OMGN

OOMYCETE MOLECULAR GENETICS NETWORK

Annual Meeting
Malmö, Sweden
15th-17th June 2016

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Annual Meeting
Sankt Gertrud Konferens
Malmö, Sweden
15th-17th June 2016

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The Oomycete Molecular Genetics Research Network (OMGN) was initially funded by an NSF Research Coordination Network grant in 2001 and continued to receive funding from the NSF for many years. More recently, the Network has received funding from the USDA--AFRI program (grant 2011- 68004- 30104).

The purpose of our annual meeting is to promote communication and collaboration, and minimize the duplication of effort within the oomycete molecular genetics community. Our community now numbers well in excess of 100 Laboratories from around the world, and research on oomycetes attracts considerable attention from outside the community as well as within. The OMGN annual meeting alternates between Asilomar, CA, and a venue outside of the USA. This year it is being hosted in Malmö Sweden, and the meeting will cover some of the latest research in the areas of Effectors, Host Interactions and Resistance, Functional Genomics, Oomycete Biology and Population Genetics and Evolution. With over 90 registrants we expect a dynamic and thought –provoking meeting with plenty of opportunity for developing existing collaborations, establishing new ones or just meeting with old friends.

To all our members, old or new a very warm welcome to the Annual OMGN Meeting, 2016, Malmö, Sweden.

Laura Grenville-Briggs
2016 Meeting Chair

2016 MEETING PROGRAMME

All Sessions Take Place In Room "Carolinahallen", at the Sankt Gertrud Conference Center, Malmö.

WEDNESDAY 15$^{\text{TH}}$ JUNE

08:15-09:00	REGISTRATION, POSTER HANGING
09:00-09:15	OPENING REMARKS, PRACTICAL INFORMATION Laura Grenville-Briggs

SESSION 1 **09:15-10:35**	**Host Interactions and Resistance I.** **Chair: Sebastian Schornack.**

09:15-09:35   Wenbo Ma
Structural and functional analyses of the RxLR effector Phytophthora Suppressor of RNA Silencing 2 (PSR2).

09:35-09:55   Ramesh Vetukuri
The role of *P. infestans* effector Avr1-like in modulating plant defense.

09:55-10:15   Sander Rodenburg
Metabolic network construction of the *Phytophthora infestans* – tomato pathosystem.

10:15-10:35   Alexandra Pelgrom
Effectors and their plant targets as leads for downy mildew resistance breeding in lettuce.

10:35-11:00   COFFEE/POSTERS

SESSION 2 **11:00-12:40**	**Effectors I.** **Chair: Eric Kemen.**

11:00-11:20   Petra Boevink
Revisiting effector translocation with RxLR effector Pi04314, which forms a phosphatase holoenzyme with host PP1c to regulate immunity.

11:20-11:40   Rebecca McDougal
Elicitin predictions from *Phytophthora pluvialis* and *P. kernoviae* and gene expression during infection of susceptible and resistant genotypes of *Pinus radiata.*

| 11:40-12:00 | Tolga Bozkurt |
| | Manipulation of host autophagy by *P. infestans* RXLR effector PexRd54. |

| 12:00-12:20 | Marek Malec |
| | Functional characterization of the *Phytophthora infestans* RXLR effector AVR2. |

| 12:20-12:40 | Joël Klein |
| | Identification and monitoring of effector proteins in the spinach downy mildew pathogen *Peronospora farinose*. |

| 12:45: 13:50 | LUNCH |

| 13:50-14:00 | GROUP PHOTO |

KEYNOTE LECTURE: Chair: Laura Grenville-Briggs

| 14:00-15:00 | **PAUL SCHULZE-LEFERT** |
| | REDUCTIONIST APPROACHES TO EXPLORE PLANT MICROBIOTA FUNCTIONS |

| 15:00-15:20 | COFFEE/POSTERS |

| **SESSION 3** | **Effectors II.** |
| **15:20-17:00** | **Chair: Petra Boevink.** |

| 15:20-15:40 | Benjamin Hall |
| | Editing a virulence target of the late blight pathogen for enhanced resistance. |

| 15:40-16:00 | Sebastian Schornack |
| | Eighteen little RXLR effectors from *Phytophthora palmivora.* |

| 16:20-16:40 | Franziska Trusch |
| | A secreted nuclease of a fish-pathogenic oomycete self-translocates into host |

| 16:40-17:00 | Brett Tyler |
| | Delivery of *Phytophthora sojae* effector Avr1b in planta requires PI3P-binding, but does not require N-terminal cleavage. |

POSTERS/PUB
17:00-19:00

| 19:00- | ATTENDEES TO FIND THEIR OWN DINNER IN MALMÖ. |

THURSDAY 16TH JUNE

SESSION 4	**Oomycete Biology.**
08:50-10:40	**Chair: Sian Deller.**

08:50-09:00   Laura Grenville-Briggs
                         Welcome, information on dinner arrangements.

09:00-09:20   Maja Brus
                         Cell wall proteins from *P. infestans* in appressorium formation and
                         field immunity.

09:20-09:40   Andrew Wagner
                         Selection of environmental isolates of *Pseudomonas* for inhibition
                         of soil oomycete pathogens.

09:40-10:00   Anna Åsman
                         Phytophthora infestans Argonaute proteins display distinct small
                         RNA binding preferences

10:00-10:20   Adeline Harant
                         Apoplastic metabolites used by *Phytophthora infestans* during
                         hyphal growth

10:20-10:40   Claire Gachon
                         The Pathogens Of Brown Algae *Anisolpidium Ectocarpii* And
                         Anisolpidium Rosenvingei Define A New Class Of Marine
                         Anteriorly Uniciliate Oomycetes

10:40-11:00   COFFEE/POSTERS

SESSION 5	**Functional Genomics.**
11:00-12:40	**Chair: Hua Wise.**

11:00-11:20   Sucheta Tripathy
                         Improved *Phytophthora ramorum* Pr102 genome assembly with
                         hybrid assembly protocol.

11:20-11:40   Theerapong Krajeajun
                         Evolution of the Sterol Biosynthetic Pathway of *Pythium
                         insidiosum* and Related Oomycetes Contributes to Antifungal Drug
                         Resistance.

11:40-12:00   Anna Gogleva
                         De-novo assembly of *Phytophthora palmivora* transcriptome and
                         characterisation of the secretome during root infection.

12:00-12:20   Arijit Panda
                         Genome Annotator light(GAL): An integrated virtual machine for
                         genome analysis and visualization.

| 12:20-12:40 | Jasmine Pham |
| | Co-regulation of gene expression in Phytophthora. |

12:45-14:00   LUNCH

SESSION 6   **Population Genetics and Evolution I.**
14:00-15:20   **Chair: Åsa Lankinen.**

14:00-14:20   Clemens Weiß
Detecting intraspecific ploidy variation in *Phytophthora infestans* using shot-gun genomics.

14:20-14:40   Melanie Montes
Rethinking reproduction in Danish *Phytophthora infestans* population genetics reveal dominantly clonal populations and alleles linked to Metalaxyl-M resistance.

14:40-15:00   Yann Dussert
De novo sequencing and population genomics of *Plasmopara viticola*, the grapevine downy mildew.

15:00-15:20   Katie Davis
Oomycete Biodiversity in the Falkland Islands.

15:20-15:50   COFFEE & POSTER REMOVAL

SESSION 7   **Effectors III.**
15:50-17:00   **Chair: Ronaldo Dalio.**

15:50-16:10   Shaista Naqvi
Potato NPH3/RPT2-like protein StNRL1, targeted by a *Phytophthora infestans* RXLR effector, is a susceptibility factor.

16:10-16:30   Mark Banfield
Structural basis of ATG8 binding by a *P. infestans* effector protein.

16:30-16:50   May Bente Brurberg
Genome sequence and analysis of the strawberry crown rot pathogen *Phytophthora cactorum.*

17:00   BUS DEPARTS TO ALNARP

17:20-18:45   CRISPR DISCUSSION GROUP/
OPTIONAL (GUIDED) WALK IN THE ALNARP PARK & GARDENS

18:45   WELCOME DRINKS RESTAURANG ALNARP DECK
19:00-22:30   CONFERENCE DINNER, RESTAURANG ALNARP
22:30   BUS DEPARTS ALNARP FOR MALMÖ.

FRIDAY 17TH JUNE

SESSION 8 **08:30-10:00**	**Host interactions and resistance II.** **Chair: Erik Alexandersson.**

08:40-09:00   Yacine Badis
Pythium porphyrae the agent of the red seaweed rot disease: a reformed plant pathogen?

09:00-09:20   Michalis Barkoulas
Probing the mechanisms of *C. elegans* infection by natural oomycete pathogens.

09:20-09:40   Harri Kokko
Transcriptional changes in roots of Fragaria vesca during *Phytophthora cactorum* challenge.

09:40-10:00   Kibrom Berhe Abreha
Non-genetic inheritance of induced resistance in a wild annual plant.

10:00-10:20   COFFEE

SESSION 9 **10:20-12:00**	**Population genetics and evolution II.** **Chair: Hernán Burbano**

10:20-10:40   Laura Masini
Wild Solanum species in Sweden and New Zealand as reservoirs of pathogenic oomycetes.

10:40- 11:00   Javier Gómez-Zeledón
Molecular characterization of *Plasmopara viticola* single sporangium strains.

11:00-11:20   Aurelien Tartar
Detection of *Lagenidium giganteum* in phytotelmata microbiomes.

11:20-11:40   Diana Minardi
Genomics-informed development of molecular markers for genotyping the crayfish plague pathogen.

11:40-12:00 CLOSING REMARKS /OMGN 2017/18
12:00 SANDWICH LUNCH AND DEPART

Abstracts for Oral Presentations

Structural and functional analyses of the RxLR effector *Phytophthora* Suppressor of RNA Silencing 2 (PSR2)

Duseok Choi | Jinqiu He | Yi Zhai | Shuyi Duan | Jinbiao Ma | Wenbo Ma

Small RNA silencing is a universal gene regulation mechanism in eukaryotes. There is an emerging body of evidence suggesting that small RNAs are integral regulators of plant immunity during the infection of filamentous eukaryotic pathogens including fungi and oomycetes. To overcome this defense, *Phytophthora* has evolved effector proteins to suppress small RNA silencing. In our earlier work, **P**hytophthora **s**uppressor of **R**NA silencing 2 (PSR2) was identified from *Phytophthora sojae* as an important virulence factor. PSR2 is an RxLR effector containing multiple L-W-Y motif modules that form tandem repeats. Here, we report the motif arrangement and the crystal structure of PSR2. The structural analysis show that each L-W-Y module folds into a five-helix bundle that is stabilized by a hydrophobic core. The overall structure looks like a string of helix-bundles linked by loops. RxLR effectors containing similar tandem repeat modules are prevalent in *Phytophthora* species, supporting a biological significance of this architecture. Using mutagenesis analysis, we found that the C-terminal L-W-Y module is required for the RNA silencing suppression activity, probably through mediating the nuclear localization of PSR2 in plant cells.

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

Ramesh R Vetukuri | Pruthyi B Kalyandurg | Maja Brus | Petra Boevink | Erik Andreasson | Stephen C Whisson | Eugene I Savenkov | Laura Grenville-Briggs

Phytophthora infestans secretes many RXLR effectors potentially involved in infection of host plants. *Avr1-like* is one such RXLR effector that acts as a virulence factor, promoting colonization. Avr1-like is closely related to Avr1 but does not trigger R1-mediated resistance. Unlike Avr1, Avr1-like is present in all the modern isolates tested in our study. High levels of transcript encoding *Avr1-like* were present throughout infection stages, suggesting that this gene is specifically induced in contact with host tissue to modulate plant defences, as seen with other known avirulence effectors such as *Avr3a* and *Avr2*. In this study we demonstrate that *Avr1-like* acts as a suppressor of RNA silencing. Using mutagenesis analysis, we found that a GW motif in Avr1-like plays a major role in its suppression activity. Subcellular localization of AVR1 in *N. benthamiana* by transient expression revealed that this RXLR effector was localized to both the nucleus and the cytoplasm.

Metabolic network construction of the *Phytophthora infestans* – tomato pathosystem

Sander Rodenburg | Michael Seidl | Dick de Ridder | Francine Govers

The primary and secondary metabolism of a pathogen reflects its relation with its host, as many pathogens lack essential metabolic reactions themselves, but instead exploit metabolites of their host. Therefore, reconstructing genome wide metabolic networks for pathogens and hosts can provide new insights into their relationship at the metabolic level. We study the interaction between the notorious plant pathogenic oomycete *Phytophthora infestans* and its host, tomato. Using network analyses, we aim to identify the metabolic interactions between the two species. This will provide the basis for a genome-wide model of this pathosystem at the metabolic level.

The metabolic network of *P. infestans* and tomato will be reconstructed based on the KEGG and MetaCyc databases. Flux balance analysis will be used to find essential reactions that characterize the metabolism of both species, and will reveal what metabolic reactions are involved by an infection. Time-series transcriptome data of the enzyme repertoire for both species will allow us to infer a dynamic representation of this pathosystem, indicating active reactions during infection stadia at different time points. Currently, methods for metabolic network modeling have been explored and draft networks have been constructed and compared. A more accurate genome sequence and annotation will provide an even higher resolution model.

Effectors and their plant targets as leads for downy mildew resistance breeding in lettuce

Alexandra Pelgrom | *Joyce Elberse* | *Thijs Koorman* |*Mike Boxem* | *Guido Van den Ackerveken*

Infection of susceptible lettuce plants by the downy mildew pathogen *Bremia lactucae* leads to major crop losses. Protection provided by classical resistance genes is usually rapidly broken by constantly evolving races of *Bremia*. Hence, there is a growing need for more durable alternatives in resistance breeding. Downy mildews, as other oomycetes, translocate effectors, e.g. RXLR proteins, into plant cells. Effectors are best known for their suppression of plant immunity by modifying and interfering with cellular host processes, thereby allowing for and promoting infection. Although RXLR effectors are ubiquitously encoded in oomycete genomes, the plant targets of only a few are known. Insight into the molecular mechanisms underlying effector-mediated suppression of immunity may provide new leads for resistance breeding. In our research project we have identified candidate plant targets of *Bremia* effectors using the yeast-two-hybrid (Y2H) system. A set of 47 previously described as well as newly discovered *Bremia* effectors was screened against a lettuce cDNA library. For 22 effectors lettuce targets were identified. The vast majority of interactions is between a single effector and lettuce target, although up to five effectors were found to interact with a single lettuce target. A subset of effector-target interactions is further investigated by microscopic and biochemical methods. Lettuce RNAi lines are under construction to validate the biological significance of the identified effector targets.

Revisiting effector translocation with RxLR effector Pi04314, which forms a phosphatase holoenzyme with host PP1c to regulate immunity

Petra Boevink | Hazel McLellan | Shumei Wang | Xiaodan Wang | Qin He | Shaista Naqvi | Miles Armstrong | Wei Zhang | Ingo Hein | Eleanor Gilroy | Steve Whisson | Zhendong Tian | Paul R J Birch

Phytophthora infestans relies heavily on its suite of secreted, translocated RXLR class of effectors for successful infection. Only a small number from the effector array have been functionally characterised. Not surprisingly effectors have been demonstrated to target defence pathways and block the functions of key host proteins; for example, inhibiting MAP kinase activity to prevent signal transduction. More recently we have shown that effectors are also targeting susceptibility factors; that is, using and promoting the activities of host proteins that assist infection. One such effector is Pi04314 which forms an active holoenzyme with plant protein phosphatase 1 catalytic subunits. We are identifying and examining the targets of the Pi04314-PP1c holoenzymes and our current findings will be presented. In addition, due to its nucleolar localisation and strong phenotypic effects on *P. infestans* growth, Pi04314 is an ideal candidate to re-examine effector translocation and, indeed, we have finally been able to visualise fluorescently-tagged effector translocation in infected tissues.

Elicitin predictions from *Phytophthora pluvialis* and *P. kernoviae* and gene expression during infection of susceptible and resistant genotypes of *Pinus radiata*

Rebecca McDougal | Lucy Macdonald | Jonah Librach | Aaron Dalusong | Brent Kronmiller | Richard Hamelin | Brett Tyler | Nari Williams

Diseases caused by *Phytophthora* species that infect the foliage of *Pinus radiata* are relatively new. These diseases are red needle cast, caused by *Phytophthora pluvialis* and *P. kernoviae*, and Daño Foliar del Pino (DFP), caused by *P. pinifolia*. The ability of these species to infect the foliage is of particular interest, when compared to infection by root-infecting species such as *P. cinnamomi*. Elicitins are apoplastic effectors that play an important role in infection of host tissues by *Phytophthora* species. In this study, elicitin gene predictions were performed from the genome sequences of *P. pluvialis* LC-9 (USA) and *P. kernoviae* CBS122049 (UK). Elicitin predictions were performed using a modified bioinformatic pipeline based on that described by Jiang et al 2006. From the *P. pluvialis* genome 33 putative elicitins were detected with 11 of those being unclassified. Using an alternative pipeline for identification 27 and 26 putative elicitins were identified in *P. pluvialis* and *P. kernoviae*, respectively. Further work is required to determine the best pipeline for prediction and consolidate these results. Resistant and susceptible genotypes of *P. radiata* needles were exposed to *P. pluvialis* and *P. kernoviae* zoospores and samples were taken over time for transcriptomics analysis. To determine the gene expression profiles of the pathogen, transcripts showing similarity to predicted elicitin genes for both of these species will be analysed. Further analysis of New Zealand strains of *P. pluvialis* and *P. kernoviae* and a Chilean strain of *P. pinifolia* is underway as well as analysis of *P. cinnamomi*.

Manipulation of host autophagy by *P. infestans* RXLR effector PexRd54

Tolga bozkurt / Pooja Pandey / Yasin / M.E. Segretin / Sophien Kamoun

Autophagy requires the formation of double-membrane vesicles named autophagosomes, which enclose the material to be degraded or reallocated. A selective form of autophagy, organized by autophagy cargo receptors contributes to immunity in plants and animals. However, we know little about how selective autophagy is regulated at the molecular level, particularly how it contributes to immunity. Recently, we discovered that *Phytophthora infestans* RXLR effector PexRD54 subverts host defenses mediated by plant autophagy cargo receptor Joka2. PexRD54 outcompetes Joka2 for binding host autophagy regulator ATG8CL, whereas it stimulates autophagosome formation. Here we investigated the mechanisms underlying PexRD54 triggered autophagosome biogenesis, cargo sorting and transport. We discovered that PexRD54 stimulates autophagosome formation by coupling host vesicle transport regulators to ATG8CL coated pre-autophagosomal membrane compartments. On the other hand, PexRD54 helped us identify several other host components that bind ATG8CL with a potential role in autophagy. Furthermore, we show that PexRD54 labeled autophagosomes are diverted towards haustoria, possibly to allocate cellular resources. Our results implicate effector-mediated employment of vesicle transport components in autophagosome biogenesis and show that effectors can serve as adaptors targeting protein complexes to co-opt host processes.

Functional characterization of the *Phytophthora infestans* RXLR effector *AVR2*

Marek Malec | Christiane Jäntsch | Yan Wang | Susan Breen | Eleanor M. Gilroy | Francine Govers | Paul R.J. Birch | Frédéric Brunner

The genome of *Phytophthora infestans* encodes a large number of RXLR effectors that are aiming to manipulate host cellular functions in order to promote disease. PiAVR2 is an RXLR effector that was shown to interact with potato BSU1-like (BSL) ser/thr phosphatase 1 (Saunders et al., Plant cell 2012), the homolog of Arabidopsis BSL1, a positive regulator of the brassinosteroid (BR) signaling pathway controlling plant growth and development. The exploitation of the large existing -omics, genetic and material resources on BR signaling in Arabidopsis could possibly help to decipher the mechanistic basis of the mode of action of PiAVR2 and guide subsequent work in solanaceous plant species, the natural hosts of *P. infestans*. Using a cell-based system, we have identified a strong interaction between PiAVR2 and AtBSL1, AtBSL2 and AtBSL3 but not with AtBSU1. In further studies, we show that, although PiAVR2 interacts with BSU1-like phosphatases, it is not affecting typical BR responses such as BR-dependent activation of BES1/BZR2 transcription factor and BR- regulated gene expression. PiAVR2 also does not affect flg22-dependent early immune responses in Arabidopsis such as the oxidative burst, MAP kinase activation, or *FRK1* induction. However, PiAVR2 enhances susceptibility to microbe infection in Arabidopsis and PiAVR2 plants are more sensitive to the (hemi)biotrophic pathogen *Pseudomonas syringae* and *Phytophthora capsici* but more resistant to the necrotroph *Alternaria brassicicola*. Future work will aim to determine how PiAVR2 impedes plant immunity through its interaction with the BSLs.

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

Joël Klein

Peronospora farinosa f. sp. *spinaciae* (*Pfs*, also known as *P. effusa*) is an obligate biotrophic oomycete pathogen of spinach (*Spinacia oleracea*). This downy mildew causes a destructive disease on spinach worldwide as it affects the harvested plant parts, the leaves. Infection can already start at the cotyledon stage and eventually results in discolored and distorted plant tissue covered with grey sporangiospores. Although resistant cultivars are being bred, new *P. farinosa* races rapidly break the employed resistance genes. This project has started with the sequencing and hybrid assembly of a high-quality reference genome of *P. farinosa* race 1 (*Pfs1*), using PacBio, and Illumina technology. Also, the sequencing of *Pfs1* mRNA at 7 different time points before and during infection has helped to identify gene models. This data can be used to identify genes that are highly induced during infection suggesting a role in the infection process. Furthermore, already 14 other *Pfs* races have been sequenced using Illumina. The genomes of these races were *de novo* assembled with a similar method. We have identified the effector genes in *Pfs1* race, these will be compared to different *Pfs* races to identify effector gene polymorphisms. Furthermore, *for Pfs1* we have assembled a full mitochondrial genome, this will be used for a reference assembly of the mitochondrial genome of other isolates and allows us to study the evolution of *Pfs* races. The increased knowledge on effectors and evolution of *Pfs* races will provide new leads to generate resistant spinach cultivars.

Editing a virulence target of the late blight pathogen for enhanced resistance.

Mark Banfield/ Richard Hughes | Stuart King

The *P. infestans* RxLR effector PexRD2 has been shown to interact with host MAPKKKe in order to suppress MAPKKKe-mediated cell death induction and promote the virulence of *P. infestans*.

Most genetic approaches to developing disease resistance have focused on the introgression of NLR genes into crops. To date, the possibility of engineering the targets of effector proteins for insensitivity to their cognate effector protein has received limited attention. Therefore, effector targets could present an untapped resource for novel disease resistance strategies.

We adopted a random mutagenesis approach to screen for variants of MAPKKKe which were insensitive to PexRD2. In transient assays we found four candidates that reproducibly evade effector mediated suppression of cell death. Subsequent yeast-2-hybrid assays have revealed that effector- insensitive kinase variants exhibit loss of, or visibly weakened, interaction with PexRD2. This provides a mechanistic basis for their insensitivity to the effector's activity.

In order to test effector-insensitive kinase variants for resistance, paired sgRNAs targeting an N- terminal region of MAPKKKe containing the catalytically active HRD-DFG motif were designed and tested transiently in *Solanum lycopersicum* for editing activity. A PCR-restriction enzyme assay was used to enrich edited DNA and confirm editing activity. Stably transformed *S. lycopersicum* plants with deleted wild type MAPKKKe genes, complemented with effector-insensitive kinase variants under the control of their native promoter, are currently being prepared. These will be infected with *P. infestans* in order to assay for resistance relative to wild type tomato plants.

Eighteen little RXLR effectors from *Phytophthora palmivora*

Edouard Evangelisti | Mehdi Doumane | Ayoub Kadoussi | Sebastian Schornack

We selected a set of 18 RXLR-EER effector candidates of two geographically distant *Phytophthora palmivora* isolates originating from Colombia and Indonesia and assessed their contribution to virulence. We found that seven of these effectors were present in several *P. palmivora* isolates isolated from different host species and countries. They are expressed in successive waves and localize to diverse subcellular locations when expressed in *Nicotiana benthamiana*. One of these effectors is able to trigger cell death in *N. benthamiana* leaves. Three other effectors are able to suppress this effector cell death, but not INF1- or BAX1-triggered cell death. Finally, one effector supports better leaf infection, and another one accelerates root infection. Elucidation of host targets should shed light on common and tissue specific target host processes.

A secreted nuclease of a fish-pathogenic oomycete self-translocates into host

Franziska Trusch | Lars Loebach | Stephan Wawra | Elaine Durward | Andreas Wuensch | Irene de Bruijn | Kevin McKenzie | Aleksandra D. Toloczko | Javier Diéguez-Uribeondo | Tim Rasmussen | Thomas Schrader | Peter Bayer | Chris J. Secombes | Pieter van West

Oomycetes are eukaryotic microbes that are among the most devastating pathogens of animals and plants with a huge economic and environmental impact in cultured as well as natural ecosystems. The fish pathogen *Saprolegnia parasitica* is responsible for the decline of natural salmonids and other fresh water fish populations as well as for disastrous losses in the aquaculture industry. Development of saprolegniosis is a comprehensive process and one strategy for establishing a successful infection is the adaption of the host immune system to reduce the host resistance and/or the metabolism for the pathogens benefits by secreting proteins which are able to enter host cells. Plant pathogenic oomycetes from the order Peronosporales secrete RxLR-effectors, characterized by the N-terminal RxLR (Arg-Xaa-Leu-Arg) motif. RxLR-proteins are thought to be delivered into host cells via the RxLR motif. However, the exact translocation mechanism of oomycete effectors into their host cells is still unclear and under debate.

We identified a novel host-targeting protein (SpHtp3) secreted by *S. parasitica* and determined its biological function as a nuclease in vitro and in vivo. The detailed elucidation of SpHtp3's translocation process via a receptor protein and the identification of its RxLR-independent translocation module allowed us to inhibit the effector protein uptake into cells with supramolecular compounds.

Delivery of *Phytophthora sojae* effector Avr1b *in planta* requires PI3P-binding, but does not require N-terminal cleavage.

Qunqing Wang / Felipe Arredondo / Yufeng Fang / Eli Perez / Shiv D. Kale / Brett M. Tyler

A major class of effectors produced by oomycetes contains RxLR motifs that mediate entry of these effectors into plant cells. We previously showed that these effectors can bind to specific lipids including phosphatidylinositol-3-phosphate (PI3P). PI3P-binding requires the RxLR motif, plus in some cases, C-terminal regions of the protein. In order to validate that PI3P-binding mediates host cell entry in planta, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RxLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. The Avr1b and various derivative mutant proteins can be specifically detected in culture supernatants after de-glycosylation, indicating that Avr1b is post-translationally modified. Some RxLR effectors such as Avr1b also undergo N-terminal cleavage following secretion, but others such as Avr4/6 do not. Cleavage of Avr1b occurs upstream of the RxLR motif, and the RxLR motif is not required for cleavage. Mutants of Avr1b that are not cleaved are delivered normally. We conclude that N-terminal cleavage is not required for delivery *in planta*. We are now using CRISPR-mediated gene replacement to refine these experiments.

Cell wall proteins from *P. infestans* in appressorium formation and field immunity.

Maja Brus | Laith Ibrahim Moushib | Marit Lenman | Erik Andreasson | Laura Grenville-Briggs

The cell wall is an essential structure for the viability of Eukaryotic cells. It is also the first point of direct contact between the pathogen and the plant and thus a potential source of Pathogen- Associated Molecular Patterns (PAMPs) and molecules involved in pathogenicity. During a proteomics screen of *P. infestans* asexual development we identified several cell wall proteins as highly expressed during appressorium formation. Transcripts of the genes encoding these proteins are highly abundant during initial contact between *P. infestans* and the potato leaf. Transient silencing of the transcripts using RNAi demonstrates an essential role for these proteins in normal appressorium formation and thus successful infection of potato.

We have also confirmed the presence of transglutaminases containing the Pep13 PAMP in the *P. infestans* appressorium cell wall. Since Pep13 is known to activate the immune response (PTI) in potatoes, expression of the peptide *in planta* should enhance resistance. Enhanced late blight resistance was observed using whole plant pathogenicity assays in growth chambers, but not under field conditions. Instead the field-grown plants showed an early senescence phenotype in relation to control plants. To determine how expression of Pep13 *in planta* triggers early senescence transcriptional changes were analysed by microarray analysis. This work highlights the importance of field analysis to fully assess the effects of resistance seen in laboratory assays.

Selection of environmental isolates of *Pseudomonas* for inhibition of soil oomycete pathogens.

Andrew Wagner | Paul Morris | Stephen Norris | Hans Wildschutte

Early seasonal planting of soybeans is directly correlated to larger yields, but planting soybeans into cold wet soils increases the risk of infection by *Pythium* isolates. Currently no broad-host seedling treatments exist, thus there is an urgent need for new approaches to reduce the economic impact of seedling diseases. *Pseudomonas*' diversity, coupled with its known anti-fungal and anti-bacterial properties make this organism a rational candidate for bio-pesticide discovery. To test this hypothesis, environmental *Pseudomonas* sp. were isolated from soil and Lake Erie. Both environments include *Pseudomonas* and oomycetes that have adapted to cold temperatures, and are hypothesized to compete for resources. In an initial screen, 90 isolates were identified as having strong inhibitory activity against the soybean pathogen *Phytophthora sojae*. High throughput hyphal growth inhibition assays have also been used to screen for growth inhibition against *Pythium irregulare*, *Pythium ultimum*, *Pythium heterothalicum*, and *Pythium sylvaticum*. Thus far, 16 genetically diverse isolates have been identified as having robust inhibitory activity against all four *Pythium* species and *P. sojae*, and several other isolates have been observed with inhibitory activity against one or more of the *Pythium* species. Pseudomonas isolates that result in inhibition of *P. sojae* and all four *Pythium* species will be screened to identify which isolates are most amenable to transposon mutagenesis. Isolates found capable of mutagenesis provide means for determining the molecular pathway responsible for their antagonistic properties, and will be selected for further DNA sequencing.

Phytophthora infestans Argonaute proteins display distinct small RNA binding preferences

Anna Åsman | Johan Fogelqvist | Ramesh Vetukuri | Chrisina Dixelius

RNA-induced gene silencing (RNA interference; RNAi) is a process of profound importance in eukaryotic cells. It is involved in mechanisms ranging from transposable element (TE) silencing, to antiviral defense, and to endogenous gene expression regulation. At the core of the RNAi machinery are Argonaute (Ago) proteins and small RNAs (sRNAs). Genomic and transcriptomic analyses have identified four Ago clades and two major classes of sRNAs in *Phytophthora* species. In the present study, we used co-immunoprecipitation (IP) and sRNA sequencing to find the sRNA populations bound by *P. infestans* Ago (PiAgo) proteins. This revealed functional specialization in the sRNA binding preferences of PiAgo1 and PiAgo4. Unexpectedly, PiAgo5 bound similar sets of sRNAs as PiAgo1, despite being closely related to PiAgo4. Transcript analyses indicated that *PiAgo3* is pseudogenized in the *P. infestans* isolate under study. Thus, the sRNA binding partners of Ago3 clade proteins in *Phytophthora* remain to be characterized. While an abundance of *CRN*-derived sRNAs was identified in the PiAgo1 IP sample, *RxLRs* generated low numbers of PiAgo-bound sRNAs. This suggests that distinct gene regulatory mechanisms govern the expression of these two effector gene families. RNAi is an important tool for functional genomics in oomycetes, so mechanistic understanding of RNA silencing will be of great value for the advancement of this research field. sRNAs are gaining recognition as key players in pathogen adaptation and host defense, and continued studies will reveal the roles of individual Ago proteins and sRNAs in oomycete-plant interactions.

Apoplastic metabolites used by *Phytophthora infestans* during hyphal growth

Adeline Harant

Phytophthora *infestans*, the plant destroyer, is the most infamous oomycete plant pathogen. Causing potato late blight, it was responsible for the Irish Potato Famine in the mid-19th century. Most molecular studies of late blight are aimed at effectors, proteins secreted by the pathogen that interact with the plant cell, and the corresponding resistance genes. Surprisingly, few studies have focused on how the pathogen uses nutrient from plants. During plant infection, the majority of *P. infestans* biomass is in the form of intercellular hyphae. We hypothesize that these hyphae can absorb nutrients from the host plant apoplast.

A mass spectrometry approach, combined with a global transcriptome analysis, is being used to identify which compounds *P. infestans* depletes from the apoplastic fluid of *Nicotiana benthamiana*, an alternative solanaceous host, during the early stage of infection and which genes are involved in these processes.

Metabolomic data analysis has revealed signals that decrease in the apoplastic fluid only during *P. infestans growth*. Amongst these signals, fatty acids, citric acid cycle intermediates and amino-acids were found. The *P. infestans* genes up-regulated during growth in apoplastic fluid were classified according to their functions. Transport and metabolism represent a large proportion of these genes, together with numerous pathogenesis-related genes. Metabolite usage data from apoplastic fluid is being further investigated using different nitrogen and carbon sources for *P. infestans*, combined with characterization of a silenced amino acid transporter. Progress towards determining the nutrients essential for *P. infestans* growth in planta will be presented.

The pathogens of brown algae *Anisolpidium ectocarpii* and *Anisolpidium rosenvingei* define a new class of marine anteriorly uniciliate oomycetes

Claire MM Gachon | Martina Strittmatter | Yacine Badis | Kyle I Fletcher | Pieter van West | Dieter G Müller

Despite their abundance in the field, and their suspected role in regulating the abundance of their host population, hyphochytrid pathogens of brown algae have been hardly studied. Using laboratory cultures, we document here the life cycle of *Anisolpidium ectocarpii*, a pathogen of *Ectocarpus* and other filamentous brown algae, and present preliminary observations on *Anisolpidium rosenvingei*. Consistent with earlier reports, the zoospores of both species have a single anterior flagellum, which justified the placement of *Anisolpidium* amongst the Hyphochytridiales (Hyphochytridiomycota). Unexpectedly, nuclear (SSU rRNA) and mitochondrial (cox 1, cox2) markers regroup *A. rosenvingei, A. ectocarpii* and other marine environmental sequences into a hitherto unrecognised monophyletic clade within the oomycetes (Oomycota), most closely related to the Olpidiopsidiales and Haliphthorales. The *Anisolpidium* genus is therefore entirely distinct from the Hyphochytridiales and represents the first unquestionable instance of an anteriorly uniciliate oomycete. We also show that *A. ectocarpii* can complete its infection cycle in a broad selection of species from various brown algal orders, suggesting that species delimitation within the genus *Anisolpidium* should not merely be based on the identity of the algal host, as is presently the case. Finally, a working hypothesis is generated in an attempt to establish a new criterion for the separation of hyphochytrids from oomycetes, based on the point of zoospore cleavage.

Improved *Phytophthora ramorum* Pr102 genome assembly with hybrid assembly protocol

Mathu Malar C | Jennifer Yuzon | Takao Kasuga | Sucheta Tripathy

Phytophthora ramorum is the causal agent of Sudden Oak Death disease that has killed over a million trees in coastal California. The earlier *P. ramorum* Pr102 genome was assembled into 65 MB and 2576 scaffolds with 12 MB gaps. We present here an updated assembly with additional PacBio (~435399 reads, coverage XX) and Illumina sequences (20942377 reads, coverage XX) with the Sanger contigs (7589 contigs, ~54.4 MB) from the 2006 assembly. We used several workflow systems with combinations of error correction protocols to produce a more refined assembly. An earlier error correction pipeline resulted in 33% (147429 reads ~ 921 MB) reads corrected, whereas the new 3 step error correction protocol resulted in 49% (206487 reads ~1.3GB) of corrected reads. We evaluated several assembly methods alone or in combination with each other for optimizing genome assembly. First, we assembled error corrected PacBio reads with Celera assembler resulting in 2735 contigs (77Mb). Second, a combination of simulated jump libraries was generated from the first round of assembly for improving the scaffolding process. Finally, the redundans pipeline was used to reduce heterozygous contigs of the simulated jump libraries of the PacBio error corrected reads (6K and 10K insert size; 118360 and 10468 reads respectively) and 2006 assembly (20K insert size having 56758 reads). The latest assembly has only 220 gaps and CEGMA analysis reveals about 95.7% COGs present. Total number of genes predicted using RNAseq hint data and Augustus pipeline is 19278. We are working on polymorphisms and synteny on this assembly.

Evolution of the Sterol Biosynthetic Pathway of *Pythium insidiosum* and Related Oomycetes Contributes to Antifungal Drug Resistance

Tassanee Lerksuthirat | Areeporn Sangcakul | Tassanee Lohnoo | Wanta Yingyong | Thidarat Rujirawat | Theerapong Krajaejun

Oomycetes form a phylogenetically-unique group of microorganisms that share microscopic features with fungi. Unlike most pathogenic oomycetes which infect plants, *P. insidiosum* is capable of infecting humans and animals, and causes a life-threatening infectious disease, called pythiosis. Direct contact of *P. insidiosum* initiates infection. Common sites of *P. insidiosum* infection include skin, eye, artery, and gastrointestinal tract. Mortality rate of pythiosis is high. Treatment for patients with pythiosis is relied on extensive surgery. Based on our experience and literature review, conventional antifungal drugs are clinically ineffective against *P. insidiosum* infection. However, *in vitro* inhibition of *P. insidiosum* by some antifungal drugs has been reported. Additionally, a patient with invasive *P. insidiosum* infection was successfully treated by two antifungal drugs: terbinafine and itraconazole. According to these inconsistent observations, the present study aims at investigating whether various strains of *P. insidiosum* are susceptible to terbinafine and itraconazole, and the pathogen harbors the drug-target enzymes and sterol biosynthetic pathway. We showed that 30 clinical isolates of *P. insidiosum* were minimally or partially inhibited *in vitro* by either terbinafine or itraconazole. Based on BLAST searches against the oomycete genomes, *P. insidiosum* and related species contained an incomplete set of enzymes (i.e., a drug-target enzyme) in the ergosterol biosynthetic pathway. GC-MS analysis identified no end-product sterol compounds of fungi, plants, and animals (i.e., cholesterol, ergosterol, stigmasterol, and fucosterol) in sterol extracts of *P. insidiosum*. In conclusion, the lack of sterol biosynthetic pathway in *Pythium insidiosum* and related oomycetes contributes to antifungal drug resistance.

De-novo assembly of *Phytophthora palmivora* transcriptome and characterisation of the secretome during root infection

Anna Gogleva | Ruth Le Fevre | Edouard Evangelisti | Sebastian Schornack

Phytophthora palmivora is a hemibiotrophic cosmopolitan broad host range pathogen, infecting shoot and roots of many economically important crops, such as cocoa, rubber, coconut and citrus. However, little is known about its genome and transcriptome and the genetic basis defining its infection potential.

We studied the *P. palmivora* transcriptome during a root infection time course on model monocot (*Hordeum vulgare*) and dicot (*Nicotiana benthamiana*) host species. Through de-novo transcriptome assembly we reconstructed 48089 clusters of transcripts (including sequence variants and alternatively spliced isoforms) and predict at least 1965 of them to encode secreted extracellular proteins. Based on orthology reconstruction and analysis of universal single-copy orthologs (BUSCOs), we propose *P. palmivora* to be an alloploid hybrid. In agreement with previously published studies on other species from the same genus we show distinct waves of expression for functional classes of genes involved in pathogenesis: RXLR- and Crinkler effectors, proteases, cell wall degrading enzymes, PAMPs, small cysteine rich proteins (SCPs) and necrosis-inducing proteins (NLPs), which align with a transition from the biotrophic towards the necrotrophic stage of infection. Furthermore, we identified transcripts and patterns of gene expression specific to either of two model hosts, which indicate plasticity of *P. palmivora* infection strategies.

Genome Annotator light(GAL): An integrated virtual machine for genome analysis and visualization

Arijit Panda | Arup Ghosh | Sucheta Tripathy

With the advent of new sequencing technologies, sequencing speed has completely outpaced the analysis process. Data analysis has become a key challenge recently. In view of this, we developed a new lightweight genome analysis and visualization tool, Genome Annotator Lite (GAL), that facilitates genome analysis in a quick and easy way. GAL enables researchers to explore, interpret and visualize data effortlessly. The database schema is a successor of Genome Unified Schema (GUS) that has been remodeled to an open source platform MySQL. GAL can take 3 different sets of input data types e.g; a simple genome fasta file; partially annotated genome data with annotation in gff format; a fully annotated sets of files with annotations such as interproscan, pathway analysis, secretome etc.. On the very first run, GAL screens for presence of database schema and shared resources such as GO, NCBI taxon data on the installing machine. Schema and shared resources are downloaded and parsed and stored in the database on the very first run of GAL. On subsequent runs, only the genome data is parsed and uploaded. The light weight front end of GAL is created with Perl/CGI and PHP is easy to install and serves a great visualization interface. GAL has 200622 lines of codes and installation of GAL takes 15 to 20 minutes. A partially annotated genome takes about
10 to 15 minutes to get fully uploaded into the system. Our current oomycetes database resource runs on GAL is available at www.eumicrobedb.org.

Co-regulation of gene expression in *Phytophthora*

Jasmine Pham | Remco Stam | Julie Squires | Christian Cole | Pieta Schofield | Peter Cock | Leighton Pritchard | Paul Birch | Michael Csukai | Steve Whisson | Edgar Huitema

Species of *Phytophthora* represent economically important plant pathogens which are notoriously difficult to control. Progression through the infection process requires the development of infection structures and production of proteins required for virulence, such as effector proteins. These changes are driven by co-ordinated regulation of gene expression, suggesting the involvement of promoter motifs and transcription factors in *Phytophthora*. The identification and characterisation of such regulatory elements may thus help understand and disrupt basic processes required for infection.

Here, we describe our efforts towards the identification of promoter motifs and transcriptional regulators in *P. capsici* and *P. infestans*. Using transcriptomics data from microarray and RNA- sequencing timecourse experiments of *P. capsici* infection on tomato, we identified clusters of co- regulated genes. Alignment and analysis of the promoter regions of clustered genes identified potential promoter motifs driving co-regulation of gene expression. We have also identified a number of transcription factors that appear to be regulated during biotrophy. Amongst those, a potential transcriptional regulator with similarity to the transcriptional repressor protein NMRA of *Aspegillus nidulans* was identified in both *P. capsici* and *P. infestans*. Over-expression of this NMRA-like protein in *P. capsici* resulted in reduced lesion formation and altered expression of the *PcHmp1* biotrophy marker gene *in planta*. Further work to determine how both transcription factors and their putative target motifs control gene expression during biotrophy is currently underway. We anticipate that a thorough understanding of transcriptional regulatory networks will help devise new and more targeted strategies to control *Phytophthora* epidemics in the field.

Detecting intraspecific ploidy variation in *Phytophthora infestans* using shot-gun genomics

Clemens L. Weiß | Marina Pais | Lida Derevnina | Liliana Cano | Sophien Kamoun | Hernán A. Burbano

Variation in ploidy plays an important role in the creation and maintenance of genetic diversity. There is evidence that polyploidy and genomic structure are crucial in the evolution and function of *Phytophthora infestans*, which is the causal agent of potato and tomato late blight. *P. infestans* has a genomic structure, where regions of low gene density harbor fast-evolving genes important for infection, and intraspecific variation in ploidy has been also detected. Diploids are common in the native range of the pathogen, where both mating types are available, whereas polyploids occur in populations reproducing asexually. Increments in ploidy level have been detected between strains from the 19th and 20th centuries. Thus, ploidy changes also fuel genetic variation that could be adaptive. Ploidy can be detected from karyotype analysis, but it is also possible to infer ploidy from shot-gun sequencing. Here, we present a method to detect ploidy using shot-gun sequencing, which is based on the distribution of base frequencies along the genome. First, we estimated the ploidy level of two deeply-sequenced *P. infestans* strains (one diploid and one triploid) based on k-mer distributions. We then benchmarked our method and estimated accuracy at different genomic coverages. Finally, we surveyed a set of *P. infestans* strains and reported their ploidy level. We showed that intraspecific variation in ploidy can be reliable estimated. Ploidy changes could play a role in the arms-race between plant and pathogen, and the joint study of ploidy and effectors repertoire can shed light on *P. infestans*' remarkable adaptability.

Rethinking reproduction in Danish *Phytophthora infestans*: population genetics reveal dominantly clonal populations and alleles linked to Metalaxyl- M resistance

Melanie S. Montes | Bent J. Nielsen | Suzanne G. Schmidt | Lars Bødker | Rasmus Kjøller | Søren Rosendahl

When the A2 mating type of *Phytophthora infestans* was introduced into Europe in the 1980s, it brought along with it the possibility for sexual reproduction. Since then, presence of sexually- derived oospores in the soil, and high genotypic diversity uncovered in microsatellite analyses have supported the idea that sexual reproduction is indeed taking place in certain regions, in particular the Nordic countries. However, the highest levels of diversity globally have sometimes been found in regions where sexual reproduction is not a possibility, and genetic diversity as such cannot prove that sexual reproduction is dominating. The population genetic structure of over 80 samples collected from seven fields throughout Denmark in the summer of 2013 was characterized using simple sequence repeat (SSR) genotypes and SNP-based mitochondrial haplotyping. Although both mating types A1 and A2 were present in most fields, tests for recombination showed that clonal reproduction still dominates in Danish populations. In addition, the metalaxyl-M resistance of each isolate was characterized, and specific SSR alleles were found to be linked to higher odds of resistance, indicating the potential for development of SNP-based markers for resistance in the future.

De novo sequencing and population genomics of *Plasmopara viticola*, the grapevine downy mildew

Yann Dussert | Jérôme Gouzy | Sylvie Richard-Cervera | Isabelle Demeaux | Ludovic Legrand | Sébastien Carrère | Pere Mestre | François Delmotte

Invasions by plant pathogens are responsible for tremendous damage in crops and are increasing in frequency, notably due to human-mediated dispersal. Consequently, population genetics studies of pathogen introductions are of great interest to understand invasive population dynamics and the processes of adaptation to new hosts and environments. Plasmopara viticola is an oomycete responsible for grapevine downy mildew, a major and costly disease worldwide. It has been introduced very recently (around 150 years ago) from North America in Europe and has subsequently invaded European vineyards in a few years. To study the genetic consequences of the introduction of *P. viticola* on its genome, we carried out a population genomics approach.

The genome of *P. viticola* has been sequenced using three Illumina libraries (paired-end and mate- pair reads) with different insert sizes. This assembly covered 70% of the estimated genome size (CEGMA pipeline: 95% completeness). Around 30% of the assembly was made up of repetitive elements, and 17,131 genes were annotated. A total of 18 strains from Europe and 2 strains from North America, *P. viticola*'s native area, have been resequenced, producing 1.08 million SNPs. The genetic diversity of the European strains was not structured geographically. Inference of the demographic history of the species using Approximate Bayesian Computation and detection of selection footprints along the genome are currently on-going, as well as a new genome assembly using the PacBio long reads.

Oomycete Biodiversity in the Falkland Islands

Katie Davis | Mohammad Nasif Sarowar | Ariane Willems | Amy Victoria Bode | Max Beckmann | Jose Vladimir Sandoval-Sierra | David Belo | Herbert van den Berg | Steve Woodward | Paul Brickle | Frithjof C. Küpper | Javier Diéguez-Uribeondo | Pieter van West

Oomycetes are well known economically important pathogenic organisms, affecting many plant and animal species within both cultured and natural environments. Little is known about the biodiversity of oomycete species within the Falkland Islands, however due to its unique geological position and ecological history, it is likely to have introduced and potentially novel oomycete species. Different baits were used to collect oomycetes from water samples from different sites during the Islands spring and summer in 2011 to 2013. Isolates were taxonomically identified by sequencing the internal transcribed spacer (ITS) region of the rRNA gene. Analysis of the ITS sequences confirmed a number of known and unique oomycete species present on the Falkland Islands covering six genera i.e. *Saprolegnia, Pythium, Phytophythium, Achlya, Leptolegnia and Phytophthora.* Seven novel species, covering five genera, were suggested by phylogenetic analysis of the ITS sequences compared to closely related species. The results of molecular data were strengthened by detailed morphological characterization of sexual and asexual structures of these isolates. Infection assays indicated *Phytopythium falklandica* is pathogenic toward the roots of ryegrass seedling. While novel *Saprolegnia* and *Leptolegnia* isolates (*S. beakesii, S. falklandica* and *L. falklandica)* were shown to have low pathogenicity towards fish eggs.

Potato NPH3/RPT2-like protein StNRL1, targeted by a *Phytophthora infestans* RXLR effector, is a susceptibility factor

Lina Yang | Hazel McLellan | Shaista Naqvi | Qin He | Petra C Boevink | Miles Armstrong | Licida M Giuliani | Wei Zhang | Zhendong Tian | Jiasui Zhan | Eleanor M Gilroy | Paul J R Birch

Plant pathogens deliver effectors to manipulate host processes. We know little about how fungal and oomycete effectors target host proteins to promote susceptibility, yet such knowledge is vital to understand crop disease. We show that either transient expression in *Nicotiana benthamiana*, or stable transgenic expression in potato (*Solanum tuberosum*), of *Phytophthora infestans* RXLR effector Pi02860 enhances leaf colonization by the pathogen. Expression of Pi02860 also attenuates cell death triggered by the *P. infestans* MAMP INF1, indicating that the effector suppresses pattern- triggered immunity (PTI). However, the effector does not attenuate cell death triggered by Cf4/Avr4 co-expression, showing that it does not suppress all cell death activated by cell surface receptors. Pi02860 interacts in yeast-2-hybrid assays with potato NPH3/RPT2-like 1 (NRL1), a predicted Cullin-3-associated ubiquitin E3 ligase. Interaction of Pi02860 in planta was confirmed by co- immunoprecipitation and bimolecular fluorescence complementation assays. Virus-induced gene silencing (VIGS) of NRL1 in *N. benthamiana* resulted in reduced P. infestans colonization and accelerated INF1-mediated cell death, indicating that this host protein acts as a negative regulator of immunity. Moreover, whereas NRL1 VIGS had no effect on the ability of *P. infestans* effector AVR3a to suppress INF1-mediated cell death, such suppression by Pi02860 was significantly attenuated, indicating that this activity of Pi02860 is mediated by NRL1. Transient overexpression of NRL1 resulted in suppression of INF1-mediated cell death and enhanced *P. infestans* leaf colonization, demonstrating that NRL1 acts as a susceptibility factor to promote late blight disease.

Structural basis of ATG8 binding by a *P. infestans* effector protein

Abbas Maqbool | Richard Hughes | Yasin Dagdas | Nicholas Tregidgo | Erin Zess | Pooja Pandey | Khaoula Belhaj | Adam Round | Tolga Bozkurt | Sophien Kamoun | Mark Banfield

Phytophthora infestans, the causative agent of potato and tomato late blight, is a devastating pathogen of historical importance that still requires significant control in modern agricultural settings. To colonise its hosts, *P. infestans* delivers effector proteins into plant cells. We have recently found that PexRD54, an RXLR-type effector protein, interacts with the potato protein ATG8CL *via* its ATG8 interacting motif (AIM) to perturb host autophagy to the benefit of the pathogen. Building on this study, we have investigated the biochemical and structural basis of the PexRD54/ATG8CL interaction. We determined the crystal structure of PexRD54, which comprises a modular architecture including five tandem repeat WY-domains, with the AIM sequence presented at the disordered C-terminus. To determine the molecular interface between PexRD54 and ATG8, we derived the crystal structure of potato ATG8CL in complex with a peptide comprising the effector's AIM sequence, and established a model of full-length PexRD54/ATG8CL interaction using solution X-ray scattering. In addition, we described the role of individual residues in the AIM region of PexRD54 for ATG8CL binding. Finally, structure-informed deletion of PexRD54's WY-domains reveals retention of ATG8CL binding *in vitro* and in plant cells, suggesting additional roles for the tandem domains. This study delivers new insights into how RXLR effector proteins engage with host cell targets to promote disease.

Genome sequence and analysis of the strawberry crown rot pathogen *Phytophthora cactorum*

Andrew Armitage | Liliana M. Cano | Erik Lysøe | Jahn Davik | Sophien Kamoun | Richard Harrison | May Bente Brurberg

Here we report the genome assembly and gene models predicted for *Phytophthora cactorum* strain 10300, originally isolated from the rhizome of a field-grown strawberry plant with crown rot symptoms in Norway. Crown rot is a serious disease resulting in big economic losses in strawberry production. *P. cactorum* 10300 causes infection in numerous cultivars of octoploid strawberry (*Fragaria x ananassa*) and accessions of wild diploid strawberry (*Fragaria vesca*).

The *P. cactorum* 10300 assembly is equivalent to 59 Mb from which 18% was identified as repetitive elements or low complexity regions. Using a combination of gene-calling algorithms and transcript evidence we predicted a total of 20,689 gene models. We performed comparative analysis of the coding space from *P. cactorum* with two other Clade 1 species (*P. parasitica* and *P. infestans*), the Clade 2 species *P. capsici* and the Clade 7 species *P. sojae*. The *P. cactorum* gene models are highly enriched in genes belonging to gene ontology categories "DNA integration" and "aspartic-type endopeptidases" in comparison to other *Phytophthora spp.* Among secreted proteins, we identified cytoplasmic effectors of both RxLR and Crinkler from six-frame translated open reading frames and protein-encoding gene models. Orthology analysis of predicted Crinkler effectors from the five species found that orthogroups separated in accordance to previously described C-terminal domains. This allowed identification of novel Crinkler C-terminal regions as well as Crinklers unique to *P. cactorum*. Similarly, orthology analysis of RxLRs effectors identified core RxLRs common to *Phytophthora spp.* as well as those expanded or found only in *P. cactorum*.

Pythium porphyrae the agent of the red seaweed rot disease: a reformed plant pathogen?

Jong Won Han | Anonios Zambounis | Tatyana A. Klochkova | Yacine Badis | Lisa Breithut | Gwang Hoon Kim | Claire Gachon

The red alga *Pyropia* (formerly *Porphyra*) sp.is the most valuable seaweed worldwide, underpinning a global industry in excess of $ 1 billion. *Pythium porphyrae*, the agent of red rot disease is responsible for devastating outbreaks in seaweed farms. Here, we investigated the gene repertoire of *P. porphyrae* and its transcriptional regulation using next-generation sequencing EST libraries obtained during a time course of infection. We focussed our annotation on the genes potentially involved in pathogenicity such as secreted proteins, toxins, and homologues of known oomycete pathogenicity effectors. In agreement with the general view that *Pythium* pathogens are opportunistic, necrotrophic pathogens less specialised than other biotrophic oomycetes, *P. porphyrae* contains a gene repertoire very similar to the one described in other *Pythium* species. Strikingly however, we could not identify any enzyme specifically involved in the degradation of red algal- cell wall components. Instead, the presence of cellulases, CBEL proteins and of a cutinase hints to *P. porphyrae* tracing its roots to a pathogen of higher plants (Embryophyta).

Probing the mechanisms of *C. elegans* infection by natural oomycete pathogens

Michalis Barkoulas | Guled Osman | Michael Fasseas

A number of microbes have evolved pathogenic and parasitic lifestyles targeting a range of eukaryotic hosts including nematodes. One such group is the oomycetes, eukaryotic organisms that are superficially similar to fungi. Oomycetes inhabit a variety of terrestrial and aquatic environments, and have evolved saprophytic or parasitic lifestyles, infecting a range of hosts including animals and plants. Although plant pathogenic oomycetes have been widely studied, research on animal pathogenic oomycetes has been much more limited, largely due to absence of tractable host systems.

To redress this, we have recently sampled and currently maintain in culture two biotrophic oomycetes naturally infecting the model organism *Caenorhabditis elegans*. These oomycetes belong to the *Myzocytiopsis* and *Haptoglossa* genus and display related but distinct infection strategies to colonise and eventually kill the nematode host. I will describe the establishment of these new pathosystems to allow comparative studies of host / oomycete interactions. I will present our results deciphering host innate immunity pathways, such as the TGF-beta and p38 MAP kinase pathways, involved in conferring resistance to infection. I will discuss transcriptomic results in *C. elegans* to address pathogen-induced changes of host gene expression and their functional role to antagonise or permit the infection. I will also present our first data characterising oomycete virulence factors utilised to overcome nematode defences. Given the wealth of molecular tools and resources available for *C. elegans* research, these new systems provide an unprecedented opportunity to establish powerful models to study animal / oomycete interactions.

Transcriptional changes in roots of *Fragaria vesca* during *Phytophthora cactorum* challenge

Anna Toljamo | Daniel Blande | Sirpa Kärenlampi | Harri Kokko

Crown rot, caused by *Phytophthora cactorum*, is a destructive strawberry disease that is most effectively controlled by using resistant cultivars. However, polygenically inherited resistance in the garden strawberry (*Fragaria × ananassa*) makes the breeding challenging, and knowledge about defense mechanisms is needed. We have employed large-scale RNA sequencing to study the transcriptional changes in the roots of *Fragaria vesca* exposed to *P. cactorum*, and show that massive reprogramming of the transcriptome occurred, involving 30 % of the genes. Some of the most characteristic features were the up-regulation of immune responses, and the down-regulation of cell wall biosynthesis and developmental processes. Also the surveillance system of the plant cells shifted from development to defense mode, as demonstrated by the GO term enrichment analysis of receptor-like kinases. Several major allergen-like genes encoding PR-10 proteins were highly expressed in the inoculated plants and genes involved in the biosynthesis of flavonoids and terpenoids were up-regulated. These new findings help to target molecular screening and breeding efforts toward more resistant strawberry cultivars.

Non-genetic inheritance of induced resistance in a wild annual plant

Kibrom B Abreha | Erik Alexandersson | Stefan Andersson | Åsa Lankinen | Erik Andreasson

Non-genetic inheritance, e.g. transgenerational epigenetic effects, has received increasing interest in recent years, particularly in plants. However, most studies have involved a few model species and relatively little is known about wild species in these respects. We investigated transgenerational induced resistance to infection by the devastating oomycete *Phytophthora infestans* in *Solanum physalifolium*, a wild relative of cultivated potato. We treated plants with β-aminobutyric acid (BABA), a non-toxic compound acting as an inducing agent, or infected plants with *P. infestans*. BABA-treatment reduced lesion size in detached leaf assays inoculated by *P. infestans* in two out of three tested genotypes, suggesting that resistance to oomycetes can be induced by BABA within a generation not only in crops or model species but also in wild species directly collected from nature. Both BABA-treatment and infection in the parental generation reduced lesions in the subsequent generation in one out of two genotypes, indicating a transgenerational influence on resistance that varies among genotypes. We did not detect treatment effects on seed traits, indicating the involvement of a mechanism unrelated to maternal effects. In conclusion, our study provides data on BABA induction and non-genetic inheritance of induced resistance in a wild relative of cultivated potato, implying that this factor might be important in the ecological and agricultural landscape.

Wild *Solanum* species in Sweden and New Zealand as reservoirs of pathogenic oomycetes

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Potato late blight, caused by the oomycete *Phytophthora infestans*, is a devastating disease that can wipe out a potato field within days. Sources of late blight inoculum between seasons include volunteer tubers or sexual oospores. However, it is still unclear if *P. infestans* can overwinter on other plant species. Wild plants can act not only as a source of pathogens, but also as a niche where pathogens can evolve. Therefore the study of wild plant pathosystems can contribute to better understanding the epidemiology of crop diseases. Wild *Solanum* species can grow in proximity of potato fields, and may harbour *P. infestans*. We carried out two rhizosphere surveys: 1) a seasonal survey of *S. dulcamara* in Sweden, and 2) a summer survey of *S. laciniatum* and *S. nigrum* in New Zealand. We isolated individual Oomycete species on selective media, followed by sequencing of ITS and Cox2 markers. We isolated *P. infestans* from symptomless *S. dulcamara* roots during winter and autumn in Sweden, and *Phytophthora undulata* from *S. nigrum* roots in New Zealand, demonstrating that wild *Solanum* species can be reservoirs of pathogenic *Phytophthora* species. Our diversity study identified a further 12 Pythiales species, and one Saprolegniales. Additionally, we investigated whether *S. dulcamara* is tolerant to *P. infestans*. By studying the relationship between plant fitness and degree of infection under controlled conditions, we observed overcompensation in different *S. dulcamara* genotypes. These genotypes will be used for gene expression studies to identify candidate genes involved in the regulation of overcompensation to *P. infestans*.

Molecular characterization of *Plasmopara viticola* single sporangium strains

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Populations of *P. viticola* are characterized by a high genetic variability, which makes its control a challenging task. Many molecular markers have been developed in the previous years, aiming for a better understanding of the population dynamic of this pathogen. For the study of genetic variation in the diploid oomycete, co-dominant and specific markers that are polymorphic at the intraspecific level are necessary. Eight microsatellites (SSRs) and eight SNPs were selected according to their level of polymorphism. In this study we aimed to achieve a molecular characterization of selected *P. viticola* strains, which would permit a better handling and understanding of their development in population studies. The strains were chosen according to their virulence on hosts with difference resistance levels. Eight single sporangium strains from a field isolate were compared among each other and with strains from other field isolates. We focused on the molecular level, looking for an explanation of the high variability observed in our previous studies at the phenotypic level. The assumption that many different genotypes might be present in a field isolate was confirmed. Different genotypes found on a field isolate were phenotypically different in terms of their ability to infect hosts with different resistance levels. The combination of available SNPs and SSRs assures a higher accuracy in genotyping and provides a reliable set of markers for population studies as well as for the characterization of interesting isolates.

Detection of *Lagenidium giganteum* in phytotelmata microbiomes

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae, but phylogenetic analyses have consistently demonstrated that it is a close relative to plant pathogens (*Phytophthora* and *Pythium* spp.). In addition, a recent transcriptome analysis showed that *L. giganteum* expresses oomycete genes that have been associated with plant infection. These observations suggest that *L. giganteum* might have evolved from a plant pathogen to an invertebrate pathogen, and have retained the ability to establish symbiotic or pathogenic interactions with plant tissues. To test this hypothesis, a metagenomic survey of plant material was initiated. Specifically, phytotelmata collected from plant axils (Bromeliaceae) were processed for metagenomic DNA extraction, and Polymerase Chain Reactions (PCR) were performed in an effort to (i) detect *L. giganteum*, and (ii) estimate the relative abundance of *L. giganteum* compared to other oomycetes. First, the presence of oomycetes in all sampled phytotelmata was confirmed by PCR reactions, using oomycete-specific *cox1* primers that were previously published and tested in a barcoding study. Next, the use of a *L. giganteum*-specific primer set demonstrated that the *L. giganteum cox1* barcode can be amplified and sequenced from phytotelmata metagenomic DNA, suggesting that this oomycete is able to colonize environments that are consistent with a close relationship to both plant tissues and mosquito hosts. Finally, the phytotelmata oomycete community was profiled by high throughput sequencing (PacBio platform) of the amplified *cox1* barcodes (800bp), in order to determine the relative abundance of *L. giganteum* among all oomycete species.

Genomics-informed development of molecular markers for genotyping the crayfish plague pathogen

Diana Minardi | Mark van der Giezen | Birgit Oidtmann | David J. Studholme

Aphanomyces spp. are water moulds, eukaryotic fungus-like organisms, belonging to the class Oomycota. This genus contains primary pathogens of plants and animals as well as opportunistic and saprotrophic species. *A. astaci* is an animal parasite and causal agent of crayfish plague, a disease listed by the World Organisation for Animal Health. It was first introduced into Italy from the U.S. in the late 19th century and rapidly spread in Europe causing the decline of native crayfish. Random amplified polymorphic DNA PCR (RAPD-PCR) on isolates of *A. astaci* distinguished five genotypes (A, B, C, D and E). No discriminatory morphological or physiological characters are available and widely used markers such as ITS, LSU and COI failed to discriminate between genotypes. There are some practical drawbacks to genotyping by RAPD-PCR, not least the requirement for pure cultures. Therefore, we used whole-genome sequencing (WGS) on multiple *A. astaci* isolates to catalogue DNA single nucleotide polymorphisms (SNPs) and unique DNA regions to be exploited as new diagnostic methods, in aid of detection and prevention of crayfish plague. By designing primers surrounding genotype-specific SNPs and unique regions, amplifying the DNA fragment by PCR and exploiting enzymatic restriction digestion, we were successfully able to distinguish genotypes on pure cultures. This approach was subsequently used on historical crayfish samples available in our laboratories to validate the reliability of this method. Once tested and validated, this method offers a new tool for diagnostics and epidemiological studies aimed at understanding the history and spread of crayfish plague in Europe.

Abstracts for Poster Presentations

Resistance to *Phytophthora infestans* in European *Solanum dulcamara*

Kibrom Berhe Abreha | Erik Alexandersson | Åsa Lankinen | Sandeep Kumar Kushwaha | Ivo Rieu | Erik Andreasson

In order to reduce potato yield loses, introduction of resistance genes against *Phytophthora infestans* (*Rpi*-genes) from wild *Solanum* species to potato cultivars is considered a compelling method. Many *Rpi*-genes have previously been cloned from various South American wild *Solanum* species, however, the genetic potential of these species from Europe have so far received little attention. Resistance screening of 164 individuals of *S. dulcamara*, collected from natural habitats in southern Sweden, shows unprecedented local variation of *P. infestans* resistance in *S. dulcamara*. A locus in *S. dulcamara* containing a putative resistance gene (*Rpi-dlc1*) has been mapped and BAC clones have been sequenced. The sequence reads were *de novo* assembled and several *Rpi*-like genes were identified using the NBSPred pipeline. Further sequence analysis shows that the *Rpi*-like genes have low sequence similarity to previously cloned *Rpi*-genes. Marker screening for presence and absence of the markers flanking the locus was performed on 45 individuals from a *S. dulcamara* mapping population. Full lengths of the *Rpi*-like genes belonging to the TIR-NBS-LRR class have been amplified from genomic DNA of *S. dulcamara* that contains both *Rpi-dlc1* locus markers. The cloning and functional analysis of the candidates is currently underway.

Silver nanoparticles: a new tool for controlling *Phytophthora* spp and insights into their control mechanism

Gul Shad Ali / Mohammad Ali / Mary Brennan / Bosung Kim / Kevin Belfield / David Norman

The synthesis and applications of nanoparticles is an emerging area in nano-technological research, and nanoparticles synthesis methods that are economical and eco-friendly are extensively investigated. In this project, we investigated the reducing potential of aqueous extract of Artemisia absinthium L. for synthesizing silver nanoparticles (AgNP). In addition, we tested the antimicrobial potential of the synthesized AgNP against Phytophthora spp., an economically important group of plant pathogens. Optimal synthesis of AgNP with desirable physical and biological properties was investigated using ultra violet-visible spectroscopy, dynamic light scattering, transmission electron microscopy and energy-dispersive X-ray analysis. Different ratios of plant extract and AgNO3 significantly affected size, stability and yield of AgNP. *In vitro* bioactivity analyses revealed that AgNP synthesized with *A. absinthium* extract were highly potent and efficacious in inhibiting mycelial growth, zoospore germination and germ tube length of different *Phytophthora spp*. Consistent with *in vitro* results, in planta AgNP treatments prevented Phytophthora infection and improved plant survival. Moreover, AgNP in *in vivo* experiments did not produce any adverse effects on plant growth. Molecular insights into the control mechanism of AgNP using RNA-seq analyses will also be discussed. These investigations provide a simple and economical method for green synthesis of highly potent and efficacious AgNP for controlling Phytophthora without affecting normal plant physiology.

Elucidation of resistance response to soil-borne necrotrophic oomycete using non- model *Zingiber-Pythium* pathosystem

Lesly Augustine | Kiran A G | Sivakumar K C | Dr. George Thomas

Zingiber zerumbet (L.) Smith (Zingiberaceae), a congener of cultivated ginger (Z. officinale Roscoe) is resistant to generalist necrotrophic oomycete Pythium, which causes soft-rot disease in several plant species including ginger. We studied the whole genome transcriptome reprogramming in Z. zerumbet and ginger in response to Pythium inoculation. RNA-seq was performed in both the species using Illumina GAIIx platform, before and after pathogen inoculation. De-novo assembly and annotation with UNIPROT database yielded 23620 and 22970 unigenes with unambiguous annotation in Z. zerumbet and ginger, respectively. Gene ontology classification of annotated transcripts yielded 16626 functionally annotated transcripts in Z. zerumbet and 16157 transcripts in ginger. The functional enrichment analysis and the temporal expression profile identified a predominant role of phenylpropanoid biosynthetic pathway against Pythium in resistant Z. zerumbet. The DGE of a set of major genes involved in the pathway were validated using RT-qPCR. Alongside robust induction of the defense related genes also seem to collectively playing against Pythium in Z. zerumbet. In ginger, the transcriptome reprogramming was targeted mainly to protect vital physiological activities. The study based on host resistance in non-model plant highlights the importance of quantitative defense response against broad host necrotrophic oomycetes.

Comparison of SNP and SSR markers in estimation of genetic diversity of isolates of *Phytophthora nicotianae* collected from different hosts

Antonio Biasi | Frank N. Martin | Ahmed Abdelfattah | Santa O. Cacciola | Leonardo Schena

Mitochondrial Single Nucleotide Polymorphisms (SNPs) and genomic Simple Sequence Repeats (SSRs) were compared for the assessment of genetic diversity and population structure of the cosmopolitan plant pathogen *Phytophthora nicotianae*. A total of 108 isolates, collected from worldwide sampling in different plant hosts, including agricultural crops (Citrus and Tobacco) and several potted ornamental species in nurseries, were analysed using 9 hypervariable SSR regions scattered throughout the genome and 2 mitochondrial SNPs, respectively. Across all samples, 71 different multilocus genotypes (MLGs) and 112 different alleles were detected. The loci showed different rates of variability varying from a minimum of 4 (locus P2039) to a maximum of 23 (locus P1509). In addition to the observed variability, some isolates showed a different degree of ploidy. As for the SNPs, 26 and 30 polymorphisms for each marker were amplified, with a total number of 42 different multilocus mitochondrial haplotypes (MMH). Phylogenetic analyses were conducted for the two datasets, and the isolates grouped into five major clusters for both SSR and SNP markers. Both markers revealed that the majority of Citrus isolates grouped together regardless of their geographical location. In contrast, isolates collected from tobacco were rather similar according to their origin, while isolates from ornamental plants were scattered within the tree (Citrus' group excluded). The present study suggests that the use of these two tools combined would facilitate the study the genetic variability of this pathogen, since both the maternal inheritance and the parental genotyping are considered.

Transcriptome and small RNA profiles during a resistant and susceptible interaction between *Pseudoperonospora cubensis* and cucumber

Alyssa Burkhardt | Brad Day

Pseudoperonospora cubensis is the causative agent of downy mildew on many cucurbits, including cucumber. Since the pathogen recently overcame host resistance, increased molecular research has focused on developing genetic resources for both the plant and the pathogen, including sequencing the genomes for each and the transcriptome of a susceptible interaction. In this study, we observed a resistant interaction between *P. cubensis* and the plant introduction line PI 197088 and found that *P. cubensis* is able to enter the host, but does not sporulate and causes minimal leaf damage. To investigate the transcriptional changes associated with resistance, we collected leaf tissue from PI 197088 and the susceptible plant line 'Vlaspik' inoculated with *P. cubensis* over a 1 to 6-day time course and extracted total RNA to analyze mRNA and small RNA in parallel. RNA-Seq data was analyzed using DESeq to identify transcripts that had significant changes in expression levels during a time course of *P. cubensis* infection within a plant line and or between plant lines. Candidate resistance-associated genes include those associated with plant defense hormones, plant nutrition, and transportation. In parallel, putative small RNAs were predicted in cucumber and *P. cubensis*, including conserved miRNA in cucumber and novel miRNA in both cucumber and *P. cubensis*. Targets for these miRNA were also bioinformatically predicted, and multiple cucumber targets were identified that could be associated with a response to the pathogen. Future work will validate the role of the candidate resistance-associated genes and the expression and targets of the small RNAs.

Morphological characteristics of *Phytophthora* spp. collected from cocoa orchards from coastal Ecuador

Gabriela Carranza / Carmita Suárez-Capello / Denny Carriel / Karina Solis / Juan Barriuso

By the end of last century, black pod disease and losses of productive cocoa (*Theobroma cacao*) trees are kept below 1% of damage and was therefore considered a very minor problem for cocoa industry in Ecuador; since then, incidence and aggressiveness of the pathogen have been increasing coinciding with the increasing crops of a single high yield clone, specially in new areas where nowdays is reported as the main sanitary problem on cocoa. *Phytophthora* isolates from cocoa have been traditionally attributed to *P. palmivora*. First results of a systematic study started by the UTEQ, with the aim of determining the *Phytophthora* species associated to cocoa trees in coastal region of Ecuador are presented. Isolation of *Phytophthora* species was done from samples taken from naturally infected pods collected from 14 localities throughout the main cocoa region. A sterile wire loop was used to transfer fungal tips onto PDA & V8 agar for pathogen description. Colony patterns were determined after seven days of growth. Morphology of the colony was recorded as pattern, nature of margin and growth rate of isolates on V8 Agar. Growth rates were evaluated based on daily records of mycelium growth for 7 days. Sporangia were observed to determine shape, length, caducity, papillation, pedicel length; chlamydospore size and position. After the study of morphological details and the classification of traits, distinct groups of isolates were separated. Results obtained support the hypothesis that in Ecuador there is more than one species of *Phytophthora* causing pod rot of cocoa

New sources of resistance to *Phytophthora* spp. among cocoa populations in Ecuador

Denny Carriel | Carmita Suárez-Capello | Karina Solis | Juan Barriuso

Phytophthora is one of the most destructive disease causal agent in cocoa (*Theobroma cacao*), producing losses between 30 to 80% of world cocoa yield, not without taking into account 10% death of plant stands. In Ecuador was considered of minor importance compared with those diseases caused by the basidiomicetes *Moniliophthora* spp., however due to climatic change and the increasing cultivation of large monoclonal farms based on the highly productive clone CCN 51 the damage by this organism has increased to the point that in some areas is becoming the main concern for the crop. Under this circumstance is imperative to incorporate an early evaluation of resistance against *Phytophthora* to breeding programs. The objective of this study was to evaluate a group of preselected cocoa cultivars from the INIAP using the leaf disc test and local isolates of *Phytophthora* spp. associated to the disease. The reliability of the test was evaluated by including the clones SCA 6 y SCA 12 (resistant) and CCN 51 (susceptible). Eleven accession of Forastero (Chalmers-INIAP collection), eight National and fourteen selected from local traditional farms were screened by using a suspension of zoospores grown on isolated from infected pods. Both SCA clones maintained their resistant condition. Those accessions from Amazonian and National origin showed good levels of resistance, best performance were presented on "Arbol No. 5", "AMAZ-2 G8" and "AMAZ-2 G10" which approached the resistance of SCA 6 and SCA12. The clone CCN51 qualified as susceptible, reached the highest value on this trial.

Fight or flight? What is the best strategy against a hemibiothrophic pathogen?

Ronaldo Dalio | Heros Maximo | Marcos Machado

Brazil and USA are the top orange producers in the world. However, the Brazilian citrus industry is constantly threatened by pests and diseases such as root rot caused by *Phytophthora parasitica*. Little is known about how the pathogen establishes interaction with the plant. During infection, the pathogen secretes an arsenal of molecules called effectors, which allows their infection. Such molecules can be recognized by plants and activate defense mechanisms which, ultimately, can lead to hypersensitivity reactions and necrosis in tissues. This strategy is quite effective against biotrophic pathogens, however, to hemibiotrophic microorganisms such protection can be disastrous, since the pathogen continues feeding of dead tissue. In this work, the physiological responses and temporal modulation of defense genes of two contrasting citrus varieties for interaction with *P. parasitica* were analyzed. Citrus Sunki is highly susceptible, whereas *Poncirus trifoliata* is tolerant. Surprisingly, it was found that the susceptible variety responds quickly to infection by activating the majority of tested defense genes, while the tolerant variety keeps its defense genes stable. These results suggest different strategies of attack and defense depending on the interaction between *P. parasitica* and *P. trifoliata* (tolerant), and Citrus sunki (susceptible) and that activation of defense mechanisms that can lead to hypersensitivity reactions are not efficient against hemibiotrophic pathogens.

Screening of Soybean F$_3$ and F$_6$ Populations to Identify Novel Genes Conferring Durable Resistance to *Phytophthora sojae* Root and Stem Rot Disease

Colin Davis | John McDowell | M.A. Saghai Maroof

Phytophthora sojae (P. sojae), the causal agent of soybean root and stem rot disease, is an oomycete pathogen responsible for over $ 400 million dollars of soybean crop damage annually in the US, and over $1 billion dollars worldwide. For successful *P. sojae* infection, the pathogen must secrete effector proteins into the host, which function to repress natural defense systems. To date, *P. sojae* has been managed through the inclusion of *P. sojae* resistance (Rps) genes and quantitative trait loci (QTL) into commercial lines. Rps genes are able to recognize specific pathogen effectors inside the host and up regulate the defense response in turn. However, the effectiveness of current R-genes is decaying as certain strains of *P. sojae* evolve to overcome the resistance. The reduction of Rps gene effectiveness can be attributed to *P. sojae's* loss or manipulation of recognized effectors. This project seeks to identify novel genes conferring durable resistance. Resistance gene screening, targeting core *P. sojae* effectors would provide durable resistance, as a loss of these effectors by the pathogen would result in a loss of pathogenicity. Using a combination of effector-based screening and detached-leaf pathogen assays, we are phenotyping recombinant inbred lines developed from crosses of resistant cultivars to susceptible "Williams". Genetic maps will be developed from the gathered data, and used to identify novel, durable, gene(s) responsible for *P.sojae* resistance in soybean.

A novel mode of action in oomycete plant pathogen control: ORONDIS™

Siân Deller | Judith Sheldon | Jill Foundling | Mathias Blum | David Beattie | Mike Csukai

Oxathiapiprolin, discovered by DuPont, is the latest oomycete-active compound with a novel mode of action to enter the market, and will be marketed by Syngenta from 2016 as ORONDIS™. DuPont initially identified the molecular target of oxathiapiprolin. This report confirms that finding by characterising forward genetics mutants with an oxathiapiprolin-resistant phenotype. Non- synonymous point mutations were found in all resistant lab strains in the oxysterol binding protein- encoding gene (OSBP). OSBP is thought to be involved in the movement of sterols and lipids between membranes, maintaining lipid homeostasis. Downstream perturbations caused by disruption of this function ultimately kill the cell. To investigate this hypothesis in the oomycete pathogen, *Phytophthora infestans*, we have used microscopy-based techniques to examine the ultrastructure of germlings treated with sublethal doses of oxathiapiprolin and compared these to control germlings. Observations of differential cytology will provide insights to better understand the role of OSBP and the way in which its inhibition by oxathiapiprolin controls oomycete plant pathogens.

New tools to identify mechanisms of nutrient transport from plants to oomycete pathogens

Kasia Dinkeloo | Guillaume Pilot | John McDowell

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

A novel family of giant cadherins in the oomycetes

Kyle IG Fletcher | Pieter van West | Claire MM Gachon

Cadherins, a group of molecules typically associated with planar cell polarity and Wnt signalling, have been little reported outside of the animal kingdom. Here, we identify a new family of cadherin in the stramenopiles, termed Nonagonal after their 9 transmembrane passes, which contrast to the one or seven passes found in other known cadherin families. Manual curation and experimental validation reveal two distinct subclasses of nonagonal cadherins, depending on the number of uninterrupted extracellular cadherin (EC) modules presented. Firstly, shorter mono-exonic, unimodular, protein models, with 3 to 12 EC domains are identified as duplicate paralogs in the saprotrophic Labyrinthulomycetes Aurantiochytrium limanicum and Schizochytrium aggregatum, the gastrointestinal Blastocystae Blastocystis hominis and a single copy gene in the autotrophic Pelagophyceae Aureococcus anophagefferens. Larger, single copy, multi-exonal, tri-modular protein models, with up to 72 EC domain in total, are found in some Oomycete genera: Albugo, Phytophthora, Pythium and Eurychasma. No homologue was found in the closely related autotrophic Phaeophyceae (brown algae) or Bacillariophyceae (diatoms) nor in several genera of plant and animal pathogenic oomycetes (Aphanomyces, Saprolegnia and Hyaloperonospora). This absence is investigated by synteny analysis of the scaffold regions flanking the gene models is highly variable. Novel to this new cadherin family is the presence of intercalated laminin and putative carbohydrate binding in tri-modular oomycete cadherins and at the N-terminus of thraustochytrid proteins. As we were unable to detect any homologs of proteins involved in signalling pathways where other cadherin families are involved, we present a conceptual hypothesis on the function of nonagonal cadherin based around the presence of putative carbohydrate binding domains.

Transcriptional dynamics of *Pythium ultimum* and *Phytophthora infestans* during tuber infection: clues tonecrotrophic and hemibiotrophic lifestyles

Audrey Ah Fong | Howard Judelson

The plant pathogenic oomycete lineage consists of genera that exhibit different parasitic lifestyles and host ranges. Amongst the notable species are the necrotroph, *Pythium ultimum,* and the hemibiotroph, *Phytophthora infestans*. Both are pathogenic on potato and yet differ in morphology, life and disease cycles. To investigate how their genetic programs are deployed during pathogenesis, RNA-Seq was used to obtain a comprehensive expression profile for both species during infection time courses on potato tubers. Transcription was highly dynamic in *P. infestans*, where 38% of the genes were significantly differentially expressed at different time points, in waves linked to pathogenic transitions. For example, many effectors and constituents of the translational apparatus were up-regulated during biotrophy, while many transporters, hydrolases, and oxidoreductases were upregulated during necrotrophy. Fewer genes (11%) were differentially regulated in *P. ultimum*, reflecting the contrasting biology of the two pathogens. Further differences between the trophic types were also revealed in the transcriptome analysis of orthologous genes where a significant proportion of orthologs showed divergent levels and patterns of expression. Underlying these differences are evolutionary diversification in *cis* and *trans*-acting elements of the transcriptional network controlling the life cycles of the two species.

Investigating how oomycetes extract iron from plant hosts

John Herlihy | Guillaume Pilot | Terri Long | John McDowell

Oomycete pathogens manipulate many aspects of host cellular machinery. For example, they secrete effectors to reprogram host pathways for immune suppression. However, little is known about the mechanisms through which oomycetes extract nutrients from susceptible host plants. Our research seeks to identify the mechanisms of iron acquisition, with emphasis on finding host susceptibility genes. Iron is a critical nutrient for all cellular life. Low iron availability is a growth limiting factor for the animal pathogen *Pythium insidiousum*. Plant pathologists have not extensively investigated the role of iron during oomycete infection. Highly alkaline or calcareous soils found in such places as, Western United States, North Africa and the Middle East have limited iron availability. Plants grown in these marginal soils may respond differently to pathogen attack. To better understand these phenomena, we are investigating the interaction of *Phytophthora capsici* or *Hyaloperonospora arabidopsidis* with *Arabidopsis thaliana*. We can leverage the genetic tools of *Arabidopsis* to screen T-DNA insertion mutants for increased susceptibility or resistance to biotrophic and hemibiotrophic oomycetes. We are using hydroponically grown plants to control both nutritional state and pathogen infection. We will describe new experimental tools that are under development to support this project. Plant pathways for iron metabolism and transport that are co-opted by pathogens, could provide promising targets for interventions aimed at limiting pathogen nutrition and growth. Farmers working in iron limited soils could use this knowledge to help control oomycete diseases.

Identification and functional characterization of a *Phytophthora infestans* G-protein γ subunit

D. Johan van den Hoogen/ Harold Meijer / Francine Govers

Signal transduction pathways lie at the base of a wide variety of cellular processes. We focus on G- protein signalling in the oomycete *Phythopthora infestans,* a notorious pathogen that causes late blight in potato and tomato. Previous research based on silencing and overexpression of the Gα and Gβ subunit genes in *P. infestans*, showed that heterotrimeric G protein signalling plays a role in zoospore motility, sporangial development and virulence (Latijnhouwers and Govers 2003; Latijnhouwers et al. 2004). Initial Contrary to Gα and Gβ genome annotation failed to identify did not reveal a typical Gγ gene, in *P. infestans* but a but recently a meta-genome study revealed a potential candidate indicated presence of a Gγ subunit in *P. infestans* (De Mendoza et al. 2014). To test the validity and elucidate its function the predicted *P. infestans* Gγ subunit gene (*Pigpg1*) was cloned and investigated in more detail. It is a two exon gene encoding a protein of 71 amino acids, shorter than most previously reported Gγ subunits. Overall the similarity with non-oomycete Gγ subunits is low, with only the most conserved amino acids maintained. In contrast, the similarity with its homologs in other oomycetes is high. *Pigpg1* is expressed in all developmental stages and shows a similar expression profile as the Gβ gene *Pigpb1*. Downregulation of *Pigpg1* in *P. infestans* was achieved by homology-dependent gene silencing. Current efforts focus at characterizing the Pigpg1- deficient mutants, assessing whether Pigpg1 is a functional Gγ subunit and elucidating its potential role in development and virulence.

Sequence diversity and an application of PCR-RFLP for detection and identification of *Pythium myriotylum* isolates recovered from *Pythium* soft rot disease of ginger

Duy Le | Mike Smith | Elizabeth Aitken

Pythium myriotylum is a wide host range Oomycote and in Australia it is responsible for severe losses to both capsicum and ginger crops. Therefore, it is proposed that intraspecific variation within the pathogens might account for aggressiveness and pathogenicity on such diverse hosts. In this study, whole genome data of five *P. myriotylum* isolates were derived from Illumina sequencer and 22 conserved loci were extracted and analysed for sequence diversity based on single nucleotide polymorphisms (SNPs). In most cases, a higher number of true and unique SNPs occurred in *P. myriotylum* isolates obtained from Pythium soft rot (PSR) ginger in Australia compared to other *P. myriotylum* isolates. Overall, SNPs were discovered more in the mitochondrial genome than those in the nuclear genome. Among the SNPs, a single substitution from the cytosine (C) to the thymine (T) in the *Cox*II gene of *P. myriotylum* isolates obtained from PSR ginger was within a restriction site of *HinP1I* enzyme, which was used in the PCR-RFLP for detection and identification of the isolates without sequencing. The enzyme specifically cut the PCR amplicons (about 600 bp) of PSR *P. myriotylum* isolates into two small fragments (about 425 and 175 bp), which were visualised under UV light, but the PCR amplicons of other *P. myriotylum* isolates and other *Pythium* spp. as well as true fungi were not digested by *HinP1I*, resulting in single bands on the gel. The PCR-RFLP was also sensitive enough to detect *P. myriotylum* directly from PSR infected ginger.

Comparative transcriptomics analysis of *Phytophthora capsici* - *Piper nigrum* phytopathosystem

Chidambareswaren Mahadevan | Manjula Sakuntala

Black pepper (*Piper nigrum* L.), a tropical spice crop, is susceptible to *Phytophthora capsici*, an oomycete pathogen which causes the highly destructive foot rot disease. A systematic understanding of this phytopathosystem has not been possible owing to lack of genome or transcriptome information. Comparative transcriptomics helps for the generation of high-throughput data which could leverage the identification and annotate novel genes from non-model organisms. In this work we explain a comparative transcriptomics pipeline to study the immune responsive components of *Piper nigrum* L. We also explain the transcripts of *P. capsici* which were identified during the foliar pathogenesis. Illumina HiSeq 2000 was employed for RNA-seq data which were assembled into transcripts through de novo assembly using SOAPdenovo-Trans. BLAST2GO pipeline was used for annotation of transcripts from black pepper as well as *P. capsici*. Further, differential expression was attempted using DESeq tool which suggests regulation of important immune components of black pepper which are important for the early detection of *P. capsici*. Critical analysis of data gives novel insights into the regulatory pathways of black pepper as well as gives a unique perspective on the diverse receptor kinases which might be involved in the detection of *P. capsici*. Our study could help in the identification of potential biomarkers, elicitor targets and novel proteins associated with various biological processes in plant disease. Detailed results will be presented at the meeting.

Functional analysis of evolutionary conserved *Phytophthora RxLR24* effector

Felix Mauch | Michael Stumpe

It is known that the ability of *Phytophthora* to successfully infect and colonize the host plant strongly depends on its virulence factors, called effectors. They are transferred to the host cell to modulate host metabolism in favor of pathogen growth. One class of such secreted factors contains a conserved RxLR amino acid motif that mediates the translocation into host cells. The precise function of these effectors is not well known. Our research aims to better understand the mode of action of conserved RxLR effectors. It is hypothesized that despite diverse host plants, homologous effectors will interfere with the same conserved process of plant immunity and target the same type of host protein. Our strategy is based on using the well-established model pathosystem Arabidopsis- *Phytophthora brassicae* to analyze a group of RxLR effectors, which share extended sequence homology between *P. infestans* and *P. brassicae*. One such example is the effector RxLR24. Co-Immunoprecipitation experiment demonstrated that the effector Pi_RxLR24 and Pb_RxLR24 target the same type of host protein in potato and Arabidopsis, respectively. The target protein is mechanistically involved in vesicle dependent secretion processes. Arabidopsis plants directly expressing RxLR24 show compromised secretion and are more susceptible to *P. brassicae*.

Crinkler effectors in the *Phytophthora parasitica*-citrus interaction: identification and global expression

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Phytophthora parasitica is a destructive pathogen causing damage in crops and natural ecosystems worldwide. In citrus crops, *P. parasitica* is related to gummosis and root rot diseases and no effective control strategies are available. *Phytophthora* species secrete hundreds of effectors that enable infection. The mechanistic molecular functioning of effectors remains poorly understood. Crinkler effectors (CRN) are known to possess conserved domains, such as LxLFLAK, DWL and HVLVXXP. These domains are used to identify CRN effectors in the pathogens genome. We aimed to identify candidate CRNs in the genome of *P. parasitica* IAC 01/95 and analyze their global expression before and after infection in two contrasting citrus rootstocks: *Citrus sunki*, higly susceptible, and *Poncirus trifoliata*, tolerant. After applying a bioinformatic pipeline, we have found 66 candidates CRNs. Some of these showed differential expression before infection, when the pathogen was only treated with root extracts, and during global expression analyses at 3, 6, 12, 24, 48, and 96 hours post infection in the roots of both citrus varieties. Remarkably, it was found that *P. parasitica* had a completely different pattern of expression of these genes comparing both varieties, suggesting that the pathogen is able to recognize different hosts and deploy different sets of candidate CRNs. The differences in CRNs expression in *P. parasitica* found after treatment of root extracts of the two genotypes might explain the susceptibility or resistance of these plants to *P. parasitica*. To shed a light on that, these genes are now target for functional characterization and silencing essays.

Pythium and *Phytophthora* diversity in *Pinus radiata* nursery soil and root samples from New Zealand

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Forest nurseries frequently face on-going issues with root-borne diseases caused by oomycetes. Typically diagnostics is based on soil baiting to isolate the organisms potentially associated with disease. This method, while good for detecting viable and active members of the soil, does have limitations including long processing time, seasonal variations and culture-based isolation bias. Next generation sequencing data provides outstanding opportunities for characterisation of microbial communities and diagnostics. This study aims to develop methods for characterisation of the prevalence of oomycetes such as *Pythium* and *Phytophthora* in nursery soils and to investigate the potential for NGS in downstream diagnostics applications. Soil samples were collected from a forest nursery and baiting was used to isolate species of oomycetes using selective media. ITS and Cox2 sequences were used to identify the isolates. *Phytophthora cinnamomi*, and *Pythium mamillatum* were the most commonly isolated species from three different nursery beds. Soil samples were also analysed to determine the pH, and total carbon, nitrogen and phosphorus levels, as well as performing DNA extraction for NGS analysis. The ability to detect taxa present but only in low numbers would be beneficial for nursery-based diagnostics, development of *Phytophthora*-free nursery practises and over all better detection of oomycetes from environmental samples. Thus we are currently assessing the ability of soil and root NGS analysis for diagnostic purposes.

Exploring *Phytophthora* Effector Functions using Yeast as a Model

William Morgan | Matthew Reeder | Matthew Sydor | Colleen Sells

The oomycete *Phytophthora sojae* causes root and stem rot of soybean. During infection, the pathogen delivers dozens, if not hundreds, of effector proteins into the plant cell to manipulate host systems. Although these pathogen proteins often have similar N-terminal delivery motifs, the C- terminal effector regions are rarely homologous to known protein domains, and consequently their biochemical function is difficult to predict. The brewer's yeast *Saccharomyces cerevisiae* has been successfully used to explore the biochemical function of pathogen effectors. As a complement to studies with the natural pathosystem, this model system has several distinct advantages, particularly in an undergraduate setting. My students and I have found that many *P. sojae* effectors when individually expressed in yeast inhibit growth, suggesting that they target conserved biological pathways. We are probing the basis of the growth inhibition using the power of yeast functional genomics, in hopes of discerning each effector's target. Transcriptome analysis of yeast over-expressing the *PsAvh172* effector gene identified hundreds of differentially expressed yeast genes, which were enriched in several biological pathways. In complementary studies, we have begun to screen for yeast knock-out mutants hypersensitive to *PsAvh172* over-expression, as well as testing for the disruption of specific pathways using simple, direct assays. In summary, the yeast model system is a promising approach for exploring pathogen effector function, especially as part of an undergraduate research program.

Toxicity evaluation of phosphite using different *Phytophtora infestans* strains

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In potato, phosphite compounds reduce the susceptibility to late blight caused by the oomycete *Phytophthora infestans*, a major pathogen challenge in potato production. However, they are reported to have both a direct toxic effect on oomycetes and function as plant resistance inducers raising the plant's own defense. To establish the toxicity level of phosphite and whether there are differences in sensitivity between strains, five *P. infestans* strains (SE-03058, 88069, Pink6_H7, BLUE13 and SE-Halland_2.5) isolated in Sweden and Europe were exposed to seven different potassium phosphite concentrations (0-6.4 mM). Direct toxicity was determined by radial growth on rye-agar plates and sporangia count after eighteen days. Furthermore, sporangia germination was tested with the same phosphite concentrations in pea broth for three of the strains (88069, Pink6_H7 and SE-Halland_2.5). The results showed a strain-specific response to phosphite with an expected negative correlation for growth with increased concentrations. At the highest concentration (6.4 mM), no growth was observed in the most sensitive strain Blue13. However, the most resistant strain, Pink6_H7, had a significantly higher half maximal effective concentration (EC_{50}) value. The sporangia numbers were very low for all strains at the three highest concentrations with no sporangia found for Pi1.4.0, Blue13 and 88069. Sporangia germination was also affected. Phosphite clearly has a direct toxic effect on *P. infestans*, but there are differences in resistance levels between strains. Whether this should impact phosphite spraying schemes in the field is yet to be evaluated.

Towards functional and comparative analysis of downy mildew effectors

Manon Neilen/ Joël Klein / Guido van den Ackerveken

The obligate biotrophic oomycete *Peronospora farinosa* (*Pfs*) is the causal agent of downy mildew on spinach (*Spinacia oleracea*). In terms of crop loss, it is the most important disease in the spinach cultivation industry (Feng et al., 2014). Sixteen official strains of *Pfs* are recognized, each potentially employing a different repertoire of effector proteins. The aim of this project is to identify proteins involved in resistance and susceptibility, both from *Pfs* and, later on, from the spinach plant. Genome and transcriptome sequences of our reference isolate *Pfs1* were used to identify effector genes. Moreover, available genomes and transcriptomes of related downy mildew species were used to compare effector repertoires. This comparative genomic approach allowed for identification of conserved effectors and effectors that are unique for *Pfs*. The conserved effectors are used to find interacting plant proteins in a yeast two hybrid screen using an Arabidopsis library and a set of *Hyaloperonospora arabidopsidis* effectors that are homologous to selected *Pfs* effectors. The function of interacting plant proteins will be validated *in planta*. Conserved effectors are interesting since they may be essential for *Pfs* and may target conserved proteins in different plant species. Therefore, knowledge on the interaction between conserved *Pfs* effectors and host proteins may yield strategies applicable to different crops. Knowledge on effectors will be used for strategic breeding of spinach cultivars with durable resistance against *Pfs*.

Glycoside hydrolases family 20 (GH20) represent putative virulence factors that are shared by all animal pathogenic oomycetes, but are absent in phytopathogens

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An on-going genomic survey for the entomopathogenic *Lagenidium giganteum* revealed novel putative virulence factors, including CRN13 orthologs, as well as Glycoside Hydrolases family 20 (hexosaminidase) and 37 (trehalase) transcripts (GH20 and GH37). Sequences analyses indicated that, unlike GH20 and GH37, the *L. giganteum* CRN13 predicted proteins did not display signal peptides. Importantly, genome mining demonstrated that GH20 genes are restricted to animal pathogenic oomycetes, and are absent from phytopathogenic oomycetes. Our analysis also revealed that the *L. giganteum* transcript is the only reported peronosporalean GH20 gene. All other oomycete GH20 homologs were retrieved from saprolegnian genomes, including the fish pathogens *S. parasitica*, *S. declina* and *Aphanomyces invadans*, the crayfish pathogen *A. astaci*, the decapod parasite *Ac. hypogyna* and the free living *Thraustotheca clavata*. Furthermore, phylogenetic analyses demonstrated that saprolegnian and peronosporalean GH20 genes clustered in unrelated clades. The saprolegnian GH20 sequences appeared as a strongly supported, monophyletic group nested within an arthropod-specific clade, suggesting that this gene was acquired via a lateral gene transfer event from an insect or crustacean genome. In contrast, the *L. giganteum* GH20 protein sequence appeared as a sister taxon to a plant-specific clade that included exochitinases with demonstrated insecticidal activities. Finally, gene expression analyses demonstrated that the *L. giganteum* GH20 gene is up-regulated in the presence of mosquito larvae. Other up-regulated genes included the carbohydrate-active GH37, GH5_27, and CBEL. Interestingly, CRNs did not show any differential expression. These results identified GH20 enzymes as potential pathogenicity factors shared by all animal pathogenic oomycetes.

Contribution to the study of Oomycetes: Morphology, Molecular biology and Phylogenetic analysis

Thiagarajan Prabha | Kasthuri Revathi | Bernard Paul

Oomycetes were historically classified as belonging to the Kingdom Fungi zoosporic organisms and cosmopolite in distribution, while some are restricted to certain geographic areas only. In the present study, Oomycetes isolation was performed by regular baiting techniques. A total of 40 Oomycetes were isolated, out of which most of the sample showed the repetitive of the genus *Pythium* and genus *Saprolegnia*. We have developed a modified DNA isolation protocol yielding good quality DNA in short time; without the need to employ CTAB, lysozyme digestion, proteinase K treatment etc. Thereby reducing the overall costs involved. We have done the molecular level characterization of the individual isolates using ITS 1 and ITS4 primer set for PCR amplification and sequencing. Using BLAST analysis we have identified each species of the isolated Oomycetes and their percentage of homology were determined. Our studies on multiple sequence alignment revealed the closely related Oomycetes species. Finally, phylogenetic trees were constructed by neighbour joining and maximum parsimony method for studying the phylogenetic relationship between closely related strains. Using morphological and molecular characterization techniques we have identified eleven Oomycetes of which nine belongs to genus *Pythium* and two from genus *Saprolegnia* Although Oomycetes has been documented well as organisms of greater economic importance, prevalence and documentation studies on individual species are very limited especially in Tamil Nadu. To our knowledge, this is the first study to document the presence many of these Oomycetes in South India.

Toward understanding how biotrophic pathogens manipulate plant amino acid transporters to acquire nutrients

Unnati Sonawala / John McDowell / Guillaume Pilot

Hyaloperonospora arabidopsidis (*Hpa*) is a naturally occurring oomycete pathogen on *Arabidopsis thaliana*. *Hpa* is congeneric with other downy mildew pathogen species that cause economically important diseases on plants belonging to the *Brassicaceae* family. Downy mildew pathogens are obligate biotrophs that extract nutrients exclusively from living plant cells. *Hpa* has lost the ability to assimilate inorganic nitrogen and sulfur. Therefore, *Hpa* can be used as a model pathogen to study how obligate biotrophs extract nutrients from plants. Analysis of publicly available transcriptome data in response to *Hpa* colonization of immune vs. susceptible Arabidopsis genotypes showed upregulation of many amino acid transporters in the susceptible accession, suggesting that the pathogen is manipulating amino acid transporters for nutrient acquisition. T-DNA knockout lines for some of these transporters display reduced growth of the pathogen on the host. Moreover, double combinations of some of these mutants show additive reduction of pathogen growth. Preliminary experiments indicate that the reduction in *Hpa* virulence is not due to heightened immunity in the mutants. We are now initiating experiments to test the hypothesis that the reduced growth of *Hpa* in these mutants is due to restricted availability of host-synthesized amino acids.

Diversity of chosen effectors in samples of Polish and Norwegian populations of *Phytophthora infestans*

Emil Stefańczyk | Sylwester Sobkowiak | May Bente Brurberg | Ragnhild Naerstad | Abdelhameed Elameen | Marta Brylińska | Jadwiga Śliwka

Late blight caused by *Phytophthora infestans* leads to high financial losses in potato production. Fungicide application used to control the disease may fail against resistant strains. The utilization of the main late blight resistance (*R*) genes in potato breeding programs began after their discovery in the 1950s. The *R* gene products recognize effectors, *P. infestans* proteins involved in pathogenesis, which leads to hypersensitive reaction and effector-triggered immunity. Several strategies to avoid recognition are confirmed for different *P. infestans* effectors and these include single nucleotides mutations, truncated effector variants or altered expression patterns.

In this work, we investigated nucleotide diversity of the *Avr-vnt1* and *AvrSmira1* effector sequences. The whole genes were sequenced from 49 Polish, 42 Norwegian and 5 control *P. infestans* isolates. Heterozygous positions were phased computationally using PHASE software. 15 and 3 different alleles were identified among the obtained 167 *AvrSmira1* and 135 *Avr-vnt1* sequences, respectively.

20 polymorphic sites were detected for the *AvrSmira1* effector, of which 7 were synonymous. All of

13 non-synonymous SNPs were located in the C-terminal domain, known to be involved in recognition specificity. 127 *Avr-vnt1* sequences differed at only one site, while the remaining 8 sequences had additional 8 polymorphic positions reported, among which 6 were non-synonymous. Further analyses to investigate population-differentiation and selection at the microevolutionary level will be conducted.

Studies of mating in *Phytophthora infestans* revealed transcriptome differences between Swedish and Dutch isolates

Georgios Tzelepis | Ramesh Vetukuri | Anna Åsman | Johan Fogelqvist | Christina Dixelius

Sexual reproduction has an important contribution in *Phytophthora infestans* epidemiology. However, many aspects of *P. infestans* mating process remained to be elucidated. In this study we are investigating its mating mechanisms using different molecular techniques. Isolates with A1 and A2 mating types from Sweden, the Netherlands and the UK, were used for this purpose because mating frequencies are known to differ among European countries. RNA was prepared from the "mating-zone" between 4 Swedish, 1 Dutch and 1 British mating-pair. Our results showed that the Dutch crossing displays significant different transcriptome patterns compared to the Swedish and British ones. Specifically, induction of genes encode for mating proteins and hydrolytic enzymes have been observed. Further, we found that some RXLR effector genes are induced during mating compared to parental strains. SNP analysis revealed insertions and deletions on the C-terminus in some of *P. infestans* isolates, resulting in RXLR sequence variation. Induction of homologous RXLR genes during mating was also observed in the related species *P. andina* and *P. mirabilabis*. The silenced strain incited milder symptoms and significant reduction of *P. infestans* DNA amount in potato leaves compared to wild type. In conclusion, our preliminary results indicate that certain RXLR effectors probably are involved in the *Phytophthora* mating process.

Defining Interactions Between Soybean U- box E3 ligase proteins and *Phytophthora sojae* Effector *Avr1b*

Hua Z. Wise | Shan Li | Regina Hanlon | Narinder Pal | Hargeet Brar | Chunyu Liao | Brett M. Tyler | Madan K. Bhattacharyya

Phytophthora sojae is an oomycete pathogen that causes rot of the roots and stem in soybean. *P. sojae* delivers many RXLR effector proteins into host cells during infection, including Avr1b. *P. sojae* Avr1b interacts with the soybean U-box E3 ligase protein GmPUB1-1 and its homologous copy GmPUB1-2, in yeast two hybrid assays, in *in vitro* pull down assays, and in bimolecular fluorescence complementation (BIFC) assays. In order to investigate if these two GmPUB1 genes are required for *P. sojae* resistance in soybean, we used an RNAi approach to jointly silence both PUB1-1 and PUB1-2 in a soybean cotyledon silencing assay. Our result showed that knock-down of the GmPUB1 genes resulted in loss of resistance against *P. sojae* isolate containing Avr1b in the resistance soybean cultivars. This further proves that Avr1b interacts with a soybean E3 ligase.

Identifying essential effectors from the soybean pathogen *Phytophthora sojae* for soybean breeding

Hua Z. Wise | Ryan G. Anderson | John M. McDowell | Brett M. Tyler

Breeding for resistance to plant pathogens is one of the most effective means of disease control. However, the ability of plant pathogens to evolve new pathogenicity factors and evade host defense mechanisms drives the continual necessity to identify new resistance genes. We are exploiting genomic technologies in an effector-directed breeding approach that augments traditional breeding efforts against *Phytophthora sojae*, the causal agent of soybean root and seedling rot. This approach is founded on identifying monomorphic *P. sojae* effector genes that are essential for virulence, and using these genes as probes to identify new sources of resistance in soybean and related legumes. These essential effectors will make excellent candidates for screening for new, durable resistance to *P. sojae*, as these genes presumably cannot be mutated or deleted without a significant fitness penalty. The majority of predicted *P. sojae* RXLR effector genes are polymorphic amongst sequenced isolates of *P. sojae*, however, a subset of *P. sojae* RXLR effectors displays little or no allelic diversity. We have established a workflow for transient gene silencing and quantitative virulence assays. To date, we have silenced and assessed the virulence contribution of 17 PsAvh genes. Among these effectors, PsAvh16, PsAvh180 and PsAvh240 showed substantially reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. These three effectors are being used as candidates in a high throughput screen system utilizing Pseudomonas Type III secretion system to screen for new resistance genes against *P. sojae*.

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