

# BOOK OF ABSTRACTS



## Oomycete Molecular Genetics Network 20<sup>th</sup> Annual Meeting

July 10-12<sup>th</sup> 2019  
SAMS, Oban



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## Keynote Speakers

### **K.1: FISHing for mRNAs to understand phytomyxea-host interactions in plants and brown algae**

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Plant-pathogen interactions follow spatial and temporal developmental dynamics where gene expression in pathogen and host undergo crucial changes. Phytomyxids are economically important, obligate biotrophic, protist parasites of plants and stramenopiles with a complex lifecycle, therefore, it is mandatory to understand spatiotemporal pattern of disease development and progression. But like many pathosystems, phytomyxids are currently not accessible for genetic amendments. Single molecule FISH techniques are a useful tool to demonstrate the presence and activity of mRNAs at the single-cell level. These methods are rapid and easily applicable. We used this method to monitor and quantify the expression of genes from the clubroot pathogen *Plasmodiophora brassicae*, several species of its *Brassica* hosts, and of several brown algae, including the genome model *Ectocarpus siliculosus*, infected with the phytomyxid *Maullinia ectocarpii*. We show that mRNAs are localised along a spatiotemporal gradient, increasing our understanding of the phytomyxid-host interaction. Our results demonstrate, therefore, the potential to rapidly increase our understanding of key, spatially- and temporally-resolved processes underpinning complex plant-microbe interactions with a resolution superior to the currently available tools to study phytomyxids.

## K.2: Evolutionary and cellular complexity of photosynthetic and autotrophic stramenopiles

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Oomycetes belong to the stramenopile, or heterokont supergroup. But what of the other members? This supergroup radiated over 700 million years in the evolutionary past, and contains both non-photosynthetic groups, such as oomycetes, and photosynthetic members, including diatoms and kelps; which may be unicellular, colonial, multicellular; phototrophs, mixotrophs, phagotrophs, and pathogens. To complicate matters further, some non-photosynthetic stramenopiles, such as the chrysophytes, are descended from photosynthetic ancestors, which retain vestigial plastids that lack photosynthetic function.

I will take you on a guided walk through the stramenopile tree, showing you the forms and functions of the different sisters of oomycetes. I will additionally demonstrate how the acquisition and evolutionary dynamics of chloroplasts by photosynthetic groups, which occurred subsequent to their divergence from oomycetes, has been directly influenced by the organelles previously present in the host, with a particular focus on the mitochondria. These include proteins that have been rerouted from the mitochondria to support chloroplast function in photosynthetic species; and conversely chloroplast-targeted proteins that have been rerouted to function in the mitochondria of species that have lost the capacity to photosynthesise. Through this, I will demonstrate how understanding oomycete biology informs our understanding of the chloroplast evolution, metabolism and physiology of some of the most important photosynthetic groups on the planet.

# Session 1: Diversity and Population Genomics of Oomycetes

## S1.1: Isolation of *Pythium insidiosum* from the environment in Thailand

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*Pythium insidiosum* is an oomycete pathogen that causes the life-threatening infectious condition called pythiosis in humans and animals living in tropical and subtropical countries. Pythiosis in human patients is mainly found in Thailand. Most pythiosis patients have their infected organs removed, and many patients die from the infection. The disease is more prevalent in agricultural workers who frequently expose to swampy areas. The current study aims at gaining more insights into epidemiology and ecological niche of *P. insidiosum* in Thailand. We attempted to isolate *P. insidiosum* from natural and recreation water reservoirs (i.e., ponds, lakes, rivers) in 7 central and southern provinces, including Bangkok, Chachoengsao, Kanchanaburi, Nakhon Pathom, Ratchaburi, Chonburi, and Trang. A total of 500 water samples were collected, baited for the organism with sterile human hairs, and inoculated on Sabouraud dextrose agar for one week. We identified suspected colonies of *P. insidiosum* in 71 samples (14.2%). Based on the established multiplex PCR and rDNA sequence homology analysis, 26 of these colonies (5.2%) were proven to be *P. insidiosum*. Phylogenetic analysis allocated the *P. insidiosum* strains into genotypes Clade-II (n = 8; 31%) and Clade-III (n = 18; 69%). It should be noted that the organism is present in the zoo and public parks in Bangkok. In conclusion, *P. insidiosum* is ubiquitous in Thailand. Public concern should be raised for prevention of this infection, especially for individuals and animals those are at risk.

## S1.2: *Pythium oligandrum*: a necrotrophic mycoparasite of *Fusarium graminearum*

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Mycoparasitism described an interaction between a fungus/oomycete parasite and a fungus/oomycete host. While mycoparasitism is considered as potential tool to control fungal/oomycete phytopathogens through biocontrol approaches, the molecular mechanisms underlying this interrelationship are poorly described. Several Pythiales species from the Oomycete kingdom have been shown mycoparasitic abilities including *P. oligandrum*. *Pythium oligandrum* is a mycoparasite oomycete, found in the rhizosphere, with a wide host range ranging from fungi to oomycetes. To study *P. oligandrum* mycoparasitism behavior, we implemented a mycoparasitism assay based on a dual-culture system using a sterol-free medium with the phytopathogenic fungus *Fusarium graminearum*. We observed that *F. graminearum* growth was stopped almost instantly after the first contact with *P. oligandrum*. Specialized hyphae of *P. oligandrum* able to enter in *F. graminearum* stopped mycelium and causing cell death were observed revealing a necrotrophic mycoparasitic behavior of *P. oligandrum*. Remaining fungal structures were dead, while sexual oospores of the oomycete were produced six days after contact, proving the transfer of nutrients crucial for *P. oligandrum* sexual lifestyle from the fungus to the oomycete. Genetic determinants of mycoparasitism identification were led by combining whole genome PacBio sequencing of the *P. oligandrum* strain, RNAseq and Pythiales comparative analysis approaches. A set of 2,222 genes specific to mycoparasite species was identified, and 323 of them are highly induced in confrontation with *F. graminearum*. Interestingly 123 genes encode putative secreted proteins such as chitinases, peptidases, and elicitors. Further studies will aim to functionally characterize genes and molecular signals involved in mycoparasitism.

### S1.3: Comparative Genomics of Downy Mildews

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We have sequenced the genomes of several tropical and temperate downy mildews including at least one representative from *Bremia*, *Peronospora*, *Sclerospora*, *Peronosclerospora* and *Perofascia*. For some genera, it was possible to sequence multiple historical and contemporary isolates. A wide range of genome sizes coupled with recent expansions of repeats, the presence of more than two haplotypes, and varying levels of heterozygosity meant that some genomes were more difficult to assemble than others. When Hi-C data was used, assemblies approached chromosome scale contiguity. There is extensive synteny between downy mildews and *Phytophthora* spp. Maximum likelihood and multispecies coalescence trees support downy mildews as being polyphyletic. Orthology analysis revealed that major adaptations to biotrophy involve the loss of genes associated with transport, carbohydrate binding, and pathogenicity. Downy mildews have reduced putative effector repertoires compared to *Phytophthora* spp.

## S1.4: Molecular basis of the *Lagenidium*-insect association: an integrated -omics approach

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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.). Recent transcriptomics and metagenomics analyses have suggested that *Lagenidium* spp. may not be restricted to invertebrate infections, but may have retained the ability to establish interactions with plant tissues. In addition to canonical oomycete effectors identified through *L. giganteum* transcriptomics, a metabarcoding analysis that used *cox1* gene sequences revealed that a previously unsampled diversity of *Lagenidium* spp. is present in bromeliad phytotelmata. To further investigate the molecular basis of *Lagenidium*-host interactions, novel *Lagenidium* growth conditions were developed with a focus on modelling phytotelmata environments. Mycelia inoculates were placed in small volumes (4mL) of minimal media, supplemented (or not) with whole insects as the unique protein source. Visual monitoring demonstrated that *Lagenidium giganteum* is able to sustain vegetative growth only while insects are present. Proteomics analyses were initiated to characterize the secretome used by *L. giganteum* to subsist on insect substrates. Analyses first consisted of protein separation and visualization through classic SDS-PAGE minigels, then transitioned to protein identification using tandem mass spectrometry (LC-MS/MS). These data represent the first assessment of an animal-pathogenic oomycete secretome, and provide a basis for comparative proteomics analyses between animal and plant pathogenic oomycetes.



## S1.5: Single-cell molecular analyses demonstrate the polyphyly of the genus *Ectrogella* and shed light on the distribution and ecology of oomycete parasites of marine diatoms

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Photoautotrophs underpin most food webs and parasites affecting them have potential cascading effects at every level in the ecosystem. In aquatic environments, diatoms are one of the dominant groups of phototrophs and are known to be infected by oomycetes traditionally assigned to the genus *Ectrogella*. Yet the assessment of the genetic diversity, evolutionary relationships and ecological importance of this oomycete genus has been seriously hindered by the limited amount of molecular information available. Here we present results from the application of single-cell (SC) molecular analyses on marine oomycete parasites of diatoms isolated from field material. SC-PCRs, SC-whole genome sequencing and *in silico* analyses have been used to retrieve marker genes and subsequently investigate the phylogeny, distribution and parasitic dynamics of these organisms. Our findings suggest that parasitic oomycetes infecting diatoms are widespread in the global ocean and highlight local swift parasitic dynamics, likely to be one of the causes of the underappreciation of their presence by molecular ecology methods. Furthermore, metabarcoding data analysis and SC approaches alike, suggested a broad host range within diatoms, even for closely related parasite species. Finally, by strengthening the link between morphological and molecular data, SC approaches granted the possibility to reassess the classical taxonomy of *Ectrogella*. Our results highlight a high genetic diversity underlying the rather homogeneous thallus morphology observed, demonstrating the polyphyly of *Ectrogella*, and demand for the reassessment of its taxonomic treatment and for the clarification of its relationships with the genera of marine oomycetes *Anisulpidium* and *Olpidiopsis*.

## **S1.6: Oomycete evolution: Novel clades of early diverging oomycetes for a new insight in the origins of biotrophy and virulence**

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The marine environment harbours a high number of oomycete parasites infecting diverse photosynthetic hosts from macroscopic seaweeds to microscopic phytoplankters. Recent molecular studies revealed a previously unsuspected diversity in these groups of oomycetes, most of which were only known from morphological descriptions in earlier taxonomic reports. Most notably, this includes an apparently monophyletic clade of obligate biotrophic parasites infecting phylogenetically distant hosts such as red algae, brown algae and diatoms. Importantly, these marine oomycetes are distant (i.e. early diverging) from the plant pathogenic models investigated to date. Therefore, genome comparisons both within this early-diverged oomycete clade and throughout the phylogenetic tree of oomycetes offer an excellent opportunity to revise our understanding of the evolution of parasitism in oomycetes. This presentation will introduce this novel diversity of marine oomycetes, their host range, and the genomic approaches currently undertaken to address these evolutionary questions.

## S1.7: Insights into the biogeography and global diversity of *Phytophthora*

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Between 2013 and 2019, within the frame of several projects aiming at unravelling global diversity and biogeography of the genus *Phytophthora*, surveys were performed in natural ecosystems of Japan, Taiwan, Vietnam, Indonesia (Borneo, Java, Sulawesi and Sumatra), Chile, Nicaragua, Panama, Curacao, Egypt and eight countries in Europe. In total 320 forest sites, 410 forest streams, 9 mangrove forests, 6 lagoons and 5 other marine sites were sampled. Baiting assays and direct plating of necrotic plant tissues were used for isolating *Phytophthora* species from forest streams, forest soils and woody plants. Isolates were identified using both classical identification and sequence analysis of ITS, *cox1* and, if necessary, further gene regions. Overall, 13242 isolates were obtained which could be assigned to 65 known and 101 previously unknown species of *Phytophthora* belonging to 11 of the 12 phylogenetic clades. In addition, an array of interspecific hybrids from *Phytophthora* Clades 6, 8 and 9, 3 known and 24 novel *Halophytophthora* species and 9 species from the novel genus *Nothophytophthora* have been isolated. These surveys contributed to pin down the origin of several invasive aggressive *Phytophthora* pathogens, including *P. cinnamomi*, *P. cambivora*, *P. lateralis*, *P. ramorum* and the *P. citricola* complex. Together with records from previous *Phytophthora* surveys conducted by the authors and other researchers in natural ecosystems of Australia, Africa, Europe, the Americas and Asia, population genetic studies, and pathogenicity data this study provides insights into the global diversity and biogeography of the different clades and subclades of *Phytophthora* which will be discussed.

## S1.8: *Phytophthora* eDNA barcoding for natural ecosystem surveillance

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The genus *Phytophthora* are a damaging group of invasive plant pathogens that can devastate crops, horticulture, forestry and plants in natural ecosystems. The 170 or so described species represent a fraction of the real diversity meaning a large potential reservoir of *Phytophthora* taxa exist in natural ecosystems. Understanding the spatial and temporal distribution of *Phytophthora* species and their ecology and impact is crucial for the accurate interpretation of plant biosecurity protocols. We have combined *in situ* water filtration, a *Phytophthora*-specific PCR test based on the rDNA ITS1 region and high-throughput Illumina sequencing to survey *Phytophthora* diversity in samples of environmental DNA (eDNA). The method has been applied to natural ecosystems as well as planting material and irrigation water in nursery production systems and although effective technical challenges and questions remain. For example, the downstream computational biology pipeline to process the data must be validated and based on a robust database of reference sequences that copes with 'fuzziness' and overlap around species boundaries. Such validation is critical to objective measures of the benefits of the technology for plant health legislation and ecosystem surveillance.

## **S1.9: The development and optimization of a TaqMan probe assay to detect *Aphanomyces invadans***

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Infection with *Aphanomyces invadans*, the causative agent for Epizootic ulcerative syndrome (EUS), is an internationally notifiable disease of fresh and brackish water fish that requires reporting to the World Trade Organisation (OIE). Following the first outbreak in 1971 in South East Asia, EUS has achieved a broad geographical distribution and host range. The spread of EUS threatens the health of natural fish populations and substantially affects the livelihood of fish farmers and subsistence fishermen. Currently the OIE recognizes four tests for EUS confirmation namely; (a) Isolation and identification of *A. invadans* culture, (b) presence of hyphae in tissue using fluorescent *in situ* hybridization (FISH), (c) visible mycotic granulomas in histological sections and (d) conventional polymerase chain reaction (PCR), of which the latter is most widely used. One common drawback of current diagnostic assays is its dependence on active outbreaks and clinically infected hosts displaying necrotic ulcerative epidermal lesions. A TaqMan probe assay could improve the sensitivity and specificity of diagnostics, by targeting a shorter DNA fragment. This study aimed to develop a TaqMan probe assay to detect *A. invadans* from infected hosts and environmental samples even when present at low concentrations. The nucleotide sequence of the internal transcribed spacer gene (ITS1-5.8S-ITS2) of *A. invadans* and other closely related oomycetes was used to design two TaqMan Probe primer sets, targeting the ITS1 and ITS2 regions, respectively. Assays were optimized and specificity was confirmed against a panel of closely related oomycetes, mostly isolated from freshwater systems around South Africa.

## S1.10: Exploring yield-limiting diseases in seaweed aquaculture to improve biosecurity practices

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Seaweed aquaculture is a rapidly growing global industry, with production doubling twice in volume and value over the last two decades. However, this global growth is being limited by multiple factors including diseases, which decrease, and in some cases cause regional collapse of the industry. For example, *Pyropia* (laver) production is widely affected by *Olpidiopsis* blight and red rot disease both caused by oomycetes, *Olpidiopsis* spp. and *Pythium porphyrae*, respectively. Along with the growth and intensification of seaweed cultivation, new diseases are increasingly being reported, but knowledge of their causative agents is limited. Here, we present our work on identifying yield-limiting pathogens in seaweed aquaculture, with an emphasis on the red algal carrageenophyte industry (*Eucheuma* spp. and *Kappaphycus* spp.). Through the international consortium GlobalSeaweed-STAR, we are conducting sampling in seaweed farms for visibly diseased seaweeds in Malaysia, Philippines, Tanzania and Madagascar. Molecular and histological analyses are being used to accelerate the discovery of pathogens and their descriptions. In parallel, we performed cross-inoculation experiments with *Olpidiopsis* pathogens from different biogeographic regions, which demonstrate the potential hazard that pathogens may cause by the uncontrolled movement of seaweeds. Collation and analysis of legislation regarding the control and spread of diseases in the carrageenophyte industry has provided evidence for a lack of seaweed-specific biosecurity measures. In the light of these results, we emphasize the need for increased efforts to improve the

understanding of seaweed diseases to develop an appropriate biosecurity framework for seaweeds globally.



## Session 2: Effectors and Virulence

### S2.1 The recognition of conserved RxLR effectors of *Phytophthora* species might help to defeat multiple oomycete diseases

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Oomycetes, especially *Phytophthora* species, cause diseases of many economically important crops. For example, potato late blight, tobacco black shank disease and strawberry crown rot are caused by *P. infestans*, *P. parasitica* and *P. cactorum* respectively. So far, many *R* genes against potato late blight have been cloned from wild *Solanum* species. However little is known about resistance against these other *Phytophthora* species.

In 2016, *Rpi-amr3* was cloned from *Solanum americanum*. Here we report a newly identified *Avr* gene *Avr-amr3* from *P. infestans*, which was identified by effectoromics. Unlike other RxLR effectors, *Avr-amr3* is located on a relatively gene-dense region, and orthologs are present in many other *Phytophthora* species. Surprisingly, we found homologs of *Avr-amr3* from *P. parasitica*, *P. cactorum*, *P. palmivora*, *P. sojae*, *P. megakarya*, *P. litchii* and *P. pluvialis* can be recognized by the same *R* protein after co-expression in *N. benthamiana*. Some of the *Phytophthora* species have a very wide host range, to further test if *Rpi-amr3* confers resistance to other *Phytophthora* species carrying the *Avr-amr3* homolog, we used *N. benthamiana* – *P. parasitica* as a model system. We found that *Rpi-amr3* transgenic *N. benthamiana* is resistant to *P. parasitica* Race 0 and Race 1, but the wild type *N. benthamiana* is highly susceptible.

This is the first report of a conserved RxLR effector from different *Phytophthora* species that can be recognized by the same *R* gene, and has the potential to enable broad spectrum resistance against many oomycete diseases.

## S2.2: Analysis of plant global alternative splicing changes during late blight infection provides new insights into plant-microbe interaction

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In both plants and animals, alternative splicing (AS) increases transcriptome and proteome diversity, and play roles in developmental and immune responses. However, the extent of host genome-wide AS changes and how these changes are modulated by adaptive pathogens during disease remain largely unknown. Here we examined AS changes in *Phytophthora infestans* infected *Solanum lycopersicum* leaves using paired-end illumina RNA-seq. We detected a total of 5125 genes exhibiting significant AS changes during three infection stages and characterize AS events of some important/novel plant immune-related genes. Quite a number of AS events are independent of gene differential expression, indicating that AS changes may be an additional layer of plant immunity against pathogens. Furthermore, we established an mRNA splicing-luciferase reporter system, and screened a few *Phytophthora infestans* splicing regulatory effectors (SREs) that are involved in plant mRNA alternative splicing process. The mode of action of SREs and their roles in plant immunity requires further investigations.

## **S2.3: Negative regulators of plant immunity derived from cinnamyl alcohol dehydrogenases are targeted by multiple *Phytophthora* Avr3a-like effectors**

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Oomycete pathogens secrete numerous effectors to manipulate host immunity. While some oomycete RXLR effectors share a conserved structural fold, it remains unclear if any have conserved host targets, especially in broad-host-range pathogens such as *Phytophthora capsici*. The family of effectors related to *P. infestans* effector PiAvr3a (Avr3a-like effectors) is widely distributed across diverse clades of *Phytophthora* species. Here, we have identified members of the plant cinnamyl alcohol dehydrogenase (CAD) subfamily as common targets of multiple Avr3a-like effectors. The CAD7 subfamily of CADs has expanded extensively in plant genomes and are induced by *Phytophthora* infection. Silencing of *AtCAD7* and *NbCAD7* significantly decreased *Phytophthora* infection, whereas overexpression of *AtCAD7* enhanced plant susceptibility. Mutations in the enzymatic active sites of *AtCAD7* neither affected its interaction with effectors, nor abolished its modulation of immune function. *AtCAD7* was stabilized by Avr3a-like effectors in a 26S proteasome-dependent manner. In *N. benthamiana*, *NbCAD7* could suppress INF1-triggered cell death and enhance suppression of INF1-triggered cell death by PiAvr3a<sup>KI</sup>. In *Arabidopsis*, increased *AtCAD7* expression suppressed the oxidative burst and callose deposition induced by flg22, and the expression of *WRKY33*. Lignin staining assays confirmed a minor role for *AtCAD7* in lignin biosynthesis. Our results reveal CAD7 sub-family proteins as negatively regulators of plant immunity that are exploited by multiple Avr3a-like effectors to promote infection.

## **S2.4: The AeSSP1256 effector, a Small Secreted Protein of the root rot pathogen *Aphanomyces euteiches*, targets a host DEAD-box RNA helicase**

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Among the oomycete lineage, the *Aphanomyces* genus includes plant and animal pathogenic species. By comparative genetic analyses, we found that the legumes pathogen *Aphanomyces euteiches* genome is characterized by a large repertoire of small secreted protein coding genes (SSP), highly induced during plant infection, and not detected in other oomycetes. Functional analyses of several AeSSPs revealed that AeSSP1256, which contains a nuclear localisation sequence, is able to bind plant RNA, to affect legumes development and increase plant susceptibility to microbial infection. Yeast two hybrid screening identify few nuclear *Medicago truncatula* proteins as putative targets for AeSSP1256 like a DEAD-box RNA helicase. FRET-FLIM analyses showed that AeSSP1256 binds the RNA helicase and prevents its association to plant RNA, probably to affect cellular processes such as pre-mRNA processing. Molecular experiments are in progress to decipher the impact of this interaction and on the outcome of the host infection.

## S2.5: An RxLR effector from *Phytophthora infestans* interacts with a lipid binding protein to regulate plant susceptibility

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*Phytophthora infestans* is an important oomycete pathogen and poses a serious threat to potato production. It secretes a range of RxLR effectors which are translocated inside plant cells and attenuate plant immunity by manipulating host target proteins. Therefore, we screened key effectors, important for pathogenicity, to identify their virulence targets in the host in order to help create new sustainable disease control strategies. The effector Pi00366 strongly suppressed the HR induced by INF1 and promoted the infection of *P. infestans*. Pi00366 interacts with the phosphoinositide binding protein Phox1 in both Y2H and Co-IP assays. Over expression of *Phox1* also suppressed INF1 cell death and significantly promoted *P. infestans* infection. In contrast, on *Nicotiana benthamiana* silenced for *Phox1* the HR induced by INF1 emerged earlier, and plants were more resistant to *P. infestans* than the GFP control. When co-expressing *Pi00366* with *Inf1* on *Phox*-VIGS plants, the ability of Pi00366 to suppress INF1 induced HR was attenuated, which indicates that this virulence function of Pi00366 is probably dependent on Phox1. Additionally, Phox1 localized to the endosome, and Pi00366 partially colocalized with Phox1 at endosomes upon co-expression. All results above indicate that Pi00366 promotes *P. infestans* colonization and suppresses PTI through interaction with Phox1 at endosomes, and Phox1 acts as a susceptibility factor to help *P. infestans* attenuate plant immunity.

## S2.6: A newly defined LWY motif as a structural and functional module in *Phytophthora* RXLR effectors

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Previous domain analysis of RxLR effectors revealed three motifs, “L”, “W”, and “Y”, named after a conserved amino acid in their respective sequences. In particular, the W-Y motifs, sometimes forming tandem repeats, are found in approximately 44% of *Phytophthora* RxLR effectors. Structural analyses discovered a three or four  $\alpha$ -helix bundle formed by each WY motif and the bundle is stabilized by a hydrophobic core. Here, we report the crystal structure of the *Phytophthora sojae* effector *Phytophthora* suppressor of RNA silencing 2 (*PsPSR2*), which consists of seven tandem repeat units including one W-Y motif and six L-W-Y motifs. Each L-W-Y motif folds into a highly conserved five  $\alpha$ -helix bundle and forms two hydrophobic cores. In addition to an internal core similar to the one formed in WY motifs, a newly defined “L” motif is essential for a second hydrophobic interaction, which results in directional linkages between adjacent units. This unique inter-unit concatenation mechanism results in an overall stick-like structure of *PsPSR2*. Genome-wide analysis revealed 293 effectors from five *Phytophthora* species that have the *PsPSR2*-like arrangement, i.e. containing a W-Y motif as the “start” unit, various numbers of L-W-Y motifs as the “middle” units, and a degenerate L-W-Y as the “end” unit. Together with functional analysis of individual WY/LWY units in *PsPSR2*, our results suggest that the L-W-Y fold is a prevalent structural and functional module that may serve as a “building block” to accelerate effector evolution in *Phytophthora*.

## S2.7: *Phytophthora sojae* effector suppresses RNA silencing and plant immunity by activating GmDCP2 mediated mRNA decay in soybean

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*Phytophthora sojae* (*P. sojae*) is a soilborne pathogen, which causes a serious *Phytophthora* root and stem rot in the soybean-growing areas of the world. RNA silencing suppressor proteins are pathogenicity determinants widely expressed by plant and animal viruses that have recently also been identified amongst *Phytophthora sojae* effectors. However, the mechanism of how *Phytophthora* effectors suppress host RNA silencing is not clear. In this study, we identified a novel RNA silencing suppressor, PSR3, in *P. sojae* using a stable transgenic system by checking the suppression activity of *GUS* gene silencing in *Arabidopsis thaliana* L1 plants instead of traditional RNA suppressor screening system in *Nicotiana benthamiana* 16C plants. Further studies showed that the expression of *PSR3* is highly up-regulated in the early infection period and *PSR3* can enhance the plant susceptibility to both PVX and *Phytophthora parasitica* in *N. benthamiana* leaves and to *P. sojae* in soybean hair root. Y2H screening showed that *PSR3* directly interacts with soybean ASYMMETRIC LEAVES like proteins GmASL2-1/2 and greatly induce those genes expression. Consistent with these findings, GmASL2-1/2 transient expression in 16C plants suppressing GFP expression also confirmed GmASL2-1/2 are involved in the RNA silencing pathway. In addition, our preliminary data showed that GmASL2-1/2 interact with soybean RNA decapping protein GmDCP2b. Collectively, our results suggested that *PSR3* may activate RNA degradation to suppress post transcription of gene silencing (PTGS) and immunity in soybean.

Key words: *Phytophthora sojae*; RNA silencing suppression; effector; mRNA decay

## **S2.8: A *Phytophthora infestans* effector promotes infection by targeting a host MAPKK protein**

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*Phytophthora infestans* is the causal agent of late blight disease on potato and tomato. It secretes a plethora of effectors to manipulate host cellular processes to promote colonization. The well-known are the RXLR effectors that are translocated into host cells to manipulate plant cell machinery. Here, we report that the RXLR effector E49 is highly expressed at early infection stage and promotes *P. infestans* colonization by suppressing PAMP-triggered immunity (PTI). By yeast two hybrid screening, we identified a host MAPKK protein as target of E49. The plant MAPK cascade proteins are known to participate in plant PTI. Silencing of MAPKK enhances plant resistance to *P. infestans* and increases PTI related gene expression, while overexpression of it promotes pathogen colonization and reduces PTI related gene expression. E49 and MAPKK co-localize in the nuclear and cytoplasm of the plant. Further investigation of the effect of E49 on MAPKK kinase activity is undergoing. Here we propose, *P. infestans* RXLR effector E49 suppresses plant PTI by targeting a PTI negative regulator MAPKK protein.

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## S2.9: Effectors of *Phytophthora agathidicida*, killer of the iconic New Zealand Kauri tree

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Oomycete pathogens of forest trees can cause devastating disease epidemics. In New Zealand, the iconic and culturally significant kauri tree (*Agathis australis*) is under serious threat from kauri dieback caused by *Phytophthora agathidicida*. A screening programme carried out at Scion (New Zealand Forest Research) suggests that some dieback tolerance is present in natural kauri populations. With the ultimate goal of identifying resistance that is durable in forests, the aim of our work was to explore the molecular basis of interactions between *P. agathidicida* and kauri, by first identifying pathogen molecules that are required for virulence or that trigger host defence. The *P. agathidicida* genome was screened for RxLR effector genes and the functions of a core set of 75 were first assessed using a model system involving transient *Agrobacterium* transformation of *Nicotiana spp.*. Eight of the RxLR proteins triggered cell death, suggestive of a hypersensitive defence response. One of these, PaRxLR24, an orthologue of the well-characterised Avh238 of *P. sojae*, was of particular interest as it was highly expressed in kauri and the cell death it elicited was suppressed by another *P. agathidicida* effector, PaRxLR40. Using an RNAi hairpin library we aim to identify cognate NB-LRR receptor(s) for PaRxLR24 in *N. benthamiana*. In future we hope to screen RxLR proteins in kauri tissue to determine if they trigger or suppress cell death responses in the natural host and to identify host targets that will help us determine the genetic basis of resistance in this very important gymnosperm species.

## S2.10: Activation and suppression of apoplastic effectors in *Phytophthora*-plant interactions

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Plants and pathogens are engaged in a dynamic co-evolutionary arms race for survival. Plants have developed innate immunity via perception of microbial molecules by immune receptors and resist infection by the majority of microbes. To achieve successful colonization, pathogenic microbes evolved special effector arsenals to modulate plant immunity. In our lab, we profiled the apoplastic effector repertoires secreted by the soybean root rot pathogen *Phytophthora sojae* using a combined proteomic and bioinformatic strategy. In this way, we identified multiple novel apoplastic effectors that are either recognized by plant immune receptors or function as virulence factors in suppressing plant defense. In addition, we found apoplastic effectors undergo post-translational modifications which are essential for the action of the apoplastic effectors in the interplay between *P. sojae*-hosts.

## S2.11: ***N*-glycosylation shield PsXEG1 from the host attack mediated by protease and inhibitor in apoplast**

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*Phytophthora* secretes a range of effectors into the extracellular and intracellular space of host. Secreted proteins of pathogens are synthesized in the rough Endoplasmic Reticulum (ER) and processed in Golgi Apparatus (AG), where they usually undergo *N*-glycosylation and/or *O*-glycosylation. But few studies have focused on this post-translational modification (PTM) in the field of plant-*Phytophthora* interacted system. Previously, we found that PsXEG1, an apoplastic effector, can trigger plant immunity and contribute to *P. sojae* virulence. In this study, Here, we identified PsXEG1 can be glycosylated in vivo. Glycosylation of PsXEG1 is essential for *P. sojae* full virulence. This means that glycosylation plays a role in PsXEG1 virulence function. Further study, glycosylation stabilizes PsXEG1 in apoplast for *P. sojae* full virulence and triggered immunity. Interesting, we found a GmGIP1 homologous protein GmGIPL1, an aspartic protease, taking part in PsXEG1 degradation. Significantly, we investigated that the glycosylation implicated in the binding-affinity of PsXEG1 to host inhibitor GmGIP1. Together, the research data highlight a novel pathogenic mechanism that pathogen use the PTM as a shield to protect effectors weapons from host counterattack and may present a promising strategy for against the Oomycetes during early infections.

## Session 3: Host Interactions and Resistance Mechanisms

### S3.1: Uncoupling growth inhibition from plant immunity in the hyperresistant *Arabidopsis dmr6 dlo1* mutant

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Plants have evolved an elaborate immune system to combat microbial pathogens. Resistance to biotrophic pathogens, which thrive on living host tissue, is predominantly mediated by the phytohormone salicylic acid (SA). Although SA stimulates immunity, it actively suppresses growth. This growth-immunity trade-off exerts itself in SA-accumulating mutants, like the *dmr6 dlo1* double mutant. *Arabidopsis* plants mutated in *DMR6* and *DLO1*, which encode 2-oxoglutarate iron-dependent SA-oxygenases, are hyperresistant to biotrophs but show growth defects. Here, we use the resistance and growth phenotypes of the *dmr6 dlo1* mutant to identify regulatory mechanisms that affect growth, but not immunity. In a forward genetics (EMS-mutagenesis) screen on the *dmr6 dlo1* mutant, we identified 104 mutants with restored growth phenotypes at seedling and adult stages, similar to Col-0. Moreover, half of these restored growth mutants had high resistance levels to the downy mildew *Hyaloperonospora arabidopsidis*. The genomes of backcrossed lines have been sequenced to map causal genes (thereafter named *MODIFIERS OF dmr6/dlo1-MEDIATED IMMUNITY (MDI)*). We will present an update on the identification of *MDI* genetics and other phenotypes and responses in these EMS mutants. In conclusion, we have identified highly resistant *Arabidopsis* mutants with a reduced growth penalty. Finding the mode of action of the *MDI* genes in coupling growth and immunity will greatly aid to our understanding of the plant immune network.

### S3.2: *Caenorhabditis elegans* as a tractable host to study natural infections by oomycetes

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Although plant-infecting oomycetes have been widely studied, research on animal-infecting species has been much more limited due to a paucity of tractable hosts. To redress this, my group has recently established new systems to study nematode-infecting oomycetes belonging to the *Myzocytiopsis* and *Haptoglossa* genera and using the well-established model organism *Caenorhabditis elegans* as a host. We will describe here these new pathosystems and present evidence that oomycetes are widespread natural pathogens of *C. elegans*. We will show that *C. elegans* senses the presence of oomycete pathogens in order to mount a pathogen-specific transcriptional response in the epidermis. This response consists of the induction of a previously uncharacterised gene family of chitinase-like (*chil*) genes. Induction of *chil* genes provides partial resistance to infection by modifying the properties of the host cuticle to hinder oomycete attachment. We will also report our recent progress aiming at dissecting the molecular pathways underlying oomycete recognition using forward genetic screens. Our results suggest that neuronal signalling is required in the host to trigger the expression of *chil* genes in the epidermis, highlighting that different tissues in the nematode share signals to orchestrate the host defence response. Our results shed light on previously unknown natural infections of *C. elegans*.

### S3.3: Multi-layered and broadly conserved defence reactions of brown algae against oomycetes and other pathogens

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Brown algae are key marine primary producers that diverged from plants and animals over a billion years ago, which like any living organism are susceptible to diseases. We have used three biotrophic, intracellular pathogens to interrogate the cellular mechanisms resulting in innate/induced disease resistance in brown algae: the two oomycetes *Eurychasma dicksonii* and *Anisolpidium ectocarpii*, and the phytomyxid *Maullinia ectocarpii*. We found that clonal brown algal strains exhibit contrasting degrees of innate resistance. Using microscopy and histochemical techniques, we also found that several defence hallmarks strongly correlate with immunity and are broadly conserved across 43 strains (27 algal species across 10 orders): i) local cell wall reinforcements (papilla) beneath the infection site, sometimes accompanied of systemic cell wall thickening across the entire individual; ii) the death of host cells challenged by a pathogen spore; iii) the upregulation of phlorotannin metabolism; iv) an inducible autophagic response of the host and the pathogen; v) hydrogen peroxide production, confirming the participation of oxidative stress in these algal-pathogen interactions. Further investigations highlight that the cell death reaction is associated to hallmarks of programmed cell death, such as controlled DNA degradation and nuclear lysis, expression of metacaspases, swollen mitochondria and obstruction of plasmodesmata. Additionally, inducible autophagy in both hosts and the pathogens restricts the pathogen propagation, thus providing the first known cellular mechanism for inducible and systemic acquired resistance in brown algae. All these responses are widely conserved across Phaeophyceae, and altogether account for innate and acquired resistance against the investigated oomycete and phytomyxid pathogens. However, we also observed several resistant algal strains where these responses were not strong, suggesting that more undescribed mechanisms are contributing to brown algal immunity.

### **S3.4: Host and pathogen autophagy are central to the inducible resistance of brown algae against intracellular parasitic water moulds**

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Brown algae encompass key primary producers of temperate ecosystems. Their wild harvest cannot supply the current demand and their cultivation is growing exponentially worldwide, with a worsening disease incidence. Both pathogen spectrum and immunity are aspects broadly under-documented in Phaeophyceae, despite their importance to inform disease management in farms/natural populations and support the development of breeding programs. Here, we investigated the time-course of the interaction between the obligate oomycete pathogen *Anisolpidium ectocarpii* and the kelp *Macrocystis pyrifera*, using TEM and *in vivo* markers. We observed that pathogen lipid droplets are abundant in the onset of the infection but gradually deplete, suggesting that the pathogen undergoes starvation as the infection progress. In response to the progressive depletion of algal resources, some *A. ectocarpii* spore initials degenerate via autophagy, supporting the continued differentiation of the others. Over time however, *A. ectocarpii* thalli undergo targeted nucleophagy and become entirely abortive, indicative of the pathogen's starvation response being subverted towards kelp defense. Infected algal cells also become autophagic: chlorophagy-like processes and chloroplast size reduction were normally observed, thus quickly mobilizing the nutrients required to mount defenses, or even directly digesting the intruder via xenophagy. Pilot experiments with autophagy inhibitors reduced significantly autophagy in *Anisolpidium*, and also stops the propagation of infection to healthy hosts. We establish a working model where several lines of inducible autophagic responses may ultimately lead to acquired disease resistance.

### S3.5: Plant recognition of *Phytophthora* apoplastic effectors and signal transduction

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*Phytophthora* species are notorious plant pathogens that cause great damage on crops. During infection, *Phytophthora* pathogens secrete a type of highly conserved glycoside hydrolase family 12 (GH12) proteins including XEG1 that can be recognized by plants as a pathogen-associated molecular pattern (PAMP). By a genome-wide assay of membrane-localized receptors, we identified the recognition receptor (RXEG1) that is responsible for response to XEG1 and multiple GH12 proteins. In addition, we dissected the defense-signal transduction pathways in plants upon recognition of the XEG1 and identified novel receptor kinases that participate in XEG1 defense-signal. Together, this study provides novel insights on plant innate immunity against *Phytophthora* pathogens and will contribute to the development of durable disease resistance.



### S3.6: Evolutionarily distinct R proteins detect *Phytophthora infestans* effector PiAVR2 through its action on different target proteins

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Understanding the roles of effectors in causing disease and the mechanism(s) by which R proteins detect them allows us to better combat plant–pathogen interactions. We recently showed that *Phytophthora infestans* effector PiAVR2 targets all BSL1-like (BSL) family members in potato and transient expression of BSL1, 2, and 3 enhances *P. infestans* infection. BSL1 and BSL3 suppress INF1-triggered cell death (ICD), showing that they negatively regulate immunity. ICD suppression is dependent on the brassinosteroid-responsive host transcription factor CHL1. Thus, these phosphatases act as susceptibility factors in late blight infection (Turnbull & Wang et al, 2019 *Plant Physiol* doi: 10.1104/pp.18.01143).

PiAVR2 is recognised by two evolutionarily distinct R proteins, R2 and MCQ1. Our data show that silencing *BSL1* significantly decreases R2 CD, whereas MCQ1 CD is compromised only in *BSL2/3* silenced plants. Interestingly, okadaic acid, a protein phosphatase (PP1 and PP2A) inhibitor suppresses both recognition events, indicating that phosphatase activities are required in each case. Transient overexpression of BSL1 increased R2 CD, whereas a BSL1 phosphatase-dead mutant suppressed it. In contrast, expression of BSL3 accelerated MCQ1 CD, whereas a BSL3 phosphatase-dead mutant did not. Our data suggest that R2 monitors the action of PiAVR2 on BSL1, whereas MCQ1 monitors its action on BSL3. We conclude that R2 and MCQ1 have evolved distinct mechanisms to detect PiAVR2 activity.

### S3.7: A pathogen turns ‘immunity on’ to ‘immunity off’ with a flick of the switch

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Plant pathogens, such as the infamous potato blight agent *Phytophthora infestans*, deliver effectors into plant cells to promote disease. Recent evidence suggests that some effectors exploit endogenous negative regulators of immunity, or so-called susceptibility (S) factors, to inactivate the immune system. Previously, we found that a *P. infestans* RXLR effector, Pi02860, uses the potato ubiquitin E3 ligase StNRL1 as an S factor to target a positive regulator of immunity, the guanine exchange factor (GEF) StSWAP70, for proteasome-mediated degradation (He and Naqvi et al. 2018 PNAS 115:E7834-E7843). StNRL1 is a member of the NPH3 and RPT2-like family of CULLIN 3-based E3 ligases that act downstream of the blue light regulators, phototropins. Remarkably, we have found that phototropins, StNRL1 and the effector Pi02860 all interact in yeast and *in planta* with 14-3-3 proteins. We have found that 14-3-3 acts to positively regulate plant immunity. 14-3-3 functions as a phosphoprotein molecular switch, interacting with StNRL1 in order to reduce or prevent its interaction with SWAP70. This promotes immunity. The effector Pi02860 potentially acts as a decoy to misdirect 14-3-3 away from its intended interaction sites in StNRL1, freeing StNRL1 to interact with and degrade SWAP70. This illustrates an example of the complex molecular battle between plant and pathogen in which an immunity ‘on’ switch is turned ‘off’ by an effector. Critically, as many plant pathogen effectors target 14-3-3 proteins, our results provide a model by which such effectors may operate.

### S3.8: Pathogen effector triggered plant immunity is modulated by light rhythm

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Plant responses to biotic stress are affected by light rhythm, however how light rhythm regulates the pathogen effector triggered plant immunity (ETI) remains largely unknown. Here, we showed that light rhythm specifically determined the activation of potato resistant gene *Rpi-vnt1* against *Phytophthora infestans* carrying cognate effector gene *Avrvnt1*. A further investigation indicates that *Avrvnt1* triggered ETI response required its associated plant protein glycerate kinase (GLYK). To adapt to light changes, plant will induce alternative promoter selection (APS) on lots of genes including *GLYK*. Interestingly, we found *Avrvnt1* physically binds to a longer isoform of *GLYK* which is generated under normal light cycle (LD) and consequently triggers ETI. However, this AVR effector fails to bind the shorter isoform produced by APS under constant dark (DD) and therefore fails to induce ETI response. This study highlights an appealing molecular basis of how plant immune response is determined by light rhythm.

### **S3.9: Exploring how plant nutrient transport affects resistance and susceptibility to oomycete pathogens**

Unnati Sonawala<sup>1</sup>, John Herlihy<sup>1</sup>, Wei Wang<sup>1</sup>, Cristian Dana<sup>2</sup>, Terri Long<sup>3</sup>, Guillaume Pilot<sup>1</sup>, **John M. McDowell**<sup>1</sup>

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All plant pathogens obtain nutrients from the plant host to fuel their own growth. At the same time, plants must reprogram their own metabolic pathways to fuel their immune responses and withhold nutrients from pathogens. These critically important functions are accomplished in environments where key nutrients are either limiting, sufficient, or in excess. Physiological experiments have demonstrated that changes in host nutrient status either promotes or retards pathogen growth or plant resistance. However, these data are fragmentary; particularly for plant interactions with oomycetes (e.g. *Phytophthora* species, downy mildews) that account for tens of billions of dollars in annual crop losses. We are investigating this knowledge gap with pathosystems comprised of *Arabidopsis* and two oomycete pathogens: the foliar pathogen *Hyaloperonospora arabidopsidis* (obligate biotroph) and the root pathogen *Phytophthora capsici* (hemi-biotroph). We have developed a hydroponic system in which we can manipulate specific nutrients to observe effects on resistance/susceptibility. We are using this system in combination with phenotyping and transcript profiling to understand how plants and pathogens respond to nutrient stress. We have also used a reverse genetic approach to identify mutant alleles of nutrient transporters that promote or retard pathogen growth, and we are currently investigating the physiological and molecular basis of these phenotypes.

### **S3.10: A ubiquitin E3 ligase Regulatory cascade controls defence by modulating the abundance of an immune-suppressive RNA binding protein**

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Ubiquitination is a post-translational modification which regulates many processes in plants. Several ubiquitin E3 ligases have been shown to act as either positive or negative regulators of immunity via the degradation of different substrates. StPUB17 is an E3 ligase which has previously been shown to be a positive regulator of immunity to the oomycete pathogen *Phytophthora infestans*. Silencing of PUB17 promotes pathogen colonisation and attenuates Cf4/avr4 cell death and flg22 signalling. Using yeast-2-hybrid and co-immunoprecipitation we identified the putative K-homology (KH) RNA binding protein, StKH17, as a target for degradation by PUB17. KH17 acts as a negative regulator of immunity which promotes *P. infestans* infection and suppresses Cf4/avr4 Cell death. As StPUB17 is a known target of the ubiquitin E3 ligase, StPOB1, we reveal the final step in an E3 ligase regulatory cascade that controls plant defence.

### **S3.11: What ‘R’ you doing here? Investigating the role of S-acylation in oomycete effector recognition**

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A crucial aspect of the plant immune response is the recognition of specific pest and pathogen ‘effector proteins’ – secreted molecules that manipulate plant processes to promote infection. Effector recognition is facilitated by plant resistance (R) proteins, largely belonging to the nucleotide-binding leucine-rich repeat (NB-LRR) family. Recent work on a group of R-proteins from potato, required for resistance to the notorious late blight pathogen *Phytophthora infestans*, has revealed that they undergo S-acylation - a reversible fatty acid-based post-translational modification. S-acylation (often referred to as palmitoylation) is largely known for its role in protein localization; particularly membrane anchoring. However, due to its reversible nature, S-acylation is also implicated in regulatory aspects of protein function such as activation, trafficking, and protein-protein interaction. Ongoing investigation has shown that S-acylation occurs at multiple conserved sites, in multiple domains, within these Solanaceous R proteins, suggesting a complex role for this dynamic post-translational modification. Our latest data on the functional consequences of R-protein S-acylation during plant defence responses will be presented, with modification shown to be essential for full immune signalling.

## S3.12: Cross-kingdom RNAi in plant-oomycete interaction

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Mobile small RNAs (sRNAs) can trigger RNA silencing across kingdoms during plant-fungal interaction, called cross-kingdom RNA interference (RNAi). Here, we found for the first time that sRNAs of a pathogen representing the oomycete kingdom invade the host plant's Argonaute (AGO)/RNA-induced silencing complex. We profiled sRNAs that bind to *Arabidopsis thaliana* AthAGO1 during *Hyaloperonospora arabidopsidis* (*Hpa*) infection by immunopurification coupled to sRNA-seq analysis and found 34 *H. arabidopsidis* sRNAs (*HpasRNAs*) that were predicted to target host genes including putative immunity factors. To demonstrate the functionality of the plant-invading *HpasRNAs*, we designed a novel CRISPR endoribonuclease Csy4/GUS repressor reporter to visualize *in situ* pathogen-induced target suppression in *A. thaliana* host plant. The significant role of *HpasRNAs*, together with AthAGO1, in virulence was demonstrated by plant *atago1* mutants and by transgenic *Arabidopsis* expressing a target mimic (STTM) to block *HpasRNAs*, that both exhibited enhanced resistance. We characterized two candidate target genes *AthWNK2* and *AthAED3* being involved in pathogen defense, because *athwnk2* and *athaed3* T-DNA insertion mutants exhibited higher *H. arabidopsidis* sporangiophore and spore production. With this, we expand the cross-kingdom RNAi model to the kingdom of oomycetes.

### **S3.13: Contrasting potato defense responses and *Phytophthora infestans* virulence between leaves and tubers.**

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Organ-specificity of plant defense is an under-represented axis of research. However, recent publications point out the importance of this concept in plant-microbe interactions. Indeed, blindly assuming the resistance status of a whole plant against a given pathogen following studies conducted only on leaves appears inappropriate. This study aims at understanding the extent of potato (*Solanum tuberosum*) defense organ-specific mechanisms. Our results show that potato leaves and tubers activate contrasted defense responses, in terms of production of reactive oxygen species (ROS) and the activation of PTI-associated genes, following treatments with the oomycete PAMP Pep-25 or the bacterial PAMP flg22. Transcriptional reprogramming following flg22 induction differed between leaves and tubers, while ROS production was similar, showing that PTI-associated genes responses do not necessarily overlap. Following Pep-25 treatment, ROS production was induced in leaves but not in tubers. Also, *StPotIx-3*, *StPR-1*, *StRbohB* and *StACRE31* exhibited organ-specific expression patterns. Since PTI appears to act differently in leaves and tubers, we also sought to identify organ-specific effectors in *Phytophthora infestans*. We thus investigated the expression level of *P. infestans* effectors (RXLR and CRN) at three time points and identified six organ-specific effectors overexpressed at all time points in tubers compared to leaves.



### S3.14: Towards marker-assisted selection for resistance to Oomycetes in Brown Algae

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The GENIALG project (Horizon 2020) aims to understand the natural diversity of the sugar kelp *Saccharina latissima* and to pursue a selective breeding program in order to improve the productivity and the composition of strains used in aquaculture. Building on more than 15 years experience, the brown algal model *Ectocarpus* is a perfect example to illustrate what could be achieved in terms of algae-variety improvement and what are the steps leading to it. Aiming to detect quantitative trait loci (QTLs) correlated with adaptation to biotic stress, we will focus on bioassays currently under development designed to phenotype, on the short term, the resistance of an *Ectocarpus* segregating population against an Oomycete pathogen (*Anisolpidium ectocarpii*) and, on the long term, to allow us to identify disease resistance genes of any brown algae species, including *S. latissima*. Acting in a complementary way to the QTLs analysis and based on quite similar analysis but on wild populations, Genome-Wide Association Study (GWAS) highlights the correlation between specific allele and a quantitative trait. To this end, the GENIALG project aims to biobank the broadest genetic diversity of *S. latissima* throughout its biogeographic range, to genotype this diversity using double digested RAD sequencing technique (ddRADseq) and to quantify the resistance of *S. latissima* gametophytes to pathogens, using on the bioassay developed on *Ectocarpus*. Hopefully, these approaches will allow us to identify in *S. latissima* some loci associated to biotic stress tolerance.

### S3.15: Who Are You Rooting For? - The Hunt For Resistance To Raspberry Root Rot Disease

Aurelia Bezanger<sup>1,2</sup>, Charlene Ogilvie<sup>1</sup>, Lydia Welsh<sup>1</sup>, Julie Graham<sup>1</sup>, Hazel McLellan<sup>2</sup>, Craig Simpson<sup>1</sup>, Steve Whisson<sup>1</sup>, Richard Harrison<sup>3</sup>, Ingo Hein<sup>1,2</sup> and **Eleanor M Gilroy**<sup>1</sup>

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*Phytophthora rubi* is a widely spread pathogen in the UK, causing raspberry root rot which leads to huge losses in raspberry crops. At present, more than 70% of the UK's soil-based raspberry production has *Phytophthora Root Rot* (PRR). With no sustainable control for PRR, the industry has been driven into a short term, mainly pot based cropping system. We are currently characterising an old non commercial raspberry cultivar 'Latham' from which we had developed a segregating population and previously identified markers that strongly associate with PRR resistance. Prior to our studies, the mechanism of resistance in 'Latham' remains elusive, and the infection process in susceptible plants was also poorly studied. We are now trying to address four major scientific questions about PRR: 1) How does *P. rubi* infect the roots of their respective hosts? 2) What is the molecular diversity in *Pr* populations in the UK and how does this relate to global pathogen population structure? 3) What are the differences in *Pr* effector content and what is their role in defining host-specificity? 4) What genes underpin resistance in raspberry and how durable are they?

## Session 4: Oomycete Biology

### S4.1: What are the impacts of microbiota-oomycete interactions on the infectious cycle?

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Microbiota has an impact on outbreak of plant diseases caused by oomycetes. On the plant side, it regulates activation of defence responses. On the oomycete side, it interferes with the pathogen growth through competition, or contribute to the success of the infectious cycle<sup>a</sup>. The structure and functions of oomycete-associated microbiota are investigated on the telluric species *Phytophthora parasitica*. The ability of the oomycete to form monospecific or mixed biofilms on the host surface is used as model. A first issue addresses the elucidation of mechanisms governing biofilm formation<sup>b</sup>. The coordinated movement of zoospores is also studied by mimicking *in vitro* the rhizospheric environment. Cellular and molecular approaches taken together with experimental physics indicate that the perception of a K<sup>+</sup> gradient induced a sequence of negative chemotaxis and bioconvection leading to zoospore aggregation. Another issue concerns the identification (16S rRNA gene sequencing) and the role (in vitro screening) of the prokaryotic rhizospheric microbiota, which both interacts with *P. parasitica* on the root, and interferes with the infectious cycle when a multi-species biofilm is constituted. The results suggest a Bacteroidetes/Proteobacteria transition associated with the presence of the oomycete. They indicate cooperation between the oomycete and opportunistic *Pseudomonas* species, via bacterial preferential adhesion to the oomycete surface, and then extension of the bacterial habitat to host plant tissues<sup>d</sup>.

a. Larousse M & Galiana E. PLoS Pathog 2017, 13(1):e1006028

b. Larousse M et al. Protist. 2014, 165:275-92

c. Galiana et al. bioRxiv 2018, 470864

d. Larousse M et al. Microbiome 2017, 16:5(1):56

## 4.2: Aggregation molecular pathways in *Phytophthora parasitica* zoospores

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This study proposes a novel view of the transcriptome of *Phytophthora* zoospores, focusing on the perception of external stimuli and the ionic gradient-mediated aggregation. Gathering of unicellular zoospores on the host surface results from the perception of signals emitted by plants or other zoospores, through detection of molecular gradients (chemotaxis) and ionic fields (electrotaxis). The mechanisms underlying intercellular communication and subsequent attraction, adhesion and multicellular behaviour are unknown. Recent studies conducted in our labs demonstrated that in *P. parasitica* the perception of a K<sup>+</sup> gradient induced coordinate motion and rapid aggregation, as result of a succession of negative chemotaxis and bioconvection. Using this model, we combined RNAseq analyses with live cell imaging and ultrastructural microscopy to propose a first definition of molecular mechanisms involved in signal perception and transduction, leading to oomycetes-directed movement and aggregation. Firstly, the RNAseq analysis defined the transcriptome of zoospores freely swimming in water, to identify sequences related to motion, perception, adhesion and pathogenesis. Moreover, it revealed that the K<sup>+</sup> gradient induced significant upregulation (up to 30-fold) of gene expression for pathogenesis, vesicular trafficking and pH homeostasis. Enzyme kinetics and pH imaging data converged toward the hypothesis of an association between membrane-bound/cytosolic upregulated enzymes playing a core role on pH balance and consequently on coordinate motion and aggregation. Finally, ultrastructural analyses showed the accumulation of a still uncharacterized intercellular filamentous matrix, likely comprising fibronectin-like proteins. Collectively, these data led us to propose a molecular model of the origin of multicellular behaviour, within oomycetes-producing zoospores.

### S4.3: Functional analysis of Argonaute3 in *Phytophthora parasitica*

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Oomycetes represent a unique group of plant pathogens, which cause severe damage to a wide range of crops and natural ecosystem. It is of importance to investigate the pathogenesis mechanism which facilitate the development of novel strategies for disease resistance. Non-coding small RNA has recently rapidly emerged as a novel class of effector, participating in the process of plant–microbe interactions. However, the biogenesis and regulation mechanisms of sRNA in *Phytophthora* are largely unknown. In this study, with the utilization of CRISPR-Cas9 system, we have knocked out the *PpAGO3*, one key element of the RNA-induced gene silencing complex (RISC) in *P. parasitica*, and obtained eleven fragment deleted *PpAGO3* mutants. Compared with the wild type strain, *PpAGO3* mutants exhibit much vigorous pathogenicity and insensitivity to abiotic stress. Combining with small RNA sequencing and RNA transcription sequencing of *PpAGO3* mutants, we found specific small RNA species change dramatically, which accompanied with the differential alternation of the target genes. Taken together, we speculate that *PpAGO3* plays a key role in the pathogenicity process and might function in the RNA silencing pathway. Results are presented to decipher the regulating mechanism as well as its biological significance for novel crop disease resistance.

## **S4.4: Differential nuclear dynamics underpin hyphal network organisation in a plant-pathogenic oomycete**

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Oomycete infections of plants involve coordinated pathogen development steps such as cyst germination, appressorium formation as well as the switch from biotrophic to necrotrophic growth. How nuclear-controlled processes are coordinated in multinucleate oomycetes remains to be fully understood. We investigated oomycete nuclear dynamics of the cocoa killer *Phytophthora palmivora* during plant infection as well as in axenic culture using time-lapse imaging. We observed coordinated bi-directional nuclear movements during plant infection and changes in nuclear dynamics upon appressorium development. During mycelial growth, active and passive movements achieve a near-equal distribution of nuclei along hyphae. This notably involves nuclei moving against the main cytoplasmic flow direction or into hyphal side branches. Such nuclei often change their shape, from near-globular up to extensively stretched. Interestingly, centrosome-labelling Centrin2 protein localizes at the stretched end of actively moving nuclei. This is supporting the hypothesis that astral microtubule-guided movements contribute to nuclear distribution within hyphae. Taken together, these results shed light on oomycete nuclear dynamics and provide a basis for computational modelling of their movement within branched hyphal networks.

## S4.5: Live cell imaging in *Phytophthora*; visualizing cytoskeleton dynamics in oomycete pathogens

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The cytoskeleton is a dynamic, well organized, intracellular network that is essential for proper functioning of eukaryotic cells. To visualize the oomycete actin and tubulin cytoskeleton by live cell imaging we generated *Phytophthora* transformants expressing Lifeact-eGFP and GFP- $\alpha$ -tubulin, respectively. As reported previously (1,2) we observed three unique actin configurations: (i) actin plaques, immobile, long-lived structures, (ii) an actin configuration associated with plug deposition in hyphae emerging from cysts to seal off the cytoplasm-depleted base after cytoplasm retraction towards the growing tip; and (iii) an actin aster in appressoria that is formed at the contact point with the underlying surface suggesting a role for plant cell penetration. We now extended our analyses by visualizing the actin cytoskeleton in protoplasts and observed several actin configurations distinct from those found previously. In regenerating protoplasts, polarity and normal actin organization was re-established within 24 hours, while cell wall formation was disturbed for at least 48 hours after protoplasting. In transformants expressing GFP- $\alpha$ -tubulin we were able to observe the dynamics of microtubules during nuclear division and in the cytoplasm radiating from microtubule organizing centers. The data presented here provide a better understanding of the structure and functioning of the *Phytophthora* cytoskeleton. The long-term goal is to uncover oomycete or *Phytophthora* specific features in the cytoskeleton that might be instrumental for drug design.

1. Meijer et al. 2014 Cell Microbiol 16, 948-962

2. Kots et al. 2016 Cell Mol Life Sci 74, 909-920

## S4.6: The role of *Phytophthora infestans* transglutaminases in appressoria formation, pathogenicity and PAMP-Triggered Immunity in potato

**Maja Brus-Szkalej**<sup>1</sup>, Ramesh Vetukuri<sup>1</sup>, Marit Lenman<sup>1</sup>, Laith Ibrahim Moushib<sup>2</sup>, Erik Alexandersson<sup>1</sup>, Hadis Mostafanezhad<sup>1</sup>, Erik Andreasson<sup>1</sup> and Laura Grenville-Briggs<sup>1</sup>

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Despite tremendous research efforts potato late blight caused by *Phytophthora infestans* remains one of the most damaging plant diseases worldwide. Our approach to the problem is to study the morphology and development of the pathogen in search of possible new targets for next generation chemical pesticides. Through large-scale proteomics study of *P. infestans* life cycle stages we identified several cell wall proteins as highly expressed during appressorium formation and during initial contact between *P. infestans* and the potato leaf (early infection). One such protein is a cell wall transglutaminase containing the Pep13 motif. Pep13 acts as a Pathogen Associated Molecular Pattern (PAMP) and an elicitor of plant defence responses. We have investigated the role of Pep13-containing transglutaminases in the development and pathogenicity of *P. infestans* and tested if *in planta* expression of the peptide enhances potato resistance to late blight. We show that Pep13-containing transglutaminases are necessary for cyst germination, formation of healthy appressoria and pathogenicity. Expression of Pep13 *in planta* reduces the severity of disease symptoms both in the controlled laboratory conditions and in field trials. Microarray gene expression analysis gives us insight into plant defence and stress responses associated with the expression of Pep13 in the field environment.



## S4.7: The use of Lab-on-a-Chip devices to study the invasive growth of oomycetes

**Ayelen Tayaqui**<sup>1,3</sup>, Yiling Sun<sup>2,3</sup>, Volker Nock<sup>2,3</sup>, Ashley Garrill<sup>1,3</sup>

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To better understand protrusive force generation and its role in the invasive growth of oomycetes, Lab-on-a-Chip (LOC) platforms have been fabricated from PDMS that enable the measurement of microNewton forces exerted at the tips of individual hyphae of both *Achlya* and *Phytophthora*. On the LOC platforms, single hyphae that emanate from a mycelial plug are grown down narrow channels that contain elastomeric micropillars. These act as force sensors as the hyphae grow into them, enabling both the magnitude and directionality of force to be measured as a function of time. The optical characteristics of the platforms allow concurrent force measurement and imaging of cellular components, such as the cytoskeleton. Inlet channels facilitate the addition of cytoskeletal inhibitors. In addition, it is possible to fill the growth channels with agar media, thereby invoking invasive growth conditions. LOC platforms have also been designed that can trap individual oomycete zoospores. Zoospores germinate in these traps and the individual germlings grow down channels that run out from them. Elastomeric micropillars in the channels have enabled the measurement of protrusive forces at the tips of these germlings. Our work designing and fabricating the LOC platforms will be described, along with our experiences growing both *Achlya* and *Phytophthora* on them, the measurement of force and our observations of the cytoskeleton. The potential of LOC devices for future research on oomycetes will also be discussed.

## Session 5: Oomycete Genomes

### S5.1: Comparative Analysis of Oomycete Genome Evolution using the Oomycete Gene Order Browser (GOB)

**Jamie McGowan**<sup>1</sup>, Kevin P. Byrne<sup>2</sup>, David A. Fitzpatrick<sup>1</sup>

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Despite the threat they pose to worldwide food security and natural ecosystems, there is a lack of tools available to study oomycete genetics and evolution. To this end, we have developed the Oomycete Gene Order Browser (GOB), a database that facilitates comparative genomic and syntenic analyses of oomycete species. GOB incorporates genomic data for 20 oomycete species including functional annotations and a number of bioinformatics tools. Here, we present the structure and function of GOB as well as a number of comparative genomic analyses we have performed.

We analyse the extent of oomycete gene duplication and identify tandem gene duplication as a driving force of the expansion of secreted oomycete genes. We identify core genes that are present and microsyntenically conserved (syntenologs) and determine the degree of microsynteny between species housed in GOB. Consistent with previous synteny analyses between a small number of oomycete species, our results reveal an extensive degree of microsyntenic conservation amongst genes with housekeeping functions. We also carried out a phylostratigraphic analysis to estimate the age and emergence of all 319,881 oomycete genes hosted in GOB. In total for the 20 species, 104,662 genes (32.7%) were placed at the origin of cellular organisms, 92,218 genes (28.8%) were eukaryotic in origin, 5,355 genes (1.7%) arose in the stramenopiles, and 65,015 genes (20.3%) arose in the oomycetes. The remaining genes were assigned as unique to particular oomycete lineages, 26,090 (8.2%) of which were determined to be unique to individual species (orphan genes).

GOB is available at <https://gob.ie>.

## **S5.2: Using cutting-edge genomics tools to study host-microbe interactions**

**Rays H.Y. Jiang<sup>1</sup>**

<sup>1</sup>Global and Planetary Health, University of South Florida, Tampa, FL, USA

Microbes are intimately associated with plant health, human health and environmental health. My current department at University of South Florida, USA, has transitioned from Global Health, into Global and Planetary health, to emphasize and address the urgent problem of sustainability of human population. My lab is using three complimentary approaches to study eukaryotic (parasitic protists) and prokaryotic (animal gut microbiota) roles in human host interactions, i.e., with 1) bioengineering, 2) cutting-edge genomics, 3) and evolutionary genetics. With bioengineering, we are developing micro-engineering technologies to recapitulate microbe-host interaction systems in miniaturized and parallelized systems in the laboratory. With novel genomics tools, we are implementing genomics tools for both real-time monitoring pathogen populations, and long-term capturing evolutionary dynamics of pathogen-host interactions. Finally, we are actively developing single cell omics technologies, powered by advanced genomics computation such as Machine Learning (ML), to unravel host-microbe interactions at single cell resolutions. Our goal is to understand and exploit microbial impact of host nutritional harvests and defense responses.

### **S5.3: New insights into mycoparasitism and microbial defence in oomycete-oomycete interactions revealed through comparative genomics and microbiome sequencing**

Dong Liang<sup>1</sup>, Charlène Faure<sup>2</sup>, Ramesh Vetukuri<sup>1</sup>, Christian B. Andersen<sup>1</sup>, Maja Brus-Szkalej<sup>1</sup>, Cassidy Million<sup>1</sup>, Dalong Dou<sup>3</sup>, Elodie Gaulin<sup>2</sup>, Bernard Dumas<sup>2</sup>, Thomas Rey<sup>4</sup>, **Laura Grenville-Briggs Didymus**<sup>1</sup>

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*Pythium oligandrum* and *Pythium periplocum* are mycoparasitic oomycetes with potential as biocontrol agents. Traits important for successful mycoparasitism are being revealed by comparative genomic and transcriptomic analyses of the hyper-aggressive mycoparasite *P. oligandrum* versus the weaker mycoparasite *P. periplocum* on oomycete and fungal hosts. These genomes lack the typical 2-speed architecture of their phytopathogenic counterparts. The CAZyome of these species are expanded compared to phytopathogens and several subfamilies of cell wall degrading enzymes are rapidly evolving indicating their importance for mycoparasitism. The presence of a novel family of GH46 enzymes may be an indication of horizontal gene transfer from bacteria. ABCb and ABCc transporter subfamilies are undergoing expansion in the stronger mycoparasite *P. oligandrum* in a strikingly similar manner to mycoparasitic fungi. Thus, there are many features of the mycoparasitic oomycetes that are distinct from the phytopathogens. Dual interaction transcriptomics reveals that the plant pathogen *Phytophthora infestans* expresses RxLRs, elicitors and protease inhibitors to defend against mycoparasitic attack by *P. oligandrum*. Microbiome sequencing reveals the effects of *P. oligandrum* on soil health. Field application of *P. oligandrum* can affect changes within bacterial and fungal communities in the potato rhizosphere, an important consideration for the ecology and application of this species as a biocontrol agent.

## S5.4: Comparative transcriptomics of a saprotrophic and several pathogenic oomycetes identifies lifestyle-specific gene expression patterns

**Sophie de Vries**<sup>1</sup>, Jan de Vries<sup>1,2</sup>, John M. Archibald<sup>1</sup>, Claudio H. Slamovits<sup>1</sup>

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Oomycetes are best known as pathogens. The diversity of oomycetes, however, encompasses non-pathogenic species with a saprotrophic lifestyle too. Oomycete saprotrophs are not only suggested to be important decomposers but also relevant partners for hybridization with pathogens, potentially facilitating host jumps. Yet molecular biological data on saprotrophy in oomycetes is sparse. We, therefore, established *Salisapilia sapeloensis* as an axenic saprotrophic system for oomycetes and gathered global gene expression data from three conditions. We show that *S. sapeloensis* responds to axenic litter with a degradation toolkit that includes CAZyme-encoding genes. Using comparative transcriptomics with eight oomycete plant pathogens colonizing different hosts and different tissues and using different pathogenic lifestyles (biotrophy, hemibiotrophy, necrotrophy) we identified fundamental differences in gene expression between the saprotroph and all pathogens. For example, CAZymes are important for the colonization of both dead and living tissues, yet, *S. sapeloensis* expresses and induces a distinct repertoire compared to the pathogenic oomycetes. Albeit only a few, *S. sapeloensis* expresses virulence-associated genes, such as *EpiC2B*, *CBEL* and *Elicitin-like* orthologs, as well as several putative RxLR-encoding and Small Secreted Protein-encoding genes. This opens the possibility that *S. sapeloensis* could - under certain environmental conditions - act as an opportunistic pathogen. This is corroborated by discordant trends in the induction of the virulence-associated orthologous genes upon infection of living and colonization of dead tissue in the pathogens and the saprotroph. Hence, a pathogen and a saprotroph may have a similar genetic potential but rely on different or altered gene regulatory networks to facilitate their lifestyle.

## S5.5: Profiling the epigenome of *Phytophthora* species provides insight into genome regulation

Han Chen<sup>1</sup>, Haidong Shu<sup>1</sup>, Liyuan Wang<sup>1</sup>, Fan Zhang<sup>1</sup>, Xi Li<sup>1</sup>, Hongyu Ma<sup>1</sup>, Yufeng Wu<sup>2</sup>,  
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Oomycetes, a distinct group of filamentous eukaryotes, include many notorious animal and plant pathogens such as *Phytophthora infestans* - the causal agent of Irish potato famine in mid-nineteenth century. Genome sequencing of oomycetes uncovers complicated genome architecture with gene sparse, repeat-rich compartments serving as a cradle for adaptive evolution. Increasing evidence suggests epigenetic regulation plays an important role in rapid adaptability of the pathogen to host plants. However, the epigenomes and their functions in oomycetes remain largely unknown. Here, we provide evidence that DNA adenine N6-methylation (6mA) and histone H3 modifications (H3K4me3, H3K9me3, H3K27me3, H3K36me3) are present in two *Phytophthora* species. Methylated DNA Immunoprecipitation Sequencing (MeDIP-seq) and Chromatin Immunoprecipitation Sequencing (ChIP-seq) are conducted to profile the epigenetic landscapes of *Phytophthora* genomes. Those epigenetic marks are clearly located in distinct genomic compartments, implicating the potential roles of the epigenome in pathogen adaptive evolution. Mapping 6mA, H3K9me3 and H3K27me3 across the genomes reveals potential roles of regulating transposon activity and maintaining chromosome stability. These epigenomic data may provide new insight into pathogen genome regulation.

## Special Session: Celebration of OMGN 20<sup>th</sup> Annual Meeting

### SS.1: Oomycete molecular genetics - a multi-decadal vision

**Brett Tyler**<sup>1</sup>

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Since 1845, *Phytophthora* species have epitomized the ferocious destructiveness and astonishing adaptivity of oomycete pathogens. By the mid-1980s, it was clear that the powerful tools of molecular genetics that had been developed in model fungi had great potential to tear away the veil of mystery from these difficult organisms. The early realization that oomycetes should be approached as evolutionarily distinct organisms, rather than as a kind of fungi, led to breakthroughs in DNA transformation and genetic mapping that laid the groundwork for the move into genomics. In 1997, oomycete genomics efforts in the US, Canada and Europe coalesced into the *Phytophthora* Genome Initiative (PGI), aimed at securing funding for genome sequencing. The PGI transformed into the *Phytophthora* Molecular Genetics Network and then the Oomycete Molecular Genetics Network through successive rounds of NSF funding. The community cooperation of the PGI and its successors enabled oomycete researchers to gain early access to large scale government funding that resulted in the sequencing and comparative analysis of numerous oomycete genomes. The sharing of these genomics resources across the community totally revolutionized our understanding of oomycetes and propelled oomycetes to the very cutting edge of molecular plant pathology. Numerous exciting questions in oomycete biology nevertheless remain to be tackled including how oomycetes integrate into plant microbiomes, the genetic and epigenetic bases for phenotypic plasticity, and how to establish truly durable resistance against the most destructive oomycete crop and forest pathogens. Solving these challenges will require the same commitments to aggressive innovation and community cooperation that have brought the field to its current point.

## SS.2: Downy mildews – difficult microbes

**Richard Michelmore<sup>1</sup>**

<sup>1</sup>The Genome Center, University of California, Davis, USA

Downy mildews (DMs) are some of the most spectacular diseases but because of their biotrophic nature, the characterization of the causal organisms has lagged behind that of culturable oomycetes. Several studies on DMs have led to discoveries generally relevant to oomycetes, others have been specific to DMs. Studies on tropical DMs in the 1960s were foundational to the realization that unlike many haploid true fungi, oomycetes were diploid. Studies initiated in the 1970s established lettuce DM as one of the genetically best-characterized gene-for-gene plant-pathogen interactions. Early unsuccessful attempts to transform *Bremia lactucae* provided vectors that have been subsequently used to successfully transform many *Phytophthora* species. Studies of Arabidopsis DM have revealed much about effector – receptor interactions and downstream molecular events leading to resistance. Genomic approaches are enabling dissection of DMs with unprecedented resolution. It is now feasible to generate chromosome-scale, whole genome assemblies of multiple isolates within a species; although assembly of highly heterozygous genomes as found in some Peronosporaceae species and generation of pan-genomes remain challenging. Recent studies of *B. lactucae* revealed the evolutionary potential of heterokaryosis. Several technical challenges remain including the development of transformation and editing technologies. Multiple major advances can be expected soon, including understanding the basis of biotrophy that could lead to axenic culture of DMs, determination of the number of times biotrophy evolved within the Peronosporales, and understanding of the molecular basis of mating type that I started working on over 40 years ago.



## SS.3: Networks work!

**Francine Govers<sup>1</sup>**

<sup>1</sup>Laboratory of Phytopathology, Wageningen University, Wageningen, the Netherlands

Musing about the **past, present** and **future** of the Oomycete Molecular Genetics Network brings up memories to my first encounters with Molecular Phytopathology, the fifth IS-MPMI meeting in 1990 in Interlaken, Switzerland. I came home with the impression that cloning of the first oomycete *Avr* gene was close; flanking RFLP markers were identified and it was just a matter of 'walking along the chromosome' to locate the gene. At that same meeting I learned that the HAM promoter is the one to use for expressing antibiotic resistance genes in *Phytophthora*. This promoter drives transcription of a 'highly abundant messenger' in *Bremia lactucae* and is still the most widely used promoter in *Phytophthora* transformation constructs. I took a leap of faith and embraced *Phytophthora* as research topic. Soon I experienced the many challenges phytophthorologists are facing and that is still the case up to the present day. Thanks to the hard work of many bright PhD students, post-doc's and researchers worldwide, and to local, national, and international networks we, as oomycete community, made substantial progress in unravelling complex networks that govern the biology, pathology and ecology of oomycetes. Yet, what we know is only a tip of the iceberg and there is still a lot to explore for future generations. In this presentation I will dwell upon our research on molecular, cellular and metabolic networks in *Phytophthora*. These networks work, but how?

## SS.4: Oomycetes - a genomicist's dream

**Sophien Kamoun<sup>1</sup>**

<sup>1</sup>The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, United Kingdom

*Phytophthora* and other oomycetes were long viewed as a “geneticist’s nightmare”. This has all changed with the advent of genome sequencing, and it’s fair to say that they have now turned into a “genomicist’s dream”. Genomics was truly the “end of the beginning” for oomycete research. Here, I will describe how genome sequences of oomycete pathogens have led to new general concepts on genome architecture and evolution, and how they have also served as an extraordinary source of scientific hypotheses. I will focus on three themes that have stemmed from the genome sequences: effectors, pathogen/host interfaces, and immunoreceptors. In each case, I will highlight concepts that originated from oomycete research and describe major unanswered questions.

## SS.5: Progress in oomycete research across two millennia

**Stephen Whisson<sup>1</sup>**

<sup>1</sup>The James Hutton Institute, Invergowrie, Dundee, United Kingdom

What was happening in the oomycete research community circa late 1980s - early 1990s when I first began working with *Phytophthora megasperma* f.sp. *glycinea* (now called *P. sojae*)? Restriction fragment length polymorphism markers were being used to assess diversity within and between species, avirulence loci were being identified in *Bremia lactucae*, and a handful of genes had been sequenced from oomycetes. Critically for work that would follow in the next quarter of a century, stable transformation of *Phytophthora* had been reported. From the early 2000s, huge strides have been made in bringing the oomycetes to the forefront of research into plant-pathogen interactions. This has been underpinned by genome sequencing, bioinformatic analyses, transcriptome analyses, and effector discovery. The roles of numerous genes in oomycete lifecycle stages and pathogenicity have now been demonstrated, often revealed through the technically challenging process of transformation and gene silencing. Emerging from many of these studies has been the diversity of ways in which the oomycetes have evolved innovative biology. Although there have been many important discoveries in oomycete biology, there remain many areas where there are opportunities to further develop our knowledge of these fascinating organisms, including, as examples: oomycete nutrient uptake and metabolism, cell biology, the host pathogen interface (especially haustoria), and cross kingdom trafficking of pathogen molecules.

## SS.6: Exploring oomycete biology: from genes to genomes and beyond

**Howard S. Judelson<sup>1</sup>**

<sup>1</sup>Department of Microbiology and Plant Pathology, University of California, Riverside USA

For decades, oomycetes were perceived as challenging if not intractable systems for molecular research of their biology. Starting in the 1980's, the characterization of the first oomycete genes led to the development of vectors and methods for DNA-mediated transformation, which enabled several species to emerge as models for studies of growth, development, and pathology. Approaches for discovering genes that are differentially-expressed during growth, development, and pathogenesis evolved from inefficient methods such as subtraction cloning to microarray and then RNA-seq technologies. Genome mining has also identified many genes for study, although we are still at a relatively early stage in understanding how sequence diversity within a species translates to phenotype. Many genes have now been tested for function, mostly by homology-based silencing in *Phytophthora*. However, our knowledge of how silencing occurs is limited and potential artifacts in silencing experiments have often been ignored. CRISPR presents a promising alternative for functional genomics, but has not yet succeeded in some species. Despite ongoing challenges, there are many opportunities for young scientists interested in studying the biology of oomycete biology.

## Poster Session 1: Wednesday July 10<sup>th</sup>

### **P1.1: Development of molecular markers to explore population dynamics of *Bremia lactucae* (lettuce downy mildew)**

**Charlotte B. Acharya**<sup>1</sup>, Melodie C. Najarro<sup>1</sup>, Kyle Fletcher<sup>1</sup>, and Richard W. Michelmore<sup>1</sup>

<sup>1</sup>The Genome Center, University of California, Davis, CA, U.S.A.

*Bremia lactucae* (Regel), the obligate biotrophic oomycete that causes downy mildew in lettuce, causes significant yield losses worldwide. Most lettuce programs are actively breeding for resistance to *B. lactucae*, but the variability of *B. lactucae* makes creating cultivars with durable resistance to *B. lactucae* challenging. We continually collect and phenotype isolates of *B. lactucae* to monitor changes in virulence in the Western US; however, this method of characterization is too labor intensive and slow to enable interventions in real time. We are currently developing molecular markers to genotype individual lesions from the field prior to culturing and phenotyping. Polymorphic simple sequence repeats (SSRs) have been identified using the largest scaffolds of the reference genome of SF5 and whole genome sequences from diverse isolates. Dye labeled, multiplex PCR is being used to quickly genotype isolates. We have so far characterized over 40 unique genotypes and are currently implementing a workflow to genotype numerous lesions within a week and then to selectively phenotype representative isolates. This will enable the characterization of large numbers of isolates and understanding of the diversity of populations within and between fields. Oligonucleotide primers for PCR amplification of these markers will be made available as a standard set for analysis of isolates worldwide.

## P1.2: Novel *Pythium* species isolated from Scottish fish farms

**Aisha Al-Shatti**<sup>1,2</sup>, Marcia Saraiva<sup>1</sup>, Pieter van West<sup>1</sup>

<sup>1</sup>Aberdeen Oomycete Laboratory, International Centre for Aquaculture Research and Development  
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<sup>2</sup>School of Biological Science, University of Aberdeen

Oomycetes have a significant impact across aquaculture, agriculture and horticulture sectors in terms of both quality and yield. These organisms are causing many waterborne diseases and are in general hard to control. One important order within the oomycetes is the Pythiaceae. It represents one of the largest orders in the oomycetes with genera included such as *Phytophthora* and *Pythium*. These pathogens tend to infect plants mostly. However, some *Pythiums* are animal or algal pathogens and some are mycoparasites. Morphologically, *Pythium* species have branching coenocytic hyaline mycelia and oogonia with thick walled oospores. Most plant pathogenic *Pythium* species are associated with pre and postemergence damping off, seed decay and stem rot. Wet environments provide ideal conditions for the development of these oomycetes and they grow among variable host range and temperature requirements. In the current project we have isolated novel *Pythium* species from infected salmon and water sources in fish farms. At this stage we are unsure if *Pythium* was the causal agent of infections on salmon or whether it was associated with a *Saprolegnia* infection. In total we have isolated 15 novel species in the farms that we are characterising and describing in detail. We have sequenced the internal transcribed spacer (ITS) regions of the rRNA and mitochondrial cytochrome c oxidase subunits 1 (Cox1) and 2 (Cox2) to place the new species in a phylogenetic tree. Furthermore, detailed microscopic observations of important stages in the life cycle of these oomycetes are in progress. Here we report on our latest findings.

### **P1.3: Molecular mechanisms of mycoparasitism, and ecological risk assessment of the biological control agent *Pythium oligandrum* against oomycete and fungal plant diseases**

**Christian B. Andersen**<sup>1</sup>, Dong Liang<sup>1</sup>, Maja Brus-Szkalej<sup>1</sup>, Ramesh R. Vetukuri<sup>1</sup>, Cassidy Million<sup>1</sup>, Åsa Lankinen<sup>1</sup>, Laura Grenville-Briggs Didymus<sup>1</sup>

<sup>1</sup>Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

The biocontrol agent *Pythium oligandrum* has proven antagonistic effects on a wide array of plant pathogens. To better formulate and use *P. oligandrum* as a biocontrol agent requires a deeper understanding of the molecular mechanisms of the mycoparasitic interaction of *P. oligandrum* and devastating plant pathogens such as *Phytophthora infestans*. Transcripts from *P. oligandrum* with predicted functions in carbohydrate binding, elicitor and lectin activity (putative CBEL proteins) are highly abundant during interactions with *P. infestans* as a host. *P. oligandrum* transcripts encoding a large number of putative cell wall degrading enzymes (CWDEs) are also highly abundant at the onset of mycoparasitism. Sensing of host cell wall carbohydrates by *P. oligandrum* may thus be important for initiation of mycoparasitism. We will utilize a transient silencing (RNAi) protocol recently developed in the lab for *P. oligandrum* and silence CBEL and the most highly expressed cell wall degrading enzymes produced by *P. oligandrum* when parasitizing diverse hosts to elucidate the molecular function of these genes. Synthetic pesticides impact soil health and ecosystem biodiversity, however little is known of the impact of biological control agents in open field agricultural systems. Thus, we are currently assessing the impact of *P. oligandrum* on the local microbiota in potato agroecosystems. Microbiome sequencing reveals that *P. oligandrum* can affect changes in the potato rhizosphere microbiome. Detailed analysis will be carried out to estimate the effects of these changes on soil and plant health.

## P1.4: Uncovering host adaptation of *Phytophthora cactorum* to strawberry and apple

**Andrew Amitage**<sup>1</sup>, Charlotte F. Nellist<sup>1</sup>, Robert J. Vickerstaff<sup>1</sup>, Maria K. Sobczyk<sup>1</sup>,  
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*Phytophthora cactorum* is an Oomycete pathogen of global significance, causing disease on over 200 host crops. In strawberry, *P. cactorum* causes crown rot leading to crop losses of up to 40% in infected fields. Phasing out of chemical fumigants is leading to increased disease pressure from soil borne diseases such as crown rot, necessitating the integration of natural resistance into new strawberry cultivars.

We investigated the basis of *P. cactorum* pathogenicity on strawberry, through genome sequencing and comparative genomics at the species (*P. cactorum* vs *P. idaei*), pathotype (strawberry vs apple) and population (within strawberry crown rot) levels. Whole genome phylogenetics showed resolution between crown rot and apple infecting isolates, providing genomic support for discrete lineages within *P. cactorum*. *P. cactorum* showed low sequence diversity within and between strawberry crown rot isolates, despite an international panel of isolates being sequenced. We further identified the gain and loss of gene complements across the *P. cactorum* phylogeny including apoplastic and cytoplasmic effectors. This identified gains and losses of previously characterised and novel effector candidates, representing putative determinants of host boundaries. Transcriptome analysis has given insight into key effectors during strawberry infection. Functional validation of these candidates, alongside integration of this information with identified QTL for *P. cactorum* resistance will lead to improved disease resistance in strawberry.



## P1.5: Plant-pathogen interaction – proteomics and metabolomics analyses of *Phytophthora* infection in poplar

**Miroslav Berka**<sup>1</sup>, Miloň Dvořák<sup>2</sup>, Ivan Milenković<sup>2</sup>, Veronika Klinkovská<sup>1</sup>, Iñigo Saiz-Fernández<sup>1</sup>, Jaroslav Ďurkovič<sup>3</sup>, Břetislav Brzobohatý<sup>1</sup>, Martin Černý<sup>1</sup>

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The elucidation of molecular mechanisms of plant-pathogen interaction is instrumental in understanding the infection process and providing means of protection. Here, we employed a set of hybrid trees (*Populus tremula* × (*P. x canescens*)) and performed a trunk inoculation with *P. plurivora* or *P. cactorum*. After 12 months, we sampled sections around the infection zone and leaf material from healthy and infected trees. We analyzed proteome and metabolome and compared it with that of control plants. In total, we identified more than 2,700 and more than 3,000 proteins in leaf and trunk proteome, respectively. We found that *P. plurivora* and *P. cactorum* elicit some overlapping responses, and that the proportion of differentially abundant proteins was much higher in samples of *P. plurivora* infected poplars. The identified putative infection markers included wound-inducible and disease resistance-responsive proteins, as well as ROS-related enzymes and metabolites.

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## **P1.6: Novel receptor tyrosine kinases contribute to oomycete recognition in *C. elegans***

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*C. elegans* has proven to be a useful model for the study of innate immunity over the past two decades, particularly in the case of bacterial and fungal pathogens. These pathogens are most commonly detected by the mechanical damage and cellular stress they inflict on their host. However, whether and how *C. elegans* senses pathogens to protect from infection is poorly understood. We have previously described a new natural infection of *C. elegans* by the oomycete *M. humicola*. We have recently shown that oomycete recognition is associated to a putative cross-tissue transcriptional response, starting from neuronal recognition of the pathogen and resulting in the up-regulation of the *chitinase-like* genes in the epidermis. Using a forward genetic approach, we have begun isolating mutants that block the induction of *chil* genes in the epidermis. I will present here my results from this screen demonstrating that two unusual receptor tyrosine kinases are involved in the oomycete recognition signalling pathway.

## **P1.7: Early diverging clades of marine obligate biotrophic oomycetes: perspectives in oomycete comparative omics**

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Pedro Murúa Andrade<sup>1</sup>, Dieter Müller<sup>2</sup>, Claire M.M. Gachon<sup>1</sup>

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The marine environment harbours a high number of oomycete parasites infecting diverse photosynthetic hosts from microscopic phytoplankters to macroscopic seaweeds. For a long time, most of these oomycetes were only known from morphological descriptions in earlier taxonomic reports. The availability of NGS-powered approaches coupled to single cell studies helped unveiling a previously underestimated molecular diversity in these morphologically and ecologically similar oomycetes. This notably includes a highly diverse, early-diverged clade of obligate biotrophic parasites infecting phylogenetically distant algae (i.e. Red algae, Brown algae and Diatoms). Evolutionarily, these oomycete parasites are strikingly far from the plant pathogenic models investigated to establish the current paradigm of oomycete-host interaction and the molecular basis of oomycete pathogenicity (e.g. effectors). Therefore, genome comparisons both within this early diverged oomycete clade and throughout the phylogenetic tree of oomycetes may provide data to revise our understanding of biotrophic lifestyles and of the evolution of parasitism in oomycetes. To cope with the paucity of data available, our working group is starting to accumulate a variety of datasets aiming at addressing these evolutionary questions.

## **P1.8: Drug repositioning of disulfiram demonstrates in vitro inhibitory effect against the oomycete *Pythium insidiosum***

**Theerapong Krajaeju**<sup>1,\*</sup>, Tassanee Lohnoo<sup>2</sup>, Wanta Yingyong<sup>2</sup>, Thidarat Rujirawat<sup>2</sup>, Yothin Kumsang<sup>2</sup>, Sirin Theerawatanasirikul<sup>3</sup>, Weerayuth Kittichotirat<sup>4</sup>, Onrapak Reamtong<sup>5</sup>

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*Pythium insidiosum* is an oomycete microorganism that causes a life-threatening infectious disease, called pythiosis, in humans and animals. The disease has been increasingly reported worldwide. Conventional antifungal drugs are ineffective against *P. insidiosum*. As to treat pythiosis, extensive removal of infected tissue (i.e., eye, leg) is required, but inadequate surgery and recurrent infection are often observed. A more effective treatment is needed for pythiosis patients. Drug repurposing is a promising strategy for the identification of an FDA-approved drug that could be used for the control of *P. insidiosum*. Disulfiram (originally approved to treat alcoholism) exhibits antimicrobial activity against various pathogens. In this study, we explored whether disulfiram possesses an anti-*P. insidiosum* activity. All 27 *P. insidiosum* strains, isolated from various hosts and geographic areas, were susceptible to disulfiram, in a dose-dependent manner. MIC range of disulfiram against *P. insidiosum* (8 - 32 mg/L) was in line with that of other pathogens. Proteogenomic analysis indicated that several potential targets of disulfiram (i.e., aldehyde dehydrogenase and urease) were present in *P. insidiosum*. By homology modelling and molecular docking, disulfiram can bind the putative aldehyde dehydrogenase and urease of *P. insidiosum* at low energies (i.e., -6.1 and -4.0 Kcal/mol, respectively). Disulfiram diminished the biochemical activities of these enzymes. In conclusion, disulfiram can inhibit the growth of many pathogenic microorganisms, including *P. insidiosum*. The drug can bind and inactivate multiple proteins of *P. insidiosum*, which may contribute to its broad antimicrobial property. Disulfiram could be repurposed as a new treatment option for pythiosis.

## **P1.9: Antifungal activity of plant extracts and its induction effect of plants against pathogenic fungi and oomycetes**

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Medicinal plants, such as the world famous sweet wormwood herb, garlic, holly and citronella, are potential sources of fungicide compounds in fields, though there is a massive application in medical science. Here, the antifungal activities of oils extracted from 4 different medicinal plants were investigated. The inhibited mycelial growth and sporangia germination of 4 tested *Phytophthora capsici*, *Peronophythora litchii*, *P. sojae* and *Fusarium oxysporum* were dose-dependent and the minimum inhibitory concentration was 500-700 µg/mL. Moreover, the mycelia were distorted, especially the hyphae polarity and branching were recorded. Cytoplasm leakage and an increase of cell membrane permeability were also being recorded, evidenced by a rise in relative electric conductivity and a decrease in reducing sugar contents, suggesting there was a disruption of plasma membrane. Furthermore, 4 oil extracts all induced a systemic acquired resistance and reduced the colonization of tested pathogenic microbiology. To explore the plant pathways triggered in response to oil extracts, phenolic compounds accumulation, peroxidase activity, expression of plant defense related genes were examined. Taken together, oil extracts can be invoked as a natural alternative to commercial fungicides or a lead compound to develop new fungicides for the control of plant fungi and Oomycete diseases.

## P1.10: Secretomic analysis of three ubiquitous *Phytophthora* species threatening global forest ecosystems

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In this study, we have used mass spectrometry to characterise the secretomes of three understudied *Phytophthora* species that are an increasing threat to global forest ecosystems: *Ph. gonapodyides*, *Ph. chlamydospora* and *Ph. pseudosyringae*. Together, *Ph. gonapodyides* and *Ph. chlamydospora* represent the two most widespread *Phytophthora* species having been found in a wide range of habitats globally. *Ph. pseudosyringae* is a more destructive pathogen and has been identified as having a role in the decline of oak and beech forests across Europe and America. To date, virtually no molecular studies have been performed on these species. Here, we profile the secretomes of these three *Phytophthora* species using an LC-MS/MS strategy. Our approach allowed for the identification of large numbers of proteins secreted into different growth media. We detected a number of important effector families including necrosis-inducing proteins and elicitors, as well as a large number of CAZymes involved in the breakdown of exogenous carbohydrates. Our results provide insights into the molecular mechanisms of *Phytophthora* infection.

## **P1.11: Development of quantitative phenotyping techniques for disease resistance in brown algae with the model pathosystem *Ectocarpus* – *Anisolpidium***

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Seaweed aquaculture is growing exponentially worldwide, yet the genetic determinism and heritability of interest traits (including disease tolerance) are virtually unknown. We used the model brown alga *Ectocarpus* to develop quantitative and parallelisable assays for disease resistance, against the oomycete *Anisolpidium ectocarpii*. Overall, we combined different techniques based on host's PAM fluorometry and chlorophyll autofluorescence, pathogen's chitin fluorescence (WGA-FITC) and DNA relative concentration (qPCR) in order to check detectable changes during the infection course. We tested two strains, Ec568f and Ec32m, which previously showed to have contrasting resistance against *A. ectocarpii* AnQU67-5. Preliminary results show that PAM fluorometry proxies normally used in physiology (e.g. quantum yield) are not very resolutive to capture subtle differences on infection progress, although sigma values (estimated cross section of the PSII) are promising because of their strong variation in *Anisolpidium*-challenged *Ectocarpus*. Contrarily, we found chlorophyll and chitin stained fluorescence are excellent to track infection progress, as long as corrections for biomass (i.e. nephelometry) are performed. In a similar way, the pathogen DNA quantification needs to be weighted with the host DNA to obtain a sensitive relative abundance. In total, four of the tested proxies are applicable to the same set up in different time points, proxies that are extrapolable to other algal pathosystems. The next steps using these phenotyping tools will include the characterization the ca. 90 individuals of a Ec 568f x Ec 32m progeny and correlate their outcome with their genetic maps, in order to identify potential loci conferring resistance against *A. ectocarpii*.

## **P1.12: Comparative expression analysis of *Phytophthora sojae* polysaccharide lyase family 3 (pectate lyase) genes during infection of the soybean *Glycine max***

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It is likely that, to initiate infection, *Phytophthora sojae* uses an appressorium-like structure to break through the plant cell wall. It is also presumed that the pathogen relies on enzymatic activity to weaken the cell wall. The polysaccharide lyase (PL) enzyme superfamily, particularly the PL family 3 (PL3), has been hypothesized to play an important role in the process of host cell penetration by contributing to the degradation of the structural polysaccharides of the cell wall. To investigate this hypothesis, we have scanned the revised version of the annotated *P. sojae* genome for the presence of putative PL3-coding genes and conducted an extensive sequence analysis of all gene models found. In addition, we have quantified the relative expression of each gene in *P. sojae* during infection of susceptible (Williams) and resistant (Williams 82) soybean cultivars over a 48-hour period. Twelve PL3-coding gene models were identified and initial results indicate that during infection of the Williams cultivar, several of these genes experience significant up-regulation during the first 48 hours of infection. An increase in transcriptional activity is also observed during infection of Williams 82; however, this expression pattern is distinct from that observed in the Williams cultivar. These results provide evidence regarding the potential involvement of PL genes in the early pathogenic processes of *P. sojae* and suggest that this pathogen expresses genes differentially, depending on whether infection is occurring on a resistant or a susceptible soybean cultivar.



**P1.13: *Phytophthora aleatoria*, a newly-described species from *Pinus radiata* is distinct from *Phytophthora cactorum* isolates based on comparative genomics**

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*Phytophthora cactorum* has long been recognised as an economically and ecologically important phytopathogenic oomycete with a broad host range and disease impacts on forest trees and horticultural crops. *Phytophthora cactorum* has caused root and collar damages in forest nurseries and shelterbelts in New Zealand since the 1970's and has now been recently re-described as *Phytophthora aleatoria*. This species grow more slowly than other *Phytophthora* species associated with *Pinus radiata* in New Zealand and is morphologically similar to isolates of *P. cactorum* previously associated with domestic horticulture productions (e.g. apple). However, this species did not infect apple twigs, unlike *P. cactorum* isolates from apple. To obtain a genome-based phylogeny for robust classification, we sequenced, assembled and annotated the genome of a representative isolate of *P. aleatoria* (NZFS 4037) from *P. radiata* in New Zealand and, nine isolates of *P. cactorum* from different conifer and angiosperm hosts in Sweden, Norway and New Zealand. Comparative genomics including whole genome phylogenetic, orthology-related and effector analyses revealed NZFS 4037 was related to but distinct from the *P. cactorum* species complex. These sequences will be a useful resource in enabling comparative analyses to identify host-plant interactions, adaptation to specific hosts or ecological niches, molecular markers, and phylogenetic studies and ultimately facilitate better control strategies.

## **P1.14: *Saprolegnia parasitica*: an UV-C resistant oomycete with an efficient DNA repair mechanism**

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UV systems in aquaculture facilities are known for significantly reducing pathogen counts in incubation and rearing facilities and have proven to be the most cost-effective disinfection technology for the inactivation of many types of bacteria, viruses and parasites that are harmful to many species of fish.

The effect of UV-B and UV-C was tested on the growth of several oomycetes including *Saprolegnia parasitica* and *Saprolegnia diclina*. These two organisms are very important fish pathogens that cause saprolegniosis, which can lead to death of fish by haemodilution and also fish eggs can be killed.

In this study different oomycetes were challenged against different UV-B and UV-C exposure times. In addition, different life stages of *S. parasitica* were challenged for 3hr against UV-C representing a UV dose of 139464Ws/cm<sup>2</sup>. All life stages tested were able to cope with UV-C and were able to complete their development. Furthermore, molecular studies were conducted using *Saprolegnia parasitica* strain CBS223.65, which revealed that it has a very efficient DNA damage repair system. Gene silencing was conducted on one gene possibly implicated in the DNA repair system. Our latest results will be presented here.

## P1.15: Towards genetic transformation of *Bremia lactucae*

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The lettuce downy mildew, *Bremia lactucae*, is one of the most threatening pathogens for lettuce cultivation and production worldwide. Over the past decades, many resistance genes have been successfully used in lettuce varieties to reduce *Bremia* infection. However, these resistances were generally quickly broken by the emergence of new virulent *Bremia* races. Detailed understanding of the *Bremia*-lettuce interaction and the identification of alternative resistance mechanisms is hampered by the lack of powerful and efficient methods to image and quantify pathogen infection. Fluorescent *Bremia* strains and knock-outs would be valuable tools to overcome this. However, genetic modification of *Bremia* has not been achieved so far. Its obligate biotrophic lifestyle poses challenges for DNA introduction and particularly for selecting and maintaining transformed strains. We aim at establishing a selection method for *Bremia* that is based on the mutation of Avr genes using CRISPR/Cas9 and growth on resistant lettuce lines. For this we will use the resistance gene *Dm2* that confers resistance against *Bremia* race 5 through recognition of effector BLG03. By introducing a CRISPR/Cas9 expression cassette that is aimed at inducing loss-of-function mutations in *BLG03*, we expect to enable selection of transformants on lettuce lines carrying the corresponding *Dm2* gene. Co-transformation with additional DNA constructs is intended to make *Bremia* accessible for various genetic modifications, e.g. introduction of genes encoding fluorescent protein. We will present the progress in establishing DNA delivery into *Bremia* and will discuss the potential of CRISPR/Cas9-mediated effector targeting as an efficient selection method for obligate biotrophic plant pathogens.

## P1.16: Infection of *Nicotiana benthamiana* by *Phytophthora kernoviae* as a model system to study tree pathogens

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Species of *Phytophthora* cause some of the most serious diseases of trees. Despite genome sequence assemblies for over ten tree pathogenic *Phytophthora* species, and improved detection methods, there are many gaps in our knowledge of how these pathogens interact with their host plants. We examined whether *Phytophthora kernoviae*, a relatively recently described tree pathogen, could infect the model plant *Nicotiana benthamiana*. *P. kernoviae* can be transformed, and we used a GFP tagged line to follow infection of plants. *P. kernoviae* forms haustoria within infected *N. benthamiana* cells, and also in infected tissue of natural hosts, *Rhododendron ponticum* and European beech (*Fagus sylvatica*). We analysed the transcriptome of *P. kernoviae* in cultured mycelium, spores, and during infection of *N. benthamiana* and detected expression of 9366 genes, of which 2734 were predicted to encode secreted proteins that may function as effectors to facilitate disease development. From these, we identified 106 expressed RXLR (Arg-any amino acid-Arg-Leu) effectors likely to be translocated into plant cells to exert their effects. We transiently expressed 12 of these as GFP fusions in *N. benthamiana* leaves and demonstrated that nine enhanced disease progression significantly by *P. kernoviae*, and localised to the cytoplasm, nucleus, nucleolus and plasma membrane. Our results show that *N. benthamiana* can be used as a model host plant for studying this tree pathogen, and that the interaction involves suppression of host immune responses by RXLR effectors. These results form a starting point to expand the understanding of tree diseases caused by *Phytophthora*.

## **P1.17: Identification of plant factors that involve in the uptake of *Phytophthora parasitica* effector PpE4 into plant cells**

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RXLR effectors secreted by the oomycete pathogen, *Phytophthora parasitica*, play essential roles in facilitating pathogenicity during the plant-pathogen interactions. Numerous RXLR effectors have been identified during the last few years, however, the mechanism that controls the internalization process of RXLR effectors into plant cells is still largely unknown. Based on the expression profiling of *P. parasitica* genes during early infection, a highly upregulated RXLR effector, PpE4, has been identified. Transient expression of PpE4 in various plant species shows indistinctive cell death, indicating that PpE4 may utilize the same pathogenic strategy during infection. Electron microscopy analysis of *Arabidopsis* root cells inoculated by *P. parasitica* indicated invaginated tubular structures at the plasma membrane and increased vesicular structures within plant cytoplasm. Together with the interaction between RXLR effectors and the membrane receptor PI3P observed in previous studies, we speculate that the plant-derived endocytosis might regulate the uptake of PpE4 into plant cells. To identify the potential endocytic pathway that mediates the plant internalization of PpE4 and the plant factors that involve in this process, four chemical inhibitors that participate in various pathways of endocytosis were tested. Moreover, twelve homologous proteins in *N. benthamiana* with known endocytosis-related functions from different species were isolated and their involvement of the uptake of PpE4 into plant cells was tested. Results from this research are expected to decipher the mechanism that controls the uptake of RXLR effectors into plant cells, which will shed light on the understanding of *Phytophthora* pathogenicity.

## P1.18: The *Phytophthora sojae* effector Avh241 modulates host immunity by targeting soybean NDR1

**Bo Yang**<sup>1</sup>, Yuyin Wang<sup>1</sup>, Baodian Guo<sup>1</sup>, Haonan Wang<sup>1</sup>, Wenwu Ye<sup>1</sup>, Yan Wang<sup>1</sup>, Suomeng Dong<sup>1</sup>, Yuanchao Wang<sup>1</sup>

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*Phytophthora* pathogens secrete an arsenal of effectors to manipulate host innate immunity and thus facilitating infection. Our previous work showed that Avh241 induces cell death in plants, but also promotes the infection of *Phytophthora*, indicating that Avh241 interacts with the plant immune system via at least two different mechanisms. By using coimmunoprecipitation (Co-IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis in *Nicotiana benthamiana*, we identified the NDR1 (non-race-specific disease resistance 1) isoforms as a host target of Avh241. We showed that Avh241 interacts with *N. benthamiana* and soybean NDR1 *in vivo*. Silencing of *NDR1* in *N. benthamiana* increase plant susceptibility to *P. capsici* infection, and overexpression of *NDR1* induce resistance to *P. capsici* in *N. benthamiana*. However, silencing of *NbNDR1* does not affect the cell death triggered by Avh241, indicating that targeting of NDR1 involves the virulence function of Avh241. The molecular mechanisms underlying how Avh241 interferes with NDR1 function will be further studied.

## **P1.19: Identification and functional analysis of a conserved RxLR effector from *Phytophthora parasitica***

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*Phytophthora* pathogens deliver an arsenal of effectors, such as RxLR proteins, to manipulate plant immunity. RxLR proteins were presumed to promote infection by targeting cytoplasmic host proteins. Identification and characterization of the RxLR effectors facilitate a better understanding of the microbial pathogenesis, which could contribute to the development of novel disease control strategies. Depending on the publicly available *P. parasitica* genomes and RNA-seq data, we identified a novel conserved RxLR effector, namely *Pp18*, which is highly upregulated during early infection stage. Further analyses indicated that *Pp18* could promote *P. parasitica* infection when transiently expressed in *Nicotiana benthamiana* leaves or stably expressed in *Arabidopsis thaliana*. Consistently, *P. parasitica* transformants overexpressing *Pp18*-flag showed enhanced virulence on *N. benthamiana* leaves as compared to the wild type. *Pp18* was localized in organelle, which has vesicle-like structure and could suppress PTI. Using CoIP-LC/MS-MS approach, we have identified a potential host target protein of *Pp18*, which is MST35, a H<sub>2</sub>O<sub>2</sub> scavenging enzyme. With further analyses by Luciferase Complementation Imaging Assay (LCIA), the interaction between *Pp18* and the target protein MST35 was confirmed. Function analysis indicated MST35 participated in resistance to *Phytophthora*.

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## **P1.20: miRNA regulates plant immunity by modulating secondary siRNA generated from NLR genes**

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Small RNAs are central players of RNA silencing in eukaryotes. These short RNA molecules (20-25 nucleotides in length) repress target gene expression based on sequence complementarity. While small RNAs are well known for their essential function in regulating growth and development, recent research has revealed that they also influence plant immunity. miRNAs are considered as the master regulator of NLR genes in soybean, which can target NLR genes and induce secondary siRNAs generated from them. Here we want to investigate the role of these secondary siRNA inducers in *Phytophthora*-soybean interactions. We found three major miRNAs that target NLR genes which is miR1507c-3p, miR1510a/b-3p and miR2109-5p. Each of them can target more than 30 NLR genes. With overexpressing and silencing these miRNAs in soybean, the abundance of the secondary siRNAs generated from these NLR genes targeted by miRNAs is significantly changed. Collectively, our findings will introduce that miRNAs can induce the production of secondary siRNAs by targeting many NLR genes, and these secondary siRNAs might be the regulator of plant immunity.



## Poster Session 2: Thursday July 11<sup>th</sup>

### **P2.1: Cell wall remodelling and phlorotannin synthesis is a conserved defence response of brown algae to biotrophic oomycete invaders**

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The cell wall is the first barrier encountered by any brown algal parasite, but the molecular and physiological events associated to a pathogen-challenged brown algal cell wall have yet to be studied. We took advantage of a pathosystem between the brown alga *Ectocarpus* sp. and the intracellular oomycete parasite *Eurychasma dicksonii* and used combinations of *Ectocarpus* and *Eurychasma* strains leading to contrasting levels of infection to identify shared components of brown algal response to biotic invaders. Transcriptome data supported the major role of oxidative stress in the course of the interaction, but also provided a biological defence context to numerous uncharacterized candidate secreted proteins with domains linked to extracellular sensing and cell wall remodelling. Complementary ultrastructural and biochemical analyses of challenged *Ectocarpus* filaments suggest that phlorotannins are accumulated, secreted and crosslinked to the algal cell wall, sometimes at the site of parasite spore encystment. The mRNA spatial regulation of the main candidates for phlorotannin synthesis (Polyketide Synthase) and crosslinking (Bromoperoxidase) was investigated at the single cell level by means of single molecule Fluorescence In Situ Hybridization, thus providing a first glance on pathogen induced mRNA compartmentalization in a brown alga.

## P2.2: Double trouble, co-inoculation of potato with both the early and late blight pathogens

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The simultaneous occurrence of multiple diseases is an understudied area in plant pathology, however, studies of animal and human diseases have shown that the presence of multiple pathogens can impact the virulence, the course of disease development, and present an important driver of epidemiological dynamics. The global potato production is plagued by multiple pathogens, amongst which are *Phytophthora infestans* and *Alternaria solani*, the causal agents of potato late and early blight respectively. Both these pathogens have different nutrient acquiring strategies and are successful pathogens on potato. However, field observations of both pathogens present at the same time led us to wonder about their effect when present together.

Thus in this study, we are investigating the effects of co-inoculation and sequential inoculation of both *P. infestans* and *A. solani* on disease development in potato. The infection process is followed using confocal microscopy, measurements of lesion development, and time-resolved transcriptomics of all three organisms during early infection. We found that leaves infected with both pathogens simultaneously, developed larger necrotic lesions morphologically resembling early blight lesions, but with both pathogens present and sporulating within the lesion. Thus, we hypothesize that the necrotroph *A. solani* benefits from the presence of the hemibiotroph *P. infestans*. Transient expression studies with effectors of *P. infestans* and *A. solani*, followed by disease tests further substantiate our observed findings and indicate altered virulence phenotypes.

## P2.3: *Phytophthora* RXLR-WY effectors cooperate to modulate host vesicle trafficking

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Upon infection, pathogens secrete effector proteins to reprogram host cells and facilitate pathogenesis. Understanding how host processes are reprogrammed during pathogen invasion is key for the development of resistant crops. In this work, we studied two *Phytophthora infestans* RXLR-WY effectors, PexRD31 and PexRD54, and their cooperative effect on vesicle trafficking. While PexRD54 has an established role in modulating selective autophagy, we report that PexRD31 was able to perturb vesicle trafficking leading to an increase in endosome numbers. Transient co-expression in *Nicotiana benthamiana* showed that these two effectors co-localized in mobile punctate bodies that accumulate at haustoria during infection. *In-planta* co-immunoprecipitation confirmed this association and revealed that both effectors had shared host interactors. We hypothesize that PexRD31 and PexRD54 act cooperatively to alter vesicle trafficking during infection. Current work focuses on further characterizing the association between these two effectors and how they function in concert. By dissecting the molecular mechanisms of these virulence proteins, we hope to gain a better understanding of how host processes are reprogrammed during infection and exploit this knowledge to engineer resistance in crops.

## **P2.4: Development of molecular methods for identification of potential strains and elucidation of phylogeographic history of *Aphanomyces invadans***

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Emerging infectious diseases (EIDs) are a threat to the health and productivity of humans, wildlife and the agricultural industry. One of the most prevalent EIDs of an important global food resource, fish, is Epizootic Ulcerative Syndrome (EUS). EUS was first described from Japanese ayu in 1971, then soon after in estuarine fish in Australia, but is now known to affect over 100 species of fish, in both wild populations and aquaculture, from Asia, Australia, Africa and North America. Fish affected by EUS display red ulcers that degenerate into open wounds of infected and dying tissue. Mortality rates are considered to be high and control options in aquaculture are limited to lime and salt. EUS is caused by the Oomycete pathogen, *Aphanomyces invadans*.

Genetic research on *A. invadans* has been hampered by the difficulties in obtaining and maintaining pure isolates from fresh infected fish tissue. The goal of my research is to develop molecular methods that are able to be applied using preserved infected fish tissue samples, not just pure isolates. I aim to determine the degree of genetic diversity and structure present in *A. invadans* and ultimately conduct a global phylogeographic study of the pathogen. This approach could provide insight into the evolutionary origin of the species (was there one or several origins?), past population size changes (bottlenecks and expansions), patterns of dispersal around the world (star-like or stepping-stone?), and the presence of local adaptations or 'strains'.

## **P2.5: Understanding the innate immune response to oomycete exposure in *C. elegans***

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We have recently identified oomycetes as a new class of natural pathogens of *C. elegans* along with a novel family of conserved resistance genes, the chil (chitinase like) genes. Members of the chil gene family are induced rapidly and specifically in response to oomycete exposure, even upon treatment with heat-killed pathogen. We have produced a water-based pathogen extract that is able to trigger induction of a GFP sensor monitoring the transcription of the highly induced chil-27 gene. This extract retains its ability to induce chil-27 expression even upon autoclaving suggesting that it may reflect an oomycete-derived PAMP that is heat-stable. Using fluorescent imaging, smFISH and RT-qPCR we have analysed the spatio-temporal dynamics of chil-27 gene induction. In order to identify the exact molecule we have fractionated the extract and recovered fractions that induced chil-27, which are currently being analysed by high resolution NMR. We also observed that animals exposed to the extract were somewhat less susceptible to oomycete infection suggesting that extract could have a 'vaccine-like' effect on nematodes. Interestingly, the progeny of extract-exposed animals showed enhanced sensitivity to extract across generations. Thus, pathogen extract provides an opportunity to explore early *C. elegans* responses directly linked to pathogen recognition as opposed to host damage upon infection and unravel novel aspects of innate immune memory in animals.

## **P2.6: Population structure and genetic diversity of *Phytophthora sojae* across Ohio and Indiana**

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Phytophthora root and stem rot, a disease of soybeans caused by the soil borne oomycete pathogen *Phytophthora sojae*, is a serious yield-limiting disease across all soybean producing regions, but especially the North Central region of the United States. Previous population surveys and reports from the past five years indicate that *P. sojae* has adapted to most of the *Rps* genes currently deployed in U.S. cultivars in Ohio and the North Central region. Currently, in a survey conducted across Ohio, Indiana and Kentucky, isolates of *P. sojae* have an average pathotype complexity of 6.72 on 15 soybean differentials based on the testing of 416 isolates. The objective of this study was to characterize the genetic diversity of these isolates with 21 simple sequence repeat (SSR) markers. For a subset of 95 *P. sojae* isolates collected from different fields in Ohio and Indiana during 2016-2017 there was an average number of 3.4 alleles per locus, with the number of alleles ranging from 2 to 6. All but one locus deviated significantly from the Hardy Weinberg Equilibrium as a result of a high degree of homozygosity. Analysis revealed 93 multi-locus genotypes (MLGs), and principal coordinate analysis (PCoA) showed no distinct clustering of Ohio and Indiana fields, which indicates a high level of diversity within these populations.

## P2.7: Nanochitin enhances plant resistance against *Phytophthora* depending on the receptors CERK1 and BAK1

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Nanochitin ssuspension (NCs) is a rodlike particle with a cationic nature and high biological activities, which our previous field trial showed that NCs has a strong synergistic effect with fungicides on inhibition of tobacco root rot disease. In this work, we explored the bioactivity of NCs to *Phytophthora*, the role and mechanism of NCs-induced plant defense against *Phytophthora in planta*. The infection assay shows that NCs enhances the resistance of *Nicotiana benthamiana* and *Arabidopsis thaliana* against *Phytophthora capsici*, but fails to directly inhibit the *Phytophthora* vegetative growth in an *in vitro* antimicrobial activity assay. Moreover, we find that NCs systemically enhances phenylalanine ammonia-lyase (PAL) activity and PR gene expression in plant, indicating that NCs enhances plant resistance through systemic acquired resistance (SAR). Furthermore, NCs enhances plant resistance and ROS generation depending on CERK1 and BAK1, suggesting CERK1 and BAK1 might be crucial co-receptors in recognition of NCs *in planta*.

## P2.8: Could rice be a source of cereal rust resistance genes?

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Most plants are resistant to most plant pathogens. The plant's ability to detect and induce defense against a potential pathogen involves cell surface immune receptors and intracellular immune receptors, the nucleotide-binding, leucine-rich repeat (NLR) proteins. NLRs activate effector-triggered immunity (ETI). Heterologous expression of surface immune receptors such as Arabidopsis EFR can elevate pathogen resistance. However, NLR genes from one plant family are often non-functional in another plant family ("restricted taxonomic functionality (RTF)"). An emerging concept of NLR function is that many "sensor" NLR proteins are paired with "helper" NLRs to initiate immune signalling. Thus, RTF for one NLR may arise from a requirement for the appropriate helper or partner NLR. *Puccinia spp.* cause major losses in cereal crops, but they do not infect rice. Rice presumably evolved from a rust-susceptible progenitor grass. Genetic and functional studies investigating resistance to rust pathogens showed the involvement of post-haustorial resistance, which suggest intracellular immune receptors may play an important role in non-host resistance (NHR) of rice to cereal rust. We investigate the basis of rice NHR to cereal rusts with the goal of transferring this rust resistance into other cereals.



## P2.9: Highly contiguous genome assemblies for three *Phytophthora* species generated from PacBio sequencing

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We have sequenced and assembled the genomes for three species of *Phytophthora* currently regarded as less damaging than their close relatives. *P. europaea* was first found associated with the rhizosphere of oak trees and is closely related to the highly damaging species *P. alni*; *P. foliorum* was first found on azalea and is closely related to *P. ramorum*; and *P. obscura* was first found in association with horse chestnut and kalmia, and is closely related to *P. austrocedri*. All three genomes were sequenced to approximately 100-fold coverage using PacBio long reads and following assembly, scaffolding and polishing produced highly contiguous assemblies for all three with N50 values of 6.40 Mbp (*P. obscura*), 7.50 Mbp (*P. foliorum*) and 10.97 Mbp (*P. europaea*). Completeness of coverage estimation using BUSCO indicated a very good coverage of the gene-space of the three organisms: of 234 BUSCOs associated with stramenopiles 98 – 99% were identified as being “complete”, with only around 1% of these classed as duplicates, suggesting that a good resolution of the haplotypes has been achieved during assembly. Repeat modelling and masking indicated repeat contents of 29 – 35% and Augustus gene prediction identified between 19,441 and 19,658 possible gene models for the three species. We believe having such highly contiguous and apparently complete genome assemblies should provide a valuable resource for studying genes associated with pathogenicity in highly damaging *Phytophthora* species.

## **P2.10: Characterization of *Avr1a* /*Avr1c* locus among *Phytophthora sojae* isolates from Ohio and Iowa**

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The resistance genes *Rps1a* and *Rps1c* have been widely deployed to manage *P. sojae*, however virulence towards these two genes is quite different among populations. The effector *Avr1a* is repeated in the locus followed by *Avr1c* in the *P. sojae* reference genome. Copy number variation, presence/absence of *Avr* genes, allelic variation and transcriptional regulation have all been proposed to contribute to the genetic and phenotypic variation towards the adaptation of *P. sojae* to the deployed resistant (*Rps*) genes. Our study aimed to elucidate the effects of allelic diversity of the *Avr1a* and *Avr1c* effector genes on the pathogenicity of *P. sojae* towards soybean using molecular tools. A total of 30 isolates from Ohio and 28 from Iowa were used in this study. Among these isolates, 83% from Ohio and 85% from Iowa were virulent on *Rps1a* while, 90% of Ohio and 67% of Iowa isolates were virulent on *Rps1c*. Almost half of the Ohio population had both effector genes present but they were not expressed. A similar trend was observed for *Avr1a* in the Iowa population however, a greater number of isolates with *Avr1c* present were expressed. Interestingly, when aggressiveness was measured, the Ohio isolates exhibited longer lesions on *Rps1c* compared to the Iowa. Our data suggests that allelic variation as well as transcriptional regulation may be playing a role for *P. sojae* to infect these differentials. In order to confirm allelic diversity, long range sequencing of the *Avr1a/Avr1c* region is in progress.

## P2.11: Novel breeding tools and effective management of *Aphanomyces cochlioides* in sugar beet

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Sugar beet (*Beta vulgaris* L.) is an important root crop and is one of the main sources of sugar production worldwide. Sugar beet fields are commonly infested by soil-borne fungal pathogens which cause poor establishment and seedlings loss (Amein, 2006). The soil-borne pathogen *Aphanomyces cochlioides* is the principal agent of black rot, one of the most serious diseases in sugar beet. When severe, *Aphanomyces* root rot disease can lead to plant degeneration death and to a drastic reduction of sugar yield (Taguchi *et al.*, 2010). *A. cochlioides* is one of the most problematic pathogens due to its persistence in the soil and the lack of effective control measures (Campbell and Klotz, 2006). Sources of resistance are scarce, thus the identification and characterization of resistance genes is a major task in sugar beet breeding. The central objectives of this work are the assessment of the population structure and pathogenicity of *A. cochlioides* populations from major sugar beet producing areas such as Europe, USA and Japan, and the enhancement of the genomic knowledge of the pathogen and the responses it induces in the host. The study seeks to conduct transcriptome analysis, using next-generation sequencing techniques, with the aim to identify genes involved in the resistance to *A. cochlioides* by comparing the defense responses in resistant and susceptible breeding lines. Association mapping of more than 1200 breeding lines at MariboHillesög has revealed the presence of several significant Quantitative Trait Loci (QTLs) in the genome. Genomic locations of QTLs and candidate genes loci will be compared in order to identify the most interesting genes.

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## P2.12: Metabolomic response of *Quercus variabilis* during *Phytophthora cinnamomi* infection

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*Quercus variabilis*, one of the major tree species in warm-temperate deciduous broad-leaved forests and subtropical evergreen broad-leaved forests, is tolerant to the infection of *Phytophthora cinnamomi*, an aggressive soilborne root pathogen of woody species which causes rot of fine feeder roots. *P. cinnamomi* zoospores attack the fast-growing areas of fine roots and, afterwards, the mycelium rapidly develops in the cortical cells, phloem and xylem of the infected roots.

The chances of survival will depend on plant defense mechanisms, which consist on physical barriers and chemical defenses. The latter include not only enzymatic defenses and *de novo* synthesis of defense-related secondary metabolites, but also the synthesis and redistribution of primary metabolites. This work focuses on the changes that plant metabolome undergoes during the first 36 hours of *Phytophthora* attack, with the aim of better understanding plant-pathogen interactions at a molecular level.

The changes observed in *Q. variabilis* root metabolome during *P. cinnamomi* infection can be grouped in two stages. The early phase (4 to 10 hours after inoculation) is highlighted by an increase in sugars, which is provably driven by the pathogen hijacking plant primary metabolism to feed himself. The second stage (16 to 36 hours) showcases a decrease in sugars and most amino acids, probably as consequence of the host blocking the transport of photoassimilates to the pathogen. In the future, this study will be repeated in *Quercus suber*, a highly susceptible species, which will help us understand the key metabolic traits that define tolerance to *P. cinnamomi*.

## P2.13: Assessing the cost of induced resistance in potato

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Plants have a diverse set of defence mechanisms against pathogens. Besides the constitutive defences, induced defence and priming of defence can be achieved by plant resistance inducers (PRIs). PRIs can improve plant protection against pathogen attack by activating the plant's own defence mechanism, known as induced resistance (IR). Activating such mechanism, however, may come with a certain fitness cost that can affect plant growth or reproduction, and, therefore, food production. Here, we will report our recent findings on the cost of induced resistance in potato plants grown in the PhenoLab at the University of Copenhagen and in the National Plant Phenotyping Infrastructure (NaPPI) at the University of Helsinki, where plants were treated with two PRIs efficient against the oomycete *Phytophthora infestans*:  $\beta$ -aminobutyric acid (BABA) and potassium phosphite. Growth rate, architecture, and changes in the plant development were measured, as well as the effect of these PRIs on photosynthesis and plant health. We will also discuss variation found in the spectral composition that was captured with thermocameras, multispectral cameras, and chlorophyll fluorescence.

## **P2.14: The sequence and *de novo* assembly of the Oomycete *Haliotricida noduliformans* isolated from South African abalones**

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Abalone Tubercle Mycosis (ATM) is the leading cause of death for South African abalones. It can result in enormous losses in the aquaculture, reducing abalone population by 90%. This disease is caused by *Haliotricida noduliformans*, an oomycete belonging to the family *Haliphthoraceae*. When compared to other oomycetes, relatively little is known about *H. noduliformans*. In order to investigate the disease in detail, we decided to sequence its genome. We are pleased to present a draft reference genome of *H. noduliformans*, with an estimated genome size of approximately 73Mb. Bioinformatic analysis was based on *H. noduliformans* samples collected from abalone farms in South Africa, which were processed using *de novo* assembly with both Illumina short reads and Oxford Nanopore long reads. Here we present the latest results of the assembly process and highlight important genes that may play a role in pathogenicity.

## **P2.15: Nitrogen acquisition is involved in pathogenicity of *Phytophthora sojae* during infection**

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Pathogen nutrient acquisition is critical for successful infection and colonization, and nitrogen is one of the main nutrients acquired from the host plants. However, the nutrient requirements and metabolic pathways related to pathogenesis in oomycete pathogens are unknown. Recently, we identified 8 ammonium transporters, 16 nitrate/peptide transporters and 78 amino acid transporters from the available genome sequences of *P. sojae*, and which are significantly higher than the other tested oomycete and fungi, indicating that transporter genes of *P. sojae* undergo the obviously expansion. Moreover, the oomycete specific ammonium transporters are identified and divided into Rh group. In contrast, oligopeptide transporters were conserved in plant and fungi, but with no homologous genes in oomycete. Meanwhile, most of transporters were up-regulated in the infection process and confirmed by qRT-PCR. Especially, the content of free amino acids in infected leaves by *P. sojae* was increased, the most significant of which was glutamate, suggesting that pathogen may somehow manipulates plant metabolism to maintain and even increase the concentration of nitrogen compounds. Furthermore, silencing of a Rh ammonium transporter PsRh1 and a cationic amino acid transporter PsCAT3, reduced *P. sojae* pathogenicity on soybean plants. These results suggest that nitrogen acquisition of *P. sojae* may play an important role during infection and colonization.

**P2.16: Effector prediction and characterization in the oomycete pathogen *Bremia lactucae* reveal host-recognized WY domain proteins that lack the canonical RXLR motif**

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Identification of effectors in pathogen genomes is foundational to understanding mechanisms of pathogenesis, for monitoring field pathogen populations, and for breeding disease resistance. We identified candidate effectors from the lettuce downy mildew pathogen, *Bremia lactucae*, using comparative genomics and bioinformatics to search for the WY domain. This conserved structural element is found in *Phytophthora* effectors and some other oomycete pathogens; it has been implicated in the immune-suppressing function of these effectors as well as their recognition by host resistance proteins. We predicted 54 WY domain containing proteins in isolate SF5 of *B. lactucae* that have substantial variation in both sequence and domain architecture. These candidate effectors exhibit several characteristics of pathogen effectors, including an N-terminal signal peptide, lineage specificity, and expression during infection. Unexpectedly, only a minority of *B. lactucae* WY effectors contain the canonical N-terminal RXLR motif, which is a conserved feature in the majority of cytoplasmic effectors reported in *Phytophthora* spp. Functional analysis effectors containing WY domains revealed eleven out of 21 that triggered necrosis, which is characteristic of the immune response on wild accessions and domesticated lettuce lines containing resistance genes. Three of the immune recognized WY effectors were found to have RNA silencing suppression activity. Only two of the eleven recognized effectors contained a canonical RXLR motif, suggesting that there has been an evolutionary divergence in sequence motifs between genera; this has major consequences for robust effector prediction in oomycete pathogens.



## P2.17: The virulence and recognition of two apoplast effectors from *Phytophthora sojae*

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For successful infection, pathogens secrete lots of effectors to build a profitable environment inside or outside of the host cell. *Phytophthora sojae* is a hemibiotrophic plant pathogen parasite of soybean. Here, we identified a novel apoplast effector PsLip1 from *P. sojae*. PsLip1 is a GX SXG-motif containing secreted lipase. Transient expressing PsLip1 in *Nicotiana benthamiana* could induce local cell death and the cell death depend on PsLip1's enzyme activity but not *NbBAK1* or *NbSOBIR1*. PsLip1 inducing cell death isn't the result of recognition but could be its virulence function. So we used CRISPR/Cas9 to knock-out PsLip1 and replaced PsLip1 with enzyme lose mutant 5A from *P. sojae* genome. Both the knock-out and replaced mutants shows decreased disease symptoms. Surprisingly, all the *P. sojae* mutants totally lost the ability to hydrolyze lipids compared to the wild type. Thus, PsLip1 proved to be an important apoplast effector of *P. sojae* and is the critical player for *P. sojae* extracellular lipids hydrolysis. At the same time, we identified a novel PAMP from *Phytophthora sojae* named PsAEP1 which can be recognized by *Nicotiana benthamiana*; *NbBAK1* is required for PsAEP1's recognition.

## P2.18: Proteomics of different immune reactions in Potato leaves

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Plants have a variety of ways to defend against pathogens. A commonly used model of the plant immune system divides it into a general response triggered by pathogen-associated molecular patterns (PAMPs), and a specific response triggered by effectors. The first type of response is known as PAMP-triggered immunity (PTI) and the second as effector-triggered immunity (ETI). We have performed comparative proteomic analysis of a PTI and two different ETI models (relating to *Phytophthora infestans*) in potato. Several proteins showed higher abundance in all immune reactions, such as a protein annotated as sterol carrier protein 2 that could be interesting since *Phytophthora* species are sterol auxotrophs. Some RNA binding proteins also showed changed abundance in the different immune reactions. Furthermore, we identified some PTI-specific changes of protein abundance, such as for a DUF26 domain-containing protein, a glyoxysomal fatty acid beta-oxidation multifunctional protein and a MAR-binding protein. The proteins specifically upregulated in ETI included several catalases. Few proteins were regulated in only one of the ETI interactions. For example, histones were only downregulated in the ETI-Avr2 interaction, and a putative multiprotein bridging factor was only upregulated in the ETI-IpiO interaction. We also analyzed protein methylation. One example of a methylated protein that increased in the ETI interactions was a serine hydroxymethyltransferase. These methods and results might be used in new pre-breeding and prediction of sustainable combinations of resistance genes.

**P2.19: *Phytophthora* suppressor of RNA silencing 2 (PSR2) targets secondary small interfering RNA accumulation by interacting with Double-stranded RNA binding protein 4 (DRB4) in *Arabidopsis***

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The genus *Phytophthora* contains important plant pathogens that cause devastating diseases of crops. Each *Phytophthora* species encodes hundreds of effectors that are presumably delivered into plant cells to enhance virulence. Previously, we discovered that the soybean pathogen *P. sojae* produces effectors with RNA silencing suppression activity. In particular, *Phytophthora* suppressor of RNA silencing 2 (PSR2) specifically affects the secondary small interfering RNAs (siRNAs) when expressed in *Arabidopsis*. In order to understand the molecular basis of PSR2 function, we characterized its associating protein(s) in plants. Our results show that PSR2 interacts with Double-stranded RNA-binding protein 4 (DRB4), which has a known function in secondary siRNA biogenesis. DRB4 partners with the endonuclease Dicer-like protein 4 (DCL4) and processes long dsRNA precursors into siRNAs. Using a series of genetic and biochemical assays, we demonstrate that PSR2 interferes with the dicing process of dsRNA substrates. Furthermore, a *drb4* mutant of *Arabidopsis* phenocopies PSR2-expressing plants with a specific, narrow leaf phenotype and enhanced susceptibility to *Phytophthora*. Taken together, these findings indicate that DRB4 is a major virulence target of PSR2, which promotes infection by targeting secondary siRNAs production.

## P2.20: Characterization of metalaxyl insensitivity in *Bremia lactucae*

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*Bremia lactucae* is the major leafy oomycete pathogen of lettuce. Agrochemicals play a key role in controlling this disease because resistance genes can be rapidly overcome when deployed singly. Metalaxyl can be highly effective against *B. lactucae*; however, insensitivity has evolved as it has in other pathogenic Peronosporales and Pythiales. The mode of action of metalaxyl and the basis of insensitivity is unclear. A single nucleotide polymorphism in *Phytophthora infestans* was associated with metalaxyl insensitivity in some but not all isolates. We analyzed the segregation of metalaxyl insensitivity in 137 sexual progeny isolates from three F<sub>1</sub> and one BC<sub>1</sub> generations of *B. lactucae*. These progeny were whole genome sequenced to medium coverage. Pseudo-testcross phase-aware linkage analysis revealed candidate loci. Genome-wide scans detected copy number variants between bulks of sensitive and insensitive progeny. Genome-wide-association analysis indicated multiple regions associated with insensitivity. Analysis of these regions for candidate genes is underway.