Phytophthora Molecular Genetics Network Workshop

Asilomar Conference Grounds, Pacific Grove, CA
March 16-18, 2003

Sequence data, Virulence Mechanisms, New Disease Threats…..

This workshop supported by:

NSF Research Coordination Networks in Biological Sciences.

Syngenta

Pioneer Hi-Bred International Inc.

***Phytophthora* Molecular Genetics Network**
Asilomar Conference Grounds, Pacific Grove, California
March 16-18, 2003

Short Schedule:

Sunday , March 16,

3:00-10:00 PM Registration, **Administration**
6:00 Dinner, **CROCKER HALL**

Monday , March 17

7:30-8:30 Breakfast, **Crocker Hall**

9:00-12:00 pm Session 1, **KILN**

12:00-1:30pm Lunch, **CROCKER HALL**

1:30-5:00 pm Session 2, **KILN**

6:00-700 DINNER, **CROCKER HALL.**

7:30-10:00 Poster session, mixer, **FIRELIGHT FORUM**

TUESDAY, **March 18,**

7:30-8:30 Breakfast, **Crocker Hall**

KILN
9:00- 9:40 **Keynote Address**: Dr. Jeffrey Boore,
9:40-10:00 Discussion

10:00-10:15 Coffee Break

10:15-10:45 Brett Tyler : *Phytophthora* EST project
10:45-11:00 Li-Jun Ma : A proposal to sequence *P. infestans.*
11:00-12:30 **Community activities and directions**

12:30 –1:00 Lunch, **CROCKER HALL**

Scientific Program

Monday March 17,

Session Chair Chris Smart.

9:00 *Phytophthora ramorum***:** between myth and reality
Matteo Garbelotto, UC Extension Specialist, Adjunct Assistant Professor, 338 Hilgard Hall University of California, Berkeley, Berkeley, CA, 9472C

9:40 –10:00 Discussion

10:00-10:20 Coffee Break

Avirulence: Mark Gijzen (Chair)

10:20 - Cloning of avirulence genes from *P. infestans*.. Miles Armstrong, Scottish Crop Research Institute, Invergowrie, Dundee, N5V 4T3,

10:40 Transcriptional profiling in *Phytophthora infestans* using cDNA-AFLP. Rays H.Y. Jiang, Wubei Dong, Maita Latijnhouwers, Jun Guo, and Francine Govers. Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands.

11:00 Responses of potato to infection by *Phytophthora infestans.* Paul Birch, Scottish Crop Research Institute, Invergowrie, Dundee, N5V 4T3,

11: 20 A comparative analysis of *Avr1a* genomic region in two different *Phytophthora sojae* race types D. Qutob, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, Canada. N5V 4T3,

11:40 Race shifts in *Phytophthora sojae* and implications in management. A.E. Dorrance, Dept. of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691.

12:00-1:00 Lunch, **CROCKER HALL**

SESSION Chair: Bill Fry

1:30 Species identification and population analysis of *Pythium* species using AFLP markers. Carla D. Garzon, David M. Geiser and Gary W. Moorman. Dept. Plant Pathology, Penn State University. cdg143@psu.edu

1:50 Stage specific gene expression in *P. infestans*. , Steve Whisson - Scottish Crop Research Institute, Invergowrie, Dundee, N5V 4T3,

2:10 A proteomic approach to identify secreted proteins from *Phytophthora infestans* relevant to the host-pathogen interaction Shuang Li[1], Pieter van West[1], Sophien Kamoun[2], Neil A.R. Gow[1]

2:30 A Data Mining Strategy to Identify *in planta* Induced Genes from the Oomycete Pathogen *Phytophthora infestans*. Luis da Cunha, Edgar Huitema, Miaoying Tian and Sophien Kamoun. Department of Plant Pathology, The Ohio State University - OARDC, Wooster, OH

2:50 Coffee Break

Session Chair : Francine Govers

3:10 Promoter analyses in *Phytophthora infestans* A. McLeod, C.D. Smart and W.E. Fry, Department of Plant Pathology, Cornell University, Ithaca NY

3:30 Structure-function relationship studies on the CBEL glycoprotein of *Phytophthora parasitica* var. *nicotianae*, by PVX expression in *N. benthamiana.*
E. Gaulin[1,2], T. Torto[2], Y. Martinez[1] M. Khatib[1], A. Bottin[1], M.T. Esquerré-Tugayé[1], S. Kamoun[2], and M. Rickauer[11]Pôle de Biotechnologie Végétale UMR5546, B.P.17 Auzeville, 31326 Castanet-Tolosan, FRANCE[2] Dept of Plant Pathology, OARDC, 1681 Madison Avenue, Wooster, OH

3:50 An extracellular protease inhibitor from *Phytophthora infestans* targets tomato serine proteases: a counter-defense mechanism? Miaoying Tian, Sophien Kamoun Department of Plant Pathology, The Ohio State University, Wooster, OH44691, USA tian.16@osu.edu

4:10 "Spore pathways in Phytophthora". Howard Judelson, University of California, Riverside.

4:25 PiCDC14 expression is specific to asexual sporulation inthe oomycete *Phytophthora infestans*. Audrey Ah Fong and Howard Judelson, University of California, Riverside.

4:40. Signaling pathways and transcription during zoosporogenesis in *Phytophthora infestans*. Shuji Tani and Howard Judelson, University of California, Riverside.

4:55 A protein kinase of *Phytophthora infestans* induced during zoosporogenesis has a novel structure. Flavio Blanco and Howard Judelson, University of California, Riverside.

Dinner: 5:30 **CROCKER HALL**

7:30-10:00 Poster Session and Mixer **FIRELIGHT FORUM**

TUESDAY MARCH 18

7:30-8:30 Breakfast, **Crocker hall**

9:00- 9:40 **Keynote Address**: The *Phytophthora* sequencing project. Dr. Jeffrey Boore, Joint Genome Institute, Walnut Creek CA
9:40-10:00 Discussion

10:00-10:15 Coffee Break

10:15-10:45 The *Phytophthora* EST project, Brett Tyler ,Virginia Bioinformatics.
10:45-11:00 A proposal to sequence *P. infestans*. .Li-Jun Ma, Whitehead Institute /MIT Center for Genome Research, 320 Charles Street Cambridge MA 02141-2023
11:00-12:30 Microarrays et al. Future directions for the PMGN, Brett Tyler, Virginia Bioinformatics.

12:30 –1:00 Lunch, **CROCKER HALL**

Abstracts of Talks.
March 17:
9:00 am KILN

***Phytophthora ramorum*: between myth and reality.** Matteo Garbelotto UC Extension Specialist, Adjunct Assistant Professor, 338 Hilgard Hall University of California, Berkeley, Berkeley, CA, 9472C matteo@nature.berkeley.edu
P. ramorum causes a highly contagious disease named sudden oak death. Since its discovery 8 years ago, significant infestations have been found in twelve counties in California and one area of Oregon. I will address how the public has responded to the emergence of a new threat to the California forests. A summary of our knowledge on this disease will be presented with particular emphasis on the comparative analysis of molecular and DNA data with phenotypic and ecological traits. A final discussion will be presented on the new role played by aerial *Phytophthora* species in the California forests. At least five species are found on leaves of trees in the state forest regions. Typical lesions from different plant species can be seen on this website. http://cemarin.ucdavis.edu/symptoms.html.

10:20
Cloning avirulence genes from *Phytophthora infestans*
Miles Armstrong, Anna Avrova, Steve Whisson, Paul Birch
Plant Pathogen Interactions, Scottish Crop Research Institute, Invergowrie, Dundee, DD25DA, UK. marmst@scri.sari.ac.uk
Phytophthora infestans is a hemi-biotrophic pathogen that exhibits gene-for-gene interactions with its host potato. Compared to bacterial pathogens, relatively few fungal avirulence genes have been cloned and only one from the oomycetes. We aim to clone and characterise avirulence genes from the oomycete pathogen *P. infestans* as a first step in studying the molecular basis of host/oomycete recognition and specificity. Two approaches are being followed: The Avr2 gene is being mapped in a segregating population of F_1 individuals derived from a sexual cross. Secondly, a number of candidate genes identified *in silico* from ESTs are being amplified and sequenced from isolates in order to identify SNPs associated with specific avirulences. This approach has identified a strong candidate for the Avr3 gene.

10:40

Transcriptional profiling in *Phytophthora infes*tans using cDNA-AFLP

Rays H.Y. Jiang, Wubei Dong, Maita Latijnhouwers, Jun Guo, and Francine Govers
Laboratory of Phytopathology, Wageningen University, and Graduate School Experimental Plant Sciences, The Netherlands. Rays.Jiang@wur.nl.

Phytophthora infestans is the causal agent of late blight, an important disease on potato. cDNA amplified fragment length polymorphism (cDNA-AFLP) was chosen as a tool to perform transcriptional profiling with the aim to identify virulence and avirulence factors of *P. infestans.* Transformants in which the gene encoding the Gα subunit of the heterotrimeric G-protein is inactivated by gene-silencing are severely impaired in virulence. By comparing the cDNA-AFLP profiles of sporangia from Gα silenced, Gα gain-of-function mutants, and wild type strains several transcript derived fragments (TDFs), representing putative downstream targets of G-protein signalling, were identified. The hypovirulent phenotype of the Gα silenced transformants merits further investigation of these targets for their role in virulence. To identify avirulence factors we previously constructed high-density linkage maps of two regions carrying *Avr* genes. Now, cDNA-AFLP analysis is performed on the parental strains and F1 progeny of the mapping population. In the cDNA-AFLP profiles obtained from germinating cyst stages we can identify TDFs, the presence of which is associated with specific avirulence phenotypes. Further analysis and mapping of these TDFs is required to determine if they are *Avr* gene candidates. Bioinformatics analysis based on EST sequences is performed to set up efficient experimental cDNA-AFLP conditions and to predict TDFs sequences.

11:00

Responses of Potato to Infection by *Phytophthora infestans*

Paul R J Birch, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.
Email pbirch@scri.sari.ac.uk

Potato is the world's fourth largest crop, global production of which is increasing at 4.5 % annually (http://www.cipotato.org/potato/POTstats.htm). The most serious constraint to potato production is the oomycete pathogen *Phytophthora infestans*, cause of late blight. Resistance in cultivated and wild potato species may be either race-specific or non race-specific. Race-specific resistance involves either direct or indirect interaction between the products of resistance (*R*) genes in the host and corresponding avirulence (*Avr*) alleles in the pathogen and is traditionally readily defeated by *P. infestans*. Non-race-specific (quantitative, field or partial resistance) is poorly defined but is genetically mapped as quantitative trait loci (QTL) and is often referred to as durable resistance. Mechanistically, all forms of resistance to oomycetes such as *P. infestans*, whether race-specific, non race-specific or non-host, may include a localised programmed cell death called the hypersensitive response (HR). In this talk, a programme for studying the potato-*P. infestans* interaction transcriptome is described. We focus on isolating potato genes with a potential role in the HR and present approaches for investigating their function in potato using virus-induced gene silencing.

11:20

A comparative analysis of *Avr1a* genomic region in two different *Phytophthora sojae* race types

D. Qutob, T. MacGregor, Y. Cui, T. Sharifian, and M. Gijzen. Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON N5V 4T3, Canada.

Phytophthora sojae is an aggressive soil-borne pathogen that causes root rot of soybean. Here we report progress on a map-based cloning study to isolate the avirulence gene, *Avr1a.* A physical map of eight overlapping BAC clones spanning 170 kb and encompassing the *Avr1a* locus has been described. A total of 119 kb of this contig has been fully sequenced from race 2 (P6497; *Avr1a*) and examined for predicted open reading frames (ORFs) and matches to expressed sequence tags. Among the 25-30 ORFs identified, there are few that show any similarity to known proteins. Three of the ORFs match expressed sequence tags from zoospore or infected plant cDNA libraries. At least four other ORFs appear to represent expressed genes, by RT-PCR analysis. A total of 10 of the potential ORFs share sequence similarities with one another, and constitute part of a large gene family that is clustered in the region. To compare this sequence to that of a virulent phenotype, 40 kb of this region was also sequenced in race 7 (P7064; *avr1a*). A total of 76 polymorphisms were noted (63 single nucleotide polymorphisms and 13 insertions or deletions), including 35 that cause amino acid changes in predicted proteins. Insertions and deletions ranged in size from 1-19 bp. The overall frequency of polymorphism was one per 477 bp. This analysis has refined our search for *Avr1a* candidates, but has not pointed to any clear and obvious targets that may encode the gene.

11:40

Race shifts in *Phytophthora sojae* and implications in management. A.E. Dorrance, Dept. of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691.

Problems with early season soybean stand establishment, pre-emergence damping-off, and an increase in incidence of *Phytophthora* root and stem rot caused by *Phytophthora sojae*, prompted a reassessment of the pathogen population in Ohio. Fifty-seven fields, part of an earlier study in 1990 and 1991, along with 29 additional fields were sampled in either 1997 or 1999. Two soybean cultivars, Sloan (*rps*) and Resnik (*Rps*1k) were used as bait in a seedling bioassay to isolate *P. sojae* from the soil samples. *P. sojae* was recovered from 82 of the 86 fields sampled. Of the 429 isolates recovered from these soils, 325 and 104 were baited with soybean cultivars Sloan and Resnik, respectively. The *P. sojae* population in Ohio increased in the number of pathotypes (races) as well as complexity since the earlier surveys. There were 72 and 202 pathotypes identified on 8 and 13 *Rps* gene differentials, respectively. When the data were compared by location, 96, 65, 73, 78, 51 and 52% of the locations had at least one isolate with virulences to *Rps*1a, *Rps*1b, *Rps*1c, *Rps*1k, *Rps*3a, and *Rps*6, respectively. The mean complexity, the number of susceptible interactions on 8 differentials, increased from 3.01 to 4.06 between 1991 and 1997/1999. In addition, the pathogenic diversity as measured by the Shannon index increased from 2.71 to 3.28 for isolates recovered from the 57 fields sampled in both surveys. Producers, whose fields were sampled, were surveyed to determine if changes in the *P. sojae* population could be linked with production practices. There was a significant correlation ($P \leq 0.05$) for reduced tillage practices and the presence of isolates that had virulence to the *Rps*1k as well as virulence to the total number of differentials

(*Rps*1a, *Rps*1b, *Rps*1c, *Rps*1d, *Rps*1k, *Rps*3a, *Rps*6 and *Rps*7). Due to the percentage of the isolates that have virulence to many of the *Rps* genes, it is questionable how long single *Rps* gene or several stacked *Rps* genes will remain viable disease management tools for *P. sojae*, unless a novel *Rps* gene is identified. More importantly, cultivars with high levels of partial resistance combined with Rps genes should now become the focus of soybean cultivar breeding and development.

12:00-1:30 Lunch: Crocker Hall

1:30 PM KILN

Species identification and population analysis of *Pythium* species using AFLP markersCarla D. Garzon, David M. Geiser and Gary W. Moorman
Dept. Plant Pathology, Penn State University. cdg143@psu.edu.
To facilitate the identification of plant pathogenic *Pythium* species and to better understand their epidemiology, we characterized isolates of several species using Amplified Fragment Length Polymorphisms (AFLP), and analyzed the population genetics of the two species most commonly found in Pennsylvania greenhouses: *P. irregulare* and *P. aphanidermatum*. We generated AFLP markers by using one selective primer combination to identify diagnostic fingerprints for 13 plant pathogenic *Pythium* species. The analysis of 89 *P. irregulare* isolates from the United States identified a species structure, with at least two groups of overlapping geographical distribution. This was supported by sequence analysis of the internal transcribed spacers (ITS) region of a sample of 31 *P. irregulare* isolates. These groups (I and II) differ in their levels of homozygocity and allele frequency. Group II has a larger number of monomorphic markers and includes a subgroup comprising isolates from *Pelargonium*. On the other hand, based on the analysis of 23 isolates from Asia, Africa, and North and South America, *P. aphanidermatum* showed little genetic variation. We identified four clusters within this species characterized by four polymorphic markers.

1:50

Stage-specific gene transcription in *Phytophthora infestans* prior to and during the early stages of potato infection. Stephen C. Whisson, Anna O. Avrova, Eduard Venter, Laura Grenville*, Pieter van West*, and Paul R. J. Birch Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, U.K.* Department of Molecular & Cell Biology, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, U.K.
Phytophthora infestans, the causal agent of potato and tomato late blight, produces several different cell types prior to penetration of the host plant and during the early stages of infection. Cell type-specific gene expression has been observed previously in zoospores, encysted zoospores, germinating cysts, appressoria, and infection vesicle-like cells. All of these cell types can be easily generated in the absence of the host plant and so form the basis for cell-type specific gene discovery. Amplified fragment length polymorphism (AFLP)-based mRNA fingerprinting (cDNA-AFLP) and suppression subtractive hybridisation (SSH) in combination with a *P. infestans* BAC library have been used to target transcripts specifically up-regulated in germinating zoospore cysts, appressoria, the biotrophic stage (15 hpi), and the necrotrophic (72 hpi) stage of infection. This has led to isolation of several genes potentially involved in different stages of the *P. infestans* interaction with potato and discovery of a new *P. infestans* necrotrophic stage-specific gene family, consisting of over a hundred members. Expression of genes identified using SSH and cDNA-AFLP was quantified by real-time RT-PCR, relative to the constitutively expressed *actA* and *actB* genes, in vegetative mycelium, germinating cysts, and at three time points post-inoculation of susceptible potato cultivar Bintje. Expression analyses allowed definition of four main transcript groups: expressed in germinating cysts only, in germinating cysts and early infection stages, in germinating cysts and late infection stages, or low expression

2:10

A proteomic approach to identify secreted proteins from *Phytophthora infestans* relevant to the host-pathogen interaction

Shuang Li[1], Pieter van West[1], Sophien Kamoun[2], Neil A.R. Gow[1].
[1] Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, Scotland UK.
[2] The Ohio State University, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA.
E-mail: p.vanwest@abdn.ac.uk

Microbial pathogens of plants secrete avirulence factors that are recognised by the products of plant resistance genes and trigger host resistance responses. In order to identify potential avirulence factors from *Phytophthora infestans*, secreted protein profiles of several strains with different avirulence phenotypes were analysed by proteomics. About 500 proteins were identified from culture filtrates of each strain. The most abundant 50 protein spots from one strain were analysed by peptide fingerprinting using MALDI-TOF mass spectrometry. Comparative analysis of the profiles of strains with known avirulence characteristics identified several proteins that were expressed differentially. The possible function of the corresponding genes could not be determined from the DNA sequences. Northern analysis showed that some of the genes were differentially expressed in the tested strains under the same or different culture conditions. Functional analysis of these genes will establish their potential as avirulence determinants of *P. infestans*.

2:30

A Data Mining Strategy to Identify *in planta* Induced Genes from the Oomycete Pathogen *Phytophthora infestans*. Luis da Cunha, Edgar Huitema, Miaoying Tian and Sophien Kamoun. Department of Plant Pathology, The Ohio State University - OARDC, Wooster, OH

The oomycete *Phytophthora infestans* causes late blight, a devastating disease of tomato and potato. A key step in understanding pathogenicity is to define the transcriptional changes that take place during colonization of host tissue. For example, *in planta*-induced (*ipi*) genes are more likely to encode virulence or avirulence factors and form attractive candidates for detailed functional analyses. Here, we describe a strategy to identify *ipi* genes from expressed sequence tags (ESTs) obtained from infected tomato tissue. To select a subset of *ipi* candidate genes, computational analyses based on GC counting and differential BLAST searches against tomato and *Phytophthora* databases were performed on 2808 "interaction" ESTs. A total of 523 ESTs (19%) were predicted to originate from *P. infestans*. Additional BLAST searches against 72.000 *in vitro P. infestans* ESTs identified a set of 55 sequences that are over-represented in the interaction. To validate this data mining strategy, we performed PCR on *P. infestans* and tomato genomic DNA, semi-quantitative RT-PCR, and northern blot analyses. All genes examined were confirmed to be from *P. infestans*. Of these, 52 were expressed during colonization of tomato and 10 were found to be up-regulated during infection using semi-quantitative RT-PCR. Current work focuses on functional analyses of the novel *ipi* genes to determine whether they play a role in pathogenesis.

3:10

Promoter analyses in *Phytophthora infestans.* A. McLeod, C.D. Smart and W.E. Fry, Department of Plant Pathology, Cornell University, Ithaca NY

The core promoter and upstream promoter regions of *Phytophthora infestans* genes were investigated. The core promoter region of two *P. infestans* genes *Piexo1* and *Piendo1*, was analyzed using a transient protoplast expression system and the reporter gene beta-glucuronidase. Mutational analyses showed that the core promoter region encompassing the transcriptional start site (-2 to +5) of the genes has high sequence and functional homology to a known core promoter element present in other eukaryotes, the initiator element (Inr). Flanking the Inr in both genes is a highly conserved oomycete core promoter region (+7 to +15), hereafter referred to as FPR (flanking promoter region), which is also important for promoter function. Together the Inr and FPR form a highly conserved 19nt core promoter region, previously identified as a 16nt core region (Pieterse *et al*., 1994). The sequence of the 19nt core promoter region is highly conserved in most published oomycete promoters. The importance of the Inr and FPR were further investigated in electrophoretic mobility shift assays (EMSA) using *P. infestans* whole-cell protein extracts, and 31-36 bp double-strand oligonucleotides containing the Inr and FPR. The EMSA studies showed that both the Inr and FPR were required for protein binding. Deletion analyses of the upstream promoter regions of *Piexo1* and *Piendo1* as well as various other *P. infestans* promoters, suggest that the promoters contain upstream regulatory regions.

3:30

Structure-function relationship studies on the CBEL glycoprotein of *Phytophthora parasitica* var. *nicotianae*, by PVX expression in *N. benthamiana.*

E. Gaulin[1,2], T. Torto[2], Y. Martinez[1] M. Khatib[1], A. Bottin[1], M.T. Esquerré-Tugayé[1], S. Kamoun[2], and M. Rickauer[11]Pôle de Biotechnologie Végétale UMR5546, B.P.17 Auzeville, 31326 Castanet-Tolosan, FRANCE[2] Dept of Plant Pathology, OARDC, 1681 Madison Avenue, Wooster, OH 44691-44096

CBEL is a cell wall glycoprotein produced by *Phytophthora parasitica* var. *nicotianae*, an oomycete pathogen of tobacco. It binds to cellulose and plant cell walls *in vitro* and induces defense reactions in the host plant (1). The protein structure of CBEL consists of two repeated domains separated by a linker region ; each domain contains a motif similar to the cellulose-binding domain (CBD) found in fungal glycanases.

In order to determine the roles of the different domains in elicitor activity of CBEL, we adopted the PVX expression system for our studies. The coding sequence of CBEL, including its proper signal peptide sequence, as well as various deletions and point mutations, were introduced into the pGR106 expression vector (2).

Production of CBEL *in planta* by means of the PVX system induces necrosis in *N. benthamiana* leaves, leading to death of the whole plant. This necrosis-inducing activity, together with western blot analysis and immunocytolocalisation of CBEL, demonstrate that the oomycete secretion signal peptide directs CBEL towards the cell wall in *N. benthamiana*. Results obtained with mutant forms of CBEL indicate that the two CBDs are involved in its necrosis-inducing activity, and hence in its perception by the plant cell.

3:50

An extracellular protease inhibitor from *Phytophthora infestans* targets tomato serine proteases: a counter-defense mechanism?

Miaoying Tian, Sophien Kamoun Department of Plant Pathology, The Ohio State University, Wooster, OH44691, USA tian.16@osu.edu. The plant intercellular space is the battlefront where complex interactions between extracellular proteins from the oomycete pathogen *Phytophthora infestans* and its host plants occur. We used data mining of *Phytophthora* sequence databases to identify eleven genes encoding putative extracellular protease inhibitors (EPIs) with one to three predicted domains of Kazal-type serine protease inhibitors that are commonly found in animals. The EPI proteins did not show similarity to protease inhibitors from plant pathogens or plants suggesting that they might reveal novel molecular mechanisms in plant-microbe interactions. *In vitro* protease inhibition assays of three purified EPI fusion proteins against several commercial serine proteases revealed that EPI1 highly inhibits bacterial subtilisin, thus confirming the protease inhibition function predicted by bioinformatics. EPI1 was further demonstrated to inhibit and interact with tomato P69 subtilisin-like proteases, among which P69B and P69C are known to be pathogenesis-related proteins involved in plant defenses. We also found that EPI1, but not EPI2 and EPI3, is resistant to degradation by tomato intercellular fluids. Interestingly, EPI1 was able to protect EPI2 from degradation suggesting that inhibition of host proteases might help secreted proteins of *Phytophthora* resist proteolytic cleavage in the plant apoplast. Overall our results suggest that inhibition of plant proteases by *P. infestans* EPI1 could form a novel type of defense-counterdefense cross talk between plants and microbial pathogens.

4:10

Identifying genes involved in asexual sporulation of *Phytophthora infestans* using cDNA macroarrays. Kyoung Su Kim and Howard Judelson, University of California, Riverside.

Asexual sporangia are the main agents of dispersal of *P. infestans,* and the main inoculum for the potato and tomato late blight. To understand the factors involved in differentiating sporangia from hyphae, and to identify spore components that participate in germination, 5200 sequenced clones from a sporangial cDNA library were spotted on membranes and hybridized with total cDNA from sporangia, hyphae, germinated spores, starved hyphae, and sporulation-defective strains. This identified 54 genes up- regulated >5-fold in sporangia. For 43 genes, searches against GENBANK predicted functions in regulation (transcription factors, protein phosphatases and kinases, signal transduction, etc.), structure, transport, and metabolism. The most common type of gene induced in spores (>10% of mRNA) were polyol dehydrogenases. These, plus selected others were also induced during starvation. The genes could also be grouped based on whether they continued to be expressed at high levels after germination, or in sporulation-defective strains. This identified genes that may participate in early versus later stages of spore biology. The cellular roles of genes with regulatory functions, such as transcriptional activators, are currently being characterized by gene silencing, yeast two-hybrid, and other methods. Fusions between GUS and spore-induced promoters are also being dissected to identify the mechanisms by which the genes are activated.

4:25

PiCDC14 expression is specific to asexual sporulation in the oomycete *Phytophthora infestans.* Audrey Ah Fong and Howard Judelson, University of California, Riverside. CDC14 protein phosphatases have been shown to control mitotic exit and/or cytokinesis in a range of ascomycetes and metazoans. Their genes are normally constitutively transcribed and regulated post-translationally. We have identified a CDC14 homologue from the oomycete *P. infestans* designated PiCDC14, which interestingly was transcribed only during sporulation. The gene was identified using an *in silico* data mining strategy for developmentally regulated phosphatases. The gene is single-copy in Phytophthora and complemented the *S. cerevisiae cdc14-ts* mutant. Northern blot and promoter::GUS fusion analyses showed that PiCDC14 is only expressed during asexual sporulation, and not in non-sporulating hyphae or cultures forming sexual spores. PiCDC14::GUS activity was detected in sporangiophore initials and along the length of sporangiophores bearing immature sporangia, and upon maturation was restricted to sporangia. PiCDC14 mRNA was present in zoospores and cysts, but disappeared from germlings a few hours before the onset of mitosis. The analysis of transformants from homology-dependent silencing experiments supported the role of PiCDC14 in sporulation. It may synchronize nuclear division during sporulation, and then help maintain sporangia in a state of dormancy by inhibiting cell division and growth. The absence of PiCDC14 during vegetative growth may explain why nuclear division is normally asynchronous in hyphae.

4:40

A protein kinase *of Phytophthora infestans* induced during zoosporogenesis has a novel structure. Flavio Blanco and Howard Judelson, University of California, Riverside.

Zoosporogenesis in *P. infestans* is a critical step in infecting plants. Proteins involved in this process are good targets for disease control. A gene expressed during zoospore formation was identified that showed high similarity to protein kinases. The predicted sequence of the protein contains all 12 domains diagnostic of Ser/Thr kinases, and the best similarity to the Ca2/CaM family. However, it lacked the long C terminal regulatory domain typical of such proteins, while having a longer N terminus. The promoter of this gene was cloned upstream of the *gus* gene and the construct was used to transform *P. infestans. Cis* elements involved in expression during zoosporogenesis will be discovered by deletion analysis and associated transcription factors will be identified through one-hybrid screening. To study the activity of the protein, it was expressed as a fusion with the maltose binding protein in *E. coli.* Antibodies were raised to study the accumulation of the kinase during life cycle, its subcellular localization and interacting proteins. At the same time, the kinase was used as bait in a two-hybrid screening of a cDNA library from different stages of zoosporogenesis (from sporangia to swimming zoospores). Several interactors were identified which may represent substrates and regulatory proteins. These studies will reveal the role and regulation of this kinase and identify other components of signal transduction pathways involved in zoosporogenesis.

4:55

Role of inositol triphosphate and calcium signalling in gene expression during zoosporogenesis in *Phytophthora infestans*. Shuji Tani, Flavio Blanco, and Howard Judelson, University of California, Riverside.

Most infections by the oomycete Phytophthora infestans, the late blight pathogen, are initiated by zoospores released from asexual sporangia. This release occurs at cool temperatures in a process known to involve Ca2+ or other cations. At higher temperatures, germ tubes emerge directly from sporangia. To better understand these pathways, we tested the effects of inhibitors on the release of zoospores, direct germination, and gene expression during zoosporogenesis. Inhibitors of ion channels, kinases, and inositol triphosphate (IP3) pathways impaired zoospore release, but not direct germination. To identify genes induced during zoosporogenesis, candidates from EST database mining approaches were tested by Northern blotting. This identified 12 genes induced in sporangia undergoing cleavage into zoospores, but not in directly germinating sporangia. Most genes were repressed by one or more inhibitors, although a broad spectrum of quantitativelly distinct responses were observed and some genes were insensitive to all inhibitors tested. The majority of genes were repressed drastically by 2APB, an inhibitor of IP3-induced Ca2+ release, and by the phospholipase C inhibitor U73122. The functions and transcriptional regulation mechanisms of the cleavage-induced genes are now being examined. Emphasis is being placed onstudying a family of transcription factor-binding proteins including the analysis of interactors identified by yeast two-hybrid analysis.

March 18

9:00
Genome resources for *Phythophthora*. Jeffrey L. Boore, Evolutionary Genomics Department Head, and Associate Adjunct Professor, University of California, Berkeley. DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598. JLBoore@LBL.gov

The DOE Joint Genome Institute (JGI), along with collaborators at the Virginia Bioinformatics Institute, have undertaken a project for determining and interpreting high-depth draft sequences of the complete genomes of *Phytophthora sojae* and *P. ramorum*. This project is funded at approximately $4 million from three different sources: the National Science Foundation, the U.S. Department of Agriculture, and the U.S. Department of Energy. The DNA sequencing is expected to be complete within a few months. These complete genome sequences will provide reagents for follow-on functional genomics studies. Further, biochemical pathway analyses and comparisons of the complete genome sequences will enable a candidate gene approach for understanding infection and disease processes and possibly provide targets for intervention. We are hoping to involve much of the *Phytophthora* community in order to see these sequences have maximum effect. I will overview JGI's sequencing, annotation, and analysis pipeline, describe intended functional genomics studies that will use these sequences, and give a brief sketch of what we've learned so far from the 300 Mb of sequence we've generated from *P. sojae*.

10:45 AM KILN
Genome sequencing of *Phytophthora infestans*
Li-Jun Ma Li-Jun Ma Whitehead Institute /MIT Center for Genome Research
320 Charles Street Cambridge MA 02141-2023 Email: lijun@genome.wi.mit.edu

Abstract: Despite the obvious scientific and economic importance in understanding the genome organization of the oomycete Phytophthora infestans, the large size of the genome relative to other filamentous fungi has delayed this work. However, the rapid evolution of sequencing methods and the dramatic reduction in sequencing costs now make a sequencing project for *P. infestans* feasible and cost effective. We will discuss a strategy for determining the genome sequence of *P. infestans* through a partnership between the Whitehead Institute / MIT, Center for Genome Research and the *Phytophthora* research community. The specific objectives of this project are to (1) produce a whole genome shotgun (WGS) assembly of one strain of P. infestans with >7X coverage comprised of plasmid, Fosmid -, and BAC-end sequences, (2) integrate the assembly with existing EST, genetic and physical mapping resources, (3) perform automated annotation and other genomic analyses on the assembly, (4) make these data publicly available through web-based tools and interfaces.

Abstracts Of Posters Not Discussed In The Oral Sessions.

Identification of race-specific avirulence genes in *Phytophthora infestans* by transciptional profiling

Rays H.Y. Jiang[1], Guo Jun[1,2] and Francine Govers[1]. [1]Laboratory of Phytopathology, Wageningen University, and Graduate School Experimental Plant Sciences, Wageningen, The Netherlands. [2]Institute of Vegetable Crops and Flowers, Chinese Academy of Agricultural Sciences., Beijing, China. Francine.Govers@wur.nl, Rays.Jiang@wur.nl

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.* Previously, we constructed high density linkage maps of two regions carrying *Avr* genes (van der Lee *et al*. 2001 Genetics 157: 949-956). Currently, cDNA-AFLP analysis is performed on F1 progeny of the mapping population. Twenty isolates with different avirulence phenotypes have been selected and mRNA has been isolated from the stage that *Avr* genes are most likely to be expressed, i.e., the germinating cyst stage. Based on the transcriptional profiles we can identify genes that are differentially expressed in different races and this may lead to identification of *Avr* genes in *P. infestans.*

Characterisation of appressoria specific proteins from Phytophthora *infestans* .

Laura J. Grenville. Catherine R. Taylor, Alison Williams,Paul Birch, and Pieter van West. University of Aberdeen, Department of Molecular and Cell Biology, Foresterhill, Aberdeen, AB25 2ZD. Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland UK. E-mail: p.vanwest@abdn.ac.uk

A thorough understanding of the molecular events taking place during early interactions between <I>P. infestans</I> and host and non-host plants is crucial for developing new control measures. The appressorial stage of the interaction is the first point in which direct contact between the pathogen and the plant occurs via the formation of highly specialised infection structures, such as the appressorium, penetration peg, and the infection vesicle. Moreover, it is during this phase that plant defence responses are initiated. Resistance is based, principally, on recognition of a particular elicitor component from the pathogen. Therefore, we anticipate that secreted and cell wall proteins from the appressorial infection stage of <I>P. infestans</I> are likely to be rich in important signalling molecules involved in disease resistance or establishing a successful infection process. A proteomic approach is employed to accelerate the discovery of novel extra-cellular and appressorial

Characterization of the glucose-6-phosphate isomerase gene in *Phytophthora infestans* reveals the presence of multiple alleles. M. D. OSPINA-GIRALDO and R. JONES. USDA/ARS/Vegetable Laboratory, BARC-WEST Building 010A/Room 309, 10300 Baltimore Avenue, Beltsville, MD 20705

Glucose-6-phosphate isomerase (GPI) plays a key role in both glycolysis and gluconeogenesis. Isoforms of GPI are common, and their isozyme patterns are widely used to characterize isolates of *P. infestans*. Despite the importance of GPI in *P. infestans* studies, the gene encoding this enzyme has not yet been characterized. Furthermore, it has been suggested that *P. infestans* contains multiple copies of the gene but this hypothesis remains to be demonstrated. We have cloned and characterized *GPI* in various isolates of *P. infestans* as well as in several species of the genus *Phytophthora*. The gene contains 1,671 bp and encodes a protein with a predicted molecular weight of 60.8 kD. Multiple different alleles were identified and Southern analysis indicated certain *P. infestans* isolates carry several copies of the gene. Phylogenetic studies revealed that *P. infestans* GPI is most closely related to sequences from plant and protozoan origin.

The Effect of Cultural Conditions on Molecular Variation in *Phytophthora infestans*. Lee C. D. Baines and James M. Bradeen. University of Minnesota. baine004@umn.edu
Observations that the long–term *in vitro* storage of *Phytophthora infestans* causes a reduction in pathogenicity have been made over many years. It is not understood if this is a physiological or genetic change. It is the aim of this study to use molecular techniques now available to identify DNA mutations brought about by post-collection storage. Isolates of *P. infestans* representing clonal lineages US-1, US-6, US-7, US-8 and US-11 have been selected and amplified fragment-length polymorphism fingerprints (AFLP) will be determined. Isolates will then be maintained under various cultural conditions representative of normal storage and experimentation, as well as conditions to induce changes in AFLP profiles. The cultural conditions will include both *in vitro* and *in planta* storage using a variety of media and potato genotypes. Several AFLP pairs will be examined and more than 300 markers are expected, providing broad genome-coverage and enhancing the likelihood of detecting polymorphisms. This is a long-term study that will evaluate AFLP as a marker system to complement other neutral-marker systems and phenotypic markers currently used in population studies. Additionally, we will determine the number of AFLP markers required to provide refined representation of natural *P. infestans* populations. To this end, we welcome contribution of naturally-occurring *P. infestans* samples collected within the US to compare the data generated from AFLP analysis with data from other, established systems

Characterisation of appressoria specific proteins from *Phytophthora infestans* Laura J. Grenville[1], Catherine R. Taylor[1], Alison Williams[1], Paul Birch[2], and Pieter van West[1].[1] University of Aberdeen, Department of Molecular and Cell Biology, Foresterhill, Aberdeen, AB25 2ZD, Scotland UK. [2] Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland UK. E-mail: p.vanwest@abdn.ac.uk
A thorough understanding of the molecular events taking place during early interactions between *P. infestans* and host and non-host plants is crucial for developing new control measures. The appressorial stage of the interaction is the first point in which direct contact between the pathogen and the plant occurs via the formation of highly specialised infection structures, such as the appressorium, penetration peg, and the infection vesicle. Moreover, it is during this phase that plant defence responses are initiated. Resistance is based, principally, on recognition of a particular elicitor component from the pathogen. Therefore, we anticipate that secreted and cell wall proteins from the appressorial infection stage of *P. infestans* are likely to be rich in important signalling molecules involved in disease resistance or establishing a successful infection process. A proteomic approach is employed to accelerate the discovery of novel extra-cellular and appressorial stage-specific proteins. Here we present our latest results.

Isolation and characterization of genes preferentially expressed during asexual sporulation in the oomycete plant pathogen *Phytophthora cinnamomi.* Reena Narayan, Weixing Shan, and Adrienne R. Hardham. Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, Canberra, Australia.
The genus *Phytophthora* contains at least 60 species, many of which are destructive pathogens causing diseases in hundreds of commercially important plants. *P. cinnamomi* is capable of infecting over a thousand plant species and causes severe economic and ecological losses to agriculture and forest industries in Australia and worldwide. Under nutrient-deprived conditions, vegetative hyphae of *P. cinnamomi* sporulate to produce multinucleate, asexual sporangia that cleave to form uninucleate, motile zoospores, which are the primary means of infection of new hosts. Our current understanding of the molecular basis of sporulation is extremely limited. Identification and characterization of *Phytophthora* genes that serve key roles in sporulation and spore function would make a significant contribution towards increasing our understanding of these processes. In this study, differential hybridization techniques were used to screen over 5000 cDNA clones from a *P. cinnamomi* cDNA library made from an early stage of sporulation, and 328 putative sporulation-specific genes were isolated and partially sequenced. Candidate genes were identified through Genbank and *Phytophthora* Genome Consortium database comparisons. Of the 328 sequenced clones, 195 were found to represent unique genes of which 27% were homologous to metabolic and structural proteins, 31% were homologous to ribosomal proteins, and 42% were unknown genes. Three candidate genes were selected for further molecular characterization. A macroarray of the unique genes was screened with probes made from mRNA isolated at nine different stages in the sporulation process and cohorts of genes expressed at different stages of sporulation were identified. The macroarray results were complemented by RNA blot analysis.

Identification of pathogenicity determinants in the interaction between the oomycete plant pathogen *Peronospora parasitica(At)* and Arabidopsis.

Jim Beynon, Rebecca Allen, Peter Bittner-Eddy, Anna Gordon, Laura Grenville, Sharon Hall and Anne Rehmany.Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK.

Peronospora parasitica is the causal agent of downy mildew on Arabidopsis and Brassica crops. We have cloned the Arabidopsis resistance genes *RPP13Nd* and *RPP1Nd* that recognise the downy mildew isolates Maks9 and Emoy2, respectively. In order to study the basis of the interaction with the pathogen and understand the molecular recognition events involved with these different genes, we are cloning the corresponding avirulence genes, *ATR13Nd* and *ATR1Nd*. We have developed a mapping cross that we have shown to be segregating for up to 15 different avirulence genes. We have used this population to locate the genes to overlapping BAC contigs. In addition, using Suppression Subtractive Hybridisation we have identified a range of genes that are specifically up-regulated on infection of Arabidopsis. An analysis of their structure and their relationship to the avirulence genes will be presented. The two resistance gene interactions result in very different interaction phenotypes. *RPP13Nd* elicits a localised necrotic lesion whereas *RPP1Nd* results in a spreading necrotic lesion. Furthermore, each of these loci has undergone very different evolutionary paths. *RPP1Nd* has developed a complex multi gene locus to generate novel gene variants, whereas *RPP13Nd* has generated an allelic series with different isolate recognition specificity. We wish to understand these differences in relation to avirulence gene evolution.

The Functions Of Gα And Gβ In The Life Cycle Of The Oomycete *Phytophthora Infestans*

MAITA LATIJNHOUWERS[1], WUBEI DONG AND FRANCINE GOVERS[1] Laboratory of Phytopathology, Wageningen University, The Netherlands

The G-protein mediated signaling pathway is a ubiquitous eukaryotic signaling pathway involved in translation of extracellular signals into intracellular ones. Previous work has shown that G-protein mediated signal transduction in plant-pathogenic fungi is indispensable for effcient adaptation to the plant environment and for the development of structures that are required for virulence.

Our aim is to study the role of G-protein signaling in pathogenesis of the oomycete *Phytophthora infestans*, the causal agent of potato late blight. We isolated and characterized *P. infestans* genes coding for Gα (*Pigpa1*) and Gβ (*Pigpb1*) protein subunits, with up to 42% and 62% identity with known G-protein subunits, respectively. Expression studies showed that both genes are differentially expressed in various stages of the life cycle. By means of homology-dependent gene silencing we obtained *P. infestans* transformants deficient in either of the two subunits. Those in which *Pigpb1* is silenced show a defect in asexual sporulation and produce a dense mat of aerial mycelium. Silencing of *Pigpa1* has a major effect on zoospore behavior. Zoospores of *Pigpa1*-silenced mutants show aberrant swimming patterns and fail to autoaggregate. The virulence of these mutants is severely impaired. To isolate downstream targets of PiGPA1, a comparison of gene expression in sporangia of wildtype and of *Pigpa1*-silenced mutants using cDNA-AFLP is in progress. In addition, to elucidate targets of PiGPA1 and PiGPB1, large scale expression profiling of wildtype silenced mutants will be performed in the near future.

Basal levels of expression of defense-related genes in soybean cultivars varying in partial resistance to *Phytophthora sojae*. Miguel E. Vega-Sánchez[1], Margaret G. Redinbaugh[2] and Anne Dorrance[1]. [1]Department of Plant Pathology, The Ohio State University and [2]USDA, Agricultural Research Service, Wooster, OH 44691, USA.

Phytophthora sojae causes root and stem rot of soybean. Quantitative or partial resistance in this pathosystem is characterized by containment of the pathogen to the lower stem and tap root, but the molecular and/or biochemical mechanisms are poorly understood. To explore the hypothesis that higher basal expression levels of defense-related genes correlate with high levels of partial resistance, Northern blot analysis was carried out using soybean roots and cotyledons. Fourteen cultivars with low, moderate and high levels of partial resistance to *P. sojae* were re-evaluated with two different root inoculation techniques and lesion length (LL), infection efficiency (IF) and percentage of dead plants (PD) were measured. Multivariate analysis of the three components (LL, IF and PD) revealed groupings consistent with previous rankings. Northern blot analysis indicated that basal levels of transcripts for the seven defense-related genes tested were detected in all fourteen soybean cultivars. Most mRNAs were at higher levels in roots than in cotyledons, especially the basic peroxidase transcript. Exceptions were the mRNAs for matrix metalloproteinase and beta 1,3-endoglucanase, with higher levels in cotyledons compared to roots, and a class I chitinase mRNA that was present at very low levels in both tissues. Interestingly, the transcript for PR1-a was detected in all cultivars in roots but only on three of the cultivars in cotyledons. Variation in basal defense gene expression levels was found among cultivars as well. Cluster analysis of gene expression profiles grouped the cultivars independently of their resistance ranking. These results suggest that no correlation between basal defense-gene expression levels and partial resistance to *P. sojae* exists in soybean roots or cotyledons.

Characterization Of The Glucose-6-Phosphate Isomerase Gene In *Phytophthora infestans*. Manuel D. Ospina-Giraldo, USDA/ARS Vegetable Laboratory, Bldg. 010A; Rm. 309,10300 Baltimore Avenue, Beltsville, MD 20705

Glucose 6 phosphate isomerase (GPI) plays a key role in both glycolysis and gluconeogenesis and GPI isozyme pattern is the most widely used approach to characterize isolates of *P. infestans*. In addition to its well-known catalytic activity, GPI is considered a neurotrophic factor. Recent studies on *Xanthomonas campestris* pv. citri indicate that GPI also plays a role in bacterial pathogenicity. Despite the crucial role of GPI in the studying of *P. infestans*, the gene encoding this enzyme has not been characterized yet. Furthermore, it has been suggested that *P. infestans* contains multiple copies of the GPI coding sequence but this hypothesis remains to be demonstrated. In an attempt to elucidate these questions, we have cloned and characterized the GPI gene in various isolates of *P. infestans* as well as in several species of the genus *Phytophthora*. The confirmed cDNA GPI sequence consists of 1,671 base pairs. The gene, which has no introns, encodes a protein of 556 amino acids with a molecular weight of 60.78 kD. Multiple different alleles were identified by cloning and sequencing and Southern analysis indicates certain *P. infestans* isolates carry several copies of the gene. Phylogenetic studies indicate that *P. infestans* GPI is most closely related to sequences from plant and protozoan origin.

TILLING: A High Throughput Method for Mutation Detection. Susan Fuerstenberg, Senior Research Scientist Anawah Inc. 215 Madson Place | Suite A | Davis, CA 95616.
sifuerst@anawah.com

TILLING (**T**argeting **I**nduced **L**ocal **L**esions **IN G**enomes) is a process originally intended to detect mutations in genes of interest in mutagenized populations. However, it can easily be used to detect existing polymorphisms (SNPs-single nucleotide polymorphisms, or even insetion /deletion events) in natural populations, provided some sequence information about the chosen locus exists.

In standard **TILLING**, a mutagenized population is sampled and DNA is extracted then mixed with unmutagenized DNA. Genes of interest (or portions thereof)) are PCR-amplified using differentially labeled primers and the products are melted and re-annealed to anneal mutagenized to unmutagenized DNA. These products are cut with a restriction enzyme that recognizes mismatches between the two species of DNA and makes a single-stranded cut at the mismatch site. These products are then run on an acrylamide gel and the PCR products are visualized by exciting the differentially labeled primers attached to each end of the PCR. Shorter than normal PCRs show up as dark spots distinct from background. Adding the lengths of the short products from the two differentially-labeled gels adds up to the full-length PCR product, for an immediate "true positive" check.

For SNP detection, the same procedure for visualizing samples is used. Samples can be pooled for SNP assignment, some species up to **eight (8) deep**. In that case, up to **768 individual samples** can be screened on a single gel. However, in our hands we have found most species can reliably be pooled at least **four (4) deep**, still allowing the screening of **384 samples** per gel.

Phytophthora Molecular Genetics Network
Asilomar Conference Center
Pacific Grove Ca,
March 16-18, 2003

List of participants

Audrey Ah Fong
Department of Plant Pathology
Webber Hall
University of California
Riverside, California 92521
aah_fong@hotmail.com

Miles Armstrong,
Scottish Crop Research Institute,
Invergowrie, Dundee, N5V 4T3,
marmst@scri.sari.ac.uk

Lee C. D. Baines
Department of Plant Pathology
University of Minnesota.
baineoo4@umn.edu

Jim Beynon
Horticulture Research International
Wellesbourne, Warwick
CV35 9EF UK
Jim.Beynon@hri.ac.uk

James M. Bradeen.
Department of Plant Pathology
University of Minnesota.
brade005@umn.edu

Andres Binder
University of Basel
CH-4332 Stein/Switzerland
binder@syngenta.com

Ursula Binder
University of Basel
CH-4332 Stein/Switzerland
binder@syngenta.com

Paul R J Birch,
Scottish Crop Research Institute,
Invergowrie, Dundee, DD2 5DA, UK.
pbirch@scri.sari.ac.uk

Flavio Blanco
Department of Plant Pathology
Webber Hall
University of California
Riverside, California 92521
flablan@yahoo.com

Jeffrey L. Boore, Ph.D.
Evolutionary Genomics Department Head
Associate Adjunct Professor,
University of California, Berkeley
DOE Joint Genome Institute
2800 Mitchell Drive
Walnut Creek, CA 94598
jlboore@imap4.lbl.gov

Luis da Cunha
OSU/OARDC - Plant Pathology
1680 Madison Avenue
Wooster, OH 44691
lcunha0@lycos.com

A.E. Dorrance,
Dept. of Plant Pathology,
The Ohio State University-OARDC,
Wooster, OH 44691.
dorrance.1@osu.edu

Bernard Dumas
UMR CNRS-UPS 5546
Pôle de Biotechnologie Végétale
24, Chemin de Borde Rouge
BP 17 Auzeville
31326 Castanet-Tolosan
FRANCE
dumas@smcv.ups-tlse.fr

James English
Department of Microbiology and plant pathology
108 Waters hall
University of Missouri
Columbia MO 65203
EnglishJ@missouri.edu

W.E. Fry,
Department of Plant Pathology,
Cornell University, Ithaca NY
wef1@postoffice.mail.cornell.edu

Susan Fuerstenberg,
Senior Research Scientist
Anawah Inc.,215 Madson Place
Davis, CA 95616
sifuerst@anawah.com

Matteo Garbelotto
UC Extension Specialist,
Adjunct Assistant Professor,
338 Hilgard Hall, University of California, Berkeley
Berkeley, CA, 9472C
matteo@nature.berkeley.edu

Carla D. Garzon,
Dept. Plant Pathology,
Penn State University.
cdg143@psu.edu.

Elodie GAULIN
Department of Plant Pathology
OSU/OARDC
1680 Madison avenue
Wooster, OH 44691
gaulin13@yahoo.fr

Mark Gijzen
Agriculture and Agri-Food Canada
1391 Sandford Street
London Ontario
Canada N5V 4T3
gijzenm@agr.gc.ca

Francine Govers
Laboratory of Phytopathology,
Wageningen University,
Wageningen, The Netherlands.
Francine.Govers@wur.nl

Rays H.Y. Jiang,
Laboratory of Phytopathology,
Wageningen University,
Wageningen, The Netherlands.

Howard Judelson, Associate Professor
Department of Plant Pathology
Webber Hall
University of California
Riverside, California 92521
judelson@ucrac1.ucr.edu

Gert Kema
Plant Research International
B.V., 6700 AA, P.O. Box 16, Wageningen
E-Mail: g.h.j.kema@plant.wag-ur.nl.

Sophien Kamoun
Dept. of Plant Pathology
The Ohio State University-OARDC,
Wooster, OH 44691.
kamoun.1@osu.edu

Maita Latijnhouwers
Laboratory of Phytopathology,
Wageningen University,
Wageningen, The Netherlands.
Maita.Latijnhouwers@wur.nl

Theo van der Lee
Plant Research International
Wageningen The Netherlands.
theo.vanderlee@wur.nl

Shuang Li
Department of Molecular & Cell Biology
University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, U.K.
s.li@abdn.ac.uk

Frank Martin
Research Plant Pathologist
USDA-ARS
1636 East Alisal St.
Salinas, CA 93905
fmartin@pw.ars.usda.gov

Adele. McLeod
Department of Plant Pathology,
Cornell University, Ithaca NY
am229@cornell.edu

Elisabeth Mueller

Syngenta
Jealott's Hill, BRACKNELL
Berkshire, UK, 44-1344-413861 (UK)
Elisabeth.Mueller@Syngenta.com

Reena Narayan
Plant Cell Biology Group
Research School of Biological Sciences
Australian National University
PO Box 475
Canberra, ACT 2601,
Narayan@rsbs.anu.edu.au
AUSTRALIA

Manuel D. Ospina-Giraldo, Ph.D.
Research Plant Pathologist
USDA/ARS Vegetable Laboratory
Bldg. 010A; Rm. 309
10300 Baltimore Avenue
Beltsville, MD 20705
ospinagm@ba.ars.usda.gov

Waraporn Prakob
Department of Plant Pathology
Webber Hall
University of California
Riverside, California 92521

Dina Qutob
Agriculture and Agri-Food Canada
1391 Sandford Street
London Ontario
Canada N5V 4T3
qutobd@agr.gc.ca

Martina Rickauer
UMR 5546 Université Paul Sabatier - CNRS
Pôle de Biotechnologie Végétale
24, Chemin de Borde-Rouge
BP 17 - AUZEVILLE
31326 CASTANET TOLOSAN
rickauer@smcv.ups-tlse.fr

Craig Richael, Ph. D.
Simplot Plant Sciences
J. R. Simplot Co.
5369 W. Irving St.
Boise, ID 83706

Craig.Richael@simplot.com

Chris Smart
Department of Plant pathology
Cornell University
Geneva, New York
cds14@cornell.edu

Kyoung Su Kim
Department of Plant Pathology
Webber Hall
University of California
Riverside, California 92521
kyoungsu@ucrac1.ucr.edu

Shuji Tani
Department of Plant Pathology
Webber Hall
University of California
Riverside, California 92521
shuji@ucrac1.ucr.edu

Miaoying Tian
Department of Plant Pathology
The Ohio State University,
OARDC, Wooster, OH44691, USA
tian.16@osu.edu

Brett Tyler
Professor
Virginia Bioinformatics Institute
Virginia Polytechnic and State University
1880 Pratt Dr., Bldg. XV
Blacksburg, VA 24061-0447
bmtyler@vt.edu

Miguel Vega-Sanchez
Graduate Research Associate
Department of Plant Pathology
The Ohio State University-OARDC
1680 Madison Ave.
Wooster, OH 44691
vega-sanchez.1@osu.edu

Pieter van West
Department of Molecular & Cell Biology
University of Aberdeen, Institute of Medical Sciences,
Foresterhill, Aberdeen AB25 2ZD, U.K.

mbi121@abdn.ac.uk

Steve Whisson
Scottish Crop Research Institute,
Invergowrie, Dundee DD2 5DA, U.K.
swhiss@scri.sari.ac.uk

John Windass
Group Leader
Molecular Genetics & Cellular Biology
Bioscience, Syngenta
Jealott's Hill, BRACKNELL
Berkshire, UK
john.windass@syngenta.com

Julie Zwiesler-Vollick
D.O.E. Plant Research Laboratory
Michigan State University
East Lansing, MI 48824
zwiesle1@msu.edu