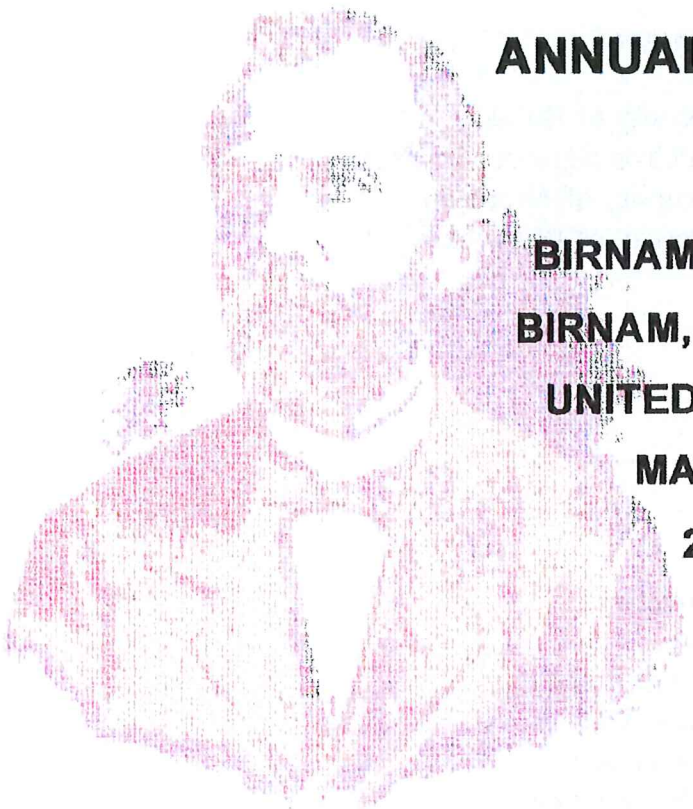
Francine

OOMYCETE MOLECULAR GENETICS NETWORK

ANNUAL MEETING



BIRNAM INSTITUTE

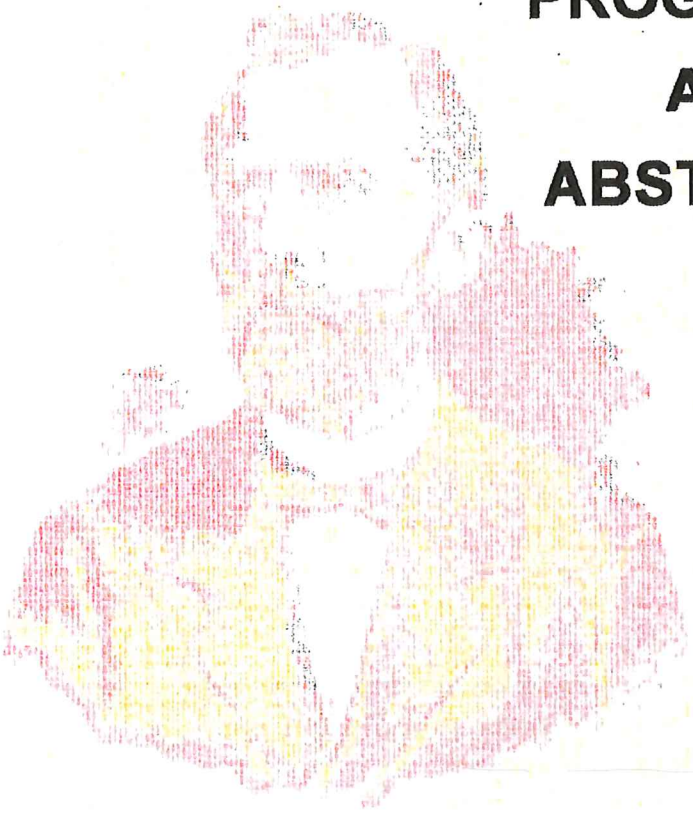
BIRNAM, SCOTLAND

UNITED KINGDOM

MAY 6 - 9

2008

PROGRAMME AND ABSTRACTS



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Cover artwork

Anton de Bary, who first described *Phytophthora infestans*, composed from the bases of the *P. infestans* genome sequence. Image generation: Dr Leighton Pritchard, Scottish Crop Research Institute.

Oomycete Molecular Genetics Network Meeting

Birnam, Perthshire, Scotland UK

6th – 8th May 2008

SCIENTIFIC PROGRAMME

6th May

4.00 pm **Registration (Birnam Institute)**

6-7:00 pm **Pre-dinner drink (Birnam Institute)**

7.30 pm **Dinner (Birnam House Hotel)**

7thMay

Birnam Institute

9.00 am Welcome by Paul Birch

9.10 am **Session 1:**
Chair: Pieter van West

9.10 am Frithjof Kuepper (SAMS Oban Scotland)
Oomycete pathogens of marine brown algae

9.20 am Claire Gachon (SAMS Oban Scotland)
A conserved immune response of brown algae against the oomycete pathogen *Eurychasma dicksonii*?

9.30 am Javier Dieguez-Uribeondo (CSIC Madrid Spain)
Phylogenetic relationships among plant and animal parasitic species in *Aphanomyces* (Oomycetes)

9.40 am Andrew Phillips (University of Aberdeen, Scotland)
Molecular studies of the *Saprolegnia*-fish interaction

10.00 am Vicky Anderson (University of Aberdeen)
A *Saprolegnia parastica* cDNA library: mining ESTs for potential vaccine candidates

10.10 am Krajaejun Theerapong (Mahidol University, Bangkok, Thailand)
Do You Know Human Pythiosis?

10.20 am **coffee/tea, posters**

10.50 am **Session 2:**
Chair: Laura Grenville-Briggs

10.50 am David Cooke (SCRI, Dundee Scotland)
Examining the drivers in a dramatic shift in *P. infestans* populations

11.10 am Josiane Chuisseu Wandji (University of the West of England, Bristol)
Differential Abundance of Proteins In Pre-invasion Stages Of The Downy Mildew Oomycete *Peronospora Viciae*

11.20 am Alon Savidor (University of Tennessee, USA)
The *Phytophthora capsici*-Tomato Interactome

11.30 am Elodie Gaulin (CNRS-Université Paul Sabatier, France)
Transcriptome of *Aphanomyces euteiches*: new oomycete pathogenicity factors and metabolic pathways

11.40 am Ryan Anderson (Virginia Tech Blacksburg USA)
Characterizing Conserved Effector Proteins from *Hyaloperonospora parasitica*

11.50 am Liliana Cano (The Sainsbury Laboratory, Norwich, England)
Exploiting transcriptome sequences to understand effector evolution in the *P. infestans* species cluster

12:00 pm Rays Jiang (Broad Institute, MIT Boston)
Genome plasticity revealed by comparative genomics of *Phytophthora* isolates, sibling species and distant relatives

12.10 pm Eric Kemen (The Sainsbury Laboratory, Norwich)
Identification of novel effectors from *Albugo candida* using Solexa cDNA sequencing techniques

12.30 pm Walid Hamada (National Agronomic Institute of Tunisia)
Monitoring *Phytophthora infestans* epidemics on potato and tomato in Tunisia using molecular tools

12.40 pm **Close of session – Lunch (Birnam House Hotel)**

1.30pm – 4.00pm free

4.00 pm **Session 3 (Birnam Institute):**
Chair: Eleanor Gilroy

4.00 pm Brett Tyler (VBI Virginia Tech, USA)
RXLR-mediated entry of *Phytophthora sojae* effector *Avr1b* into soybean cells does not require pathogen encoded machinery

4.20 pm Miles Armstrong (SCRI, Dundee, Scotland)
Identification of the host targets of *Phytophthora infestans* intracellular effector AVR3a

4.40 pm Jorunn Bos (The Sainsbury Laboratory, Norwich, England)
Molecular basis of cell death suppression by the *P. infestans* RXLR effector AVR3a.

4.50 pm Ros Taylor (University of Glasgow, Scotland)
The Possible Role of Avr3a in Ubiquitination

5.00 pm Shiv Kale (Virginia Tech Blacksburg USA)
Conserved C-terminal motifs required for avirulence and suppression of cell death by Phytophthora sojae effector Avr1b

5.20 pm Sebastian Schornack (The Sainsbury Laboratory, Norwich, England)
Regulation of host genes by Nuk10, a nuclear localised RXLR-effector of *P. infestans.*

5.30 pm Volkan Cevik (Warwick HRI, University of Warwick, England)
Genetic analysis of the *RPP13/ATR13* interaction complex between downy mildew and Arabidopsis

5.40 pm **close of session**

6.00 pm **keynote lecture (Birnam Institute)**: Gordon Beakes (Newcastle University)
NATURAL BORN KILLERS: insights into oomycete origins and infection diversity gained from studies on pathogens of algae and animals.

7.00 pm **Dinner followed by ceilidh (Birnam House Hotel)**

8th May

Birnam Institute

9.00 am	**Session 5:** Chair: Edgar Huitema
9.00 am	Isabell Kuefner (University of Tuebingen, Germany) NLPs – Virulence Factors from Oomycetes, Fungi and Bacteria that Affect the Integrity of the Plasma Membrane of Dicots
9.20 am	Guido van den Ackerveken (Utrecht University, The Netherlands) Why are the Arabidopsis *dmr* mutants resistant to downy mildew?
9.40 am	Harold Meijer (Wageningen University, The Netherlands) *Phytophthora* phospholipase D genes and their role in plant cell degradation
10.00 am	Frederic Brunner (ZMBP-Eberhard Karls University, Tübingen, Germany) Toward the Identification of Oomycete Effectors suppressing PAMP-triggered Immunity in *Arabidopsis thaliana*
10.10 am	Patricia Manosalva (Boyce Thompson Institute for Plant Research, Ithaca, USA) Potato's SA-binding protein 2 ortholog (StSABP2) is required for SAR against *Phytophthora infestans* induced by prior treatment with arachidonic acid
10.30 am	Joe Win (The Sainsbury Laboratory, Norwich, England) Identification of plant proteins targeted by oomycete RXLR effectors using *in planta* co-immunoprecipitation.
10.40 am	**coffee/tea, Posters**
11.10 am	**Session 6:** Chair: Elodie Gaulin
11.10 am	Nicholas Champouret (Wageningen University, The Netherlands) (A)virulence on *Rpi-blb1* expressing plants is determined by the *ipiO* effector family from *Phytophthora infestans*
11.20 am	Edgar Huitema (The Sainsbury Laboratory, Norwich, England) A multi-faceted approach towards understanding the function of the *Phytophthora infestans* Crinkler protein family, particularly the CRN16 class
11.40 am	Amin Madoui (CNRS-Université Paul Sabatier, France) Sterols biosynthesis In *Aphanomyces euteiches*, new target for pea root rot management.
11.50 am	Hendrik Rietman (Wageningen University, The Netherlands) Avirulence mining in the secretome of *Phytophthora infestans*: Implications for durable resistance.

12.00 pm Paul Morris (Bowling Green State Univ., Ohio, USA)
In Silico Identification of Regulatory Elements of the ABCA Gene Family and the Sulfate Assimilation Genes of Oomycete.

12.10 am Leighton Pritchard (SCRI, Dundee Scotland)
Bioimaging of *P. infestans* stages and computational toolbox

12.20 am Close of session

12.30 pm Lunch (Birnam House Hotel)

2.00 pm **Session 7 (Birnam Institute):**
Chair: Dinah Qutob

2.00pm Ping Kong (HRAREC, Virginia Tech, USA)
Quorum sensing controls zoospore behaviour of *Phytophthora*

2.20 pm Ilham Badreddine (CNRS-Université Paul Sabatier, France)
Chitosaccharides are cell-wall structural components and exposed patterns of the phytopathogenic oomycete *Aphanomyces euteiches*

2.30 pm Howard Judelson (University of California, Riverside, USA)
Dissecting transcriptional networks controlling the development of *Phytophthora infestans* through bioinformatic and functional approaches

2.50 pm Laura Grenville-Briggs (University of Aberdeen, Scotland)
Appressorium formation in *Phytophthora infestans.*

3.10 pm Mireille van Damme (The Sainsbury Laboratory, Norwich, England)
Deciphering the role of *Phytophthora* CRN8 effectors in pathogenicity

3.20pm Sucheta Tripathy (VBI Virginia Tech, USA)
Pathogenicity islands and putative alien genes in the genomes of *P. sojae* and *P. ramorum.*

3.30 pm coffee/tea, Posters

4.00 pm **Session 8:**
Chair: Leighton Pritchard

4.00 pm Ramesh Vetukuri
Components of gene silencing in *Phytophthora infestans*

4.10 pm Johnathan Hulvey (University of Tennessee, Knoxville, USA)
ENU induced mitotic gene conversion in *Phytophthora capsici.*

4.20 pm Dinah Qutob (Agriculture Canada, London Canada)
Copy number polymorphisms and transcriptional silencing of *Avr1a* and *Avr3a* effector genes in *Phytophthora sojae*

4.40 pm	Joann Mudge (NCGR, Santa Fe, USA) *De novo* Hybrid 454/Sanger Genome Assembly of the eukaryotic pathogen, *Phytophthora capsici*
5.00 pm	Laura Baxter (Warwick HRI, University of Warwick, England) *Hyaloperonospora* genome
5.20 pm	Mike Zody (The Broad Institute MIT Boston USA) Progress on Analysis of the *Phytophthora Infestans* Genome
5.40 pm	Close of session
6.00 pm	Discussion Session
7. 00 pm	Close of meeting
7.30 pm	**Conference Dinner (Birnam House Hotel)**

Session 1

Oomycete pathogens of marine brown algae

Küpper FC[1], Gachon CMM[1], Strittmatter M[1], Sekimoto S[2], Honda D[2], Beakes GW[3], Müller DG[4]

[1] The Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Argyll, PA37 1QA, Scotland UK; [2] Department of Biology, Faculty of Science and Engineering, Konan University 8-9-1 Okamoto, Higashinada, Kobe City 658-8501, Japan; [3]School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; [4]Universität Konstanz, Germany.

The seaweed oomycete pathogen *Eurychasma dicksonii* is most abundant eukaryotic pathogen of marine brown algae, and also the most basal member of the oomycete lineage. It not only has the largest reported host range among marine pathogens - infecting virtually every brown algal species tested so far, but it is also the most prevalent eukaryotic pathogen in natural brown macroalgal populations worldwide. Remarkably, virtually nothing is known about many fundamental aspects of its pathogenicity, biology, epidemiology, and ecology of *Eurychasma*.

Due to its availability in culture and the recently-completed sequencing of the genome of one of its main brown algal hosts (*Ectocarpus siliculosus*), it is a particularly attractive model to study oomycete infection strategies and algal defense mechanisms. Our lab is therefore developing tools to study the impact of *Eurychasma* epidemics in coastal ecosystems and the molecular mechanisms of infection. Our results show that *Ectocarpus* strains exhibit a differential susceptibility to the same *Eurychasma* strain, suggesting a genetically-determined basis for resistance in the alga. The talk will also provide a short overview of other eukaryotic (fungal, oomycete, plasmodiophoralean) pathogens of marine brown algae.

A conserved immune response of brown algae against the oomycete pathogen *Eurychasma dicksonii*?

Claire MM Gachon[1], Dieter G Müller[2], Martina Strittmatter[1], Frithjof C Küpper [1]

1: Scottish Association for Marine Science; Dunstaffnage Marine Laboratory; PA37 1QA Oban; United Kingdom

2: Fachbereich Biologie der Universitaet; D-78457 Konstanz, Germany

The seaweed oomycete pathogen *Eurychasma dicksonii* is most abundant eukaryotic pathogen of marine brown algae, and also the most basal member of the oomycete lineage. We currently hold over 300 *Ectocarpus* and 10 *Eurychasma* strains from all over the world, most of them being freely available from the Culture Collection for Algae and Protozoa (http://www.ccap.ac.uk/).

We have set up a pathosystem between the *Eurychasma* and the genomic brown algal model *Ectocarpus siliculosus*. Our results show that the reaction of different algal strains against *Eurychasma* range from extreme susceptibility to complete resistance against infection. In all cases investigated, resistance is associated with the early death of the challenged algal cell, which prevents further spread of the disease. The most frequent infection outcome however is an intermediate resistance phenotype, whereby a fraction of algal cells get successfully infected, whereas others undergo cell death before the pathogen completes its development cycle. Significantly, we have observed such responses in 8 different algal species, encompassing 4 brown algal orders (Ectocarpales, Laminariales, Tilopteridales, Discosporangiales). This broad distribution suggests that resistance-associated cell death might be a conserved immune mechanism of brown algae.

We are now focusing our efforts on the molecular characterization of this cell death response in *Ectocarpus* and related species, testing the hypothesis that it might be the outcome of a genetically-programmed mechanism similar to animal apoptosis or the hypersensitive response in higher plants.

broad host range ~ every brown algae tested so far

Ectocarpus genes: large introns

There are LRR gene proteins but Unclear if they are NBS-LRR

Phylogenetic relationships among plant and animal parasitic species in *Aphanomyces* (Oomycetes)

Javier Diéguez-Uribeondo, Miguel A. García, Lage Cerenius, Eva Kozubíková, Carol Windels, John Weiland, Howard Kator, and María P. Martín, M.P.

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USDA-ARS-Northern Crop Science Laboratory, Fargo, N.D. 58105-5677 weilandj@fargo.ars.usda.gov
Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, College of William and Mary, PO BOX 1346, Gloucester Point, Virginia 23062

The genus *Aphanomyces* (Oomycetes) contains species that are among the most damaging parasites in plants and animals. In this investigation we analyzed phylogenetic relationships among 12 species of *Aphanomyces* based on 120 ITS sequences of nuclear rDNA. Sequences used in the analysis belong to the major species currently available in pure culture and Genbank sequences. Both Bayesian and maximum parsimony analyses revealed that the genus *Aphanomyces* appears to constitute a monophyletic group. Three independent lineages were found: (i) animal parasitic, (ii) plant parasitic, and (iii) saprobiotic. Among the physiological characters that were studied, repeated zoospore emergence appears to be an advantageous property for the parasitic mode of life. The predominance of asexual reproduction seems to be advantageous for exploiting specialization in animal parasitism while sexual reproduction is critical in plant parasites for surviving in wet soil environments. In addition, specialization on secretion of host-specific barrier breaking enzymes, i.e. chitinases, cellulases, pectinase, appears to represent a critical property in the evolution of the separate plant and animal parasites lineages.

Molecular studies of the *Saprolegnia*-fish interaction

Andrew Phillips[1], Victoria Anderson[1,2], Sam Martin[2], Chris Secombes[2] and Pieter van West[1]

1. *Aberdeen Oomycete Group*, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK.
2. *Scottish Fish and Immunology Research Centre*, University of Aberdeen, Zoology Building, Aberdeen, Scotland, UK.
Contact: andrew.phillips@abdn.ac.uk

The pathogenic-oomycete, *Saprolegnia parasitica* infects freshwater fish and is a particular problem in the aquaculture industry where it is estimated that 10% of all hatched salmon succumb to *Saprolegnia*-infection. In addition, *Saprolegnia* species have been associated with the decline of wild salmon populations around the world. The impact of the disease to aquaculture was previously minimized by the use of an organic dye, malachite green. However, the use of this compound has been banned resulting in a dramatic recrudescence of *Saprolegnia* infection, and as a result has increased the need to study and understand this important host-pathogen interaction.
To enable us to study the fish-*Saprolegnia* interaction we have developed an *in-vitro* infection model, where a cultured-monolayer of a primary fish cell-line (RTG-2) is infected with cysts of *S. parasitica*. This model has enabled us to harvested material from several stages of the interaction between fish and *Saprolegnia*, allowing us to investigate the kinetics of the infection using a range of molecular, microscopic and biochemical techniques. This integrated approach has allowed us to begin addressing the molecular mechanisms, which enable *Saprolegnia* to successfully infect fish, the processes that suppress host defenses during infection, and the nature of the pathogen/host interaction. Our latest findings will be presented.

A *SAPROLEGNIA PARASITICA* cDNA LIBRARY: MINING ESTs FOR POTENTIAL VACCINE CANDIDATES

Vicky Anderson[1,2], Chris Secombes[2] and Pieter van West[1]

1. *Aberdeen Oomycete Group*, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK.
2. Scottish Fish and Immunology Research Centre, University of Aberdeen, Zoology Building, Aberdeen, Scotland, UK.
Contact: *v.anderson@abdn.ac.uk*

Saprolegnia parasitica is a fish pathogenic oomycete capable of causing disease in freshwater fish species. Since the global ban of the preferred control agent malachite green, *S. parasitica* infections have become a re-emerging problem for aquaculture, causing major losses worldwide. It is therefore essential to find a new and effective control strategy. Our work is aimed towards the development of a vaccine against *S. parasitica*. An infection model was established using Atlantic salmon *Salmo salar* and a cDNA library was constructed using *S. parasitica* mycelia interacting with the Atlantic salmon host. Sequencing of 3000 ESTs from this library has given an insight into the genes transcribed at this crucial point in the *S. parasitica* lifecycle. Both *S. parasitica* and *S. salar* genes identified in the library are of interest to our research. To assess the *S. salar* response to a *S. parasitica* infection, qualitative PCR has been performed on genes sequenced from the library and revealed numerous genes that are significantly up-regulated during infection. The *S. parasitica* genes sequenced from the library form the basis of the selection of vaccine candidates. By expressing these genes in salmonids, we hope to identify a protein from *Saprolegnia* that induces an immune response in the host. Ultimately, this work may lead to the development of a commercial vaccine that has the potential to alleviate the problems caused to the aquaculture industry due to *S. parasitica*.

Do You Know Human Pythiosis?

Theerapong Krajaejun

Department of Pathology
Faculty of Medicine-Ramathibodi Hospital
Mahidol University
Rama 6 road, Bangkok 10400, Thailand.

Human pythiosis is an emerging, life-threatening infectious disease caused by *Pythium insidiosum*, which is the only oomycete member known to infect humans and some animals in tropic and subtropic countries. The disease has high level of mortality and morbidity. Human pythiosis is endemic in Thailand and mostly presented with arteritis resulting in limb amputation or death, and cornea ulcer leading to enucleation. Diagnosis of pythiosis is time consuming, and needs expertise. Radical surgery is a main option for treatment of pythiosis because medical treatment is ineffective. New cases have been increasingly reported. Thalassemia and agriculture-related careers are predisposing factors. It is apparent that more needs to be done in the way of basic research to provide insights into *P. insidiosum*'s biology and pathogenesis, and thereby lead to the discovery of novel strategies for infection control. Molecular genetic research of *P. insidiosum* is just in an initial step. Some research progressions on *P. insidiosum* will be shared in the meeting, which include: (i) Identification of the encoding gene of the 74-kDa immunodominant antigen of *P. insidiosum*, a candidate for diagnostic test and vaccine development; (ii) Phylogenetic analysis of clinical and environmental isolates of *P. insidiosum* from Thailand and around the world; and (iii) Sequence identification of *P. insidiosum* genes (ESTs).

Session 2

Examining the drivers in a dramatic shift in *P. infestans* populations

David Cooke[1], Alison Lees[1], Ruairidh Bain[2], Nick Bradshaw[3], David Shaw[4] and Moray Taylor[5]

[1]SCRI, Invergowrie, Dundee, DD2 5DA, Scotland
[2] Scottish Agricultural College (SAC), Auchincruive, Ayr, KA6 5HW, Scotland
[3]ADAS, Henstaff Court Business Centre, Groesfaen, Cardiff, CF72 8NG, Wales
[4]Sárvári Research Trust (SRT), Henfaes Research Centre, Abergwyngregyn, Llanfairfechan, LL33 0LB, Wales
[5]Central Science Laboratory (CSL), Sand Hutton, York, YO41 1LZ, England

Understanding the nature of genetic variation in natural populations and the evolutionary forces that shape such populations is of great scientific interest. When the species involved is *Phytophthora infestans* the cause of serious late blight disease of potato and tomato then the focus is sharper with the outcomes likely to have practical implications on the success of short and long-term strategies of disease management. In general, populations of *P. infestans* have been characterized by the localized occurrence of sexual populations generating 'hopeful monsters' from which a limited number of highly successful clones have emerged that may dominate crops on a regional to global scale for decades until their eventual displacement by new clonal lineages. Gaining a detailed view of the mechanisms and processes behind such transitions has been challenging but the current study provides such an opportunity. In this British Potato Council funded project we are monitoring the *P. infestans* population (approximately 3000 isolates, to date) in GB crops using neutral SSR markers. We have shown that the population is dominated by fewer than eight clones and a dramatic population shift is underway due to the migration of a single clonal lineage. Detailed comparisons of the different clonal lineages have identified increases in aggressiveness and fitness as principal drivers of this transition. This study, in combination with the wealth of information emerging on *P. infestans* effector diversity, provides an excellent opportunity to examine associations between genotype and phenotype. That is, the repertoire of pathogen effectors in relation to the aggressiveness and fitness of different *P. infestans* lineages.

Differential Abundance Of Proteins In Pre-invasion Stages Of The Downy Mildew Oomycete *Peronospora Viciae*

Josiane Chuisseu, Judith Harrison, Heather Macdonald and Peter Spencer-Phillips

Faculty of Applied Sciences, University of the West of England, Bristol, Frenchay Campus Coldharbour Lane BS16 1QY

The plant pathogenic oomycete *Peronospora viciae* causes downy mildew of pea (*Pisum sativum*), resulting in up to 55% yield losses in the UK. Proteomics has been used to analyse the host-pathogen interaction, with the specific aim of identifying key marker and target proteins that may enable the development of novel methods for detection and control. We focus here on the application of two dimensional (2D) gel electrophoresis and mass spectrometry to proteins extracted from three pre-invasion developmental stages of the pathogen: 1) conidia from sporulating infections; 2) germinating conidia; 3) conidia with germ tubes and appressoria. Over 700 protein spots were present on 2D gels from each stage following staining with Coomassie blue. Data from MALDI-TOF peptide mass fingerprints and Q-TOF analysis of amino acid sequences has enabled identification of 11 proteins. These include 6 that matched other oomycete proteins: actin 1, actin 2, calmodulin, two glyceraldehyde-3-phosphate dehydrogenase (GAPDH) proteins and heat shock protein 70 (hsp70). A further 53 did not match any proteins in the Matrix Science (MASCOT) database. Calmodulin showed a similar abundance in all three pre-invasion stages, whilst actin 2, the two GAPDH proteins and hsp70 showed at least a two-fold decrease in abundance between the conidial and the appressorium stages. In contrast, actin 1 remained unchanged in abundance between un-germinated and germinated conidia, but decreased by two-fold at the appressorium stage. A further 9 unidentified proteins showed a two- to three-fold alteration in abundance between the different stages. These results suggest that the proteins with a differential abundance may play an important role in the pre-invasion stages of pathogen development, and therefore could have potential as novel targets for control. Proteins that did not alter in abundance (e.g. calmodulin and some of the unidentified proteins) have potential as markers for detection of early stages of infection using biosensors.

The *Phytophthora capsici*-Tomato Interactome
Alon Savidor[1], Oscar Hurtado-Gonzales[2], W. Hayes McDonald[3] and Kurt H. Lamour[2] [1]Genome Science & Technology, The University of Tennessee [2]Entomology & Plant Pathology Department, The University of Tennessee [3]Organic & Biological Mass Spectrometry Group, Oak Ridge National Laboratory
Organisms in the genus *Phytophthora* are destructive plant pathogens that infect many agriculturally and ornamentally important plants. *Phytophthora capsici* is a vegetable pathogen that causes significant damages to tomato, pepper, and a wide variety of cucurbit crops. Our objective is to identify *P. capsici* proteins that play a key role in the infection process. As a first step towards this goal, multidimensional protein identification technology (MudPIT) was used to measure the "interactome" (the protein complement) of *P. capsici*-infected tomato plants over the course of 3 days of infection. Multiple *P. capsici* proteins were identified that may be involved in infection, as well as tomato proteins that may be involved in the response to infection.

Transcriptome of Aphanomyces euteiches : new oomycete pathogenicity factors and metabolic pathways
Gaulin E., Madoui A., Dumas B. UMR5546 CNRS-Université Paul Sabatier Pole de Biotechnologie Végétale 24 Chemin de Borde-Rouge BP4617 auzeville 31326 Castanet-tolosan
Aphanomyces euteiches is an oomycete pathogen that causes seedling blight and root rot of legumes, such as alfalfa and pea. The genus *Aphanomyces* is phylogenically distinct from well-studied oomycetes such as *Phytophthora* sp., and contains species pathogenic on plants and aquatic animals (for review, Gaulin etal., 2007). To provide the first foray into gene diversity of *A. euteiches*, two cDNA libraries were constructed using mRNA extracted from mycelium grown in an artificial liquid medium or in contact to plant roots. A unigene set of 7,977 sequences was obtained from 18,864 high-quality expressed sequenced tags (ESTs) and characterized for potential functions. Sequences are stored in a publicly available databse called AphanoDB (http://www.polebio.scsv.ups-tlse.fr/aphano/) (Madoui et al., 2007) Comparisons with oomycete proteomes revealed major differences between the gene content of *A. euteiches* and those of *Phytophthora* species, leading to the identification of biosynthetic pathways absent in *Phytophthora*, of new putative pathogenicity genes and expansion of gene families encoding extracellular proteins, notably different classes of proteases (Gaulin et al., 2008). Among the genes specific of *A. euteiches* are members of a new family of extracellular proteins putatively involved in adhesion, containing up to four protein domains similar to fungal cellulose binding domains. Comparative analysis with the proteomes of fully sequenced eukaryotic pathogens, including fungi, apicomplexa and trypanosomatids, allowed the identification of *A. euteiches* genes with close orthologs in these microorganisms but absent in other oomycetes sequenced so far, notably peptide transporters and non-ribosomal peptide synthetases, and suggests the presence of a defense mechanism against oxidative stress which was initially characterized in the pathogenic trypanosomatids Gaulin et al. (2008) : Transcriptome of *Aphanomyces euteiches* : new oomycete pathogenicity factors and metabolic pathways. Plos One (in Press). Gaulin et al. (2007) Root rot disease of legumes caused by *Aphanomyces euteiches*. Mol. Plant Pathol. 8: 539-548 Madoui et al. (2007) AphanoDB: a genomic resource for *Aphanomyces* pathogens. BMC Genomics 8:471

HpAvh98 ~ B163
↳ Induces cell death in N. benth
↳ localize to ~~the~~ onion nuclei

Characterizing Conserved Effector Proteins from *Hyaloperonospora parasitica*

R. G. Anderson[1], R. A. Fee[1], P. Thakur[1], R. H. Y Jiang[2,3], D. Dou[2], X. Wang[2], B.M. Tyler[2], J.M. McDowell[1]

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Many types of plant pathogens utilize effector proteins that are secreted to the inside of host cells, where they interact with host targets to promote disease. Oömycete effectors carry a host targeting sequence that is required for translocation into the host cell. We are using the interaction between the model plant *Arabidopsis* and its downy mildew pathogen *Hyaloperonospora parasitica (Hp)* to understand how oömycete effectors manipulate plant cells. We have recently assembled a draft sequence of the *Hp* genome. Bioinformatic analyses have revealed over 190 candidate effector genes with a secretory leader, RXLR, and dEER motifs. We are focusing on a small subset of these genes that have moderately conserved homologs in the soybean pathogen *Phytophthora sojae*. Secreted effectors conserved between *Hp* and *Phytophthora* may have important functions in oömycete pathogenicity. We have determined *in planta* expression for each candidate effector during the course of the *Hp* interaction with *Arabidopsis*. In addition, we are examining subcellular localization of these effectors *in planta* and are carrying out various assays to test potential virulence functions. One *Hp* effector (HpAvh98) can suppress programmed cell death (PCD) triggered *in planta* by mammalian BAX. A *P. sojae* homolog of HpAvh98 also suppresses PCD *in planta*, suggesting that these proteins share a conserved function in plant defense suppression.

Exploiting transcriptome sequences to understand effector evolution in the *P. infestans* species cluster

Liliana M. Cano, Joe Win, David Studholme, Carla Garzon, Maria E. Hernandez, Brian Haas, Michael Zody, Chad Nusbaum, and Sophien Kamoun

The highlands of Central Mexico are the center of origin of the potato late blight pathogen *Phytophthora infestans*. Additionally, they are home to several species very closely related to *P. infestans* namely *P. mirabilis* (causes leaf blight on *Mirabilis jalapa*) and *P. ipomoeae* (causes leaf blight on *Ipomoea longipedunculata*), respectively. These species have evolved by host switching followed by adaptation and specialization on distinct host plants belonging to three different botanical families. Like other oomycetes, *P. infestans* secretes a large repertoire of effector proteins that have been noted to evolve rapidly through birth-and-death evolution and typically exhibit the hallmarks of adaptive (Darwinian) selection. Studying this evolutionary dynamics of effector genes in the *P. infestans* species cluster is crucial for understanding how these species adapted to their respective hosts. Our objective is to characterize the variations in structure, evolution and function of effectors in *P. infestans* and its two sister species using transcriptome sequence analysis. We used both traditional Sanger and Illumina/Solexa technologies to sequence the transcriptomes of *P. mirabilis* isolate PIC99114 and *P. ipomoeae* isolate PIC99167 represented in normalized cDNA libraries constructed from mixed developmental stages (mycelia and germinated cysts). Sequence analysis of assembled contigs enabled us to identify similarities and variations in several candidate effectors including protease inhibitors, Crinklers and RxLR effectors. These analyses identified several cases of positive selection, pseudogenization, and gene loss in the *P. infestans* species cluster. We are currently examining the distribution of the identified polymorphisms among a larger set of isolates to reveal associations with effector functionality and performing activity analysis using plant assays. Our study highlights the value of comparing transcriptomes from closely related species to identify and study fast evolving gene families. The information generated in this study will greatly facilitate follow-up functional analyses to connect the discovered polymorphisms with effector activities and interaction with host targets.

pseudogenization Ψ

Genome plasticity revealed by comparative genomics of *Phytophthora* isolates, sibling species and distant relatives

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The destructive late blight pathogen *Phytophthora infestans* is notorious for its rapidly evolving virulence, which correlates with a dynamic genome and rapidly changing gene set.
Large numbers of retrotransposons are present in the repeat rich genome of *P. infestans*. These mobile elements appear to play roles in two aspects of remodeling the genome: paralog generation and large-scale structural changes. Genome-wide comparative analyses showed that retrotransposons are enriched in regions containing expanded gene families. Blocks of conserved synteny tend to be generally poor in mobile elements, while regions lacking synteny tend to be enriched in specific retrotransposons. These results indicate that abundant retrotransposons are associated with increased genome fluidity.
Comparative genomic analysis revealed a surprising variety of dynamic patterns of effector gene evolution, including rapid duplication, frequent genome rearrangement, extensive domain shuffling and large-scale pseudogene formation. The RXLR effectors belong to one of the most rapidly evolving families. In the example of one 800 kb genome region, all 7 RXLR effector genes show copy number variation in a set of field isolates, indicating that these genes are generated and deleted at a rapid rate. We have demonstrated that one of these genes, the newly cloned Avr1 gene, is prone to copy number changes and that these structural changes correlate with changes in virulence.
New sequencing technology gives us the opportunity to address these questions comprehensively in the genome. We have recently generated whole genome shotgun sequence data of two field isolates of *P. infestans* and four closely related sibling species, using the Illumina Genome Analyzer. These data enable us to study sequence variation and copy number changes, offering insight into the scale, nature, scale and mechanisms of genome dynamics, and in particular evolution of effector genes and their impact on virulence.

Identification of novel effectors from *Albugo candida* using Solexa cDNA sequencing techniques

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It was hypothesised (Jones and Dangl, 2006) that some pathogens fail on a non-host because their effectors have not been selected to evade recognition by the plant and thus trigger resistance (non-host resistance, NHR). In order to make use of this kind of resistance for plant protection it is important to know how pathogens evade recognition or how they suppress the defence reactions.
An ideal candidate to analyse defence suppression is *Albugo candida* (white rust, Oomycete). After infection of *Arabidopsis thaliana* by a compatible *A. candida* strain, the plant becomes susceptible to formerly incompatible downy mildew pathogens (Cooper et al., 2002). This indicates a suppression of non-host resistance.
To find these important suppressors, the in planta transcriptome of *A. candida* strains is being sequenced and assembled using Solexa/Illumina sequencing technique. To cover the entire in planta transcriptome of the pathogen a method was established to normalize, enrich and concatemerize pathogen mRNA in order to reach homogenous sequence coverage. After assembly, the sequence data can be used to computationally analyse for effectors. Fast evolution of effector genes due to arms race between pathogen and host (Dodds et al., 2006) facilitates the screen for important effectors. An effector data base will be build up and related effectors that nevertheless exhibit sequence diversity will be inferred.
To verify the computational analyses, potential effectors will be tested for their functionality to repress non host resistance using the effector detector system (Sohn et al, 2007).

Ac suppress defence against Hp

SSAHE
VELVET

Monitoring *Phytophthora infestans* epidemics on potato and tomato in Tunisia using molecular tools

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Laboratory of Plant genetics, Department of biotechnology
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Late blight caused by *Phytophthora infestans* in Tunisia is always present during the year on potato or tomato since weather conditions and plant host are permitting. Indeed, potato is planted and harvested three times from august to June and tomato is planted in field and in greenhouse. Collection of several isolates from these plants, different locations and among various seasons was achieved. Molecular analysis performed on either pure isolates or infected material allowed us to highlight the presence of the A2 type in Tunisia with a stable frequency of 12% on three years of observation. This could be explained by a asexual multiplication rather than the sexual one which is rare. These A2 strains exhibited high levels of resistance to the fungicide metalaxyl. However, no cross resistance was observed with the new fungicide mandipropamid. They also showed more severe symptoms when inoculated on the host. The characterization of the avirulence genes is compulsory to dress a suitable genetic control to the pathogen. On another hand, analysis of genes involved in plant - pathogen interaction revealed that expression level is different according to the origin plant host (potato or tomato) of the strain and the organ infected (leaves, stems or tubers). Some adaptation of the pathogen to the plant material could be possible. All these characterization are important to consider for more efficient control of late blight.

Rays

- transposons (Mariner / Pogo)
- LTR retro (Gypsi Copia)
- Rolling-circle (Helitrons)
- Cyclin recombinase mediated (Cryptons)

Session 3

RXLR-mediated entry of *Phytophthora sojae* effector *Avr1b* into soybean cells does not require pathogen encoded machinery

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Effector proteins secreted by oomycete and fungal pathogens have been inferred to enter host cells. Using the effector protein Avr1b of the oomycete pathogen of soybean, *Phytophthora sojae,* we have shown that a pair of sequence motifs, RXLR and dEER, plus surrounding sequences, are required to deliver the protein into plant cells, and can be replaced by heterologous autonomous protein transduction signals, such as those from the HIV TAT protein. Particle bombardment experiments demonstrate that the motifs function in the absence of the pathogen, indicating that no additional pathogen encoded machinery is required for effector protein entry into host cells. Fusion of the Avr1b RXLR-dEER domain to green fluorescent protein (GFP) enables GFP to enter soybean root cells autonomously. The conclusion that RXLR and dEER serve to transduce oomycete effectors into host cells indicates that the more than 370 RXLR-dEER containing proteins encoded in each of the genome sequences of *P. sojae* and *P. ramorum* are candidate effectors. We have also shown that the RXLR and dEER motifs can be replaced by the erythrocyte targeting signals of *Plasmodium* effector proteins, which the motifs closely resemble. Thus the machinery of the hosts (soybean and human) targeted by the effectors may be very ancient. Mutational analysis of the RXLR motif shows that the first and third positions in the motif is highly sequence specific. For example, FRLR, RFRL, QFLR and RFVR are non-functional. One the other hand RFLQ is almost fully functional. KFLR is partially functional. The sequence specificity of the RXLR motif suggests that it binds to a receptor.

Identification of the host targets of *Phytophthora infestans* intracellular effector AVR3a

Miles R Armstrong", Rosalind Taylor*, Ari Sadanandom*, Jorunn Bos#, Sophien Kamoun#, Paul Birch".

" Plant Pathology, SCRI
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The oomycete *Phytophthora infestans* causes late blight, the potato disease that precipitated the Irish famines of 1846 and 1847. It represents a re-emerging threat to potato production and is one of over 70 oomycete species which are arguably the most devastating pathogens of dicotyledonous plants. The AVR3a protein is a member of the RXLR class of cytoplasmic effectors. Two alleles of AVR3a, AVR3a^{KI} and AVR3a^{EM}, are typically found. AVR3a^{KI}, but not AVR3a^{EM}, activates plant defense triggered by the potato resistance protein R3a and suppresses the cell death response initiated by the *P. infestans* elicitin INF1. R3a activation and INF1 suppression activities appear to be conditioned by distinct amino acids in AVR3a. This suggests that these diverse functions may be modulated by separate host targets. We have screened an infected potato Y2H library with both alleles of AVR3a. Fourteen potential interactors have been identified, some of which appear to be specific to the AVR3a^{KI} allele. There is also evidence that some of the interacting proteins participate in a protein complex. Progress towards validating these interacting proteins will be discussed.

— α SNARE
— TRIP

CMPG1 is an E3 ligase

Molecular basis of cell death suppression by the *P. infestans* RXLR effector AVR3a

Jorunn I.B. Bos[1], Miles R. Armstrong[2], Rosalind M. Taylor[3], Joan Wong[4], Alex M.E. Jones[1], Richard W. Michelmore[4], Ari Sadanandom[3], Paul R.J. Birch[2] and Sophien Kamoun[1]

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The AVR3a effector of *P. infestans* is a member of the RXLR family of cytoplasmic effectors and exhibits dual effector activities. AVR3a induces hypersensitivity mediated by the resistance protein R3a and suppresses cell death induced by *P. infestans* INF1 elicitin. We utilized extensive structure-function analyses to gain insight in the molecular basis of AVR3a effector activities. This approach identified AVR3a mutants that activate R3a but lack cell death suppression activity suggesting that distinct amino acids condition the effector activities of AVR3a. One mutant of particular interest is the AVR3a^{KI} Y147 deletion mutant that is missing the C-terminal tyrosine residue and shows loss of cell death suppression activity but not R3a activation. In addition, results from our mutant analyses pointed to a model that involves the interaction of AVR3a with one or more host proteins and is not consistent with the recognition of AVR3a through an enzymatic activity. To identify candidate virulence targets of AVR3a we used transient *in planta* expression in combination with immunoprecipitations and mass spectrometry. In addition, the Birch and Michelmore labs found that AVR3a interacts with the E3 ligase CMPG1 in yeast-two-hybrid assays. Interestingly, CMPG1 is known to be required for INF1 induced cell death, suggesting that this protein could be involved in AVR3a cell death suppression activity. Our objective is to validate the AVR3a interacting proteins *in planta* and to determine if these proteins mediate cell death suppression by AVR3a. For this purpose, we used transient expression assays in *Nicotiana benthamiana* and CMPG1 transgenic tobacco lines as well as biochemical approaches. Finally, we aim to investigate the contribution of AVR3a to virulence using transgenic tomato, *Nicotiana benthamiana* and *Arabidopsis thaliana* lines expressing AVR3a. These efforts will provide new insights into the contribution of AVR3a to *P. infestans* virulence.

The Possible Role of Avr3a in Ubiquitination

Ros Taylor, Miles R. Armstrong, Eleanor Gilroy, Jorunn I.B. Bos, Sophien Kamoun, Petra Birch, Paul R.J. Birch, Ari Sadanandom

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AVR3a is a member of the RXLR family of cytoplasmic effectors and induces hypersensitivity mediated by the resistance protein R3a. Avr3a also suppresses cell death induced by *P. infestans* INF1 elicitin. Previous work using AVR3a mutants that activate R3a but lack cell death suppression activity suggest that a distinct amino acids cause the effector activities of AVR3a. Previous work by the Birch and Michelmore labs found that AVR3a interacts with CMPG1; an E3 Ligase required for INF1 induced cell death in yeast-two-hybrid assays. From these data the role of Avr3a in the Ubiquitination pathway as part of plant defense suppression is being investigated. The two forms of Avr3a: Avr3aEM and Avr3aKI are believed to have different roles in the ubiquitin pathway. Recent work involves the use of Ubiquitination assays to show the role of Avr3aEM and KI in ubiquitination as well as mutated ubiquitin to show the pathway used by Avr3aEM and KI as part of the interaction with CMPG1. Future work involves the expression of CMPG1, Avr3aEM and Avr3aKI in *Nicotiana benthamiana* for split YFP experiments to show *inplanta* interactions.

Ubiquitin

Pub17 } E3 ligase
AvrPtoB }

Is Avr3a an E2?
↳ E2 conjugating enzyme

Bax induced cell death PNAS 1999 July C. Lacomme

Conserved C-terminal motifs required for avirulence and suppression of cell death by Phytophthora sojae effector Avr1b

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The sequenced genomes of oomycete plant pathogens contain large superfamilies of effector proteins containing the protein transduction motif RXLR-dEER. However, the contributions of these effectors to pathogenicity remain poorly understood. We show here that the *Phytophthora sojae* effector protein Avr1b contributes positively to virulence and can suppress programmed cell death (PCD) triggered by the mouse BAX protein in yeast, soybean and *Nicotiana benthamiana* cells. We identify three conserved motifs (K, W and Y) in the carboxy-terminus of the Avr1b protein and show that mutations in the conserved residues of the W and Y motifs reduce or abolish the ability of Avr1b to suppress PCD and also abolish the avirulence interaction of Avr1b with Rps1b. W and Y motifs are present in at least half the identified oomycete RXLR-dEER effector candidates, and we show that three of these candidates also suppress PCD in soybean. Together these results indicate that the W and Y motifs are critical for the interaction of Avr1b with host plant target proteins and support the hypothesis that these motifs are critical for functions of the very large number of predicted oomycete effectors that contain them.

Regulation of host genes by Nuk10, a nuclear localised RXLR-effector of *P. infestans*

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Phytophthora infestans secretes RXLR-type effectors that were shown to be translocated inside host cells where they modulate plant immunity. Recent availability of five oomycete genome sequences enabled the prediction of proteins that carry a secretion signal, an RXLR translocation motif and nuclar localisation sequences (NLS)
Microarray and RT-PCR analyses identified Nuk10, a modular effector with nuclear localisation motif (NLS) that was expressed during colonization of tomato.

This work aims at identification of host genes that are regulated by nuclear localised effectors, specifically Nuk10, and to characterise their impact on the plant-pathogen interaction.

We applied transient Agrobacterium-mediated expression in tomato and *N. benthamiana* to analyse phenotype and localisation of Nuk10 *in planta*.

Nuclear localization of Nuk10-GFP suggests a function in modulation of the host transcriptome. Nuclear targeting was dependent on presence of the NLS motif. Microarray studies identified at least two tomato genes that are altered in their transcription levels by Nuk10 but not by its NLS-deletion derivative. These differentially expressed host genes were subsequently analysed by RT-PCR. A Nuk10 regulated gene that is conserved in tomato and *N. benthamiana* is now being studied by silencing, overexpression and promoter-reporter gene fusions and recent progress will be presented.

Genetic analysis of the *RPP13/ATR13* interaction complex between downy mildew and Arabidopsis

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Elucidation of complex mechanisms driving host-pathogen co-evolution has been of great interest and presents a challenge. The studies involving the *Arabidopsis-Hyaloperonospora parasitica* pathosystem have contributed extensively to our understanding of the genetics of host-pathogen co-evolution. We have been studying a co-evolutionary model based on the *Arabidopsis RPP13* resistance gene with the highest reported level of sequence diversity among known R-genes, and the highly variable cognate effector gene *ATR13* from *H. parasitica*. Previously, sequence analysis of the *RPP13* gene resulted in numerous distinct clades, and the recognition of *ATR13* by *RPP13-Nd* has been attributed to the alleles in a single clade. In addition, ATR13 independent recognition of various *H. parasitica* isolates by an *RPP13* (Rld-2) allele in a different clade suggested the presence of novel ATR molecules. Furthermore, recognition of various alleles of *ATR13* by resistance genes other than the *RPP13* has been shown. Overall, this has revealed the presence of a complex co-evolution between *Arabidopsis* and *H. parasitica*. In order to provide an in-depth view of such a complex system, we are currently using map-based cloning approaches both in *Arabidopsis* and *H. parasitica* to identify and characterize the genetic components of the RPP13/ATR13 complex. Recent progress towards the cloning of these genes will be presented.

Session 4. KEYNOTE LECTURE

***NATURAL BORN KILLERS*: insights into oomycete origins and infection diversity gained from studies on pathogens of algae and animals.**

Gordon W. Beakes

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The oomycetes are usually thought of as either benign saprotrophic water moulds or as potent pathogens of higher plants. However, there are many species which are pathogens of algae, lower animals (nematodes, crustacea, insects etc.) and even vertebrates (notably fish but also some mammals). We now know that oomycetes reside within the chromalveolate lineage, which encompass a diverse assemblage of protists, algae and fungal-like organisms. Many of the morphologically simple, often marine, pathogens make up the earliest branching clades within the monophyletic oomycete lineage, diverging before the two main terrestrial lines. Even within the latter, parasites of nematodes and insects seem to occur in basal or early branching clades. This suggests the intriguing possibility that the oomycetes may have moved from marine to terrestrial environments along with their animal or algal hosts, before adopting a more typical fungal 'lifestyle' and becoming pathogens of land plants. Many of the species which infect these diverse hosts have simple non-mycelial thalli and show a much greater morphological diversity in their infection cells and infection mechanisms than the more familiar plant pathogenic species. Some of this diversity will be illustrated with reference to parasites of nematodes and fish. One of the major conclusions is that, without exception, all of the early diverging oomycete species are pathogens and it now seems certain that this lineage arose in the sea and came 'hard-wired' for parasitism – indeed they appear to be truly 'natural born killers'. It is hoped that this broad overview of the biological diversity of the lesser known oomycete species might provoke some new questions that might be formulated and tested by the application of molecular genetics and genomics.

38 years in oomycetes 1976 Saprolegnia
1996 nematode infecting oomycetes
Marine oomycetes

Session 5

NLPs – Virulence Factors from Oomycetes, Fungi and Bacteria that Affect the Integrity of the Plasma Membrane of Dicots

Isabell Küfner, Christian Ottmann, Borries Luberacki, Hanns Ulrich Seitz, Gregor Anderluh, Minna Pirhonen, Claudia Oecking, Frédéric Brunner and Thorsten Nürnberger

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The Nep1-like Protein (NLP) superfamily is named after its first identified member from *Fusarium oxysporum*, Nep1 (Necrosis and Ethylene inducing Peptide 1). It comprises proteins from oomycetes, fungi and bacteria but no homologous proteins are present in animals or plants.

Proteins of this family have been shown to trigger cell death and various immune responses in dicotyledonous plants, but until now not in monocotyledonous plants or other organisms. During plant-*Phytophthora spp.* interactions, NLPs show an enhanced production with the onset of the necrotrophic phase. Furthermore, a virulence promoting activity has been shown e. g. for the NLP of *Pectobacterium carotovora* (NLP_{Pc}). Thus we consider NLPs as toxin-like virulence factors. Activity of NLPs was also observed in protoplasts and thus does not require an intact plant cell wall. In addition, the protein acts on the extracytoplasmic side of the plant plasma membrane.

The 3D structure of a NLP from *Pythium aphanidermatum* reveals a cation-containing cavity that turns out to be crucial for the cell death-inducing activity and hence for the virulence of the pathogen. Otherwise, structural analysis of the NLPs did not reveal the presence of any characterized functional domain. Pore formation or the disruption of the plasma membrane integrity seems to be responsible for the cell death, although the molecular mechanism underlying the mode of action remains to be elucidated.

Why are the Arabidopsis *dmr* mutants resistant to downy mildew?

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The Arabidopsis *dmr* (downy mildew resistant) mutants carry recessive mutations that result in loss of susceptibility to the oomycete *Hyaloperonospora parasitica*. We have map-base-cloned four of these genes (*DMR1*, *DMR3*, *DMR5*, and *DMR6*). In the *dmr3* and *dmr5* mutants the activated defense responses are the probable cause of resistance to *H. parasitica*. In the *dmr6* mutant defense responses are only slightly activated. We are currently analysing *dmr6/npr1* and *dmr6/sid2* double mutants to determine if the *NPR1* or *SID2* is required for *dmr6*-mediated resistance. As *DMR6* encodes an oxidoreductase of unknown function we are performing untargeted metabolomics to identify its potential substrate and product. The *dmr1* mutants have defects in homoserine kinase that result in the accumulation of homoserine. We will present data that show that *dmr1*- resistance to downy mildew is mediated via, an as yet, unknown pathway and mechanism. Genetic suppressors of *dmr1*-resistance have been identified as well as additional TILLING mutants. The preliminary characterization of these mutants will be discussed. The *dmr* mutants have led us to the identification of *DMR* genes that were previously not associated to plant disease or resistance. Their functional analysis will provide us with new insights into hitherto unknown mechanisms of resistance to oomycete pathogens.

Phytophthora phospholipase D genes and their role in plant cell degradation

Harold J.G. Meijer, Shutong Wang, Klaas Bouwmeester and Francine Govers

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Phospholipase D (PLD) catalyzes the hydrolysis of structural phospholipids, such as phosphatidylcholine, leading to the production of phosphatidic acid and a free headgroup. Phosphatidic acid is a keyplayer in the arena of cellular signalling. It is involved in many processes, including G-protein regulation, protein phosphorylation, transcription, cell proliferation and growth. PLD activity in plants is correlated with membrane degradation during senescence and wounding. High levels of phosphatidic acid induce loss of cell membrane integrity and subsequently cell viability.

In comparison to other eukaryotes, *Phytophthora* spp. have a more complex and diverse set of PLD genes. Of the 18 PLD genes that are found only one encodes a universal PLD that is present in all eukaryotes. In this PXPH-PLD two lipid-binding domains, PX and PH, precede the catalytic PLD domain. The other seventeen are divided over four novel PLD sub-families. Three of the sub-families encode small PLD-like proteins. They are divided based on their catalytic domains and the absence or presence of a signal peptide. We have previously shown that the PLD activity present in exudates of *Phytophthora* cultures is capable of degrading lipid vesicles derived from plant material and exhibits substrate specificity. To further test the hypothesis that the PLD-like proteins play a role in pathogenesis we are currently analyzing the effects caused by transient expression of PLD-like genes in plants. Here we will present the latest results.

Toward the Identification of Oomycete Effectors suppressing PAMP-triggered Immunity in _Arabidopsis thaliana_

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Text of abstract, approximately 300 words

Both plants and animals possess innate defence mechanisms to resist microbial infection. The primary plant immune response is referred to as PAMP-triggered immunity and has evolved to recognize invariant microbial structures termed pathogen or microbe-associated molecular patterns (PAMPs/MAMPs). Typical PAMPs/MAMPs include flagellin and lipopolysaccharides from bacteria or the Pep-13-containing transglutaminase and Necrosis and Ethylene inducing–like Protein from *Phytophthora spp.* Microbial interactions with host cells frequently involve the utilization of effectors that help infection and cause disease in the plant. Recent studies have identified a host-targeting signal centered on a RXLR-EER motif present in major, known, oomycete (a)virulence proteins. How these eukaryotic effectors are translocated in the host cell is largely unknown. *In silico* analysis of the *Phytophthora spp* and *Hyaloperonospora parasitica* genomes predicts hundreds of putative RXLR-EER effectors.

An *Arabidopsis* protoplast-based system was developed to screen for oomycete (priority is given to *H.parasitica*)-RXLR-EER effectors that suppress PAMPs/MAMPs-mediated defence gene activation. A major advantage of the system is that it simplifies the complex pathogen-plant interactions to pure individual signals and synchronized cell-autonomous responses. It potentially allows performing an epistatic analysis of PAMP/MAMP signalling suppression via gain- or loss-of function experiments.

He et al Cell (2006) } now able to reproduce the results in this paper ← High transformation rate > 60% of Arabidopsis protoplast is needed

PAMP-inducable promotors

WRKY29

FRK1

PR-1

TetraFA is an inhibitor for esterase
↳ Structural analog of SA

Potato's SA-binding protein 2 ortholog (StSABP2) is required for SAR against *Phytophthora infestans* induced by prior treatment with arachidonic acid

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After pathogen attack plants activate a variety of defenses to restrict pathogen growth and spread. Systemic acquired resistance (SAR) is a physiological state of enhanced defense induced by an initial local infection, whereby the plant's innate defenses are potentiated throughout the plant to subsequent biotic challenges. Studies in tobacco and Arabidopsis have shown that salicylic acid (SA) is a critical component requires for SAR activation but it is not the mobile signal, which is translocated from the site of infection to systemic tissue. Our group recently identified methyl salicylate (MeSA) as a mobile signal for SAR in tobacco and Arabidopsis. In tobacco, MeSA synthesized in the primary infected leaves is a phloem-mobile signal for SAR that must be converted into biologically active SA in the systemic tissue by the esterase activity of SABP2. High SA levels in primary infected tissue inhibit SABP2's esterase activity, thereby facilitating buildup of MeSA for translocation.
Potato is the world's fourth largest crop and is very susceptible to devastating pathogens like *P. infestans*. In contrast to tobacco and Arabidopsis, which have very low levels of SA, potato contain high basal levels of SA and the role of SA during SAR is not well established. SAR in potato can be induced by pathogens as well as by treatment with fatty acids, such as arachidonic acid (AA). In this study we identified a putative potato ortholog of tobacco *SABP2* (*StSABP2*) and show that it has MeSA esterase activity, which is inhibited by SA binding. A synthetic SA analogue 2,2,2,2',-tetrafluoroacetophenone (tetraFA), which competitively inhibits its esterase activity *in vitro*, blocked SAR against *P. infestans* induced by prior treatment of lower potato leaves with AA. Moreover, RNAi-mediated silencing of *StSABP2* compromised SAR induced by AA. Together these findings argue that StSABP2 and MeSA are critical components for SAR in potato.

Identification of plant proteins targeted by oomycete RXLR effectors using *in planta* co-immunoprecipitation

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Oomycete plant pathogens establish parasitic colonization by modulating host defences via an array of secreted effector proteins. Our research focuses on RXLR effectors that were shown to be translocated into the plant cells. The function of these effectors may involve physical interactions with plant proteins leading to inhibition, interfering with post-translational modifications, transport, and stability of the targeted proteins. We aim at identifying these plant proteins targeted by oomycete effectors. We selected 23 validated oomycete RXLR effectors including several avirulence proteins, effectors that cause cell death, and those under positive selection. We made expression constructs based on high-expression vector pTRBO, a binary plasmid containing a modified *Tobacco mosaic virus* with its coat protein gene replaced by cDNAs coding for mature oomycete effectors fused to N-terminal FLAG tags. The modified viruses with the effector payloads were delivered into the leaves of *Nicotiana benthamiana* by agroinfiltration and effectors were expressed *in planta* to high levels under the control of the viral coat protein promoter. The leaves were harvested 2-3 days after infiltration and total proteins were extracted. FLAG-tagged effector proteins and their putative interactors from the plant were co-immunoprecipitated (co-IP) with anti-FLAG resins under non-denaturing conditions. Bound proteins were specifically eluted using 3X FLAG epitope peptides and separated by SDS-PAGE. The proteins in the gels were visualized by colloidal Coomassie blue. Protein bands were excised, digested with trypsin, and peptide species were identified by LC-MS/MS using the LTQ-Orbitrap mass spectrometer. We were able to express 22 out of 23 effectors to sufficient levels for co-IP and subsequent MS identification of precipitated proteins. We are currently analysing the MS data using both pattern matching and *de novo* sequencing techniques. We will present and discuss the roles of the putative interacting plant proteins and putative functions of the effectors.

Session 6

(A)virulence on *Rpi-blb1* expressing plants is determined by the *ipiO* effector family from *Phytophthora infestans*

Nicolas Champouret, Hendrik Rietman, Klaas Bouwmeester, Francine Govers, Evert Jacobsen, Richard Visser, Edwin van der Vossen, and Vivianne Vleeshouwers

Profiling the spectrum of resistance (*R*) genes in potato and avirulence (*Avr*) genes in *Phytophthora infestans* can lead to significant progress in acquiring long term genetic resistance against late blight, the major disease on cultivated potato. The 11 *R* genes introgressed from *Solanum demissum* were broken rapidly in the field, but recently, renewed hope for *R*-gene based breeding emerged with the discovery and cloning of so-called broad-spectrum genes, e.g. *Rpi-blb1* from *S. bulbocastanum* (van der Vossen et al., 2003). Is this resistance indeed broad-spectrum? The recent cloning of *Avr-blb1 (ipiO)* enables addressing these questions, and in this study we examined a highly diverse collection of 15 *P. infestans* isolates for genetic variation of *ipiO* and for virulence on *Rpi-blb1* (Vleeshouwers et al., submitted). We detected 7 different *ipiO* genes in this set, which were classified in 4 groups by phylogenetic analyses. Class I and II contain the highly similar *ipiO1* and *ipiO2*, respectively, whereas class III and IV contain genetically more divergent *ipiO* genes. We found that most *P. infestans* strains contain *ipiO* genes from class I/II, except two Mexican strains that were originally isolated from *S. stoloniferum*. Detached leaf tests on potato cultivars and wild *Solanum* species containing *Rpi-blb1* genes, i.e, *S. bulbocastanum, S. stoloniferum* and *S. papita,* showed that most strains were indeed avirulent on *Rpi-blb1*, but virulence was evident for the two Mexican strains that lack the class I/II *ipiO* genes. The findings suggest that (a)virulence on *Rpi-blb1* could be determined by *ipiO* variation in *P. infestans* strains. In addition to virulence, some other highly aggressive strains were able to cause symptoms on the leaves of the transgenic cultivars but not on the wild species, suggesting that *Rpi-blb1*mediated resistance levels in a cultivated potato background are lower than in the wild species. Future large scale studies on genetic variation of *ipiO* in contempary *P. infestans* populations as well as field tests of transgenic potatoes expressing *Rpi-blb1* will inform likely durability of *Rpi-blb1*.

van der Vossen, E.A.G. et al. (2003). TPJ 36, 867-882.

A multi-faceted approach towards understanding the function of the *Phytophthora infestans* Crinkler protein family, particularly the CRN16 class

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The plant pathogenic oomycete *Phytophthora infestans* forms intimate associations with its host, a feat that requires extensive reprogramming and suppression of defense responses. Perturbation of defense signaling is achieved by delivery of a suite of effector molecules that modify, mimic or eliminate host signaling events during infection. The *crinkler* (*crn*) gene family encodes a large class of secreted proteins. Computational analyses unveiled a conserved N-terminal LxLFLAK motif, followed by diverse C-terminal domains with markedly different levels of conservation in related oomycete species. Transient expression of these domains in plants result in cell death in some but not all cases, reflective of diverse functions and roles in virulence. These findings have led to the proposition that the *crinkler* genes encode a large family of effector proteins in *Phytophthora*. To substantiate this hypothesis, we chose to characterize *crn16*. Computational analyses indicate that CRN16 is a modular protein with the signal peptide and LxLFLAK domain followed by a predicted ATP-binding P-loop motif and a C-terminal region that is conserved in oomycetes. Ectopic expression of the C-terminal domain in plants does not induce cell death, suggesting a distinct role for this protein compared to other Crinklers. To unearth CRN16 function and its homologs, we embarked on an approach that combines transient expression studies with biochemistry, cell biology as well as genetics in the *P. capsici-Nicotiana benthamiana* interaction system. Towards these goals our current efforts are aimed at (i) developing novel reporter systems that detect protein translocation and delivery, (ii) localization studies of CRN proteins during infection, (iii) identification of CRN host targets and (iv) functional characterization of CRN proteins *in planta*. Here we will report on our progress and comment on our current understanding of the function of the Crinkler protein family in oomycetes.

STEROLS BIOSYNTHESIS IN *APHANOMYCES EUTEICHES*, NEW TARGET FOR PEA ROOT ROT MANAGEMENT

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A major biochemical target of fungicides is the cytochrome p450 CYP51 enzyme involved in sterol biosynthesis. However these fungicides are inefficient on oomycetes like *Phytophthora* since CYP51 genes are absent in their genomes. Recently, we have performed a large scale analysis of *Aphanomyces euteiches* ESTs which revealed the presence of transcripts showing a high similarity to plant cytochrome p450 CYP51 and other sterol biosynthesis enzymes. This suggests that *A. euteiches* may be able to synthesize its own sterols and that this pathway could be a new target for anti-*Aphanomyces* strategies. Here we present a genomic and biochemical characterization of *A. euteiches* sterol biosynthesis pathway and the efficiency of CYP51 inhibitors on *A. euteiches* growth.

Molecular and biochemical analyses showed that a sterol biosynthetic pathway is present and active in *A. euteiches*. Specific genes that code for sterol biosynthesis pathway enzymes may be lost in *Phytophthora* but kept by *A. euteiches*. Biochemical analyses showed a sterol prototrophy of *A. euteiches*. *In vitro* assays revealed the efficiency of triazoles to inhibit mycelial growth and could help pea root rot management

Avirulence mining in the secretome of *Phytophthora infestans*: implications for durable resistance.

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Phytophthora infestans, the causal agent of late blight in potato (*Solanum tuberosum*), secretes numerous effector proteins, which are believed to interact with host plants. Botanic *Solanum* species growing in the center of origin of *P. infestans* evolved an arsenal of resistance (*R*) genes, and an abundance of cognate avirulence (*AVR*) effectors are expected to have co-evolved in the pathogen. All known *AVR* genes of *P. infestans* contain a signal peptide for secretion into the apoplast, and RxLR or RxLR-EER motifs for subsequent host cell internalization. To identify novel R-Avr interactions we screened a diverse set of resistant botanic potatoes by PVX agroinfection with an initial set of candidate genes coding for Phytophthora extracellular (Pex) proteins, comprising both RxLR(-EER) and apoplastic proteins. We observed three classes of effectors based on the responses in *Solanum*:

1) Effectors that induce cell death responses in most or all *Solanum* species assayed. This group includes the proteins CRN8, PiNPP1.1, CRN2 (positive control) and 5 RxLR proteins.

2) Effectors that induce cell death in specific phylogenetic petota clades, suggesting that *P. infestans* has undergone coevolution with phylogenetic groups. These interactions are genotype-specific, suggesting that genes leading to cell death can be identified through genetic analyses.

3) Effectors that induce cell death in many genotypes, belonging to most or all phylogenetic clades. Some of these suggest an ancient interaction between *Solanum* and *P. infestans* before Petota speciation, e.g. the *Avr3a* homologue *PEX 147-3* (Armstrong et al. 2005).

Some RxLR proteins showed no response at all, suggesting that either these proteins may not induce cell dead, or a macroscopically cell death is not visible since these proteins are expressed at such high levels, thus resulting in extreme resistance (ER). For some allelic RxLR effectors we observed differential responses in *Solanum*, suggesting that one of the tested alleles might specifically interact with an *R* gene, and genetic studies are underway. With up to 425 RxLR-EER genes identified in the *P. infestans* genome (Whisson et al. 2007), extending this functional genomics approach may result in detecting a substantial amount of *R* genes evolved in *Solanum*. Moreover, this study provides insight into functional R-AVR interactions, and promises to aid in judicious exploitation of *R* genes for durable late blight disease management

Armstrong, M. et al. (2005). PNAS 102: 7766-71.Whisson, S. et al. (2007). Nature 450: 115-8.

***In Silico* Identification of Regulatory Elements in Oomycete genomes using WordSeeker**
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Both high throughput experimental approaches and computational strategies are now applied to develop pictures of protein-protein interactions in model genomes. A predicted interactome has now been produced for *Arabidopsis* that is based on the assumption of evolutionary conservation of protein-protein interactions across plant, animal and fungal kingdoms. However a similar strategy for oomycetes may be less useful given their unique evolutionary history. Unique features of oomycete genomes include the absence of whole genome duplication events, and the transfer of several nuclear and plastid genes from the red algal symbiont to the nucleus of its protozoan ancestor. At least 2% of the oomycete genome also consists of novel multifunctional proteins that are derived from domain swapping and fusion events, and horizontal introgression of bacterial genes is also a prominent feature of the oomycete genome. In this study we have investigated whether the sequences immediately upstream of orthologous genes in the genomes of *P. sojae, P. ramorum* and *P. infestans* contain conserved sequence repeats that could serve as transcription binding sites for regulatory elements. The authors have applied this strategy to the genes involved in sulfate assimilation (18 genes) in the three genomes, and to five sets of orthologues in the ABCA family (15 genes). Motif discovery analysis was conducted using WordSeeker, which consists of an enumerative motif discovery algorithm and a Markov-model-based word scoring function. For genes in the sulfate assimilation *(SA)* pathway. the word "CCAAT" and its reverse complement (ATTGG) were detected as highly conserved words, with CCAAT occurring times 41 times in 17 of the sequences (denoted as (41/17)) and ATTGG occurring (16/37) times. Furthermore, the reverse complement of CAAT is conserved in all 18 sequences. Additional significant words in the SA set are GCAAA (16/31), TCAAG (16/29), CACGC (16/27), ATCAC (16/21), and TTCGT (16/24). In the analysis of the five sets of three *ABCA orthologues,* several different conserved words were found. In set one GATTGCC (3/4) and GTAATCG (3/3) marked the most overrepresented words, while set two is defined by CAGTGTAG (3/3) and CTCACACC (3/3); for set three CCTATTCCA (3/3); for set four CCAGCTTC (3/3); for set five CAGAATCC (3/3).

Bioimaging of *P. infestans* stages and computational toolbox

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We describe the use of imaging infrared transmission spectroscopy using a focal plane array to resolve chemical information from potato leaf samples infected with *P. infestans*. We demonstrate that the captured spectra are able to resolve chemically-distinct regions of an infected leaf, and are potentially able to resolve *P. infestans* infective tissue against the leaf background. We also describe the development of a MatLab computational toolbox to process and analyse the generated data.

IR and Raman spectroscopy permit chemical imaging at 1-10 μm

Bruker IR-FTIR microscope

Session 7

Quorum sensing controls zoospore behavior of Phytophthora

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The term quorum sensing was introduced to describe the control of gene expression in bacteria species in response to cell density. Bacteria produce, release, detect and respond to hormone-like signal molecules called autoinducers to coordinate communal behaviors. Here we show quorum sensing operation in *P. nicotianae* and *P. sojae*. Zoospore aggregation and plant infections that require a high concentration of zoospores occurred on a low concentration or single spore level when provided with zoospore free fluid from a highly concentrated suspension (ZFF). Moreover, with supplement of ZFF, zoospores were able to make directional movement to plant tissue. Bioassay and chemical analyses revealed an AI-2 –like molecule in the ZFF. AI-2 is a bacterial universal autoinducer regulating gene expression of many important behaviors including virulence. As in quorum-sensing bacteria, AI-2 affected a number of steps during the zoospore homing process and virulence. These results indicated *Phytophthora* may share similar quorum sensing mechanisms with bacteria although the autoinducers may be produced from different pathways. This mechanism may allow *Phytophthora* species to maximize infection potential by taking advantage of the widespread bacterial autoinducers in nature.

Chitosaccharides are cell-wall structural components and exposed patterns of the phytopathogenic oomycete *Aphanomyces euteiches*

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Chitin is an essential skeletal component of the fungal cell wall, where it forms a crystalline scaffold. Oomycetes are fungal-like microorganisms which usually contain cellulose instead of chitin. evidence was obtained that *A. euteiches* cell-walls contain a chitin-like material. This finding is based on (i) presence of *N*-acetyl-glucosamine in the cell-walls, that can be released by chitinase treatment, (ii) inhibition of growth by the chitin synthase (CHS) inhibitor Nikkomycine Z, (iii) presence and expression of two CHS genes identified through EST and Southern blot analyses. In contrast to true fungi, *A. euteiches* does not contain crystalline chitin as shown by biophysical analyses. Most of the chitosaccharidic material could be solubilized by mild chemical hydrolysis, or by enzymatic hydrolysis of the other polysaccharides, namely cellulose and (1-3)-b-glucans. These observations suggest that chitosaccharides are involved in cell-wall function in *A. euteiches* by acting as an amorphous component, possibly linked to the other glucans, and not as a crystalline scaffold. The other salient feature that distinguish *A. euteiches* from true fungi is that the chitosaccharides are readily exposed at the cell surface, as shown by intense labelling of the cell surfaces by wheat germ agglutinin. Our data show that the involvement of cell-wall chitosaccharides in plant-*Aphanomyces* interactions should be considered, and open new perspectives concerning anti-oomycete drug development.

Nikkomycin Z is a CHS inhibitor
IC50 ~ 50uM 100uM → strain in effect
N2 effect is relieved by [redacted] inositol or sulbitol
Effect of N2 on gene expression → AeCHS2 is induced
not AeCHS1

Dissecting transcriptional networks controlling the development of *Phytophthora infestans* through bioinformatic and functional approaches

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The success of oomycetes such as *Phytophthora infestans* are dependent on their abilities to transition through their life cycles in response to biotic and abiotic stimuli. For example, disease requires coordination of processes such as sporulation, spore germination, and appressorium development. To understand these pathways, transcriptional networks involved in development are being characterized. A starting point has been microarray analyses that identified genes expressed during certain life-stages. Bioinformatics and functional studies are now identifying the transcription factor binding sites (TFBSs) regulating their expression, along with the cognate transcription factors (TFs). Promoters from co-regulated genes were searched for over-represented motifs using Gibbs sampling, expectation maximization, and enumerative search approaches, using algorithms set to identify motifs of differing sizes as well as gapped motifs. High-priority candidates for TFBSs were identified by parsing output from the programs, and sorting and growing candidate motifs to optimal size. Secondary analyses of motifs has involved phylogenetic footprinting, in which conservation between species of *Phytophthora* helps identify high-priority candidates for functional analyses. In summary, bioinformatics identifies many potential TFBSs for each developmental transition, suggesting complexity in the transcriptional networks. Selected TFBSs are now being analyzed through functional studies such as mutagenesis and gel-shift (EMSA) assays. Some promoters appear to be relatively simple. For example, *Pks1* appears to be largely regulated by one TF that recognizes a tandemly repeated motif. Other promoters appear regulated by multiple pathways, such as *Cdc14*. One TF activates *Cdc14* during early sporulation, and a second TF between the sporangia and germinated cyst stages. Data also suggest spatial diversity in the activity of the two TFs. Chromatographic methods are being used to purify the TFs, using EMSA to follow binding activity. Candidate proteins have been identified and sequenced.

Appressorium formation in *Phytophthora infestans*.

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Appressorium formation represents an important event in the establishment of disease by *Phytophthora infestans*. An understanding of molecular events occurring in appressorium development could suggest new strategies for the control of late blight. Unlike fungal appressoria, the structure and precise functions of oomycete appressoria are not well defined. Upon encystment, *P. infestans* spores produce a cellulosic-cell wall, which is extended and thickened upon germination and appressorium formation. Inhibition of cellulose synthesis, using 2,6,Dichlorobenzonitrile (DCB) leads to a dramatic reduction in the number of normal-looking appressoria, severe disruption of the cell wall, and a complete loss of pathogenicity. Here, we also report the identification and characterization of a family of four *CES* genes within *Phytophthora* species. Silencing of this gene family in *P. infestans* leads to the disruption of the cell wall surrounding appressoria, an inability to form typical functional appressoria and a marked decrease in cellulose. Our *in vitro* and ultrastructural studies show that the production of normal appressoria and specifically, the uniformity and thickness of the cell wall is disturbed in *CES1-4* silenced lines or in the presence of the inhibitor DCB.

Indirect measurements of turgor pressure, by incipient plasmolysis, suggest that *P. infestans* appressoria build up significant turgor to aid in penetration of the host plant. We hypothesise that proline may have a role in the generation of turgor pressure. Free proline increases significantly in appressoria, and genes encoding enzymes involved in proline biosynthesis are highly upregulated during zoospore release and appressoria formation. Silencing of the proline biosynthetic enzymes results in partial sporangial cleavage, incomplete zoospore release and bursting of zoospores in water, as well as a decrease in appressoria production and an increase in germ-tube branching under starvation conditions. Here we present our recent findings and hypotheses for the role of cellulose and proline in *Phytophthora infestans* appressoria.

Deciphering the role of *Phytophthora* CRN8 effectors in pathogenicity

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Phytophthora spp. cause some of the most destructive plant diseases in the world. The most notable species is *P. infestans,* causing late blight on potato and tomato. *P. infestans* is an oomycete, and it is now well established that oomycetes secrete an arsenal of effector proteins that modulate plant innate immunity to enable infection. Effectors can be classified based on their target site in the host as apoplastic effectors and cytoplasmic effectors. The N-terminus of cytoplasmic effectors consists of a conserved motif behind the signal peptide. Cytoplasmic effectors are translocated inside the plant cell where they target different subcellular compartments. Cytoplasmic effectors with a conserved RXLR motif have been studied intensively, but the CRN (Crinkle) family containing the LXLFLAK motif is relatively unknown. They were identified by an *in planta* functional expression screen of candidate *P. infestans* secreted proteins. Similar to RXLR effectors the N-terminal region of the CRNs is dispensable for cell death induction *in planta*, indicating the presence of modular domains that are involved in distinct processes. One of the *crn* genes, *crn8*, encodes a secreted protein with a C-terminus that has a predicted serine/threonine RD kinase domain. Secreted kinases have not been reported from microbial plant pathogens, so far. Mutation of the catalytic residues RD into AA abolishes cell death induction suggesting that kinase activity is important. CRN8 has a striking similarity to plant kinase-like proteins. This intriguing finding raises the possibility that CRN8 mimics a specific class of plant enzymes, a feature that was noted for other pathogen effectors. Results obtained by a combination of genetic and biochemical methods of the CRN8 effector protein and the plant proteins that CRN8 targets and/or mimics will be presented. These new findings will enable us to decipher how pathogens successfully colonize and reproduce on their host plants.

The title of your presentation here: Pathogenicity islands and putative alien genes in the genomes of *P.sojae* and *P.ramorum*

Sucheta Tripathy and Brett M Tyler

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Pathogenicity islands (PAIs) are distinct genomic segments of pathogens encoding virulence factors represent a subgroup of genomic islands (GIs). The virulence genes present in the PAIs are also sometimes referred to as putative aliens. Although this plays a major role in bacterial pathogenesis, its role in eukaryotic pathogens is yet to be established. In bacteria often the site of location of genomic islands are the 3'ends of tRNAs harboring direct repeats. In *P.sojae* 27(11%) out of 238 tRNA genes had direct repeats, whereas in *P.ramorum* 36(23%) out of 155 tRNA genes had direct repeats in the 3' end. Large genomic islands varying from 5KB to 10 KB are found near the tRNA genes in both the organisms on the basis of their average absolute dinucleotide relative abundance difference. There are 39(16%) genomic islands found bordering tRNA genes of *P.sojae* out of which 2 had pathogenesis related genes. Only 6(2.5%) such islands were flanked by direct repeats in *P.sojae*. In *P.ramorum* the number of genomic islands associated with tRNA genes was relatively higher 45(29%). 16 such islands had direct repeats and 4 pathogenicity related genes were located within this. Out of the 968 and 881 infection related genes from *P.sojae* and *P.ramorum* only 18(1.8%) and 4(0.4%) were linked to the tRNA genes. About 39 pathogenesis related genes from both the organisms were found to be located in different genomic islands other than the tRNA islands. This indicates unlike bacterial pathogens, presence of direct repeats near the tRNA genes are not very crucial for integration of foreign genetic element in *Phytophthora*. Although there is large number of genomic islands found near the tRNA genes, there is equally large number of GIs located in different parts of the genome. Only 4% of the pathogenicity related genes appear to be putative aliens. This indicates the rest of the pathogenicity genes are "ameliorated", thereby adjusting to the base composition and codon usage of resident genome. The acquisition of knowledge about PAI, their structure, their mobility, their origin and the pathogenicity factors they encode will be helpful in gaining a better understanding of evolution and interactions of pathogens with eukaryotic host cells.

GC plotting method

siRNA against A484854 SINE element SINEM
A484

Session 8

Components of RNA mediated gene silencing in *Phytophtora infestans*

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Gene silencing is a conserved phenomenon in many eukaryotes and plays a role in numerous cellular processes, from defence against transposon activity, virus control, aberrant RNA degradation, to gene regulation through micro RNAs. The core proteins involved in RNA silencing in animals, plants and fungi are well characterized, as are their roles in the biogenesis of small interfering RNAs (siRNAs). From the *Phytophthora infestans* genome database, we have identified genes encoding the putative components of the gene silencing machinery. Since 'silencing genes' are often developmentally regulated, we have determined their expression profiles using real-time RT-PCR in pre-infection stages and during the infection of potato leaves. In *P. infestans,* like in most other organisms, the majority of the silencing genes are represented by gene families with different members up-regulated in different lifecycle stages.
The *P. infestans* genome is saturated with repetitive DNA families, including SINEs, LINEs, Gypsy and Copia families of retrotransposons. Most eukaryotes control the frequency of transposition of retroelements by employing gene silencing, generating siRNAs. To determine if retroelements are controlled by siRNAs in *P. infestans,* we used retrotransposon sequences (SINEs, LINEs and Gypsy) as probes for Northern blots to detect siRNAs from the low molecular weight RNA fraction extracted from different strains of *P. infestans* cultured under different growth conditions. Our data indicate significant accumulation of siRNAs specific to these elements, confirming the fact that there are indeed siRNAs employed against transposable elements in *P. infestans*. We aim to exploit aspects of gene silencing in *P. infestans* to develop robust stable gene silencing strategies for this organism. Progress towards this goal will be presented.

ENU induced mitotic gene conversion in *Phytophthora capsici.*

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Gene conversion is defined as the non-reciprocal transfer of genetic information from a DNA duplex to a homologous duplex (Petes *et al.* 1991). Mitotic gene conversion (MGC) events can occur during mitosis, resulting in homozygosity at previously heterozygous alleles. MGC has been reported to spontaneously occur in *Phytophthora sojae* (Chamnonput *et al.* 2001) at an avirulence locus and other unlinked loci. Such widespread switching to homozygosity is likely an important mechanism of generating genotypic change in *Phytophthora spp.* MGC events are also reported to occur also occur as a result of exposure to mutagenizing agents (Tsang *et al.* 1999, Freeman *et al.* 2007). MGC was observed in ENU mutagenized isolates of *P. capsici* at the phosphate lipase D locus (*PLD*) at a rate of 1 X 10^{-2}. MGC mutants displayed switching to homozygosity at 3 alleles, spanning ~600 bp of the *PLD* locus (unpublished data). Homozygosis occurred bi-directionally with all three heterozygous nucleotide sites coconverting to homozygosity. Switching to homozygosity has also been observed for ENU mutants at 2 other loci, an un-annotated locus possibly involved in surface adhesion, and *Dicer*, a component of the RNAi gene silencing machinery (unpublished data). Further experiments are underway to determine rates and the extent of MGC events in ENU mutagenized and non-mutagenized *P. capsici* isolates using a high-throughput genotyping assay (LightScanner, Idaho Tech.). Thus far, coconversion of alleles to homozygosity was found to span up to at least several kilobases. Current findings will be presented along with future directions for investigation of MGC in *P. capsici.*

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Copy number polymorphisms and transcriptional silencing of *Avr1a* and *Avr3a* effector genes in *Phytophthora sojae*

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We have identified two *P. sojae* effectors, *Avr1a* and *Avr3a*, by map-based cloning and transcript profiling technologies, respectively. Both *Avr1a* and *Avr3a* are predicted to encode small, secreted RxLR-dEER proteins of 121 and 111 amino acids each. Analysis of the 8X draft assembly of the *P. sojae* genome localized each *Avr* gene to separate, unlinked contigs. Further work was required to resolve the arrangement and configuration of genes at each locus because of sequence ambiguities in the vicinity of both *Avr1a* and *Avr3a*. Analysis of genomic DNA by PCR and by RFLP, together with the trace files from the whole genome shotgun sequencing, enabled the re-assembly of the *Avr1a* and *Avr3a* regions. Each locus is characterized by four copies of nearly perfect repeats in *P. sojae* race 2 (P6497). For *Avr1a*, each 5.2 kb repetitive unit includes a single ORF for the RxLR effector. For *Avr3a*, each 10.8 kb per repetitive unit includes four different ORFs, one of which corresponds to the RxLR effector. The structure of the *Avr1a* locus and the copy number of the gene itself varies among *P. sojae* strains. The *Avr1a* transcript is expressed during infection but only in *P. sojae* strains that trigger *Rps1a* resistance; strains that are virulent on *Rps1a* do not express the gene. These transcript differences are due to *Avr1a* deletions or to gene silencing, depending on the *P. sojae* strain. The *Avr3a* locus also displays copy number variants, depending on *P. sojae* strain. Allelic diversity within the coding sequence of *Avr3a*, and evidence of diversifying selection, is apparent among *P. sojae* strains. Evasion of *Rps3a*-mediated immunity may be accomplished by amino acid substitutions or by transcriptional silencing. These results demonstrate the variety of mechanisms that have enabled *P. sojae* to adapt to *Rps*-gene deployment in soybean cultivars.

De novo hybrid 454/Sanger genome assembly of the eukaryotic pathogen, *Phytophthora capsici*

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Next generation sequencing technologies have created sequencing opportunities by increasing throughput and decreasing costs. They have also introduced several challenges compared to traditional sequencing technology. In *de novo* assembly, assembly strategies must take into account the large amount of sequencing data as well as the short read lengths and distinctive error profiles of the technology. Hybrid assemblies have been used to mitigate some of these issues by combining next generation sequencing with traditional Sanger sequence and have been particularly effective in *de novo* sequencing of prokaryotic genomes. We looked at the feasibility of using a hybrid sequencing approach for *de novo* eukaryotic genome sequencing by creating a draft genome sequence for *Pythophthora capsici,* an oomycete and devastating pathogen of vegetable crops. Its highly repetitive, 60 Mb genome, with characteristic eukaryotic complexity, as well as the availability of closely related Sanger-sequenced genomes make *P. capsici* an excellent arena for benchmarking hybrid sequencing in eukaryotes. We generated 23X 454 GS20 pyrosequencing singleton reads, 5X Sanger paired reads, and 2M 454 GS20 paired reads. In addition, 20,000 sequencing reactions have been completed to obtain sequence within captured gaps. We assembled these reads using Forge, modified to accommodate the disparate read lengths and pair spacings of the two technologies as well as the unique error profiles of the Sanger and 454 reads. For comparison purposes, a Sanger-only (Arachne) and 454-only (Newbler) assemblies were also generated. Finally, Solexa cDNA libraries from nine life stages have been sequenced to improve gene calls, identify SNPs, and compare gene expression levels. The successes and challenges of doing a 454-based genome sequencing project will be discussed.

Alphaw tools for SNPs

Solexa run 1 run, 9 samples
↓ 56k Sanger ESTs
for transcriptome

Hyaloperonospora parasitica: genome assembly and annotation

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The obligate oomycete downy mildew pathogen of Arabidopsis, *Hyalopeonopsora parasitica,* is at the centre of a rapid growth in research projects aimed at understanding host pathogen interactions. To enable expansion of these research efforts, Virginia Tech and Warwick University have been collaborating to sequence and make publicly available the genome of *H. parasitica*. The Washington University Genome Sequencing and Sanger Centres have generated data that has allowed us to present version 6 of the draft assembly with sequence coverage of 9.2X with a projected genome size of 75 Mb.

Here we will present the latest information on the genome project and the collaborative efforts of gene annotation and discuss the state of the search for pathogenicity effectors.

Progress on Analysis of the *Phytophthora Infestans* Genome

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In 2007 we released a version 1 assembly and annotation of the *P. infestans* genome, consisting of 190 Mb of assembled sequence and ~22,500 genes. We have now generated a second release of the *P. infestans* gene set based on comparative and de novo methods and refined by manual curation and community input. The revised set contains ~20,000 protein coding genes. Comparison of the proteome to *P. sojae* and *P. ramorum* shows a conserved core proteome of ~8000 genes. *P. infestans* is distinguished by much greater expansion of gene families, particularly within secreted protein families involved in host interaction, such as RxLRs and Crinklers.

We also find that most of what appeared to be the largest gene families in *P. infestans* are actually repeats. In addition to the previously identified Gypsy and Copia LTR elements, we find evidence of unprecedented numbers of less common repeat types, including piggyBacs, helitrons, cryptons, mutator-like proteins, and others. In some cases, these elements have inactivated other genes by recent insertion, and appear to play an active role in remodeling the genome.

We have also completed pilot sequencing by Illumina (Solexa) of whole genome shotgun from the closely related species *P. andina*, *P. ipomoeae*, *P. mirabilis*, and *P. phaseoli*, as well as two additional *P. infestans* isolates. We expect these sequences to provide insight into recent variation in pseudogene content and gene copy number and also to help study recent expansion of specific repeat elements.

Poster abstracts

A brown algal superbug: the oomycete *Eurychasma dicksonii*

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Eurychasma dicksonii has the largest known host range among pathogens infecting marine algae, and it occupies the most basal position in the oomycete lineage. It occurs in epidemics in temperate and cold seas in both hemispheres. Due to its availability in culture and the ongoing sequencing of the genome of one of its main brown algal hosts (*Ectocarpus siliculosus*), it is a particularly attractive model to study oomycete infection strategies and algal defense mechanisms. Preliminary results show that *Ectocarpus* strains exhibit a differential susceptibility to the same *Eurychasma* strain, suggesting a genetically-determined basis for resistance in the alga. With the view to support ongoing
cell and molecular biology studies, we have performed a detailed ultrastructural study of *Eurychasma* infection in two filamentous brown algal species. We are also trying to clarify further the phylogeny and biogeography of this pathogen.

Reference: S. Sekimoto et al. (2008) ***Protist*** The development, ultrastructural cytology, and molecular phylogeny of the basal oomycete *Eurychasma dicksonii*, infecting the filamentous phaeophyte algae *Ectocarpus siliculosus* and *Pylaiella littoralis*.

Differential susceptibility of *Ectocarpus* (Phaeophyceae) to the oomycete pathogen *Eurychasma dicksonii* - a real time PCR study

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Real-Time PCR is a fast method to amplify and quantify DNA simultaneously. The method is based on the monitoring of amplified target DNA after each cycle during PCR by measuring fluorescence emission, which also allows working with a limited amount of template DNA, as it is often the case in phycopathology.

Here we describe the application of Real-Time PCR to detect oomycete infections in brown algae - using our model system of *Ectocarpus siliculosus* and the pathogen *Eurychasma dicksonii*: we have observed that different degrees occur in the severity of *Eurychasma* infection in *Ectocarpus*, depending on the combination of host and pathogen strains used. In microscopic observations, this ranges from complete absence of infection symptoms (resistant *Ectocarpus*) over intermediate phenotypes to complete sensitivity of the alga. We have designed a Real-Time PCR assay suitable to rapidly screen a number of various *Ectocarpus* strains for the presence and severity of *Eurychasma* infection in *Ectocarpus*. First developed on defined laboratory cultures, this technique also has the potential of accurately monitoring the prevalence and abundance of pathogens in natural algal populations in the field.

Effectoromics of *Hyaloperonospora parasitica*

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Some of the most devastating plant diseases are caused by oomycetes, yet little is currently known about how these pathogens colonize their host plants. Genetic analysis has identified several oomycete effector proteins, which are delivered to the cytoplasm of the plant cell where they act as virulence factors or are recognized by the plant immune receptors to initiate a defence response. The RXLR motif required for delivery into the plant cell is highly similar to a motif in effectors of the distantly related malaria pathogen *Plasmodium falciparum*, indicating evolutionary conservation of this process in both animal and plant pathogens. Recently, the genomes of several oomycetes, including the potato blight pathogen *Phytophthora infestans* and the *Arabidopsis thaliana* pathogen *Hyaloperonospora parasitica* have been sequenced. This has allowed to employ computational approaches to identify putative effectors and to study their roles in promoting disease.
Using a stringent bioinformatics approach, a large number of putative effector proteins in *H. parasitica* have been identified, which possess an amino terminal secretion signal followed by the RXLR motif and variable carboxy terminal effector domains. Among these are several proteins with homology to the known effectors ATR1 and ATR13, the CRN proteins from *P. infestans* or other annotated proteins. The majority of the putative effectors, however, encode proteins of unknown function and their roles in disease promotion and defence remain elusive.
We are currently investigating the *H. parasitica* effectorome using a variety of approaches. Since the obligate biotroph pathogen is not amenable to genetic manipulation, we are using surrogate strategies utilizing bacterial vectors as well as transient expression experiments to determine potential functions of the putative effectors. We are assessing the ability of the putative effectors to manipulate basal and specific defences, and are thus obtaining novel insights into oomycete pathogenesis. Our preliminary results of these studies will be presented.

Gene specific mutants through the Oomycete Tilling Resource

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Whole genome sequences are available for four species in the genus Phytophthora (*P. sojae, P. ramorum, P. infestans* and *P. capsici*). Currently there are few tools available to support functional genomic investigations on a large scale. Reverse genetics using the TILLING (Targeting induced local lesions in genomes) methodology has proven to be a useful approach to isolate gene specific mutants for plant pathogens in the genus Phytophthora and we are currently proposing to expand our capabilities to include *P. capsici* as well as *P. sojae*. We will provide an updated overview of the process required for choosing gene targets, development of appropriate gene specific PCR primers, and sequencing protocols to confirm target quality. In addition, a detailed overview of the process required to convert heterozygous mutants to homozygosity will be provided. Mutant libraries of *P. sojae* and *P. capsici* are available for screening and all mutants discovered will be made freely available to the scientific community.

Heterogeneous organization and expression of polyubiquitin genes in the oomycete plant pathogen *Phytophthora parasitica*

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Text of abstract, approximately 300 words

Oomycetes contain some of the most devastating plant pathogens worldwide. These fungus-like eukaryotes belong to the Stramenopiles, closely related to photosynthetic organisms such as brown algae and diatoms. We investigated the mechanisms underlying pathogenicity of the broad host range oomycete *Phytophthora parasitica* through construction of cDNA libraries and generation of expressed sequence tags (EST).

Mining these libraries allowed identifying several genes encoding ubiquitin and ubiquitin-like proteins. We report here that *P. parasitica* ubiquitin genes constitute a multigene family that displays an unusual highly diverse intraspecific organization. We also show that these genes undergo differential expression during several stress conditions and during host infection. Intra- and interspecific comparisons show that distinct mechanisms have driven the evolution of *Phytophthora* polyubiquitin genes and monomer-encoding genes.

EST libraries from the *Albugo candida – Brassica juncea* host pathogen interaction.

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White rust caused by the obligate oomycete *Albugo candida* (Pers. ex. Fr.) O. Kuntze affects Brassicaceae species including cultivated Brassica crops and Arabidopsis. To understand the mechanisms of plant defence regulation it is important to identify key plant and pathogen effector molecules involved in the host-pathogen interaction. Arabidopsis and *Brassica juncea* were used to study the interaction with two isolates of *A. candida*, Ac2 and Ac7, which infect *B. juncea* and *B. rapa,* respectively.

To identify *A. candida* effector molecules that regulate the *RAC4* initiated defence response, cDNA libraries were made from AC2V sporangia and from Ac2v infected *B. juncea* cultivar 'Cutlass'. Approximately 36,000 clones from Ac2V infected Cutlass were sequenced and compared with plant and pathogen sequences in public databases as well as the Brassica EST databases developed at Saskatoon Research Centre. This analysis revealed that nearly 80% of the ESTs were of plant origin and the rest were potentially from *A. candida* Ac2V. Analysis of the Ac2V cDNA sequences for potential signal peptide sequences and the RXLR motif, common in oomycete plant pathogens, identified 288 ESTs that encode secreted proteins. Among the candidate secreted proteins 10 proteins were identified that contained the RXLR motif. Functional analyses of these proteins are being carried out using a transient assay in tobacco and Arabidopsis. Sequencing of an AC2V sporangial cDNA library is in progress and 10,000 clones from this library have been sequenced from both directions.

The cell death regulator AtPUB17 directly interacts with the BTB/POZ domain transcriptional repressor, AtBTB1 to control disease resistance in plants.

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Programmed cell death or hypersensitive cell death and disease resistance are intimately connected and crucial in plant defence against pathogens. Although resistance proteins are known to be important players in pathogen recognition little is known of the molecular mechanisms downstream of this recognition event leading to hypersensitive cell death. We have established the Ubox protein NtACRE276 and its functional homolog, AtPUB17 as key proteins required for multiple resistance proteins mediated hypersensitive cell death in tobacco and *Arabidopsis*. E3 ligase activity of both NtACRE276 and AtPUB17 is required for ubiquitinating key negative regulators of programmed cell death. Our data identify the presence of a conserved class of U-box ARM repeat E3 ligases across the Solanaceous and Brassica genera that act as positive regulators of cell death and defence.We have identified AtBTB1, a BTB/POZ domain transcriptional repressor, as a direct interactor of AtPUB17. AtBTB1 is located in the nucleus during the hypersensitive response. AtBTB1 knockout plants produce spontaneous cell death in the absence of pathogen infection indicating that AtBTB1 has a critical role in cell death signalling. We detail experiments which show that the BTB/POZ domain of AtBTB1 is required for its interaction with AtPUB17 and how this interaction regulates its transcriptional repression role during defence. We show that AtPUB17 interacts with AtBTB1 to regulate disease resistance in *Arabidopsis* and tobacco. The target genes whose expression is repressed by AtBTB1 are key molecules which regulate defence against pathogenic Pseudomonads. By focussing on the biochemical function of AtBTB1 we reveal specific molecular events which bring about cellular signalling during disease resistance. The data yields new insights into our understanding of ubiquitin mediated signal-transduction mechanisms controlling defence against pathogenic Pseudomonads and its relevance will extend to all biotrophic pathogens.

Unravelling *Hyaloperonospora arabidopsis* effector functions in *A. thaliana*.

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Infection of plant by pathogenic microbes involves the complex interplay between the interacting organisms. While plant pathogens use a wide range of effectors to gain access to resources available in their hosts, plants simultaneously respond with a defence network aimed at preventing invasion and eliminating infecting pathogens. This co-evolutionary interaction imposes an intense selective pressure on both organisms. A very suitable pathosystem to study this interplay between host and pathogen is the interaction of the oomycete *Hyaloperonospora arabidopsis* (*Hpa*, formerly *H. parasitica*) with its naturally occurring host, *Arabidopsis thaliana*. As an obligate biotroph, *Hpa* requires living plant tissue for growth and reproduction implicating in a precise modulation of host processes. Little is still known about how oomycete pathogens manipulate their hosts to establish a successful infection. To gain insight into this process, we initiated the characterization of *Hpa* effector proteins. We focused our analyses on the class of secreted proteins that are characterized by the presence of a RXLR motif, and that are predicted to be translocated to the plant cell cytoplasm. *Hpa* genome mining and EST-sequencing allowed the identification of about 200 RXLR candidate effectors (*Hpa* genome annotation jamboree). A total of 105 RXLR genes expressed during infection have already been cloned for functional studies. These genes are now being screened for possible "*in planta*" effector function by delivery of RXLR proteins into the plant cytoplasm using the type III secretion system (TTSS) of the bacterial plant pathogen *Pseudomonas syringae* (*Pst*) (assay established by Sohn et al., 2007). We will report on the results of potential effector activities related to enhanced bacterial virulence and/or suppression of PAMP triggered immunity, as well as possible avirulence functions.

Silencing of Cysteine protease, acidic chitinase or PR1-a individually, does not hamper BTH mediated resistance to *P. infestans* in tomato

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Induced resistance by chemicals such as benzothiadiazole BTH (Syngenta Inc) mimics the biological activation of Systemic Acquired Resistance (SAR) by necrogenic pathogens. BTH takes the place of salicylic acid (SA) in the SAR signal pathway, inducing the same molecular markers and range of resistance. Previous work in our laboratory found that BTH activates resistance against late blight caused by *P. infestans*, on petunias and tomatoes while it did not activate resistance against the same pathogen on potatoes, suggesting that the spectra of resistances activated by BTH are crop and pathogen specific. The goal of our work was to understand the molecular mechanism by which BTH mimics the SAR response and further understanding why BTH works in some plants and not others. To address this question we used microarray technology to identify the genes expressed in response to BTH in tomatoes. Of these we selected three candidate genes (cysteine protease, acidic chitinase and PR1-a) to characterize further by silencing using Virus Induced Gene Silencing (VIGS). Our hypothesis was that silencing of these genes will reduce the resistance response observed in plants after BTH treatment. However, silencing of cysteine protease, PR1-a or acidic chitinase II individually did not reduce the effect of BTH on plants. The lack of phenotype after silencing PR1-a supports previous conclusions from our lab that partial resistance to *P. infestans* in tomato is not dependent of the SA pathway.

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

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Bremia lactucae is an obligate biotrophic pathogen of lettuce (*Lactuca sativa*). As other downy mildews and *Phytophthora* species it belongs to the oomycetes (kingdom Stramenopiles). During infection Bremia grows intercellular hyphae and forms haustoria in host cells. Bremia is a major problem in lettuce cultivation. Bremia is mainly controlled by dominant resistance genes that are rapidly overcome by new isolates. Durable resistance is more desirable than ever, as Bremia is also becoming increasingly resistant to fungicides. The aim of this project is to identify Bremia effector proteins and to study their role in the infection process and in disease susceptibility. To identify large numbers of effectors we will use Next Generation Sequencing (NGS) of the Bremia transcriptome. Spores and Bremia-infected lettuce leaves will be used to generate cDNA for NGS. The assembled cDNA sequences will be bioinformatically mined for potential effectors by homology searches in other oomycetes and by screening for key features of oomycete effectors; Nterminal signal peptides and RXLR-motifs. Potential disease-promoting activities of candidate effectors will then be assessed. The knowledge gained will be used to identify lettuce breeding lines that are insensitive to the action of important effector proteins.

Functional characterization of *Phytophthora sojae* RXLR-dEER effectors

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Phytophthora species are thought to release a diverse array of effectors into host cells, where they manipulate host metabolism and suppress host defenses. These effectors may induce hypersensitive reaction (HR) in the plant, if they are recognized by a resistance protein within plant cell.

Several well-characterized effectors from plant pathogenic *Phytophthora* species share a conserved N-terminal domain (RXLR-dEER) required for entry of the effectors into host cells. This motif was demonstrated to be functional even in human malaria parasite (*Plasmodium falciparum*). A number of effectors identified by bioinformatics analysis, termed Avh (Avirulence Homolog) proteins, possess RXLR-dEER motifs. There are more 390 genes encoding Avh proteins in the genome of *Phytophthora sojae* and more than 370 in *Phytophthora ramorum*.

Our microarray analysis *P. sojae* genes during infection of soybean, shows a number of Avh genes are highly induced during the early hours of infection (3-12 hrs). To further investigate their functions, we have characterized three of the most up-regulated genes, Avh238, Avh240 and Avh181. RT-PCR confirmed that all three genes were up-regulated within 24hrs, and also showed that their expression level dropped after 24 hr. Transient assays in soybean leaves showed that none of the three Avh genes could suppress BAX-triggered programmed cell death. In contrast, both Avh238 and Avh181 triggered HR on soybean.

Polymorphisms were identified in the three Avh genes in five isolates of *P. sojae* representing the four major genotypes, P6497(race 2; genotype I), P7064 (Race7, genotype IV), P7074 (Race17, genotype III), P7076 (Race19, genotype II) and PT2004C2.S1 (Race30, genotype IV). The predicted Avh238 protein contains several sequence polymorphisms at its C-terminus. The five predicted Avh181 proteins display strong polymorphisms and also vary in size at their C-terminus. Both genotype IV Avh181 proteins contain 70-amino acid internal duplication.

Towards cloning Arabidopsis resistance gene *RHPC1* that recognize a 'cryptic' avirulence gene in *H. parasitica*

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In the *Arabidopsis-Hyaloperonospora parasitica* model system, the interaction phenotype is determined by the pathogen originated *ATR* (***A****rabidopsis* ***t****haliana* recognized) genes and the corresponding host *RPP* (recognition of ***P****eronospora* ***p****arasitica*) genes. The Columbia accession (Col-0) carries the *RPP2* gene and recognizes the *H. parasitica* isolate Cala2. Similarly, the Landsberg accession (La-*er*) carries the *RPP5* gene and recognizes the isolate Noks1. A cross has been generated between these 2 isolates designated CaNo and an F_2 population (CaNo F_2) was produced to map *ATR2* and *ATR5* genes. We have used the parental isolates, Cala2 and Noks1, to screen the Nordborg/Bergelson (NB) worldwide diversity collection of 96 Arabidopsis accessions. Eighteen accessions were identified to be susceptible to both Cala2 and Noks1. These accessions were then screened with CaNo F_1 and 13 CaNo F_2 isolates with *atr2 atr2/atr5 atr5* genotypes. Eight of these 18 accessions showed resistant phenotypes to some of the Cano F_2 isolates, indicating the presence of 'cryptic' avirulence gene(s), revealed through transgressive segregation. Testing of one of the NB accessions, RMX-A02, has revealed that this avirulence gene segregates as a single dominant gene (based on a chi-square test of an expected ratio 3:1 avirulence:virulence). Mapping and isolation of the corresponding *R*-gene designated *RHPC1* (recognizing ***H****. parasitica* cryptic avirulence gene product) would help the identification of this novel *ATR* gene. In order to identify and clone *RHPC1*, we generated an F_2 mapping population from a cross between RMX-A02 and the susceptible accession Col-0. The segregation data suggests the presence of a single dominant gene. *RHPC1* was mapped onto chromosome 4 and the progress towards the cloning of this gene will be presented.

Inventory and analysis of ABC membrane transporters in Oomycete Genomes.

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In previous work, our manual curation of the ABC superfamily in the *Phytophthora sojae* and *P ramorum* genomes has identified 117 membrane localized transporters in each genome, almost two fold higher than that seen in fungal genomes and comparable in size to that of *Arabidopsis* and rice genomes. We have extended this analysis to the genomes of *Hyaloperonospora parasitica* and *Phytophthora infestans* where we identified 36 and 111 membrane localized transporters, respectively. Comparative analysis of the H. parasitica genome with that of the three Phytophtora genomes indicates that almost all of its transporters are ortholgous to those in *Phytophthora* sp. suggesting that the expansion of this family is an ancient event. ABC transporters are grouped into different families based on the order of transmembrane (TM) domains and associated ATP Binding Cassette regions (ABC). In other kingdoms, the ABC domains associated with a particular family have a common phylogenetic origin. In *Phytophthora* sp., ABC domains within the same family do not always share common phylogenetic origins, due to horizontal gene acquisition events from bacterial genomes (ABCB family). In the *P infestans* genome there is also EST data confirming the conversion of full transporters to half transporters (PDR to ABCG families). This represents a novel strategy for expanding the functional capabilities of membrane transporters in addition to gene duplication and divergence.

VMD: a community annotation database for oomycetes and microbial genomes

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The VBI Microbial Database (VMD) is a database system designed to host a range of microbial genome sequences. At present, the database contains genome sequences of three plant pathogens *Phytophthora sojae*, *Phytophthora ramorum* and *Hyaloperonospora parasitica*. After the completion of the draft genome sequences of these pathogens in collaboration with the DOE Joint Genome Institute (JGI) and Washington University (WashU),we have created this resource to make the sequences publicly available. For *H.parasitica* and *P.sojae* different genome assembly versions are stored in the database. Gene calls are transferred to the latest assembly versions wherever possible. The genome sequences (95 MB for *P.sojae* and 65 MB for *P.ramorum* and 77 MB *H.parasitica*) were annotated with 19000, 16000 and 15800 gene models, respectively. We used two different statistical methods to validate these gene models, Fickett's and a log-likelihood method. Functional annotation of the gene models is done on the basis of results from BlastX , InterProScan, SignalP, TMHMM and TargetP. KEGG and KO annotations are newly added to the database. A number of modifications are made to the user interface of the database, that include a more user friendly annotation interface and browser. The genome browser is modified to show models in different colors if the gene models are already reviewed . The community annotation interface is now modified so that the users will be able to see the newly created/modified models immediately on the browser. On the main annotation page, the EST unigenes are mapped to the gene models. A new search sequence feature is added to the toolkit that enables the user to fetch a subsequence from any of the listed blastable database. The toolkit blast currently has around 40 blastable databases. A number of community annotation data has been added to the database. This includes around 2200 new gene models for *P.sojae* and 700 gene models *for P.ramorum* from different gene families. VMD is publicly available at http://vmd.vbi.vt.edu/.

Gene family reduction and expansion in the obligate biotrophic plant pathogen *Hyaloperonospora parasitica* detected with ortholog- and paralog-grouping approaches.

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Gene duplication is one mechanism that enables genes to acquire new functions, whereby paralogous genes are released from selective pressures exerted on single genes. Conversely, gene loss can occur when selective pressures to maintain an array of genes are removed, as may be the case in niche specialists such as obligate biotrophic symbionts. Both gene family expansion and reduction are being studied in the recently sequenced genome of *Hyaloperonospora parasitica*, an obligate biotrophic pathogen of *Arabidopsis*. To facilitate grouping of paralogs within *H. parasitica* and orthologs among related organisms, whole-genome protein comparisons were performed using GeneTrees, a phylogenomic algorithm developed at Virginia Bioinformatics Institute, and InParanoid, an algorithm based on all-vs-all BLAST, developed at the Stockholm Bioinformatics Centre. Translated gene models (protein sequences) were compared among nine organisms: oomycetes *H. parasitica, Phytophthora ramorum, P. sojae*, and *P. infestans*; diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*; and three outgroups, *Arabidopsis thaliana, Saccharomyces cerevisiae*, and *Plasmodium falciparum* isolate Dd2. Initial findings from GeneTrees showed roughly 2,000 multiple sequence alignments containing proteins from some or all organisms. 1,500 alignments contained only four proteins, 440 had greater than 30 sequences, and one alignment had more than 250 proteins. Approximately 29% of the *H. parasitica* genome was represented in GeneTrees alignments. The InParanoid algorithm detected 4,435 ortholog groups between *H. parasitica* and *P. ramorum*, and 4,332 between *H. parasitica* and *P. sojae*, compared to 9,671 between *P. sojae* and *P. ramorum*. Between *H. parasitica* and *P. ramorum,* 102 ortholog groups had multiple *H. parasitica* proteins compared to one *P. ramorum* protein (versus 529 with one *H. parasitica* to multiple *P. ramorum*; 3,804 with one *H. parasitica* to one *P. ramorum*; and 76 where both organisms had multiple proteins). Re-analysis and annotation are underway with the newest gene call of the *H. parasitica* genome.

Bioinformatic and experimental identification of microRNAs in *Phytophthora infestans*

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MicroRNAs have been reported in animals and plants. They are involved in fundamental gene regulations in theses organisms. They are also involved in disease pathogenesis (such as cancer) in animals and stress/disease resistance in plants. Some viruses have been found to produce microRNA to manipulate host genes. We are using experimental and bioinformatic approaches to identify potential microRNA in *Phytophthora* species. In the bioinformatic approach, we compared the genome sequences of three *Phytophthora* species (*P. infestans*, *P. sojae*, and *P ramorum*) and used secondary structure (stem_loop), minimal free energy, and conservation among these three species to identify candidate microRNAs. A total of 617 candidates have been predicted and algorithms are being developed to further filter and rank these candidates. A subset of the candidates will be validated experimentally. In the experimental approach, a small RNA library of *P. infestans* is being sequenced with the Solexa system and the sequences will be analyzed with a bioinformatic pipeline we have built.

Translocation of effector proteins from the oomycete *Phytophthtora infestans* into plant cells.

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Like bacteria and fungi, the potato blight pathogen *Phytophthtora infestans* translocates effector proteins into host plant cells during infection. Whereas bacteria possess the well characterized type III secretion system, the mechanism used by eukaryotic plant pathogens for delivering effector proteins into the host cell remains unclear. In oomycetes this process depends on a short conserved amino acid sequence (RxLR) located near the signal peptide of many secreted proteins. This motif, and its position within secreted proteins, is specific to oomycetes but resembles the host cell targeting-signal found in virulence proteins from the malaria parasite *Plasmodium falciparum*. Recently, it was shown in our laboratory that the RxLR, and a downstream conserved EER motif, are required for the translocation of the avirulence protein AVR3a into the host cell; AVR3a is recognised by the cognate R3a resistance gene in potato to trigger a hypersensitive resistance response. A recent study showed that the RxLR motif from AVR3a was sufficient to export the green fluorescent protein (GFP) from *P. falciparum* to the erythrocyte, suggesting a conserved mechanism to deliver effector/virulence proteins into host cells. Potentially, the host targeting signal used by the malaria parasite could function in *P. infestans*. Moreover, we may expect that the RxLR motifs found in avirulence proteins from other oomycetes to also function in *P. infestans*, but these hypotheses remain to be demonstrated. We have used the AVR3a-R3a interaction as a reporter for translocation in *P. infestans* transformants and replaced the AVR3a RxLR-EER motif with the motifs from a related oomycete or from the malaria parasite. Transformation of a virulent *P. infestans* isolate with the various *Avr3a* constructs yielded avirulent transformants, implying that the alternative sequences are functionally similar to the native RxLR-EER. Fluorescent protein fusions to translocated effectors to examine the host subcellular targeting of *P. infestans* effectors are also being performed and progress will be presented.

Glycoside hydrolase gene families in *Phytophthora infestans* genome.

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Phytophthora infestans infection mechanism may rely on cell wall degrading enzymes (CWDE) that specifically target the carbohydrates of the plant cell wall. Among the CWDE enzymes secreted by plant pathogens are glycoside hydrolases (GH), which catalyze the hydrolysis of the glycosidic bonds that form cellulose. *Phytophthora infestans* is believed to secrete these enzymes to degrade cellulose into smaller, less complex sugars, allowing for infection through the plant cell wall. Thus far, only GH family 5 and family 12 genes have been identified in *P. infestans*. Using homologous sequences obtained from the Carbohydrate Active Enzyme (CAZY) database, a wide scale analysis of the *P. infestans* genome was conducted to identify putative genes coding for other GH families, and a total of 281 genes belonging to 34 families were identified. Amplification of genes belonging to families 7 and 18 from 10 different isolates of *P. infestans* was attempted and 90% of the targeted glycoside hydrolase genes were successfully amplified. Six of the ten isolates contained all six glycoside hydrolase genes, while all but one included the majority of the genes. US-940-SO1 was the only isolate to clearly lack glycoside hydrolase genes of families 7 and 18. Pathogenicity tests will be conducted in order to assess the potential correlation of virulence and GH copy number in *P. infestans*. The presence or absence of specific glycoside hydrolase genes may play a significant role in the pathogenicity of *P. infestans*.

Is cutinase required for *P. infestans* pathogenicity?

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Cutinases are enzymes thought to play a role in plant invasion by fungal and oomycete pathogens. Using homology searches and comparative genomics, we have identified four genes encoding putative cutinases in *Phytophthora infestans* genome. Using primers specific for each sequence we attempted to PCR-amplify each putative gene from eleven isolates of *P. infestans*. All but four isolates contained all four putative cutinase-coding genes. A phylogenetic analysis including these and the cutinase sequences available from *P. sojae* and *P. ramorum* was conducted. The analysis revealed that the sequences from *P. ramorum* and *P. sojae* are grouped together, and located between clusters of sequences isolated from *P. infestans*, possibly suggesting a recombination or lateral gene transfer event between the three species. A detached leaf assay was performed to determine if there is a correlation between the number of putative cutinases present and the relative virulence of each isolate. Results revealed no correlation between these variables, suggesting there are other factors involved in the infection process of *P. infestans*.

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