infestans* and the role of these pathways in pathogenicity, (ii) elicitors, in particular elicitins, and their role in inducing defence responses in Solanaceous plants, (iii) characterisation of race-specific avirulence genes and their role in the gene-for-gene interaction, (iv) comparative genomics in the genus *Phytophthora* and (v) optimisation of DNA transformation procedures and gene silencing.
An overview of the current status of the research will be presented and a few topics will be discussed in depth.

Phytophthora Genome Update

Brett Tyler. Virginia Bioinformatics Institute. Blacksburg, VA24060.

The *Phytophthora* Genome Initiative was founded in 1997 with the long term goal of fostering the development of genome resources for *Phytophthora* species. With the arrival of federal funding for microbial genomics and the drop in sequencing costs, the development of research resources for *Phytophthora* has moved a ahead rapidly. In the 2000 we received funding from USDA for 33,000 *P. sojae* ESTs and 11,000 from *P. infestans*, to expand upon the 2000-3000 *P. sojae* and *P. infestans* ESTs already in the public domain. So far about 16,000 new *P. sojae* ESTs have been produced from existing and new mycelial, zoospore and infected-plant libraries. An additional 17,000 are currently under production. These sequences are currently publicly available at https://xgi.ncgr.org/pgc/. By the end of 2002, the sequences will also be available at www.vbi.vt.edu. More recently, funding has be awarded to create cDNA microarrays containing a set of 1000 sequences chosen by the *Phytophthora* community, and a larger set of 10,000 P. sojae sequences. Efforts are currently underway to obtain funding for sequencing the genomes of several *Phytophthora* species including *P. sojae*, *P. ramorum* and *P. infestans*

Session 2; Population Genetics
Moderator: Sophien Kamoun

A Window to the Past: Tracking Historic Migrations of the Irish Potato Famine Pathogen using herbarium specimens.

Jean B. Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695. Email: Jean_Ristaino@ncsu.edu

The plant pathogen *Phytophthora infestans* causes late blight, a devastating disease on potato that led to the Irish potato famine during 1845-1847. The disease is considered a reemerging problem and still causes major epidemics on both potato and tomato crops worldwide. Theories on the source of initial inoculum for 19th century potato late blight epidemics and research that is underway with mitochondrial and nuclear gene sequences and genealogies to examine the genetic structure of *P. infestans* populations from historic and modern day epidemics will be described. The dispersal of the pathogen in relation to the relative importance of human-mediated transport of inoculum and alternative hosts for *P. infestans* in the 19th century will also be discussed. Our data indicate that some of the oldest herbarium samples from potato late blight epidemics from the US, Britain, Ireland and France were infected with the Ia mtDNA haplotype of *P. infestans* and not the Ib mtDNA haplotype as previously believed. Population genomic studies are planned to improve genotype diagnostics, help enhance forecasting systems and disease management strategies for this devastating pathogen.

Rapid discovery of Simple Nucleotide Polymorphisms in *Phytophthora capsici* using Expressed Sequence Tags from mixed isolates.

Lamour, K. H., Torto, T., Hausbeck, M. K., and Kamoun, S. , Department of Plant Pathology, Michigan State University, East Lansing, Michigan 48824.

We devised a strategy to rapidly identify single nucleotide polymorphisms (SNP's) from expressed sequence tag (EST) data. A pooled cDNA library was constructed using ten *Phytophthora capsici* isolates recovered from various locations and hosts in the United States. The isolates differ based on sensitivity to mefenoxam, mating type, and represent a diverse set based on amplified fragment length polymorphism (AFLP) markers. 1152 expressed sequence tags (EST) were sequenced. Seventy-four percent (N = 854) of the EST's passed quality controls and were assembled using Sequencher and phrap. Of these 528 ESTs were present in a single copy, whereas the remaining 326 assembled into 104 contigs. Of these contigs, 64 were found to contain a total of 284 single nucleotide polymorphisms (SNP's). The SNPs encoded by twenty cDNA fragments were then confirmed by PCR amplification and DNA sequencing of the original isolates plus two additional isolates. *Phytophthora capsici* is known to be actively sexual in the United States and migration appears to be infrequent. SNP markers provide a powerful tool for investigating the population structure of *P. capsici* as well as investigating phenotype-genotype associations.

Molecular Detection of *Phytophthora ramorum*, the causal agent of Sudden Oak Death

Kelly Ivors(1), Katy Hayden(1), Matteo Garbelotto(1), and David Rizzo(2). (1) Department of ESPM-ES, University of California, Berkeley, CA 94720; (2) Department of Plant Pathology, University of California, Davis, CA 95616.

Phytophthora ramorum is a newly described plant pathogen that has been confirmed to cause a deadly canker disease of *Lithocarpus densiflora*, *Quercus agrifolia*, *Q. kellogii* and *Q. parvula* var. *shrevei* in California and Oregon. Diagnosis of *P. ramorum* was initially attempted via direct isolation from symptomatic plant tissue. However, *Phytophthora* species are often difficult to culture from plants, which can lead to false-negative isolations and misdiagnosis of infected plant material. We have developed four specific PCR primers based on sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) of *P. ramorum*. The first set of primers, Phyto1 and Phyto4, was designed to amplify a 687 bp fragment of the ribosomal operon including portions of the ITS1 and ITS2, and the entire 5.8S rDNA region. The second set of primers, Phyto2 and Phyto3, was designed to amplify a 291 bp portion of the ITS2 region, fully nested within the Phyto1-Phyto4 amplicon. This nested PCR approach was constructed to amplify *P. ramorum* DNA directly from plant tissue. SYBR-green mediated Real Time PCR and DNA melt curve profiles were used to confirm that the 291 bp amplicon was the target *P. ramorum* fragment. In the case of new hosts or newly detected geographic areas, a full sequence of the Phyto1-Phyto4 amplicon was also generated. Using these taxon specific PCR primers, it was determined that *Phytophthora ramorum* is not restricted to oaks, but has a host range including at least 11 families and 18 plant species.

Phytophthora infestans genome contains transposon elements of both *SINE* and *TC1/mariner* families.

Bagirova S. F. Moscow State University, Moscow, Russia . slana@sbagirova.home.bio.msu.ru and bagirova@herba.msu.ru

Transposable-like elements were identified by PCR amplification from *Phytophthora infestans* genomic DNA with specific primers. The primers flanked internal fragments of *SINE*-like elements (including RNA polymerase III promoter) or *TC1/mariner*-like elements (including putative transposase gene). PCR products were isolated and sequenced. The elements were found to share significant structural similarities with transposable elements of *short interspersed elements (SINE)* or *Tc1/mariner* families. The elements could constitute tools for modification of the genome, might be good clad markers and their analysis is alternative approach to molecular phylogenetics

Characterization of *Phytophthora capsici* populations in Massachusetts and Development of PCR-based protocols for rapid identification of Isolates.

Bess Dicklow Department of Plant Pathology, Univ. of Massachusetts, Amherst, MA 01003. mbdicklo@pltpath.umass.edu

Phytophthora blight of cucurbits caused by *Phytophthora capsici* (Leonian) is a devastating disease of pumpkin, squash, cucumber and watermelon. Symptoms include seedling damping-off, root and crown rot, tip blight, stem lesions, foliar blight, leaf spots and fruit rot (13). *P. capsici* also infects pepper, tomato, and eggplant, although the host specificity of isolates is unclear (20). No effective management strategy has been identified. A better understanding of the dispersal and survival of the causal agent is critical to minimize further spread and necessary for the development of satisfactory management programs. Possible mechanisms of dispersal of *P. capsici* include wind driven storms, infested seed, infected transplants, or infested soil. The importance of seed-borne inoculum to the survival and dispersal of *P. capsici* is not known. How the fungus overwinters in New England under field conditions has not been determined, but is predicted to be as oospores. Genetic variation within and among populations of *P. capsici* can answer questions about population structure, relatedness of populations, routes of travel to sites, and age of infestations. Specific traits to be analyzed include mating types, phenylamide fungicide resistance, host specificity of isolates, and DNA polymorphisms. Answers to these questions about the biology of the pathogen will contribute to the development of effective management strategies for growers in the Northeast.

Molecular characterization of *pimar1*, a *mariner*-like element in the *P. infestans* genome. William Morgan[1], Sophien Kamoun[2] [1] College of Wooster; [2]Ohio State University - OARDC, Wooster, OH, USA

Transposable elements of the *Tc1-mariner* family transpose via a DNA intermediate and exhibit extensive homology in the catalytic domain of the encoded transposase enzyme. EST sequencing initially identified an element in the *P. infestans* genome that exhibits significant homology to *soymar1,* a mariner-like transposable element present in the soybean genome. We named the *P. infestans* element *pimar1* and have extensively characterized its structure, contribution to rapid variation in *P. infestans*, and possible evolutionary origin. Screening of a *P. infestans* genomic library with the *pimar1* cDNA identified two distinct copies of a 1.7 kilobase (kb) element. One of these was bounded by inverted terminal repeats (ITRs) and a TA direct repeat, as expected for a member of the *Tc1/mariner* family. As seen with other *mariner*-like elements, the genomic elements possess multiple inactivating mutations in the transposase gene. To identify additional *pimar1* elements in the *P. infestans* genome, we performed PCR using an ITR primer. In addition to the expected 1.7 kb product, a 0.7 kb fragment was also amplified. Subsequent analyses indicated that the 0.7 kb fragment corresponds to internally deleted elements. Southern (DNA) blot analysis using various *pimar1* probes revealed just the two 1.7 kb elements and multiple copies of the 0.7 kb elements in the *P. infestans* genome. Most of the detected restriction fragments are common to all *P. infestans* isolates analyzed, while others are unique to a particular isolate(s). Southern blot analysis of a parent strain and four transgenic derivatives detected no variation in the location and number of *pimar1* elements. This lack of variation among the transgenic derivatives, the apparent absence of a functional *pimar1* transposase gene, and the low number of elements in all strains examined argue that *pimar1* transposition is not a major contributor to the rapid rate of phenotypic variation characteristic of *P. infestans.* To elucidate the evolutionary origin of *pimar1*, we conducted a phylogenetic analysis after correcting the frame-shift and missense mutations. This analysis indicates that *pimar1* belongs to the recently identified DD39D subfamily. This monophyletic group consists exclusively of plant *mariner*-like elements, including *soymar1*, suggesting the possibility of horizontal transfer between this plant pathogen and a host.

Session 3 Resistance and Avirulence Genes

Moderator: Brett Tyler

Rps1-k is comprised of multiple functional _Phytophthora_ resistance genes.
Madan K. Bhattacharyya, Agronomy Department, Iowa State University.

Soybean [*Glycine max* L. (Merrill)] suffers yield loss valued about 0.12 billion dollars annually from the root and stem rot disease caused by *Phytophthora sojae*. *Rps1*-k, the most widely used monogenic resistance gene, has been providing stable resistance in cultivars grown in the North-Central United States. We have applied a positional cloning strategy for isolating this gene. Previously we identified AFLP markers that encompass the *Rps1*-k gene in a 0.13 cM interval. The *Rps1*-k gene is mapped to a recombination-suppressed region. We have mapped this gene to a locus physically spanned by 3 overlapping bacterial artificial chromosomes (BAC). Through sequencing and fingerprint analyses, ten coiled coil-NBS-LRR-type genes were identified from these three BAC clones. Three of these genes were expressed in stable transgenic soybean plants. Surprisingly all three genes have been shown to confer resistance against *P. sojae* race 4 in transgenic plants. These paralogous sequences share very high identity at both nucleic acid and amino acid levels. In fact two paralogs are identical at the nucleic acid level. We speculate that unequal crossing over may be the main mechanism of gene duplication in the *Rps1*-k region.

Map-based cloning of the *Avr1a* locus from *Phytophthora sojae*. T. MacGregor[1], D. Qutob[1], Y. Cui[1], B. Tyler[2], M. Bhattacharyya[3], and M. Gijzen[1]. [1] *Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON N5V 4T3, Canada;* [2] *Department of Plant Pathology, University of California, Davis, CA 95616, U.S.A.;* [3] *G303 Agronomy Hall, Iowa State University, Ames, IA 50011-1010, U.S.A.* gijzenm@agr.gc.ca

Phytophthora sojae is a soil-borne pathogen that causes root rot of soybean. Host resistance (*Rps*) genes and pathogen avirulence (*Avr*) genes control race-cultivar specificity in this interaction. To isolate the *Avr1a* locus, a high-resolution genetic map of the region was constructed using DNA markers. A marker co-segregating with *Avr1a* was used to screen a bacterial artificial chromosome (BAC) library, and a contiguous region of eight BAC clones covering 170 kb and encompassing *Avr1a* was assembled. Genetic markers derived from the BAC contig were screened in 486 F2 progeny, and an average genetic to physical distance ratio of 391 kb per cM was estimated for the region. Two overlapping BAC clones of 74 and 51 kb were fully sequenced, resulting in a contig of 119 kb. This sequence was examined for predicted open reading frames and matches to expressed sequence tags to produce an annotated map of the locus, and candidate genes have been identified for functional analysis.

Nonhost Resistance Of Arabidopsis To The Oomycete Pathogen *Phytophthora Infestans*

Edgar Huitema and Sophien Kamoun *Ohio State University-OARDC, Wooster 44691 OH, USA*
huitema.1@osu.edu

Phytophthora infestans, an oomycete plant pathogen, causes the late blight disease on some solanaceous plants causing considerable economic losses. Accumulating biochemical and genetic data indicate that plant pathogenic oomycetes have acquired pathogenicity independently from fungi and therefore may interact differently with their hosts. A plant species can be fully resistant to all known strains of a pathogen. This type of resistance, which is also called nonhost resistance, is thought to operate through a variety of mechanisms, depending on the organism involved. Despite our increased understanding of plant pathogen interactions on the molecular level, little progress towards dissecting nonhost resistance has been made. In order to understand nonhost resistance to oomycete pathogens, we initiated an integrated approach towards establishing and exploiting the *P. infestans- Arabidopsis* interaction as a key model. *Arabidopsis* plants were assayed for resistance to a range of *P. infestans* isolates and other *Phytophthora* species. Inoculation of *Arabidopsis* rosette leaves with zoospores of *P. infestans*, resulted in penetration of epidermal cells, followed by active defense responses including the Hypersensitive Response (HR). Induction of *BGL2* gene expression, a marker for the salicylic acid (SA) mediated defense pathway, was recorded. Interestingly, *Arabidopsis* mutant genotypes compromised in their SA mediated defense response, did not display a susceptible phenotype, indicating the presence of alternative defense pathways. Screenings of known defense response mutants for enhanced susceptibility using kinetic quantitative (realtime) PCR experiments, *Arabidopsis* gene expression profiling experiments and *Phytophthora infestans* elicitor identification in *Arabidopsis* are the components of a comprehensive approach aimed at enhancing our understanding of nonhost resistance to oomycetes.

Session 4. Functional Genomics.
Moderator :James English

Use of Combinatorial Phage Display for Selection of Peptides that Affect Receptor-Mediated Development in *Phytophthora*. James English, Plant Pathology, University of Missouri,Columbia ,MO. EnglishJ@missouri.edu

Many *Phytophthora* species develop through a well-defined sequence of life stages under the direction of environmental signals. Understanding of signal recognition and subsequent development has been hindered by the difficulty of identifying cell-surface receptors that mediate the transfer of environmental information to the cell. In studies with Phytophthora capsici we have developed methods that successfully identify cell-surface receptors based on affinity-selection of synthetic peptide ligands from combinatorial phage libraries.

Phage-display libraries provide a vast array of random peptides from which to select ligands. Phage-displayed random peptide libraries are mixtures of filamentous phage clones, each of which displays a single, random foreign peptide sequence on the virion surface. The displayed peptide is physically linked with its coding DNA in the phage genome. Thus, the peptide can be easily and quickly identified and transferred to other vectors or display systems.

We recently used phage-display methods to select peptides with affinity for surface molecules of P. capsici zoospores. By this whole-cell screening approach, we identified affinity-selected peptides that induce zoospore encystment whether in phage-displayed format or free in solution. The pattern of zoospore encystment induced by varying concentrations of peptides suggests their roles as encystment agonists. An obvious advantage of the phage display ligand selection system is that prior knowledge of receptor structure or functional properties was not required. We are using affinity-selected peptides to define zoospore surface molecules involved in encystment and components of the signaling pathway. We are also applying phage library screening to identify peptide ligands that mimic environmental signals relevant to other stages of development.

Identification and characterization of a *Phytophthora sojae* necrosis inducing protein expressed during the necrotrophic phase of disease development. D. Qutob[1], S. Kamoun[2] and M. Gijzen[1], *Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Center[1], 1391 Sandford Street, London, ON, Canada, N5V 4T3; Department of Plant Pathology, The Ohio State University-OARDC[2], Wooster, OH, USA, 44691.*

Phytophthora sojae is an aggressive hemibiotroph that causes stem and root rot of soybean. In this pathosystem, host cell death may occur as an early response to restrict pathogen spread, or late in infection as consequence of the disease process. To identify pathogen factors that induce plant cell death, *P. sojae* secretory proteins were assayed for expression analysis in *Nicotiana bentemiana* using a heterologous potato virus X (PVX) based expression system. This led to the identification of a 25.6 kD *P. sojae* necrosis-inducing protein (PsojNIP). The activity of PsojNIP was compared to proteins of similar sequence from *Fusarium oxysporum*, *Bacillus halodurans* and *Streptomyces coelicolor* by PVX expression in *N. bentemiana* and by transient expression in soybean tissues by particle bombardment. Results showed that PsojNIP was the most powerful inducer of plant cell death in both assays, followed by the *F. oxysporum* protein, NEP1. Similar proteins from *B. halodurans* and *S. coelicolor* varied in their necrosis inducing activity, despite that the *B. halodurans* protein shares greater sequence identity with PsojNIP than does *F. oxysporum* NEP1. Furthermore, analysis of *PsojNIP* by RNA blot hybridization and by RT-PCR showed that this gene is expressed during the necrotrophic phase of growth but not in zoospores nor early infection stages. We suggest that PsojNIP is an elicitor-toxin that facilitates the colonization of host tissues during the necrotrophic growth phase.

***Phytophthora infestans* 1,3-β-glucanases: The genes and their regulation**

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Three putative exo-1,3-ß-glucanase genes (*Piexo1*, *Piexo2*, *Piexo3*), one endo-1,3-ß-glucanase gene (*Piendo1*) and one endo-1,3;1,4-ß-glucanse gene (*Piendo2)* were cloned and characterized from the oomycete *Phytophthora infestans*. The genes have no introns and are single copy, except for *Piendo1* that is present as two copies. The 1,3-ß-glucanases are differentially expressed in the different developmental stages of the pathogen, and are also expressed during infection of potatoes. Nucleotide sequence analysis of the glucanase promoter regions identified a conserved motif with 56-81% similarity to a 16-nt core sequence hypothesized to be the transcriptional start point in oomycete genes. Primer extension analyses of *Piexo1, Piexo3* and *Piendo1* showed that their transcriptional start points mapped to nucleotides within the 16-nt core sequence. Subsequent analysis of the 16-nt core sequence of the glucanases and several published oomycete promoters revealed the presence of a putative core promoter element, the initiator (Inr), that directs accurate transcription in the absence of a TATA box. The putative oomycete Inr has a consensus sequence (YCAYT, where Y is a pyrimidine) similar to the Inr consensus sequence of metazoans (YYAN(T/A)N), *Drosophila* (TCA(G/T)T(T/C)) and early branching protists (TCAY(T/A). The function of the putative oomycete Inr was further investigated by creating deletions and mutations in a vector containing the 993 bp promoter region of *Piexo1* fused to the ß-glucuronidase (GUS) gene. Transient expression analyses of these vectors showed that (1) deletion of the 16nt core sequence results in complete abolishment of promoter activity, (2) three or four consecutive base mutations within the Inr results in complete abolishment of promoter activity and (3) spacing of the Inr relative to the ATG start codon is important for promoter activity. These results show that the Inr identified in *Piexo1* is an authentic core promoter element that is also present in all known oomycete promoters.

Extracellular protease inhibitors: possible weapons of *Phytophthora infestans* involved in overcoming plant defenses. Miaoying Tian and Sophien Kamoun (kamoun.1@osu.edu). Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH44691, USA. The plant intercellular space is the battlefront where complex *P.infestans*-plant interaction events occur. The extracellular proteins secreted by *P.infestans* are likely to play important roles during this interaction. In our lab, we developed the program PexFinder V1.0 to identify cDNAs encoding extracellular proteins (Torto *et al*, 2001). Data mining from *Phytophthora* genome databases identified nine genes encoding putative extracellular protease inhibitors (EPIs). Each of them has one or two predicted domains of Kazal-type serine protease inhibitors that are found in animal protease inhibitors. Domain alignment analyses demonstrated that these genes form a diverse gene family that may exhibit different substrate specificity when interacting with serine proteases. The genes do not show similarity to protease inhibitors from plant pathogens or plants. Therefore, the epi genes might reveal novel molecular mechanisms in plant-microbe interactions. To confirm the protease inhibition function predicted by bioinformatics, we expressed the fused proteins of three *epi* genes with the FLAG epitope tag in *E.coli* and purified the fused proteins by affinity chromatography. The result of *in vitro* protease inhibition assays showed that EPI1 highly inhibits Subtilisin Carlsberg, but not trypsin and chymotrypsin. The *epi* genes are likely to be involved in overcoming plant defenses by inhibiting plant serine proteases because there are at least 15 subtilisin-like extracellular proteases in tomato (Meichtry *et al*, 1999), among which two were reported to be pathogenesis-related proteins involved in plant defenses (Jorda *et al*, 1999).

POSTER PRESENTATIONS

Engineered Plants as Biosensors

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The possibility of using green fluorescent protein (GFP) as a biosensor for a real time expression marker to monitor plant pathogens has been demonstrated in tobacco and Arabidopsis plants. The plants were transformed with two different binary vectors containing the nucleotide sequences of pathogen-inducible promoters 1) pathogenesis-related protein1 (PR1) fused with a gene encoding GFP and 2) glucanase1 (GN1) fused with a gene encoding GFP. The confirmation of the presence of the transgene was carried out using polymerase chain reaction (PCR) and Southern blot analysis. The plants were inoculated with different pathogens, including *Phytophthora infestans, Peronospora tabacina, Macrophomina phaseolina*, and were treated with chemical activators that have been reported to induce systemic acquired resistance (SAR), including salicylic acid (SA) and its analogue, benzothiadiazole (BTH). The expression of GFP after treatment was detected under UV/blue light and Western blot analysis. These results suggest that this approach of using GFP as a self-reporting marker is a useful tool to track pathogens, including *Phytophthora* sp.

Genetic Structure of *Phytophthora infestans* populations from Costa Rica. L. Gomez, A.C. Café-Filho and J.B. Ristaino. NC State University. jean_ristaino@ncsu.edu

Phytophthora infestans causes late blight and is the most devastating disease of potato worldwide. In Costa Rica, ca. 3000 ha of potato are grown every year mostly under agro-climatic conditions conducive for the disease. In recent years, disease management has become increasingly difficult and could be associated with changes in the population structure of this pathogen. Forty-one isolates of *P. infestans*, including three isolates from wild *Solanum*, were analyzed from two geographically distinct potato growing regions of Costa Rica to examine the genetic diversity and structure of pathogen populations using mating type analysis, allozyme genotyping with *Glucose-6-phosphate isomerase* (*Gpi*) and *Peptidase* (*Pep*), mitochondrial DNA (mtDNA) haplotyping, and DNA fingerprinting with probe RG-57. All isolates tested thus far were of the A1 mating type, *Gpi* 100/100, *Pep* 94/100, and the Ia mtDNA haplotype. No variation among tested isolates was observed for RG57 DNA fingerprint. Isolates from wild *Solanum* species showed the same characteristics as isolates from commercial potato fields.

***PDR5*-like transporters in the soybean pathogen *Phytophtora sojae*.** Mary S. Connolly Kena Allsion, Anita, Zhao, and Paul F. Morris, Department of Biolgoical Sciences, Bowling Green State University, Bowling Green OH 43403.
A family of proteins termed **A**TP **B**inding **C**assette (ABC) proteins are found in all organisms and are responsible for the active transport of a wide variety of compounds including ions, phospholipids, sterols, organic acids, and xenobiotics. In plant pathogens, ABC transporters may contribute to biological fitness by controlling lipid asymmetry of membranes, providing protection against plant phytoalexins, or delivering toxins to host tissues.
The primary focus of our interest is the Pleiotropic Drug Resistance family (*PDR5*) of ABC transporters in the soybean pathogen. *Phytophthora sojae. PsABC1* the first member of this sub-cluster to be cloned from *P. sojae*, encodes a 1310 aa protein that is constitutively expressed at high levels by the free swimming zoospores stage and is the only *PDR5*-like transporter expressed by this stage. This transporter is **not** expressed by hyphae grown on V8 plates. Nor does it appear to be expressed during infection of soybean tissues. Progress in cloning elated members of the *PDR5* family is described, and a strategy to assign function to individual members is outlined.

A mating-induced gene of *Phytophthora infestans* encodes an elicitor-like protein with proline-rich repeats. Anna-Liisa Fabritius and Howard S. Judelson, Department of Plant Pathology, University of California, Riverside, CA 92521 USA.
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We have cloned several genes from the late blight pathogen, *Phytophthora infestans,* that are specifically expressed during mating. The predicted protein products of about two-thirds of the genes have similarity to previously characterized sequences. One gene, named M-81, encodes an unusual protein with two distinct domains. These are a N-terminal region resembling a previously identified glycoprotein elicitor produced by *P. megasperma*, and a C-terminal region encoding more than 90 repeats of a proline-rich sequence. The features of the protein and its timing of expression suggested that it may serve a role in the adhesion of mating structures or the development of the oospore wall. The length of the proline-rich region varied substantially within *P. infestans,* apparently resulting in *M-81* transcripts ranging from 4 .0 to 5.1 kb in different isolates. Genes similar to the elicitor- and repeat domains of *M-81* were detected in other species of *Phytophthora*, including a small family of highly diverged sequences in *P. infestans.* At least some members of this family were tightly clustered with M-81 in the genome, but not all showed mating-specific patterns of expression. Further studies of *M-81* should help reveal the steps involved in mating and explain the origins of elicitor genes within plant pathogens

The mitochondrial DNA haplotype of *Phytophthora infestans* in 19th century herbarium specimens. K. J. MAY and J. B. Ristaino. NC State University, Raleigh, NC 27695. kjmay2@unity.ncsu.edu

PCR and DNA sequencing was used to identify the mitochondrial DNA (mtDNA) haplotype present in 19th and 20th century herbarium specimens infected with *P. infestans*. DNA was extracted from lesions in dried leaves from 187 specimens from 7 herbaria. A 100 bp fragment of rDNA was successfully amplified from 87% of the leaves using the PINF/HERB1 primers indicating they were infected with *P. infestans*. Primers P3F1/R1/P3F2/R3 and primers P4F2/P4R3 amplify variable regions in the *Cox*1 and *Rpl*5 genes that contain *EcoR*1 sites that distinguishes type I from type II haplotypes. The mtDNA primers P2F4/R4 amplify a variable region in the *Nad*4 gene that distinguishes Ib haplotypes from Ia, IIa, and IIb haplotypes. The sequence and *EcoR*1 digestion data from these three regions of the mitochondrial genome indicate that all samples for which sequence data was obtained were infected with the Ia haplotype of *P. infestans* with the exception of one specimen from Ecuador that was infected by a Ib haplotype, and one from Nicaragua that was infected by a type II haplotype. Our data indicate that some of the oldest samples from the US, Britain, Ireland and France were infected with the Ia haplotype of *P. infestans* and that this is the predominant haplotype in the oldest historic specimens.

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