Index:

Molecular Biology of Plant Pathogens (MBPP) Conference 2017

29th-30th March 2017
Lindisfarne Conference Centre
St Aidan's College
University of Durham

Venue: Lindisfarne Centre, St Aidan's College for talks, St Aidan's College dining hall for lunch and refreshments.
Grey College dining hall for poster session, wine reception and dinner

Day One: 29th March 2017 Arrival and Registration from 11am - Lindisfarne Conference Centre

Lunch: 12:30: St. Aidan's College

13.30: Session 1, Chair: Jack Lee

13.35: Opening Address: **Ari Sadanandom** - SUMO and plant immunity

Pathogen i

13.50: **Aron N. Horvath** – Resistance to QoI fungicides in the grape black rot pathogen, *Guignardia Bidwellii*, and related species, in the light of the *CYTB* gene structure.
14.05: **Alexandra Pintye** - Powdery mildew phenology as a driver of differentiation in generalist fungal mycoparasites
14.20: **Daniel de Vega Perez** - Molecular characterisation of Chitosan-induced priming for resistance against *Botrytis cinerea*
14.35: **Joseph D. Payne** - Integron-like elements found in *Pseudomonas syringae*.
14.50: **Gareth Thomas** - Crops and Robbers (and Beneficial Fungi); Characterisation of the Volatile Signalling from the Beneficial Soil Fungus *Trichoderma hamatum*

15:05: ***Refreshments***

15:35: Session 2, Chair: Friederike Gross-Holz

Pathogen ii

15.35: **Osman Telli** - Circadian regulations of effectors in the oomycete pathogen *Hyaloperonospora arabidopsidis***.**
15.50: **Daniela Sueldo** – Role (and inhibition) of plant apoplastic subtilases during *Pseudomonas syringae* infection
16:05 **Elspeth Ransom** - Elucidating mechanisms of plant and necrotrophic fungal interactions.
16.20: **Pierre Buscaill** - A novel natural product produced by Pseudomonas syringae pv. tomato DC3000 specifically inhibits a host-secreted β-galactosidase (BGAL) in the apoplast upon infection

16.35: **Mark Z. Nemeth** - Intracellular mycoparasitism as a biotic stress for powdery mildews: how to make intruders more visible?
16.50: **Ray Chai** - Activation of $ToxIN_{Pa}$, a Type III toxin-antitoxin/abortive infection system in the phytopathogen, *Pectobacterium atrosepticum*

Plant Pathogens and alcohol seminar
17.05: **Richard Oliver** - Building a career in plant pathology; don't collaborate, don't be multidisciplinary and don't tweet

17.30: Wine reception and poster session at Grey College

19.30: Dinner at Grey College

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Venue: Lindisfarne Centre, St Aidan's College for talks, St Aidan's College dining hall for refreshments and lunch.

Day Two: 30th March 2017 09:00: Arrival. St Aidan's College dining hall

09.15: Session 3, Chair: Helen Pennington

Host Pathogen interactions i

09.15: **Steven Spoel -** Post-translational control of plant immune signalling
09.45: **Satish Kulasekaran** - Enhancing plant disease resistance through synthetic re-engineering of ABA signalling and catabolism
10.00: **Judith K. Paulus** - Activation of the Rcr3 immune protease by secreted Ser proteases
10.15: **Baptiste Castel** - Optimize CRISPR in Arabidopsis and apply the method to investigate immunity
10.30: **Helder Pedro -** PhytoPath

11.00: ***Refreshments***

11:30: Session 4, Chair: Anjil Srivastava

Host Pathogen interactions i

11.30: **Trupti P. Gaikwad** - Investigating induction of SAR in during gene- for-gene interactions between *Arabidopsis thaliana* and *Pseudomonas syringae*
11.45: **Chris Dutton -** The Interaction between plant disease and stomatal density
12.00: **Catherine Jacott** - Disease resistance and mycorrhizal colonisation
12.15: **Stephanie Kancy** - The role of histone acetylation in plant growth and immunity

12.30: Closing remarks and next meeting venue announcement

12.40: ***Lunch and Depart***

Speaker:

Professor. Ari Sadanandom

Professor of Plant Pathology

Prof. Ari Sadanandom completed his PhD in 1999 at the John Innes Centre for Plant Science research. Progressing rapidly through two postdoctoral positions at The Sainsbury Laboratory for Plant Pathology at Norwich, He took up a lectureship position at the University of Glasgow in 2003, and then as senior lecturer at Warwick University in 2009. Ari moved to Durham at 2011 where he is currently Professor of Plant Molecular Sciences.

Ari's research group focus is on the topic of protein modification and how these modifications control how plants grow and interact with their environment. The current focus of his research is to understand how protein modifications influences how plants respond to pathogen attack. When plants are under pathogen attack, lots of processes are halted and the plant stops growth and development. This has a big impact on productivity. If we understood more about how regulatory mechanisms like protein modification influence this growth arrest, this would enable us to develop crops with better yield without compromising crop immunity.

As the academic lead of the N8 AgriFood programme in Durham, Ari oversees research projects into resilience and sustainability of food production. He is currently the director of the Durham Centre for Crop improvement Technology (DCCIT) since 2014. DCCIT is a cross-disciplinary research centre that has multiple links with Agriculture industry to develop solutions that are more effective in field conditions.

Guest Speaker:

Professor. Richard Oliver

Chief Scientist, Centre for Crop Disease Management

Prof. Richard Oliver was trained in biochemistry at Bristol University from 1976 and obtained a PhD in 1982 on the enzymology and spectroscopy of the light-activated enzyme required for chlorophyll biosynthesis. Richard then obtained an EMBO postdoctoral fellowship (1982-84) at the Carlsberg Laboratory, Denmark under Diter von Wettstein. There he learnt molecular biology techniques whilst analysing the sequence of the barley chloroplast genome. Richard next accepted a lectureship at the University of East Anglia and began a research program that developed new molecular tools to study fungal plant pathology. During the next 12 years, he established widely used technologies for molecular analysis of fungal pathogens and pioneered the use of Arabidopsis to study fungal disease resistance.

At UEA, Richard led the teaching of molecular biology and coordinated courses in plant biology, pathology and genetics. He initiated the Norwich Research Park Graduate School post-graduate taught course program and started a highly successful MSc in Plant Breeding and Genetics.

Richard was then invited in 1996 to return to Carlsberg Laboratory as the Head of Department and Professor of Physiology. He initiated large scale genomics of powdery mildew, developed transient assay techniques to test antifungal genes and expanded metabolomics analyses of infection processes. He was a member of the research board of Carlsberg Brewery. This group of five led the technical development of the world's fifth largest brewer. In 1999 Carlsberg withdrew from basic plant science research and Richard resigned to become briefly a consultant at Zeneca, specialising in bioinformatic analyses of fungal pathogens.

Subsequently he was appointed to lead the Australian Centre for Necrotrophic Fungal Pathogens (ACNFP) at Murdoch University, Australia, an initiative of the Grains Research and Development Corporation (GRDC). By 2006, the ACNFP was established as a major international centre for research in plant-fungal interactions. In 2010 he moved the ACNFP, by then a group of 25, to Curtin University. New research areas in fungicide resistance and barley mildew had been developed. Recently, Curtin and the GRDC have established a bilateral research program called the Centre for Crop Disease Management. This is a 5 year program initially with total funding of $96m. The scope includes agronomic and economic aspects of crop disease management and a new program in Sclerotinia. The group size is about 70. Richard is the Centre Chief Scientist,

reporting to the Board with a brief to oversee the science/research programs and projects, develop new research proposals, monitor research quality and to build and maintain international research linkages. Richard's primary role is to build and manage overseas collaborations and to this end he work's closely with research institutes in the UK (NIAB, Earlham, JIC, RRes, University of Nottingham and Exeter), continental Europe (Aarhus University, University of Life Sciences Oslo, Bioger) and the USA (USDA North Dakota).

Guest Speaker:

Dr. Steven Spoel

Reader in Molecular Plant Sciences
Royal Society Research Fellow

Dr. Steven Spoel did his undergraduate and MSc degree at Utrecht University, Netherlands. He then moved to Duke University (USA) where he received his PhD degree in 2008 and was subsequently awarded an *EMBO Long-Term Fellowship* and a *Netherlands Science Foundation Rubicon Fellowship*, which saw him move to the University of Edinburgh, UK. In 2010 he was awarded a 5-year *Royal Society University Research Fellowship* that allowed him to set up an independent laboratory. His lab aims to understand how upon exposure to stress, cells orchestrate dramatic reprogramming of gene expression to favour defence responses over normal cellular household functions. In 2015 he was promoted to Reader and received fellowship renewal from The Royal Society. In 2016 he became Chair of GARNet, a BBSRC-sponsored network that represents the Arabidopsis and wider plant communities in the UK.

During his career Steven has been recipient of several prestigious awards, including the *New Phytologist Tansley Medal for Excellence in Plant Science* (2010), the *Early Excellence in Science Award* from Bayer Crop Science & Healthcare (2013), and a *European Research Council Starting Grant* (2016).

Guest Speaker:

Dr Helder Pedro

Bioinformatician

Dr Helder Pedro studied Biological Engineering in Instituto Superior Tecnico in Lisbon where he concluded his masters. He then did some research on HIV sequencing in UC Berkeley with Prof. Adam Arkin.

Over the past 6 years Helder has been working on PhytoPath where he is expanding the number of fungi, oomycetes and bacterial phytopathogens genomes in Ensembl. Helder is also supporting the community manual curation of gene models using WebApollo.

Session 1:

Chair: Jack Lee

5 Talks (12 min presentation + 3 min questions)

Aron N. Horvath
RESISTANCE TO QOI FUNGICIDES IN THE GRAPE BLACK ROT PATHOGEN, *GUIGNARDIA BIDWELLII*, AND RELATED SPECIES, IN THE LIGHT OF THE *CYTB* GENE STRUCTURE

Alexandra Pintye
POWDERY MILDEW PHENOLOGY AS A DRIVER OF DIFFERENTIATION IN GENERALIST FUNGAL MYCOPARASITES

Daniel de Vega Perez
MOLECULAR CHARACTERISATION OF CHITOSAN-INDUCED PRIMING FOR RESISTANCE AGAINST *BOTRYTIS CINEREA*

Joseph D. Payne
INTEGRON-LIKE ELEMENTS FOUND IN *PSEUDOMONAS SYRINGAE*

Gareth Thomas
CROPS AND ROBBERS (AND BENEFICIAL FUNGI); CHARACTERISATION OF THE VOLATILE SIGNALLING FROM THE BENEFICIAL SOIL FUNGUS *TRICHODERMA HAMATUM*

RESISTANCE TO QoI FUNGICIDES IN THE GRAPE BLACK ROT PATHOGEN, *GUIGNARDIA BIDWELLII*, AND RELATED SPECIES, IN THE LIGHT OF THE *CYTB* GENE STRUCTURE: PRELIMINARY RESULTS

ÁRON N. HORVÁTH[1], LEVENTE KISS[1,2], KÁLMÁN Z. VÁCZY[3], ZSUZSANNA VÁCZY[3], GIOVANNI ONESTI[4], MÁRK Z. NÉMETH[1], GÁBOR M. KOVÁCS[1,5], ALEXANDRA PINTYE[1] and ZSOLT BERECZKY[1]

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[3] Food and Wine Research Institute, Eszterházy Károly University, Eger, Hungary

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Strobilurins, belonging to the group of Quinone outside Inhibitors (QoIs), are considered as single-site of action fungicides which inhibit the electron transfer in mitochondria by binding to the cytochrome *bc1* enzyme complex. It has repeatedly been shown that a single point mutation in codon 143 of the mitochondrial gene *CYTB*, which encodes cytochrome b, confers complete resistance to QoI fungicides in many plant pathogenic fungi. However, in some species, such as *Puccinia* spp., neither QoI resistance nor this mutation, designated as G143A, have been detected so far. This was explained by the presence of an intron in the *CYTB* gene right after codon 143 in these plant pathogens: it was predicted that a G143A mutation would prevent the splicing of this intron and, thus, the production of functional cytochrome b proteins. Consequently, in these intron-containing species the G143A mutation is considered to be lethal and the risk for QoI resistance is predicted to be low.

Guignardia bidwellii (anamorph: *Phyllosticta ampelicida*), the causal agent of grape black rot, is considered as a *CYTB* intron-containing species with low risk for the development of QoI resistance in the field. We amplified and cloned *CYTB* fragments in several *G. bidwellii* strains, and also in some other *Guignardia* spp., including authentic strains of *G. citricarpa*, the causal agent of citrus black spot, and also *G. gaultheriae, G. mangiferae* and *G. aesculi* obtained from CBS, to sequence the intron located after codon 143. Surprisingly, no intron was detected in the predicted position in several *G. bidwellii* strains isolated from different grape varieties in Hungarian vineyards. Also, the intron was not found in either an authentic *G. bidwellii* strain obtained from LGC ATCC, or the *G. aesculi* and a *G. gaultheriae* strains included in this study, while the intron was identified, and sequenced, in all other *Guignardia* spp. strains examined by us. *In vitro* fungicide resistance tests did not show a clear correlation between the presence/absence of the intron in *Guignardia* spp. strains and their sensibility to QoI compounds. This might suggest that other mechanisms may also be involved in their QoI resistance. So far, our results indicate that at least some *G. bidwellii* strains causing grape black rot could contain the G143A mutation and might be able to develop QoI resistance in this way in the field.

This work was funded by the Széchenyi 2020 programme, the European Regional Development Fund and the Hungarian Government (GINOP-2.3.2-15-2016-00028). Zsolt

Bereczky acknowledges the support of a Janos Bolyai Research Fellowship and a grant (NKFIH PD-100724) of the Hungarian Research, Development and Innovation Office.

POWDERY MILDEW PHENOLOGY AS A DRIVER OF DIFFERENTIATION IN GENERALIST FUNGAL MYCOPARASITES

ALEXANDRA PINTYE[1], MÁRK NÉMETH[1], GÁBOR M. KOVÁCS [1,2], LEVENTE KISS[1,3]

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Ampelomyces spp. are natural intracellular mycoparasites, and also commercialized biocontrol agents (BCAs), of powdery mildews (the Erysiphales). These fungi sporulate inside powdery mildew colonies by producing their pycnidia mostly inside conidiophores and sexual fruiting bodies of their mycohosts. All known *Ampelomyces* spp. are strictly specialized to powdery mildews. Powdery mildew species, in turn, are each specialized to one or a few host plant species. In fact, these are well defined natural tritrophic relationships where one of the most interesting basic questions is whether *Ampelomyces* strains isolated from certain species of the Erysiphales are narrowly specialized to their original mycohosts or are generalist mycoparasites of many powdery mildew fungi. Understanding this relationship is also important for the use of *Ampelomyces* strains as BCAs against economically important powdery mildews because some recent studies explained low field performance of an *Ampelomyces*-based biofungicide in terms of strain-specific differences in mycohost range. Other studies did not support such *Ampelomyces*–mycohost associations.

In Europe, most powdery mildew hosts of *Ampelomyces* cause epidemics on their host plants mainly in summer and autumn. Apple powdery mildew (*Podosphaera leucotricha*, APM) is a notable exception because epidemics occur in spring, soon after bud burst. We isolated and genotyped >600 *Ampelomyces* strains from APM in spring, and from many other powdery mildew species in autumn, using 13 microsatellite markers and nrDNA ITS, *ACT1, RPB1* and/or *EukNR* sequences. All these data revealed that the strains coming from APM were distinct from those isolated in autumn, which, in turn, were genetically diverse, but did not group according to their mycohost species of origin. However, our field and laboratory cross-inoculation experiments showed that all the *Ampelomyces* strains, regardless of their mycohost species, or date of isolation, were able to heavily parasitize several other powdery mildew species, as well. All these data indicate that *Ampelomyces* spp. are genuine generalist mycoparasites and their genetic differentiation is driven by mycohost phenology rather than strict mycohost specialization.

This work was supported by a grant of the Hungarian Research, Development and Innovation Office (NKFIH NN100415). Alexandra Pintye acknowledges the support of a Janos Bolyai Research Fellowship.

MOLECULAR CHARACTERISATION OF CHITOSAN-INDUCED PRIMING FOR RESISTANCE AGAINST *BOTRYTIS CINEREA*

DE VEGA PEREZ D.[1], HOLDEN N.H.[1], HEIN I.[1], LUNA E.[1], HEDLEY P.[1], AND NEWTON A.C.[1]

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Conventional crop protectants (pesticides) can lose their efficacy due to selection pressure for pathogen resistance caused by their widespread use1. To date, there is a lack of genetic resistance in commercial crop varieties against necrotrophic fungal pathogens2 such as Botrytis cinerea and Sclerotinia sclerotium. The aggressive fungal pathogen Botrytis cinerea infects almost all vegetable and fruit crops3 (>1400 plant species), killing the host by inducing necrosis with degradation enzymes (virulence factors) and manipulating its host defences. Non-specific inducing agents, called resistance elicitors, are able to stimulate pathogen-induced defence mechanisms in the plant4 and induce plant defences for increased and more efficient resistance (priming) against pathogens such as Botrytis cinerea. Priming is based on a fine tuned and enhanced resistance to biotic/abiotic stress that results in a faster and stronger expression of resistance upon pathogen attack5. The aim of this PhD project is to determine the mode of action of specific elicitors, to characterise their molecular function and to investigate their role in priming crops against Botrytis cinerea. Resistance phenotypic assays have revealed that the pathogen-associated molecular pattern (PAMP) chitosan was able to induced resistance in Arabidopsis thaliana, Solanum melongena, Nicotiana benthamiana and Solanum lycopersicum by significantly decreasing necrotic lesion sizes and inducing callose deposition. Furthermore, large scale transcriptomic analysis has unveiled that chitosan was able to prime 1,745 tomato transcripts during asymptomatic stages of Botrytis infection. Gene ontology (GO) enrichment analyses revealed redox state, receptor kinases, cell-wall modification, auxins, jasmonate and ethylene, and phenylpropanoid pathways were enriched by chitosan. Finally, functional analysis indicated that two transiently-overexpressed chitosan-primed Avr9/Cf-9 rapidly elicited (ACRE) genes were able to induced resistance of N. benthamiana against B. cinerea. These results can ultimately facilitate new antifungal strategies by including resistance elicitors into integrated crop protection protocols.

References: 1Pappas, 1997; 2Smith et al. 2014; 3Weiberg et al. 2013; 4Aranega-Bou et al. 2014; 5Conrath, 2011

INTEGRON-LIKE ELEMENTS FOUND IN *PSEUDOMONAS SYRINGAE.*

JOSEPH D. PAYNE*[1], HELEN C. NEALE[1], JOHN T. HANCOCK[1], ROBERT W. JACKSON[2] and DAWN L. ARNOLD[1]

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Plant pathogenic Pseudomonads are responsible for the loss of millions of pounds in crop revenue each year. Pseudomonads export effector molecules into the plants' cells in order to supress immune responses such as the plant hypersensitive response (HR). The plants immune system can recognise certain effector molecules and trigger the HR preventing bacterial infection. Pseudomonads can evade HR by potentially gaining different effector molecules captured within integrons. There are genes conserved within integrons that can be identified such as *xerC* and the UV damage repair gene *rulB,* encoding a DNA polymerase V, appears to be a hotspot for integron insertion. Using these features it has been possible to identify a number of integron-like elements (ILEs) within *Pseudomonas syringae* pathovars. The three regions used to identify potential ILEs were the *rulAB* operon, the *xerC* gene and the ILE insertion junction, *rulB-xerC*. The screening of 164 strains revealed new uncharacterised ILEs from 22 strains all containing at least one type three effector molecule. The screening also revealed that the XerC integrase is conserved across multiple ILEs within plant pathogens. Research has also been carried on UV tolerance of ILE strains to ascertain whether the disrupted *rulB* gene is still functional. The conditions required for ILE gene expression has also been assessed.

CROPS AND ROBBERS (AND BENEFICIAL FUNGI); CHARACTERISATION OF THE VOLATILE SIGNALLING FROM THE BENEFICIAL SOIL FUNGUS *TRICHODERMA HAMATUM*

GARETH THOMAS[1,2], PROFESSOR MURRAY GRANT[3] DR CHRISTOPHER THORNTON[2], PROFESSOR JOHN PICKETT[1], DR JOZSEF VUTS[1], REBECCA WINSBURY[4], DR JOHN SIDDA[3] and DR MICHAEL BIRKETT[1]

[1] Rothamsted Research
[2] The University of Exeter, UK
[3] The University of Warwick, UK
[4] John Innes Centre, UK

Sustainable intensification of agricultural systems requires a new generation of interventions that reduces over-reliance on broad spectrum toxicant synthetic pesticides for pest, weed and disease management, and over-use of synthetic fertilizers that impact negatively upon the environment through greenhouse gas production. *Trichoderma* species comprise beneficial fungi that are capable of controlling plant pathogens and enhancing plant growth promotion (PGP), and are therefore of interest to the agricultural community. However, the application of *Trichoderma* species in farming systems requires an understanding of the mechanisms underpinning their biocontrol and PGP properties. The aim of this PhD is to test the hypothesis that these beneficial properties are mediated by small lipophilic molecule chemical signals produced and emitted by *Trichoderma* species, using *T. hamatum* GD12, a non-pathogenic soil borne fungus, and □*hepA*, a mutant of GD12 which lacks the chromatin remodelling protein hep1, involved in the activation and repression of ordinarily silent metabolomic clusters in the *T. hamatum* genome. Non-contact 'sandwich plate' bioassays are being used to investigate the role of volatile organic compounds (VOCs) of *T. hamatum* GD12 and □*hepA* in the inhibition of growth of the cosmopolitan soil pathogen *Sclerotinia sclerotiorum*. Dynamic headspace collection, along with coupled gas chromatography-mass spectrometry (GC-MS) is being used to identify and quantify the VOCs produced both by the GD12 and □*hepA* strains, which could be involved in the observed inhibition. In-soil assays using lettuce plants, *Lactuca sativa,* in the presence of either GD12 or □*hepA* and subsequent challenge with *S. sclerotiorum* are being undertaken to investigate PGP properties of GD12 and □*hepA*. To date, results suggest there is a role for *T. hamatum* VOCs in *S. sclerotorium* inhibition and, for □*hepA*, an increase in PGP of lettuce plants in the presence of *S. sclerotorium* compared to GD12 and non-challenged controls. Quantitative analysis of volatile compounds demonstrates significant upregulation in some compounds in □*hepA* relative to GD12, including the coconut-odour producing antifungal compound 6-pentyl-2H-pyran-2-one. Furthermore, plate-based dual-culture confrontation assays suggest that □*hepA* is able to suppress growth of various fungal pathogens via chemically-mediated inhibition compared to GD12. Moreover, headspace VOC changes occur as a result of the plate-based *Trichoderma*-pathogen mycelial interactions. The significance of these results to date, in conjunction with newly generated comparative RNA-seq data for GD12 and □*hepA*, will be discussed.

Session 2:

Chair: Friederike Gross-Holz

6 Talks (12 min presentation + 3 min questions)

Osman Telli
CIRCADIAN REGULATIONS OF EFFECTORS IN THE OOMYCETE PATHOGEN *HYALOPERONOSPORA ARABIDOPSIDIS***.**

Daniela Sueldo
ROLE (AND INHIBITION) OF PLANT APOPLASTIC SUBTILASES DURING *PSEUDOMONAS SYRINGAE* INFECTION

Elspeth Ransom
ELUCIDATING MECHANISMS OF PLANT AND NECROTROPHIC FUNGAL INTERACTIONS

Pierre Buscaill
A NOVEL NATURAL PRODUCT PRODUCED BY PSEUDOMONAS SYRINGAE PV. TOMATO DC3000 SPECIFICALLY INHIBITS A HOST-SECRETED B-GALACTOSIDASE (BGAL) IN THE APOPLAST UPON INFECTION

Mark Z. Nemeth
INTRACELLULAR MYCOPARASITISM AS A BIOTIC STRESS FOR POWDERY MILDEWS: HOW TO MAKE INTRUDERS MORE VISIBLE?

Ray Chai
ACTIVATION OF TOXIN$_{PA}$, A TYPE III TOXIN-ANTITOXIN/ABORTIVE INFECTION SYSTEM IN THE PHYTOPATHOGEN, *PECTOBACTERIUM ATROSEPTICUM*

CIRCADIAN REGULATIONS OF EFFECTORS IN THE OOMYCETE PATHOGEN *HYALOPERONOSPORA ARABIDOPSIDIS*

OSMAN TELLİ[1] DAVID STUDHOLME[2] AND MAHMUT TÖR[1]

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Most organisms have an internal circadian clock that also called the circadian rhythm, which regulates and imparts a survival advantage by enabling an organism to anticipate daily environmental changes. The biological clocks have three basic properties; a period length of about 24 hours, can be reset by environmental factors such as light and temperature, and has at least one internal autonomous circadian oscillator. The biological clock mechanisms are outwardly very similar in all species but the genes that make up the clock mechanisms can be quite different.

The identification of clock-regulated genes has led to the determination of common elements that regulate these genes. If these common elements can also be identified in pathogens, this would give us information on whether the virulence factors are also regulated by the circadian rhythm. Recently, a link between the plant immune system and the biological clock has been identified and, therefore, it is imperative that we identify the link between the pathogenicity factors and the biological clock.

Arabidopsis thaliana and its natural biotrophic pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) have been used as a model system for this study. The main aims were to determine whether *Hpa* is affected by the biological clock and, if so, how the pathogen regulates this rhythm; whether the rhythm is important for its pathogenicity, and more importantly whether selected virulent factors show circadian regulated rhythmic expression patterns. Pathogen growth has been investigated under normal and different light conditions to understand whether the circadian rhythm has an effect on pathogenicity. In addition, RNA-seq experiments were performed to elucidate differentially expressed genes in both the plant and the pathogen. The latest data will be presented.

ROLE (AND INHIBITION) OF PLANT APOPLASTIC SUBTILASES DURING *PSEUDOMONAS SYRINGAE* INFECTION

DANIELA SUELDO[1], NATTAPONG SANGUANKIATTICHAI [1], TRAM NGOC HONG[1,2], RENIER A. L. VAN DER HOORN[1,2]

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Apoplastic immune proteases play an instrumental role in plant defence against invading pathogens. Subtilases are abundant apoplastic serine proteases, whose accumulation is increased during pathogen attack, though their role in plant defence is still unclear. Activity-Based Protein Profiling (ABPP) is a technique that allows monitoring of protein activities without need of purification or previous knowledge of substrates. Using ABPP we have detected that the activity of apoplastic subtilases is suppressed in *N. benthamiana* upon infection by *Pseudomonas syringae* pv. *tomato* (PtoDC3000) by an inhibitor that is larger than 3kDa and heat-stable. To determine the identity of the inhibitor, apoplastic fluid obtained from PtoDC3000-infected *N. benthamiana* leaves, which contains the inhibitor, was fractionated by gel filtration. Fractions were tested for the presence of subtilase-inhibitory activity using competitive ABPP and analysed by mass spectrometry to determine their protein composition. In parallel, we performed immunoprecipitation assays to identify proteins that interact with apoplastic subtilases of *N. benthamiana* during infection with PtoDC3000. Preliminary results of both strategies will be presented at this meeting. With these experiments, we aim at identifying and disabling the suppression mechanism of apoplastic subtilases in wild tobacco during infection with PtoDC3000 to reveal their true role in plant defence.

ELUCIDATING MECHANISMS OF PLANT AND NECROTROPHIC FUNGAL INTERACTIONS

ELSPETH RANSOM[1], JOHN CLARKSON[1], SASCHA OTT[2] AND KATHERINE DENBY[3]

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Fungal pathogens cause devastating damage to crops globally. In the UK lettuce losses up to 50% pre-harvest have been reported as a result of necrotrophic fungal infection. However, currently available fungicide control methods are both expensive and unsustainable stressing the need for further research into alternative management strategies. The development of new lettuce varieties with fungal resistance has the potential to save growers £7 million per annum. My PhD aims to elucidate core mechanisms of necrotroph-plant interactions and identify key regulators of the defence response underlying Lettuce (*Lactucasativa*) infection by *Sclerotinia sclerotiorum* using a plant and pathogen approach.

Analysis of novel dual RNAseq timecourse data, generated from lettuce leaves inoculated with *S. sclerotiorum*, has captured the pattern of genes differentially expressed over 48 hours post infection in both the host and pathogen. Functional analysis of the time series has shown the chronology of lettuce gene expression changes from initial fungal perception to the defence response, including the activation of pathogenesis related genes. Additionally, we found temporal regulation of hormonal and transcriptional signalling. Using genes with known *Arabidopsis thaliana* orthologs, core conserved defence responses to nectroph infection in Lettuce and *Arabidopsis* can be observed. Furthermore, *S.sclerotiorum* pathogenesis genes including those involved in the oxalic acid pathway, carbohydrate active enzymes and potential effector candidates were identified.

During the defence response large scale regulatory reprogramming is a response to pathogen infection occurring before visible symptoms. Network modelling of differentially expressed transcription factors has identified key lettuce regulatory hub genes, which are up-regulated in response to *S. sclerotiorum*. To elucidate the impact of these key hub genes upon *S. sclerotiorum* resistance I intend to use CRISPR-Cas9 knockouts, generated through the regeneration of transformed lettuce protoplasts, and over-expression mutants. Examining these results in combination with disease resistance QTL data, will establish whether network analysis can be used to enhance the identification of candidate genes underlying resistance for breeding programs.

A NOVEL NATURAL PRODUCT PRODUCED BY PSEUDOMONAS SYRINGAE PV. TOMATO DC3000 SPECIFICALLY INHIBITS A HOST-SECRETED B-GALACTOSIDASE (BGAL) IN THE APOPLAST UPON INFECTION

BALAKUMARAN CHANDRASEKAR[1], PIERRE BUSCAILL[1], WENDY OFFEN[2], GIDEON DAVIES[2], RICHARD VAN DE BERG[3], HERMEN OVERKLEEFT[3], DAVID MIRON[1], GAIL PRESTON[1] AND RENIER VAN DER HOORN[1]

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The extracellular space in plant tissues (the apoplast) is an important battleground in plant-pathogen interactions. Using an activity-based probe for β-glycosidases [1], we discovered that the activity of a host-secreted β-galactosidase (BGAL) is reduced during infection of *N. benthamiana* with *Pseudomonas syringae* pv. *tomato* DC3000 (PtoDC3000) and its disease-causing derivative (mutant lacking T3 effector HopQ1-1 [2]). BGAL produced by agroinfiltration is able to cleave a classical galactosidase substrate (XGAL), demonstrating that BGAL is a true β-galactosidase. Quantitative proteomics demonstrated that BGAL protein levels are unaltered during infection, indicating the presence of an inhibitor. This novel BGAL inhibitor, called 'galactosyrin', is also produced by the ΔhrcC mutant of PtoDC3000, demonstrating that inhibitor production is independent of T3 secretion.
Bacterial mutant strains that no longer secrete the BGAL inhibitor are unable to suppress BGAL activity during infection and are hence called BGAL inhibitor mutants (*bim*). Preliminary infection assays demonstrated that all tested *bim* mutants have reduced bacterial growth on *N. benthamiana*. In addition, depletion of BGAL by virus-induced gene silencing (VIGS) does not cause developmental phenotypes but results in the depletion of the BGAL signal from the activity profile and an increased susceptibility for PtoDC3000(ΔhQ). Because BGAL suppresses PtoDC3000 growth *in planta*, we hypothesise that BGAL acts on extracellular glycans of PtoDC3000, either to disintegrate them, and/or to create elicitors that are perceived at the cell surface. Importantly, our preliminary experiments support this second hypothesis: PtoDC3000 bacteria treated with BGAL induce an oxidative burst in Arabidopsis leaf disks, and this response is suppressed by BGAL inhibitor galactostatin. Taken together, this study have elucidated a novel host manipulation mechanism by the model pathogen PtoDC3000, which suppresses the activity of a host-secreted galactosidase by a novel phytotoxin, e.g. to prevent the release of elicitors. These discoveries implicate a previously uncharacterized role for BGAL in defence and reveal a novel biosynthesis pathway in bacteria.

[1] Chandrasekar et al. 2014 Mol. Cell. Proteomics 13:2787-800. [2] Wei et al. 2007 Plant J. 51:32-46.

INTRACELLULAR MYCOPARASITISM AS A BIOTIC STRESS FOR POWDERY MILDEWS: HOW TO MAKE INTRUDERS MORE VISIBLE?

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Powdery mildew fungi (*Erysiphales*) are obligate biotrophic plant pathogens, infecting around 10,000 dicot species and also some members of the *Poaceae*. Important crops, including wheat, barley, grape, apple and a number of vegetables and ornamentals, are amongst the major targets of powdery mildew fungi. Pycnidial fungi belonging to the genus *Ampelomyces* are commonly found in powdery mildew colonies in the field, and some selected strains have also been developed as biocontrol agents of grape powdery mildew, because the parasitized colonies are slowly destroyed by these intruders.

To better understand the details of the interactions between *Ampelomyces* spp. and their mycohosts, we produced GFP expressing *Ampelomyces* transformants using *Agrobacterium*-mediated transformation using *Agrobacterium tumefaciens* strain AGL1 carrying a plasmid with the hygromycin resistance and GFP genes. Transformants were selected on hygromycin-containing medium and were checked for fluorescence after being grown in culture. Selected transformants were used in mycoparasitic tests using the following powdery mildew species: *Podosphaera xanthii* infecting cucumber, *Blumeria graminis* f. sp. *hordei* infecting barley, *Pseudoidium neolycopersici* infecting tobacco and *Leveillula taurica* infecting pepper. In these experiments, sporulating powdery mildew colonies were inoculated with spore suspensions of transformants. Inoculated colonies were examined with fluorescence microscopy 7-10 days following treatments.

The transformation method was effective as several transformants emerged on the selective medium and also exhibited strong green fluorescent signal, demonstrating the expression of GFP. We successfully transformed strains from two phylogenetically distinct *Ampelomyces* lineages. Transformants were genetically stable as they emitted strong green fluorescence after several subculturing in the absence of selective pressure. In mycoparasitic tests we observed extensive intracellular colonization of powdery mildew hyphae, conidiophores and conidia; intracellular *Ampelomyces* hyphae, as well as pycnidia and conidia produced in powdery mildew conidiophores exhibited strong green signals when examined with fluorescence microscopy.

This work showed that *Ampelomyces* is amenable to *Agrobacterium tumefaciens*-mediated transformation and that commonly used heterologous marker and reporter genes like hygromycin resistance and GFP can be efficiently used. No impairment of the

mycoparasitism could be observed. Transformation with GFP is useful in the direct observation of mycohost-mycoparasite interactions. Further studies are needed to reveal the details of the biotic stress caused by these mycoparasites in the powdery mildew mycelium. These newly developed tools will allow us to study the mycoparasitic interaction between the powdery mildew hosts and *Ampelomyces* at the molecular level.

This work was supported by a grant of the Hungarian Research, Development and Innovation Office (NKFIH NN100415) and a grant of the Austrian-Hungarian Action Foundation (90öu16).

ACTIVATION OF TOXIN$_{PA}$, A TYPE III TOXIN-ANTITOXIN/ABORTIVE INFECTION SYSTEM IN THE PHYTOPATHOGEN, *PECTOBACTERIUM ATROSEPTICUM*

RAY CHAI[1], TIM R. BLOWER[1], RITA PRZYBILSKI[1], SHAHZAD CHINDHY[1], XINZHE FANG[1], SAMUEL E. KIDMAN[1], HUI TAN[1], BEN F. LUISI[1], PETER C. FINERAN[2], GEORGE P. C. SALMOND[1]

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Pectobacterium atrosepticum is a Gram-negative bacterium which causes blackleg and soft rot diseases in economically important crops, such as potato. One possible option for blackleg and soft rot disease control may be the use of bacteriophages - the viral predators of bacteria - to treat these infections. Advantages of the use of bacteriophages are that they can be highly specific to their bacterial hosts and are easy to isolate and propagate. To use phages in bacterial infection control, it is imperative that we understand as much as we can about the biology and ecology of phage-host interactions. One such interaction that is poorly understood is abortive infection. This is an anti-phage bacterial defence system that leads to the precocious death of infected bacteria. While the infected bacterial cells die prematurely, this also prevents the release of potential progeny phage, thus protecting the rest of the bacterial population. One abortive infection system (ToxIN$_{Pa}$) is also a Type III toxin-antitoxin system, which was first discovered in *P. atrosepticum*. To try to understand how phages activate these systems novel phages from the environment were first isolated and tested for susceptibility to Type III toxin-antitoxin systems - including ToxIN$_{Pa}$ from *P. atrosepticum*. The data presented will show how certain phages evolve specific mutations in order to circumvent the bacterial defence systems in the plant pathogen.

Session 3:

Chair: Helen Pennington

3 Talks (12 min presentation + 3 min questions)

Satish Kulasekaran
ENHANCING PLANT DISEASE RESISTANCE THROUGH SYNTHETIC RE-ENGINEERING OF ABA SIGNALLING AND CATABOLISM

Judith K. Paulus
ACTIVATION OF THE RCR3 IMMUNE PROTEASE BY SECRETED SER PROTEASES

Baptiste Castel
OPTIMIZE CRISPR IN ARABIDOPSIS AND APPLY THE METHOD TO INVESTIGATE IMMUNITY

ENHANCING PLANT DISEASE RESISTANCE THROUGH SYNTHETIC RE-ENGINEERING OF ABA SIGNALLING AND CATABOLISM

SATISH KULASEKARAN[1], MARTA DE TORRES ZABALA[1], DEBORA SHOEMARK[2], ROB SESSIONS[2] AND MURRAY GRANT[1]

[1]School of Life Sciences, University of Warwick, Coventry
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Using a high resolution, time-resolved microarray dataset of *Pseudomonas syringae* pv. tomato DC3000 infected *Arabidopsis thaliana* leaves we identified early response genes that are specifically targeted by one or more of the 28 DC3000 effector, while remaining unresponsive to abiotic stresses, wounding and a disarmed DC3000*hrp* mutant. We designed strategies to re-engineer hormone signaling pathways utilizing these "effector responsive" promoters to create conditionally activated synthetic constructs which can neutralize pathogen virulence. Here we present results focusing on synthetic constructs designed modulate ABA signaling and ABA catabolism during susceptible interactions. Using modelling informed approaches, we mutated key residues in the PYL5 and PYL9 ABA receptors to enhance binding of pathogen induced ABA without activating the downstream signaling components. Transgenic plants carrying the mutant PYL proteins under the control of the effector responsive promoters showed markedly reduced symptom development and were more resistant to DC3000. Moreover, in re-engineered *PYL9* expressing plants, the levels of ABA responsive transcript, the protein phosphatase 2C, *HAB1*, remained significantly less in comparison to wild type plants at 6 h after DC3000 infection. To validate these results we demonstrate that 35S CaMV over expression the mutated PYL5 generated plants which where more insensitive to exogenous ABA application, indicating that the ABA signaling pathway is disrupted in these mutant PYL5 lines. Concurrently, we generated transgenic lines designed to catabolize pathogen generated ABA driving the ABA catabolic enzyme, CYPA3, under the control of an effector responsive promoter. Transgenic plants carrying the conditionally activated CYPA3 gene were more resistant to DC3000 infection in comparison to wild type Col-0.

ACTIVATION OF THE RCR3 IMMUNE PROTEASE BY SECRETED SER PROTEASES

JUDITH K. PAULUS, SELVA KUMARI, ANJA HOERGER, RENIER A. L. VAN DER HOORN

The Plant Chemetics Laboratory, University of Oxford, UK

Rcr3 (Required for Cladosporium Resistance-3) is a secreted papain-like cysteine protease (PLCP) in the apoplast of tomato [1]. The fungal leaf mold pathogen *Cladosporium fulvum* secretes a small molecule Avr2 that inhibits Rcr3. Tomato cultivars that are harbouring the Cf-2 (*C. fulvum* resistance 2) immune receptor recognize the manipulation of Rcr3 and trigger a hypersensitive cell death response that stops fungal invasion [2]. Like other PLCPs, Rcr3 is expressed with an inhibitory pro-domain that is cleaved off in a maturation process to generate active Rcr3 [1]. It is not known where and how this maturation process takes place in tomato and if maturation is autocatalytic. We found that heterologously expressed and purified proRcr3 slowly matures itself *in vitro* under reducing conditions at low pH [3]. However, the kinetics of autocatalytic Rcr3 activation seems to be too slow compared to the maturation of Rcr3 in the apoplast. I will present experiments that indicate that secreted serine proteases are processing proRcr3 in the apoplast, implying that Rcr3 is activated by a proteolytic cascade. While regulation of proteases by proteolytic cascades is well described in the animal field e.g. during apoptosis [4] or cancer [5], to our knowledge it has not been described in plants.

[1] Krüger J et al. (2003) A tomato cysteine protease required for Cf-2-dependent Disease resistance and suppression of autonecrosis. Science 296, 744–747. [2] Rooney HCE et al. (2005) Cladosporium Avr2 inhibits tomato Rcr3 protease required for Cf-2-dependent disease resistance. Science 308, 1783–1786. [3] Ramasubramanian SK (2012) Biochemical and Structural Characterization of RCR3-AVR2: A model for protease-inhibitor interactions at the plant-pathogen interface. (Universität zu Köln). [4] Slee EA et al. (1999) Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J. Cell Biol. 144, 281–292. [5] Mason SD & Joyce JA (2011) Proteolytic networks in cancer. *Trends Cell Biol.* 21, 228-237.

OPTIMIZE CRISPR IN ARABIDOPSIS AND APPLY THE METHOD TO INVESTIGATE IMMUNITY

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CRISPR is a genome editing method that can generate indels, DNA insertion and deletion *in vivo*. In Arabidopsis, this method consists in the expression of a RNA-guided protein (*eg* Cas9, Cpf1) in complex with a user-designed guide RNA (gRNA). The ribonucleoprotein complex binds DNA homologous to the gRNA and executes double-strand break, resulting in knockout. However, in Arabidopsis the efficiency to generate knockout lines remains inconsistent. We are comparing several components of the CRISPR/Cas9 system in order to optimize the strategy. We found that the nucleotide sequence of *Cas9* and the 3' regulatory sequences of Cas9 and the sgRNA greatly influence the overall efficiency. We even obtained some stable homozygous mutants in the first generation after transformation. We also applied CRISPR to knockout putative immunity-related genes while mutants are not available from the public collections. Thereby, we confirmed the role of a TIR-NB-LRR encoding gene in White Rust Resistance in Ws-2 and revealed the role of two redundant genes involved in Effector-Triggered Immunity. Our results (i) highlight the convenience of CRISPR to study genetic traits when natural variation is not available and (ii) demonstrate that the transcriptional regulation of the CRISPR components is crucial to generate stable knockouts in Arabidopsis.

Session 4:

Chair: Anjil Srivastava

4 Talks (12 min presentation + 3 min questions)

Trupti P. Gaikwad
INVESTIGATING INDUCTION OF SAR IN DURING GENE- FOR-GENE INTERACTIONS BETWEEN *ARABIDOPSIS THALIANA* AND *PSEUDOMONAS SYRINGAE*

Chris Dutton
THE INTERACTION BETWEEN PLANT DISEASE AND STOMATAL DENSITY

Catherine Jacott
DISEASE RESISTANCE AND MYCORRHIZAL COLONISATION

Stephanie Kancy
THE ROLE OF HISTONE ACETYLATION IN PLANT GROWTH AND IMMUNITY

INVESTIGATING INDUCTION OF SAR IN DURING GENE- FOR-GENE INTERACTIONS BETWEEN *ARABIDOPSIS THALIANA* AND *PSEUDOMONAS SYRINGAE*

TRUPTI P. GAIKWAD[1], MARTA DE TORRES-ZABALA[1], STEPHEN GREEN[2], PETER WINLOVE[2], DAVID HORSELL[2], MURRAY GRANT[3].

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[3]Biosciences, School of Life Sciences, University of Warwick, UK

Plants deploy two key active defensive strategies to combat microbial pathogens; (i) Recognition of Pathogen-Associated Molecular Patterns (PAMPs) by extracellular surface receptors leading to the activation of PAMP-Triggered Immunity (PTI); (ii) Recognition of pathogen effector activity, usually intracellularly, by host Resistance (R) proteins leading to Effector-Triggered Immunity (ETI). ETI is characterised by a rapid localised Hypersensitive Response (HR). HR induces Systemic Acquired Resistance (SAR) through the production of an inducible immune signal(s), leading to broad spectrum systemic resistance. We investigated the earliest events associated with SAR signalling using plant electrophysiology, SAR mutants and a unique promoter-luciferase fusion that captures early systemic transcriptional events underlying initiation of systemic immune signal(s). We describe the transcriptional dynamics of *A70* (At5g56980), a gene of unknown function (Truman *et al.* 2007), in local and systemic tissue following challenge with different elicitors and virulent or avirulent pathogen challenges. We provide evidence that *A70* responds to a jasmonate (JA) related signal that is rapidly generated following ETI recognition. We further evaluate *A70::LUC* reporter activity in response to JA stimulus and correlate activity with histological expression of a JA repressor reporter (*JAZ10::GUS*) and *A70::GFP* reporter in systemically responding leaves following avirulent pathogen challenges. Finally, we examine changes in electrophysiological signals following ETI in local and systemic leaves. Focussing on events underpinning initiation, propagation and perception of SAR-inducing signals within the first 6-8 h of pathogen challenge we provide new insight into the integrated signalling mechanisms, dynamics and connectivity underpinning systemic immune responses. We conclude that their multicomponent signals that link ETI induced transcriptional and electrical signals, with a COI1 receptor propagative transcriptional wave the leads to rapid temporal spatial transcriptional activation of jasmonate responsive genes in systemic responding leaves.

THE INTERACTION BETWEEN PLANT DISEASE AND STOMATAL DENSITY

C. DUTTON, L. HUNT, J. TON & J. E. GRAY

University of Sheffield

Stomata are recognised as important entry sites for many microbial pathogens. Since the discovery that stomata on host plant leaves close in order to restrict pathogen entry (Melloto et al. 2006) much work has focused on understanding the mechanisms and signalling pathways that enhance immunity through stomatal closure.

In this study we have asked whether, in addition to inducing stomatal closure, plants might also adjust their stomatal frequency to restrict pathogen entry following bacterial infection. Our results indicate that (1) wild-type Arabidopsis plants have the capacity to adjust the frequency of stomata that develop on their new leaves following *Pseudomonas syringae* infection; and (2) that Arabidopsis plants that have been manipulated to have altered stomatal densities have correspondingly altered levels of pathogen colonisation. Thus, we propose that following infection, plants can regulate their stomatal development in order to restrict the number of entry points for bacterial pathogens. The systemic signals and transduction pathways that could potentially mediate such pathogen-induced alterations in stomatal density will be discussed.

DISEASE RESISTANCE AND MYCORRHIZAL COLONISATION

CATHERINE JACOTT

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There is an increasing need to develop disease resistant crops and reduce fertilizer usage. Mutations at the *Hvmlo1* locus in barley confer broad-spectrum resistance to fungal pathogen, *Blumeria graminis* f. sp. *Hordei.* Possible trade-offs between *Hvmlo1* and colonisation by arbuscular mycorrhizal (AM) fungi have been previously documented. Barley, wheat and *Medicago truncatula* are being used to determine whether *mlo* resistance genes differentially modulate root colonisation by mycorrhizal fungi.

Our findings from phylogenetic analyses suggest that *HvMlo1* is in a clade with only other plant species that form mycorrhizal symbiosis. Previous transcriptome studies have also shown that orthologues of *HvMlo1* in *M. truncatula*, rice and *Lotus japonicus* are upregulated upon mycorrhization. However, colonisation assays in barley, wheat and *M. truncatula* have not shown a reduction in colonisation frequency in the *mlo1* mutants. qPCR results have also shown no differential expression of *Mlo* during root colonisation by AM fungi.

During powdery mildew infection, a paralogue of *HvMlo1* is induced in the *mlo1* mutant compared to the wild-type. *Mlo* belongs to a large family of genes, which may collectively contribute towards phenotypes. The expression of genes in the *Mlo* family needs to be investigated during mycorrhization in the wild-type and *mlo1* mutant background. This may determine whether the observed lack of mycorrhizal phenotype is due to the compensating effect of other *Mlo* genes.

Mycorrhizal colonisation in different barley cultivars is also being measured to determine the extent of variation and effect on grain quality parameters for the malting process. Interestingly, colonisation frequencies in five cultivars with wild-type *Mlo* alleles have shown positive correlation with susceptibility to powdery mildew infection. This suggests an overlap between disease resistance and mycorrhizal colonisation. Overall, the results will inform future breeding strategies by determining the contribution of mycorrhization to nutrient uptake, disease resistance and grain quality.

THE ROLE OF HISTONE ACETYLATION IN PLANT GROWTH AND IMMUNITY

STEPHANIE KANCY

University of Warwick
Supervisor: Vardis Ntoukakis
Co-supervisor: Katherine Denby

Activation of plant defence responses requires significant transcriptional reprogramming and is often associated with growth suppression and interruption of developmental processes[1]. Despite its agricultural importance, knowledge regarding the balance between immunity and development at the transcriptional level is limited. Histone acetyltransferases (HATs) are key regulators of chromatin remodelling by catalysing the transfer of an acetyl moiety to specific histone tail lysine residues leading to transcriptionally active and structurally relaxed chromatin regions[2].

In a reverse genetic screen of *Arabidopsis HAT* mutants, a negative regulator (*HAT5*) of defence against the plant pathogen *P. syringae* was identified. Whilst the negative regulator demonstrates enhanced resistance to *P. syringae*, it's susceptibility to the necrotrophic pathogen *B. cinerea* is unchanged. Alongside the immunity phenotype, *hat5* plants exhibit increased adult leaf surface area, fresh weight, root length and a greater number of viable seeds in the siliques.

Since *hat5* is the only known mutant with increased immunity and growth, it represents a promising target in an agricultural context. Homology models of *A. thaliana, B. napus* and *S. lycopersicum* HAT5 were created, supported by a series of cheminformatics and *in silico* docking methods, to identify chemical inhibitors for future agricultural applications.

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[2] Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*(4), 693–705.

Poster Listings

1. **Adam Talbot**

A SYSTEMS APPROACH TO BREEDING DISEASE RESISTANCE IN LETTUCE AGAINST NECROTROPHIC FUNGAL PATHOGENS

2. **Alexandra Puertolas**

A MULTI-LOCUS ILLUMINA ASSAY DEMONSTRATES OOMYCETE MOVEMENT THROUGH THE INTERNATIONAL TRADE IN ORNAMENTAL PLANTS

3. **Alonso Pardal Bermejo**

CHROMATIN REMODELLING COMPLEXES CENTRAL SUBUNITS AND PLANT DEFENCE

4. **Andrew Day**

ENVIRONMENTAL BACTERIOPHAGES ISOLATED AGAINST THE POTATO PATHOGEN, *DICKEYA SOLANI*

5. **Andrew J. Foster**

DEVELOPMENT OF CRISPR/CAS9 GENE EDITING TOOLS FOR THE RICE BLAST FUNGUS

6. **Antonio Gomez Cortecero**

THE MOLECULAR BASIS OF PATHOGENICITY OF NEONECTRIA DITISSIMA

7. **Arsheed Sheikh**

MITOGEN-ACTIVATED PROTEIN KINASES ACTIVATION POST EFFECTOR RECOGNITION IN TOMATO

8. **Brian O'Loinsigh**

DEVELOPMENT OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR THE DETECTION OF *APHANOMYCES EUTEICHES* – A ROOT ROT PATHOGEN OF PEAS

9. **Catherine Walker**

EFFECTOR DISCOVERY AND CHARACTERISATION IN THE *FUSARIUM GRAMINEARUM*-WHEAT INTERACTION

10. **Clara Rodriguez-Herrero**

INVESTIGATING THE BIOLOGY OF EFFECTOR DELIVERY DURING RICE INFECTION BY THE BLAST FUNGUS *MAGNAPORTHE ORYZAE*

11. **Corinne Arnold**

EVOLUTION OF FUNGICIDE RESISTANCE IN WHEAT POWDERY MILDEW

12. **Eleanor Brant**

IDENTIFYING R-GENES IN *ARABIDOPSIS THALIANA* USING CRISP-R TECHNOLOGY

13. **Emma Thomas**

PROTEIN PROCESSING IN *NICOTIANA BENTHAMIANA* HYPERSENSITIVE RESPONSE

14. Freya Varden

ARMED AND DANGEROUS: MOLECULAR BASIS OF RICE BLAST EFFECTOR RECOGNITION BY AN IMMUNE RECEPTOR

15. Friederike Grosse-Holz

MAPPING THE PROTEASE REPERTOIRE OF NICOTIANA BENTHAMIANA

16. Guilherme Rossato Augusti

INVESTIGATING THE CURRENT STATUS AND PREDICTING FURTHER EVOLUTION OF AZOLE FUNGICIDE RESISTANCE IN THE WHEAT PATHOGEN *ZYMOSEPTORIA TRITICI*

17. Hannah Brown

HOW DOES A PLANT IMMUNE RECEPTOR COMPLEX CONFER DISEASE RESISTANCE?

18. Helen Brabham

DISSECTING MULTIPLE RESISTANCE SPECIFICITIES AT THE MLA LOCUS IN BARLEY

19. Helen Pennington

GENETIC BASIS OF TRANSLOCATION OF *MAGNAPORTHE ORYZAE* AVR-Pik INTO RICE CELLS

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PAMP-TRIGGERED IMMUNITY & QUANTITATIVE DISEASE RESISTANCE IN BRASSICAS

21. James Doonan

GENE EXPRESSION ANALYSES OF THE BACTERIAL TREE PATHOGENS *GIBBSIELLA QUERCINECANS* AND *BRENNERIA GOODWINII IN VITRO* AND *IN PLANTA*

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IDENTIFYING LIFE-STAGE SPECIFIC SURFACE PROTEINS OF THE GOLDEN POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS*

23. Jiorgos Kourelis

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DEVELOPMENT OF MYCOVIRUS BASED VECTORS TO SILENCE DOTHISTROMA SEPTOSPORUM GENES

25. Kathryn Hales

UNDERSTANDING THE ECOLOGY AND EPIDEMIOLOGY OF *PYTHIUM VIOLAE* CAUSING CAVITY SPOT ON CARROT

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30. Mahira Al Zadjali
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33. Marcel Bach-Pages
DISSECTING THE ROLE OF RNA-BINDING PROTEINS IN PLANT IMMUNITY

34. Matevz Papp-Rupar
LIGHTING UP PLANT DEFENCES; FROM OUR LAB TO YOUR FRIDGE

35. Nattapong Sanguankiattichai
INTERPLAY BETWEEN PLANT APOPLASTIC PROTEASE AND INHIBITOR DURING INFECTION

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INVESTIGATING THE ROLE OF NADPH OXIDASES DURING INFECTION RELATED DEVELOPMENT OF THE RICE BLAST FUNGUS *MAGNAPORTHE ORYZAE*

37. Sascha Jenkins
EXAMINING IDENTITY, PHYLOGENY AND PATHOGENICITY FACTORS IN FUSARIUM SPECIES AFFECTING PEA

38. Samuel W Wilkinson
UNDERSTANDING PRIMING OF THE JASMONIC ACID DEFENCE PATHWAY: A MULTISPECIES APPROACH

39. Shaoli Das Gupta

BLUMERIA METALLO-PROTEASE LIKE EFFECTOR: A VIRULENCE FACTOR FOR CEREAL POWDERY MILDEWS OR A UNIVERSAL VIRULENCE FACTOR IN FUNGI?

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EVOLUTION OF SWIMMING MOTILITY IN AFLAGELLATED STRAINS OF *PSEUDOMONAS FLUORESCENS*

#1

A SYSTEMS APPROACH TO BREEDING DISEASE RESISTANCE IN LETTUCE AGAINST NECROTROPHIC FUNGAL PATHOGENS

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Botrytis cinerea and *Sclerotinia sclerotiorum* are fungal pathogens of global importance, each causing multi-million pound annual crop losses pre- and post-harvest on many dicotyledonous crops including field-grown and protected lettuce crops. Chemical control is and problematic with increased regulation and the emergence of resistance within the microbial population. Development of host resistance is a more sustainable solution, but has been an intransigent problem for breeders.

We are taking a novel approach to breeding for disease resistance against *B. cinerea* and *S. sclerotiorum* in lettuce, combining systems biology and quantitative genetics. Genetic variation in susceptibility to these pathogens was identified in a set of diverse lettuce accessions, based on lesion size following leaf inoculation. Susceptibility to both pathogens was also correlated across different accessions thereby increasing the potential of identifying alleles conferring broad resistance. The validity of the detached leaf assay data was investigated in polytunnel trials, which demonstrated correlation between the susceptibility levels of detached leaves and infected lettuce plants.

To identify genetic loci that contribute to disease resistance, a quantitative genetics approach was employed. Recombinant inbred lines (RILs) from a mapping population were assessed for disease susceptibility using the detached leaf assay and QTLs indicating potential genetic loci contributing to resistance were identified. Further work is being initiated to fine map and validate these QTLs.

We have developed a network analysis gene disco very strategy in Arabidopsis exploiting time series transcriptome data and are now applying this methodology in lettuce to predict genes conferring disease resistance against *B. cinerea*. Using RNAseq we generated a time series of gene expression from 9 to 54 hours post infection in lettuce leaves after inoculation with *B. cinerea* or mock inoculation. From this time series data we will investigate the chronology of transcriptional reprogramming in lettuce following infection, infer regulatory networks mediating this response and predict key regulators of disease resistance within these networks. The expression of these key genes will be tested in diversity set lines showing extreme resistance phenotypes to examine correlation of expression with resistance. Co-localisation of these key regulators with resistance QTL would fast-track the identification of causal genes and associated markers for integration into lettuce breeding programmes.

#2

A MULTI-LOCUS ILLUMINA ASSAY DEMONSTRATES OOMYCETE MOVEMENT THROUGH THE INTERNATIONAL TRADE IN ORNAMENTAL PLANTS

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The dispersal and introduction of plant pathogens has increased with the internationalization of trade in ornamental plants. More potted plants are being moved around Europe than ever before and the rise in Internet plant sales is of particular concern. We screened 99 woody ornamentals bought in the UK and the Netherlands for major oomycete plant pathogens (Phytophthora, Pythium and Phytopythium) from soil, roots and water from baiting assays. Two approaches were used and species diversity compared: Next Generation Sequencing (NGS) using a multi-locus Illumina MiSeq assay for four molecular markers (ITS, COXI, β-tubulin and trnM-trnP-trnM genes); and classical isolation techniques. Approximately 90% of tested plants contained at least one species of *Phytophthora*, *Pythium* or *Phytopythium*. More worryingly, 86% of asymptomatic plants tested positive for oomycetes in the growth substrate. In total we isolated 10 *Phytophthora* species, 17 *Pythium* spp. and five *Phytopythium* spp. A much higher diversity of oomycete species was found with multi-locus NGS, which detected potential pathogens in all plants tested, including asymptomatic plants. Several potentially damaging species were detected at high frequency, including *Phytophthora ramorum* and *P. alni*. These results provide strong evidence for the cryptic movement of plant pathogens between European countries through trade in ornamental plants. The findings highlight the need for stronger vigilance and regulation to reduce biosecurity threats and safeguard a valuable trade.

#3

CHROMATIN REMODELLING COMPLEXES CENTRAL SUBUNITS AND PLANT DEFENCE

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Plants can detect pathogen microorganisms and build up defences accordingly. In order to survive and guarantee the provision of seeds for the next generation, the limited plant resources need to be finely tuned in the trade-off between growth and defence upon pathogen perception. Gene reprogramming is therefore a major component of the innate plant defence. Chromatin remodelling complexes have been pinpointed as regulators of immunity. Here we present a susceptibility screening, using the *Arabidopsis* – *Pseudomonas siryngae* pathosystem, for chromatin remodelling ATPases as novel regulators of plant immunity. We also characterise the biological function and we are interested in describing its molecular mechanism of action. From the results of our work it could be speculated that chromatin remodelling may act as a negative feed-back loop mechanism, allowing the plant to recover pre-defensive genetic program in order to re-gain successful growth and reproduction.

#4

ENVIRONMENTAL BACTERIOPHAGES ISOLATED AGAINST THE POTATO PATHOGEN, *DICKEYA SOLANI*

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Dickeya solani is an enterobacterial phytopathogen responsible for significant economic losses in the European potato industry. Identified a decade ago, it is considered to be more virulent than other bacterial potato pathogens, such as taxonomically-related *Pectobacterium* species, and has been named as a pathogen of increasing economic importance. Whilst widespread in Europe, *Dickeya solani* is not yet fully environmentally established throughout the United Kingdom, and therefore there are efforts to prevent this from occurring.

Bacteriophages have been suggested as potential biocontrol agents for *Dickeya solani* in the field, with a small number of published papers discussing this possibility. Here we present over 60 bacteriophages of *Dickeya solani* isolated from environmental sources in the UK and discuss their characteristics, possible suitability for use in biocontrol and the ecological and biological questions their discovery raises.

#5

DEVELOPMENT OF CRISPR/CAS9 GENE EDITING TOOLS FOR THE RICE BLAST FUNGUS

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CRISPR/Cas9 gene editing is a rapid developing field which holds great promise for accelerating the pace of targeted mutagenesis in fungi. Currently, although very many fungal genes are predicted based on genome sequencing, the process of specifically mutating selected genes, even in the best studied fungi, requires months rather than weeks. The rice blast fungus *Magnaporthe oryzae* is the most serious pathogen of rice. To accelerate the process of targeted mutation and specific gene edits in this important species we are working towards the development of a rapid Crispr/Cas9 based gene editing technology. Vectors facilitating rapid recombination of oligonucleotide-based adapters to target specific genes for mutation have been constructed potentially making functional genomics realistic and affordable. Furthermore vectors allowing multiple genes to be targeted by a single construct exploiting ribozyme mediated excision of active guide RNAs are under test and will open the door to analysis of gene families or subsets of target genes. Latest results will be discussed.

#6

THE MOLECULAR BASIS OF PATHOGENICITY OF *NEONECTRIA DITISSIMA.*

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European canker, caused by the phytopathogenic fungus *Neonectria ditissima*, is one of the most destructive diseases of apple and pear. In the orchard this fungus is able to infect a wide range of apple varieties causing canker and die back of young shoots, resulting in significant losses of fruiting wood. This pathogen has been reported in many apple-producing regions of the world, being especially common in the North-Western European countries. Modern varieties suffer most and in extreme cases do not survive establishment in the orchard. Canker control is difficult to achieve due to the pathogen's lifestyle which is able to infect trees all year-round through wounds, either natural such as bud-scale scars, leaf scars, fruit scars or artificial such as pruning wounds. Resistance breeding is underway in many global breeding programmes, but nevertheless, a total resistance to canker has not yet been demonstrated in either fruit or woody tissue. There is no known race structure of the pathogen and the global level of genetic diversity of the pathogen population is unknown. Plant resistance is a promising alternative to largely ineffective cultural control, but is time consuming to deploy due the long breeding cycle in apple. A deeper understanding of the host-pathogen interactions, and how host resistance and pathogen avirulence are linked is key to the deployment of durable resistance in the field.

Through several inoculation tests in apple seedlings and young trees, it was possible to assess how the pathogen varies in its virulence and how apple varieties differ in the responses. In most of the cases, these responses were consistent among the cultivars regardless the inoculation method. Necrotrophic pathogens, like *N. ditissima,* rely on an arsenal of lytic and cell wall degrading enzymes to damage the host cell and acquire nutrients. Recent research suggests that the mechanism of infection might not be as 'simple' as producing phytotoxic compounds to induce host cell death but rather requires an intricate balance of programmed cell death orchestrated through the secretion of proteins, called effectors, which suppress the immune response in the host and allow colonisation. The genome of *N. ditissima* facilitates the identification of putative effectors and pathogenicity genes through bioinformatics analysis of the secretome and through comparisons to other pathogens. We annotated a previously sequenced *N. ditissima* genome assembly using RNA-Seq data and have now improved this assembly using PacBio long-reads sequencing, allowing us to present an updated analysis of the predicted secretome of this pathogen. In due course RNA from infected host tissue will be extracted and further RNA-Seq data will be generated. Differentially expressed transcripts will be sought. The identification of specific candidate genes controlling pathogen virulence will allow a better understanding of the mechanism of infection. Similar comparisons will be carried out on the host, utilising the

apple genome to identify putative resistance genes, differentially expressed between resistant and susceptible cultivars. Furthermore, knowledge of effector targets in the pathogen could lead to novel opportunities for control by targeted disruption of the pathogen.

#7

MITOGEN-ACTIVATED PROTEIN KINASES ACTIVATION POST EFFECTOR RECOGNITION IN TOMATO.

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The major virulence strategy of the phytopathogenic bacteria *Pseudomonas syringae* pv. tomato DC3000 is to secrete effector proteins into the tomato cells to target the immune machinery. AvrPto and AvrPtoB are two such effectors from *P. syringae*, which disable a range of kinases in tomato. Both effectors target surface-localized receptor-kinases to avoid bacterial recognition. In turn, tomato has evolved an intracellular effector-recognition complex composed of the NBLRR protein Prf and the Pto kinase. AvrPto is an inhibitor of Pto kinase activity, but paradoxically, this kinase activity is a prerequisite for defence activation by AvrPto. Following P+1 loop disruption and transphosphorylation the Pto/Prf complex dissociate leading to downstream signalling through mitogen-activated protein kinases (MAPKs). Hence, the Pto/Prf complex is a sophisticated molecular trap for effectors and provides an excellent model to study the mechanism of MAPKs activation. In the current study we sought to investigate the mechanism of MAPKs activation post Pto/Prf recognition of AvrPto/AvrPtoB effectors. We identify 14-3-3 proteins as part of the Pto/Prf resistance complex acting as regulators of MAPKs activation. We also show that an additional *P. syringae* effector acts act in concert to avert the activation of MAPKs cascade thereby dampening the immune responses.

#8

DEVELOPMENT OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR THE DETECTION OF *APHANOMYCES EUTEICHES* – A ROOT ROT PATHOGEN OF PEAS

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Aphanomyces root rot, caused by *Aphanomyces euteiches* is one of the most damaging diseases of pea (*Pisum sativum*) and is one of the major constraints to pea production globally. *A. euteiches* is an oomycete, a strict soil borne pathogen surviving as oospores in the soil, and completes its entire lifecycle in the host roots and surrounding soil. The initial symptoms start as water soaked lesions on the roots, developing into a yellow coloured soft-rot of the cortex which, in severe cases can affect the entire root system. The infection of the roots leads to chlorosis, stunted plant growth, and wilting symptoms. In severe cases *Aphanomyces* root rot can cause up to 80% losses per annum in infected regions and is prevalent in Europe, North America, Japan and Australia.

Due to the devastating effect *Aphanomyces* root rot can have on yield and a lack of effective control methods, growers find it useful to obtain information on the degree of infestation within their fields to avoid those at high risk. Traditionally, in the UK, this is achieved through the growth of peas in a representative soil sample in the glasshouse. This is followed by the laborious job of visually inspecting the roots for symptoms and the presence/absence of oospores on the infected roots; providing the grower with a root rot potential index. More modern techniques such as Polymerase Chain Reaction (PCR) and Quantitative PCR (qPCR) have become an attractive option for the detection and quantification of pathogenic organisms of plants. However, these methods tend to be expensive and need specialised equipment and compared to other substrates, soils are problematic, since many substances in soil can inhibit the PCR reaction.

Therefore, the need for a rapid, simple, and inexpensive diagnostic detection tool of soil borne pathogens is vital. Loop-mediated isothermal amplification (LAMP) has these capabilities; it is less prone to enzyme inhibition, does not require gel electrophoresis to separate or visualise the products, nor costly laboratory equipment. In this current study, a LAMP assay for the specific detection and evaluation of A. euteiches from both soil and pea plate baiting experiments was developed. The LAMP assay was established with six primers targeting known specific ITS regions of *A. euteiches.* The LAMP assay efficiently amplified the target gene in 30min at 60^{o}C. Specificity was evaluated against *A. cochlioides, A. cladogamus, Phoma medicaginis* and *Fusarium spp*. These results suggest that this LAMP assay can be used to detect extracted *A. euteiches* DNA within both soil samples directly from the field and on the roots of infected seedlings that has been baited with soil within days of receiving the samples. Following this, the protocol was used to determine the spread of *A. euteiches* across the pea growing regions of the UK.

#9

EFFECTOR DISCOVERY AND CHARACTERISATION IN THE FUSARIUM *GRAMINEARUM*-WHEAT INTERACTION

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Fusarium Head Blight (FHB) is recognised as one of the most devastating diseases of cereal crops globally, significantly hampering agricultural productivity and threatening food security. The main causal pathogen, *Fusarium graminearum* (*Fg*), is an ascomycete fungus with a hemibiotrophic lifestyle facilitated by the biphasic secretion of an arsenal of proteins which modify living host plants, aiding colonisation and infection. *Fg* infection begins with an initial symptomless phase during which it is hypothesised that the secretion of small, cysteine-rich effector proteins subvert recognition by the host and/or suppress host defences [1]. A second wave of effectors are secreted, alongside cell-wall degrading enzymes and virulence factors, coincident with the onset of disease symptoms.

In order to identify candidate effectors, a species-specific microarray analysis (Brown et al., submitted) was used to interrogate the *in vitro* and *in planta* expression patterns of genes belonging to the *Fg* secretome. This pipeline, used in conjunction with additional bioinformatic analyses, yielded over twenty putative Fusarium effectors which have begun to be characterised using the *Barley-Stripe Mosaic Virus*-mediated overexpression system (BSMV-VOX), stable transformation of Arabidopsis and via the generation of *Fg* gene deletion mutants [2].

Initial data suggests that a number of candidate proteins, characterised using BSMV-VOX, are biologically active in the *Fg*-wheat interaction. These initial experiments also indicate that viral titre is critical to the manifestation of a disease phenotype when overexpressing an *Fg* protein. Further studies are ongoing to determine the effect of viral titre on protein expression and subsequent impacts on the *Fg*-wheat interaction.

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#10

INVESTIGATING THE BIOLOGY OF EFFECTOR DELIVERY DURING RICE INFECTION BY THE BLAST FUNGUS *MAGNAPORTHE ORYZAE*

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Magnaporthe oryzae is the causal agent of rice blast disease, the most destructive disease of cultivated rice. The life cycle of *M. oryzae* starts when a spore lands on the hydrophobic surface of a leaf and attaches tightly to the cuticle by secretion of spore tip mucilage. The spore germinates to form a polarized germ tube which generates aspecialized infection structure, called an appressorium. The appressorium generates enormous turgor pressure that it is translated into physical force at the base of the infection cell to facilitate emergence of a penetration peg which ruptures the rice leaf cuticle. The fungus then develops invasive hyphae (IH), which rapidly invade and colonize plant tissue. *M. oryzae* is a hemi-biotrophic fungus and during its biotrophic phase, the fungus feeds and develops within living plant cells. To facilitate this state, the fungus secretes effectors that suppress plant immunity and modify host cell structure, metabolism and function. Effectors in *M. oryzae* can be classified as either symplastic (cytoplasmic) effectors or apoplastic effectors depending on where they are delivered. Symplastic effectors can be seen to accumulate in a membrane-rich plant structure called the biotrophic interfacial complex (BIC), while apoplastic effectors are found between the fungal cell wall and the extra-invasive hyphal membrane which surrounds the IH. The BIC is thought to be involved in translocation of effectors into the rice cytoplasm. Two different secretion pathways are believed to operate to drive effector secretion from fungal hyphae. Apoplastic effectors are secreted by the conventional ER-Golgi secretory pathway, while symplastic effectors are secreted via an unconventional route that is Golgi-independent (Giraldo et al, 2013 *Nature Comms* 4, 1996). However, little is known about how the secreted effectors are delivered to the correct domains and, in particular, how symplastic effectors cross the extra-invasive hyphal membrane. We are currently investigating how effectors translocate to the plant cell. First, we are trying to identify a host targeting sequence by generating a library of chimeric effectors that are being systematically tested for translocation ability. Second, we are observing invasive hyphae in live cell imaging experiments and testing the role of secretion mutants in final effector delivery. Finally, we are generating stable transgenic rice plants in which we have GFP-tagged both early and late endosomal compartments to determine the potential role of plant endocytosis in effector uptake.

#11

EVOLUTION OF FUNGICIDE RESISTANCE IN WHEAT POWDERY MILDEW

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Blumeria graminis is a powdery mildew pathogen which infects many wild grasses and cereals across the world. If left uncontrolled, it can cause significant loss of yield in cereals, such as wheat and barley. Resistance to strobilurin and older triazole fungicides already occurs in mildew, and there is a limited selection of fungicides which still control it. I have characterised isolates from an outbreak of wheat mildew which was not controlled by cyflufenamid (Cyflamid), an eradicant, mildew-specific fungicide with an unknown mode of action. All these isolates of *B. graminis* f.sp. *tritici* (*Bgt*) were completely resistant to Cyflamid sprayed at the recommended field rate and very much less sensitive than wild-type isolates in a laboratory test. Over the following two years, the *Bgt* population at this location recovered sensitivity to cyflufenamid, suggesting that resistance may have a significant fitness penalty. This may allow cyflufenamid to continue to be used as part of an integrated disease control programme, despite the risk of resistance. Genetic and genomic analysis is in progress, with the ultimate aim of identifying candidate genes for cyflufenamid-resistance. I also studied resistance to fenpropimorph (Corbel) in the same population of *Bgt*. Fenpropimorph, a morpholine fungicide which inhibits double-bond reduction in sterol synthesis, is not as effective as when it was introduced over 30 years ago but it still provides moderate control of wheat mildew. Higher resistance has evolved on occasions when Corbel has been used excessively. At the study site, Cyflamid was not sprayed after cyflufenamid-resistance appeared but following two sprays of Corbel, isolates of *Bgt* with significantly lower sensitivity to fenpropimorph than field isolates had emerged. Overuse of fenpropimorph would almost certainly lead to strong resistance in mildew, so it should be used as part of a rotation of fungicides with different modes of action.

#12

IDENTIFYING R-GENES IN *ARABIDOPSIS THALIANA* USING CRISP-R TECHNOLOGY

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With recent advances in targeted genome editing holding the potential to create faster and more efficient methods of trait selection than conventional techniques, the term ‘it’s all in your genes’ has never been more relevant. In particular, with the rate of plant pathogen emergence showing a steady incline, increasing understanding of the genes (*R*-genes) which enable innate immunity in plant species could be a crucial component of adapting to the trending rise in agricultural strain observed across the globe. One of the current most promising avenues for targeted genome editing stands with the CRISPR/Cas9 system, which although still novel, could be utilised in model plants to give a greater indication of its latent uses in more complex plant species. Although in the early stages, this study aims to explore the potential of using the CRISPR/Cas9 system for selected disease resistance by identifying the causal gene for susceptibility to *Hyaloperonospora arabidopsidis* in the model species *Arabidopsis thaliana* L.. The project revolves around six *R*-genes, found on Chromosome 4 of the RMX-A02 accession (resistant to *H. arabidopsidis*), which will be silenced and any resulting changes in susceptibility observed. If successful, this method could be replicated for use on species of a greater ecological or agricultural importance in order to initiate selected disease resistance.

#13

PROTEIN PROCESSING IN *NICOTIANA BENTHAMIANA* HYPERSENSITIVE RESPONSE

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The immune response of plants to pathogens is associated with localised cell death called hypersensitive response (HR) at the primary infection site. Very little is known regarding programmed cell death in plants in comparison to animals. Whilst animal apoptosis is dependent on a processing cascade mediated by capsases, plants lack caspases in their genomes. However, studies of inhibitors and activity against substrates indicate plants do possess caspase like activity. Vacuolar processing enzyme (VPE) and cathepsin B have been shown to exhibit caspase-1 and caspase-3 activity respectively, and when silenced or knocked out block HR development in both *Arabidopsis thaliana* and *Nicotiana benthamiana*. I will present my PhD plan to investigate the biological importance of VPEs and cathepsin B during HR, starting with the use of high throughput proteomic techniques to identify protease substrates.

#14

ARMED AND DANGEROUS: MOLECULAR BASIS OF RICE BLAST EFFECTOR RECOGNITION BY AN IMMUNE RECEPTOR

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John Innes Centre

The rice blast pathogen *Magnaporthe oryzae* employs effector proteins to undermine host resistance and promote pathogen growth. However, a number of these effector proteins can be recognised by host intracellular immune receptors known as NLRs (Nucleotide-binding, Leucine-rich Repeat proteins), enabling the plant to launch a defence response. In some cases, such as for the rice NLRs Pikp1/Pikp2, these proteins function in pairs, with one of the NLRs being responsible for directly binding to the effector protein. Pikp1 achieves direct binding to the effector AVR-Pik through its unconventional integrated HMA (Heavy-Metal Associated) domain. Interestingly, another NLR pair from rice, RGA5/RGA4, also recognises effectors through direct binding to an integrated HMA domain, although this only shares 53% sequence identity to the Pikp1-HMA. In the RGA5/RGA4 case, the NLR pair recognises the effectors AVR-Pia and AVR1-CO39. Despite sharing little sequence similarity, the *M. oryzae* effectors AVR-Pik, AVR-Pia, and AVR1-CO39 share a similar conserved protein fold. This work aims to unravel how these structurally similar but sequence divergent components of the plant/pathogen battleground retain their individual activities, and whether there is potential to use any shared features to engineer bespoke NLRs with the ability to confer improved disease resistance in host plants.

#15

MAPPING THE PROTEASE REPERTOIRE OF *NICOTIANA BENTHAMIANA*

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Nicotiana benthamiana is a key expression system used both in plant research and for the production of pharmaceuticals in plants (molecular farming). Infiltration of *N. benthamiana* leaves with *Agrobacterium tumefaciens* (agroinfiltration) is a versatile, yet quickly scalable technique to obtain transiently transgenic tissues. In molecular farming, degradation of the target protein by plant proteases has been identified as a bottleneck limiting recombinant protein yield [1]. When studying localization or interaction partners of a protein of interest, knowing the physiology of the expression can help interpret results correctly [2].

To inform decisions, both on how to overcome the degradation bottleneck in molecular farming and on how to design and interpret overexpression studies, we have assembled a comprehensive physiological map of both naïve and agroinfiltrated *N. benthamiana*. We characterize the *N. benthamiana* response to *A. tumefaciens* and highlight proteases that likely play key roles in recombinant protein degradation.

A time-resolved transcriptome dataset sets the scene, allowing us to understand the physiological state of the plant in molecular farming conditions. The transcriptome also provides the basis for detailed manual curation and annotation of the *N. benthamiana* proteome with a special focus on proteases. Harnessing the power of the annotated database, a time-resolved apoplastic proteome dataset provides a second level of information. We thus reveal the post-transcriptional regulation and secretion processes shaping the protease repertoire of the plant apoplast, the target compartment for most biologics produced in *N. benthamiana*. Finally, active proteases are pointed out within the repertoire, using activity-based protein profiling [3] coupled with protein mass spectrometry.
Our study provides the community with a comprehensive dataset of the proteases that need to be taken into consideration when aiming to improve recombinant protein production in *N. benthamiana*.

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#16

INVESTIGATING THE CURRENT STATUS AND PREDICTING FURTHER EVOLUTION OF AZOLE FUNGICIDE RESISTANCE IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Plant pathogens are a major challenge to crop production and food security around the world. One of the most important diseases in wheat-growing countries is *Septoria tritici* blotch (STB), caused by *Zymoseptoria tritici* (formerly known as *Mycosphaerella graminicola*). Although there are wheat varieties with resistance to STB, the pathogen can overcome plant resistance [1].

Septoria tritici blotch is managed mainly through fungicide applications, although the pathogen can adapt resulting in fungicide resistance. Resistance to the Methyl Benzimidazole Carbamate fungicides (MBCs) occurred by 1984 [2]. The G143A mutation in cytochrome *b*, resulting in resistance to the QoI fungicides, emerged in the UK and Ireland in 2002 [3] and is now widespread throughout the UK. A large number of mutations in the gene that encodes the azole fungicides target site protein have been identified, resulting in populations of *Z. tritici* less sensitive to most azole fungicides. Some field isolates with mutations conferring reduced sensitivity to the SDHI fungicides have already been detected [4]. On the other hand, the multi-site inhibitors have not been affected by resistance in *Z. tritici*, due to their low risk resistance status. It is important to keep monitoring *Z. tritici* populations to design management programs to reduce risk of establishment of fungicide resistance.

Once the fungicide resistance status of *Z. tritici* populations from South America is not known, my research will establish if fungicide resistance has evolved in *Z. tritici* populations from Argentina, Chile and Uruguay. In addition, the mechanisms of fungicide adaptation in the South American populations will be compared with those found in NW-European populations, with a well-known history of fungicide resistance evolution. The potential for future azole resistance evolution in Z. tritici will also be explored, through experimental evolution.

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#17

HOW DOES A PLANT IMMUNE RECEPTOR COMPLEX CONFER DISEASE RESISTANCE?

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Plants employ a range of intracellular immune receptors for the detection of pathogens through the recognition of effector proteins. One such example of these immune receptors are the paired resistance proteins RRS1 and RPS4. These proteins function as a complex with RRS1 responsible for the detection of the bacterial effectors AvrRPS4 and PopP2, via its integrated WRKY domain, and RPS4 acting as the signalling component transducing RRS1's effector perception to the activation of downstream plant defence pathways. The exact mechanism through which effector recognition results in the conversion of RRS1/RPS4 to a defence-activating complex however still remains elusive.

Previous *in planta* studies have demonstrated a number of interactions between the domains of RRS1, RPS4 and bacterial effectors but structural evidence is required in order to truly understand and validate the signalling events involved in RRS1/RPS4 activation upon recognition of effector proteins.

Through heterologous expression of the modular domains of RPS4 and RSS1 we hope to be able to utilise biophysical techniques such as X-ray crystallography and surface plasmon resonance (SPR) in order to define at the structural level both the inter and intra molecular interactions that occur between these paired resistance proteins. Further to this we shall investigate how these interactions change upon recognition of bacterial effectors AvrRPS4 and PopP2 and how these reconfigurations result in the conversion of RPS4 and RRS1 to a defence-activating complex. This knowledge will provide crucial insights for future engineering of such paired resistance proteins for the development of durable broad-spectrum resistance crops.

#18

DISSECTING MULTIPLE RESISTANCE SPECIFICITIES AT THE MLA LOCUS IN BARLEY

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In host-pathogen interactions, disease resistance is often conditioned in a gene-for-gene manner involving the direct or indirect interaction of a avirulence and R gene. In contrast, nonhost resistance is known to be broadly functional against a range of microbes. It remains unclear how a plant can be nonhost to the majority of pathogens but encode a finite number of immune receptors. We propose the model of multiple pathogen recognition specificity (MPRS) to explain the broad recognition of nonhost pathogens. Several examples are known, including Arabidopsis RPS4/RRS1 NB-LRR genes [1] and tomato Mi-1 [2] which confer resistance to taxonomically distinct plant pathogens. In our investigation of nonhost resistance in barley to wheat yellow rust, we found that resistance colocalises with Mla, which is a major resistance locus in barley to the host pathogen, barley powdery mildew (Blumeria graminis f. sp. hordei) [3]. Over 30 alleles of Mla have been identified to confer isolate specific B. graminis f. sp. hordei resistance, and these belong to the NB-LRR class of resistance genes [4]. Resistance to multiple pathogens has been associated with the Mla locus of barley, including B. graminis f. sp. hordei, Puccinia striiformis f. sp. tritici (Rpst1), Puccinia striiformis f. sp. hordei [5] and Magnaporthe oryzae (Rmo1) [6]. Sensitivity to the host selective toxin victorin produced by Cochliobolus victoriae (LOV1) is also in coupling with Mla. Previous work in Arabidopsis found LOV1 to be conditioned by an NB-LRR, highlighting the trade-off between biotrophic pathogen resistance, and necrotrophic pathogen susceptibility [7]. Isolation of victorin allowed for the identification of cross-species toxin sensitivity, and work is underway to identify a shared susceptibility pathway between Arabidopsis, Brachypodium and barley. Current work will elucidate the relationship of Mla3, Rmo1, and LOV1 including forward genetic (high-resolution recombination screens) and reverse genetic (transformation of candidate gene) approaches.

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4 Wei, F., et al. The Mla (powdery mildew) resistance cluster is associated with three NBS-LRR gene familes and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics, 153: 1929-1948 (1999) 5 Verhoeven, E. C., et al., Registration of the BISON Genetic Stocks in Hordeum vulgare L. Plant Registrations (2011) 6 Inukai, T. RMo1 confers blast resistance in barley and is located within the complex of resistance genes containing Mla, a powdery mildew resistance gene. MPMI, 19:9, 1034-1041 (2006) 7 Lorang, J. et al., Tricking the Guard: Exploiting Plant Defense for Disease Susceptibility. Science, 338, 659 (2012)

#19

GENETIC BASIS OF TRANSLOCATION OF *MAGNAPORTHE ORYZAE* AVR-Pik INTO RICE CELLS

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Fungi and other filamentous pathogens secrete effectors inside host cells to help establish a successful infection in the host plant. However, the mechanism by which effectors translocate into the plant is unknown (Petre and Kamoun, 2014, PLOS Biology, 12:e1001801). The effector AVR-Pik is produced by the rice blast pathogen *Magnaporthe oryzae* and binds to the rice intracellular NLR immune receptor Pikp-1, which subsequently activates Pikp-2. The allele AVR-PikD binds the HMA domain of the Pikp-1 protein with nanomolar affinity, indicating that AVR-Pik is translocated inside the host cell. In addition, the crystal structure of the AVR-PikD/Pikp-HMA complex has been resolved (Maqbool *et al*. 2015, eLife, 4:e08709) revealing the contact points between the effector and the NLR protein. I have performed an alanine mutant scan for single amino acid mutants of AVR-PikD, and investigated the activity of the mutants by performing a Hypersensitive Response (HR) screen in *Nicotiana benthamiana*. I have adapted *M. oryzae* transformation plasmids to work with the Golden Gate cloning system, and have used these to transform the fungus. Work is ongoing to further characterise the active mutants, and to determine which are translocated from *M. oryzae* into Pikp rice. The objective of this project is to take advantage of this basic knowledge to identify AVR-PikD residues that are required for translocation inside rice cells but do not affect detection by Pikp-1. This will help us to determine the genetic basis of translocation of AVR-Pik into rice cells.

#20

PAMP-TRIGGERED IMMUNITY & QUANTITATIVE DISEASE RESISTANCE IN BRASSICAS

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Brassica species are important crops used in bioenergy production as well as being an important food source consumed widely around the world. Plant diseases cause significant losses to farmers, average of 26% of worldwide crop production and this amount of lose directly threatening our main life sources. More Durable and broad-spectrum disease resistant crop varieties are needed to ensure food security and stable production. Improved understanding of disease resistance mechanisms involved in plant-pathogen interactions will enable more efficient and environmentally-friendly solutions to crop improvement.

The first level of the plant immune response is PAMP-triggered immunity (PTI). PAMPs (Pathogen associated molecular pattern) are conserved molecules of a fungal or bacterial pathogens that elicits host`s defence response. There is increasing evidence that PTI may contribute to durable disease resistance. I am studying PAMPs called NLPs (Necrosis & Ethylene-inducing like peptide 1-like proteins) which were recently identified in Arabidopsis (Oome et al., 2014). NLPs are found in a wide range of pathogenic fungi infecting Brassica species, so improved understanding of their recognition could enable more disease resistant crops to be developed. To identify genes controlling NLP response I am undertaking Genome Wide Association Study (GWAS) using a diversity set of Brassica napus lines. The production of reactive oxygen species (ROS) in response to NLPs derived from Brassica pathogens will be measured in 192 cultivars and GWAS will be performed. Candidate gene loci will be functionally tested in Arabidopsis and Brassicas. Results will enable identification of gene markers that could be used to develop more durable disease resistance in Brassicas.

#21

GENE EXPRESSION ANALYSES OF THE BACTERIAL TREE PATHOGENS GIBBSIELLA QUERCINECANS AND BRENNERIA GOODWINII IN VITRO AND IN PLANTA

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Acute Oak Decline (AOD) is an increasingly prevalent, recently described Decline-disease affecting native British oak; similar Declines are also reported in continental Europe. Two bacterial species, *Brenneria goodwinii* and *Gibbsiella quercinecans* are isolated consistently from necrotic lesions on affected oaks in Britain, but are rarely identified on healthy oaks. Gene expression analyses provide insights of lifestyle alterations within bacterial phytopathogens, which frequently exist as harmless commensals before switching to a pathogenic phenotype. Here, RNA-seq was used to characterise gene expression of *G. quercinecans* and *B. goodwinii* in laboratory created microcosms, supplemented with oak phloem or sapwood, as well as in AOD lesions. *G. quercinecans* demonstrated substantial upregulation of putative virulence genes in phloem microcosms, whereas *B. goodwinii* virulence genes were highly expressed in sapwood microcosms. These data indicate that *G. quercinecans* preferentially utilises phloem, whereas *B. goodwinii* utilises sapwood as a nutrient source. Metatranscriptome sequencing of AOD field lesions revealed expression of the same phytopathogenic virulence genes, but also transcripts of *B. goodwinii* and *G. quercinecans* indicated that these genes were more abundant *in planta* when compared to *in vitro* microcosms. These results indicate that *B. goodwinii* and *G. quercinecans* express phytopathogenic genes on oak tissue, thereby implicating them in maceration of oak tissue. This work has characterised the phenotype of *B. goodwinii* and *G. quercinecans* on oak tissue, identifying key virulence factors both in the field and in laboratory trials providing compelling evidence of their role in lesion formation in AOD. Ultimately, this information will inform management aimed at halting the spread of a serious Decline of Britain's native oak.

#22

IDENTIFYING LIFE-STAGE SPECIFIC SURFACE PROTEINS OF THE GOLDEN POTATO CYST NEMATODE GLOBODERA ROSTOCHIENSIS.

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Potato cyst nematodes (PCN) are estimated to cause over £50 million worth of crop losses in the U.K. each year. It has been shown that the infective juveniles are able to alter their surface composition to avoid damage from host defence mechanisms. However, relatively few proteins secreted from the cuticle surface of PCN juveniles have been identified. This project will identify the surface proteome of infective juveniles and will identify changes to these surface components throughout infection of the host. We have developed methods that allow labelling of proteins present on the cuticle surface of PCN. Isolated proteins can then be purified and analysed using mass spectrometry; this information is used to allow identification of proteins from the genome of the nematode. Genes of interest are cloned and protein expression trials will identify protein function *in vitro* and *in planta*.
Surface protein removal techniques have identified a variety of proteins some of which have previously been described as members of the *G. rostochiensis* surface secretome. This presentation will focus on three previously undescribed proteins; Galectin-5, Cri-2 (conserved regulator of innate immunity) and a nematode specific hypothetical protein.

#23

UTILIZING EXTENDED NATURAL VARIATION TO ENGINEER THE CF-2/RCR3 PERCEPTION MECHANISM

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The extracellular space of leaves is the location of many interactions between pathogens and host. These interactions include the inhibition of host-secreted hydrolytic enzymes by pathogen-derived inhibitors. Rcr3, a secreted Papain-like cysteine protease from tomato, is inhibited by Avr2, a small cysteine-rich effector from the fungal pathogen Cladosporium fulvum. Tomato varieties containing the Cf-2 resistance gene, which encodes for a receptor-like protein, are resistant to C. fulvum races expressing Avr2. Rather than perceiving Avr2 directly, Cf-2 perceives the Avr2-Rcr3 complex, ultimately resulting in a hypersensitive cell-death response (HR). RCR3 has been proposed to act as a decoy for the perception of Avr2, while Pip1, an ancient paralog of Rcr3, would be the true target of Avr2. We found that Rcr3 and Pip1 are conserved in a syntenic block in all analysed Solanaceae thus far. We were able to transiently express Cf-2, Rcr3, and Avr2 in Nicotiana benthamiana and trigger a specific immune response. This is possible because N. benthamiana is a natural KO for Rcr3 and Pip1. In addition to tomato Rcr3, all other tested Rcr3 orthologues from other Solanaceae species are able to signal through Cf-2. We are using this information, together with important residues identified in a series of hybrids of Rcr3 and Pip1, to engineer Pip1 to signal the perception of Avr2 through Cf-2. Ultimately we aim to engineer the Cf-2/Rcr3 system to perceive additional effectors from other pathogens.

#24

DEVELOPMENT OF MYCOVIRUS BASED VECTORS TO SILENCE DOTHISTROMA SEPTOSPORUM GENES

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Mycoviruses specifically infect and replicate in fungi, they are cryptic in nature and usually associated with hypovirulence (reduced virulence) or more unusually hypervirulence. It is suggested that, with the discovery of mycoviruses in Aspergillus fumigatus, hypovirulence can be induced through gene silencing. Dothistroma septosporum is the causal agent of Dothistroma needle blight affecting Pinus spp. Its origin is unclear with suggestions that it originated from South America and Nepal, with early reports in 1911 in Eastern Europe and in the 1920's in Western Europe and North America. Following a screen of over 49 isolates we discovered one isolate infected with a tetra partite mycovirus reminiscent of a chrysovirus which we have named Dothistroma septosporum chrysovirus (DsCV-1), belonging to the family Chrysoviridae. DsCV-1 shares similar phenotypic and genomic properties to Aspergillus fumigatus chrysovirus possessing a tetra partite, double-stranded RNA (dsRNA) genome, RNAs 1-4 with respective accession numbers (FN168512, -13, -14 and -15) and sizes 3560, 3159, 3006 and 2863 bp. The aim of this project is to construct full-length clones of DsCV-1 characterising in detail the proteins involved in gene silencing which is currently underdeveloped in fungi and to construct gene silencing vectors. At this early stage of the project, approximately 57% of the tetra partite DsCV-1 dsRNA genome has been cloned and sequenced using genome walking and RLM-RACE recombinant DNA technology.

#25

UNDERSTANDING THE ECOLOGY AND EPIDEMIOLOGY OF PYTHIUM VIOLAE CAUSING CAVITY SPOT ON CARROT.

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Cavity spot is a major disease of carrot. In the UK it's caused primarily by the soilborne oomycete *Pythium violae*. Infection results in small black lesions and an unmarketable crop with high-value losses occurring every year. Disease management is challenging due to variable fungicide efficacy and difficulty in implementing long rotations. A lack of effective research tools including diagnostics for *P.violae* and the absence of a reproducible inoculation procedure has hampered research to understand factors conducive to disease.

Initially, the identity of *Pythium* spp. associated with cavity spot was determined using a collection of 127 isolates from diseased carrots. Following ITS sequencing, 60% were identified as *P.violae*, 15% *P.intermedium* and 14% *P.sulcatum.* Further characterisation through sequencing of housekeeping genes and pathogenicity tests is being completed to understand variation in the pathogen. Furthermore, an amplicon sequencing approach will be used to investigate *Pythium* communities and their interaction with other microbiota.

In order to quantify *P.violae* in soil and roots, a specific PCR test has been developed which, in combination with an oospore-capture procedure, potentially allows testing of larger soil samples. Initial results suggest that detection of less than 10 oospores in 10g may be possible. Testing of qPCR is currently underway.

#26

FUNCTIONAL GENOMICS OF VIRULENCE FACTORS AND SUSCEPTIBILITY GENES IN THE BARLEY-POWDERY MILDEW INTERACTION

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Short antisense oligonucleotides have been shown to induce gene silencing in both animal and plant systems. We first adapted this technology to investigate effectors of *Blumeria graminis* f.sp. *hordei* the causal agent of barley powdery mildew. We referred to the system as “Silencing Targeted Effectors in Planta” or STEP.

STEP is advantageous in its simplicity compared to other transient Host induced Gene Silencing (HIGS) methods and in allowing the treatment of whole tissues rather than single cells. Moreover, the use of short antisense oligonucleotides for the STEP system allowed for more specificity in silencing a single gene target. STEP was first validated by targeting known *Blumeria* effectors previously identified by HIGS in an alternative transient biolistic transformation assay, leading to comparable results.

STEP has also been successful in decreasing *Blumeria* infection when targeting *Blumeria* housekeeping genes and barley susceptibility genes such as MLO and Blufensin.

#27

CORE EFFECTORS OF PLANT-PARASITIC NEMATODES AND THEIR HOST TARGETS

Kerry Leslie

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Plant parasitic nematodes (PPN) parasitise many major food crops including potato, tomato and soybean. PPNs cause damage valued at ~80 billion dollars in crop loss per year. Many nematode species must form a feeding site called a syncytium in the roots of their host plant. The nematode can only form one syncytium in their life time. The aim of this project is to identify a core set of effectors present and expressed in all nematode species that form syncytia. This analysis will be conducted on nematode species; *Globodera rostochiensis, Globodera pallida, Rotylenchulus reniformis* and *Nacobbus aberrans*. This will allow for the characterisation of a basal set of effectors that these nematodes need in order to successfully surpass the plant immune responses and to create and maintain a syncytium. Comparisons between the effectors present in these species suggests that 37 gene families are conserved across these nematodes. A subset of these core effectors will be functionally characterised, with the aim of determining their function. Current work is focused on a novel cell wall degrading enzyme that is present in each of these species.

#28

HYPER-REACTIVE CYSTEINE PROFILING IN ARABIDOPSIS CELL CULTURES

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Hyper-reactive Cys residues are relevant for sensing and transducing changes in cellular redox status. The unique property that sulphur can have multiple oxidation states permits a variety of reversible post-translational modification of Cys residues. Consequently, Cys residues are often found in the catalytic or regulatory domains of important regulatory enzymes such as ubiquitin-activating/conjugating enzymes, chaperones, transcription factors, kinases, phosphatases, proteases, and RNA-binding proteins. However, the underlying mechanisms of redox-dependent regulation of these proteins are still poorly understood. In this study, we employ Hyper-reactive Cys Profiling, a sensitive and powerful quantitative method, based on labelling native proteomes with a biotin tagged alkylating reagent, in Arabidopsis cell cultures to identify hyper-reactive Cys residues on genome-wide scale. We demonstrate that low concentrations of probes label the proteome of Arabidopsis cell cultures at cytonuclear pH (pH 7.5) and confirm that the probe labels a broad range of Cys-containing proteins. Sequencing labelled peptides has revealed positions of novel hyper-reactive Cys residues in Arabidopsis. We will discuss our findings in terms of potential redox signalling in plants.

#29

IDENTIFICATION OF AVIRULENCE GENES IN POTATO CYST NEMATODES AND ASSESSMENT OF DURABILITY OF RESISTANCE

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The potato cyst nematodes (PCN) Globodera rostochiensis and G. pallida are the most economically important nematode problems of UK arable agriculture. They impose an annual cost of over than £50 million. Both species include different genotypes (pathotypes) that originate from different sites in South America where it is presumed that have been independently introduced from into Europe in the 19th century along with wild potato material. The most effective way to control PCN is through the use of natural resistance. The major resistance gene H1 has been used to control G. rostochiensis which has led to a predominance of G. pallida. Two wild potato species, Solanum vernei and S. tuberosum ssp. andigena CPC2802 have been widely used in PCN resistance breeding programmes. The main goal of this project is to identify candidate Avirulence (Avr) genes in both PCN. Previous studies showed that continuous rearing of G. pallida field populations on partially resistant potato lines allowed selection for increased virulence against these two resistance sources. We aim to analyse diversity in effector sequences from G. pallida in order to identify candidate Avr genes recognised by the commercially important QTLs from Solanum vernei and S. tuberosum ssp. andigena CPC2802. Screening of these G. pallida selected populations confirmed significant differences in their reproduction rate depending on the initial selection source selected on. Moreover, the genome assembly for G. rostochiensis revealed differences in the sequences of two potential effectors between virulent and avirulent pathotypes. Functional validation of these genes is now ongoing.

#30

CHARACTERISING THE INFLUENCE OF DIFFERENT WHEAT CULTIVAR RHIZOSPHERES ON VARIATIONS IN MICROBIOME DIVERSITY AND FUNCTIONALITY.

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In agricultural systems plant diseases caused by soil borne fungi are regarded as the most devastating. Wheat is recognized as an important crop worldwide. However, it is highly susceptible to Take-all disease caused by the soil ascomycete fungus *Gaeumannomyces graminis var. tritici* (*Ggt*). Interest in biological control of Take-all has increased due to a lack of resistant wheat cultivars and chemical pesticides. Although this disease has been extensively studied it is still regarded as an excellent model for biological control of plant root diseases. *Pseudomonas fluorescens* is well recognized for its plant growth promoting and disease suppressive properties as it is often found to be prevalent in controlling Take-all. A preliminary investigation by Mauchline et al., (2015) highlighted a possible effect of wheat cultivar on the selection of plant associated fluorescent *Pseudomonas* spp. I have used a dot-blot approach to screen a larger number of *Pseudomonas* isolates for key plant colonisation genes to validate these earlier findings and carry out a deeper analysis. This talk will discuss these findings in relation to the previous study and consider future plans.

#31

RAPID IDENTIFICATION OF *ARMILLARIA* SPECIES ASSOCIATED WITH OAK DECLINES IN BRITAIN

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Members of the genus *Armillaria* are basidiomycete fungi, comprising of approximately 40 described species with representatives found in all regions of the world. Some *Armillaria* species are soil-borne plant pathogens that cause necrosis of living tree tissue in a wide variety of host species. *Armillaria* species have been associated with tree declines across Europe, however, it is difficult to assess the role of *Armillaria* within oak declines, as this is confounded by variations in host specificity and virulence. In Britain, four *Armillaria* species are associated with disease in oak, *A. mellea, A. gallica, A. ostoyae,* and *A. tabescens*. These species have been found on the root systems of trees that are affected by acute oak decline (AOD) and chronic oak decline (COD). Rapid species detection is an important factor in understanding the role and ecology of *Armillaria* species in oak declines. Traditional diagnostic techniques are time consuming and consist of fungal isolation and cultivation, DNA extraction, sequencing and phylogenetic analysis of marker gene regions such as ITS, IGS and EF1-α. This project aims to utilise High Resolution melt curve (HRM) analysis to develop a rapid diagnostic test for species-level identification of *Armillaria*. HRM is a PCR-based method that detects differences in DNA sequences based on the differential melting temperature of amplicon templates, providing a melt curve profile to discriminate between the *Armillaria* species. To test the applicability of this technique, three primer pairs with a range of amplicon sizes were designed using the using the EF1-a gene region. End-point PCR was used to validate primer specificity and one primer set was taken forward for optimisation and validation using HRM analysis. Preliminary results show that HRM can be used to discriminate between *A. ostoyae* and *A. tabescens*, however, *A. gallica* and *A. mellea* are currently more complex to differentiate. The primer set has recently been modified and testing is currently underway to determine if the modifications allow for better discrimination between *A. gallica* and *A. mellea*.

#32

COMPARATIVE AND FUNCTIONAL ANALYSIS OF DOWNY MILDEW EFFECTORS

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Peronospora farinosa (Pfs) is an obligate biotrophic oomycete that causes downy mildew in spinach. In terms of crop loss, it is the most important disease in the spinach cultivation industry. Upon infection, downy mildews secrete effector proteins that either function inside or outside the host cell. Effectors manipulate plant cell processes and can suppress defence responses of the plant. The aim of my project is to identify effector proteins that are conserved in downy mildews/oomycetes and to study their role in the infection process. A reference genome and transcriptome of Pfs were generated and used to predict the secretome of Pfs1. In a comparative approach using OrthoMCL, the secreted proteins of Pfs were compared to the predicted secretomes of eight other plant pathogenic oomycetes (downy mildew and Phytophthora species). From the OrthoMCL clusters, conserved proteins that have known effector domains, like RXLR and crinkler motifs, were selected for further functional studies. Conserved effector proteins are likely to be of crucial importance to the infection strategy of downy mildews/oomycetes and are, therefore, an appealing target for resistance breeding. Moreover, this approach allows for the identification of unique effectors that are species- or genus-specific and that have evolved more recently, possibly to adapt to specific hosts.

The virulence function of selected Pfs effectors is now being studied by identifying interacting host proteins using the yeast two hybrid (Y2H) system. Interaction screens of Pfs effectors with an oligo-dT-primed Arabidopsis cDNA Y2H library revealed candidate target proteins. Among the interaction candidates are NAC transcription factors and other proteins that have a potential link to plant defense. The interactions between effectors and candidate targets will be validated in planta and their possible role in plant resistance or susceptibility will be further functionally analyzed.

#33

DISSECTING THE ROLE OF RNA-BINDING PROTEINS IN PLANT IMMUNITY

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RNA-Binding Proteins (RBPs) have emerged as master regulators of gene expression and are essential for cellular RNA homeostasis. Even though the function of RBPs in several cellular processes has been extensively studied for metazoans, bacteria and viruses, plant RBPs have received little attention in the literature. Moreover, although recent studies have pinpointed the importance of RBPs in plant immunity, the evidences that RBPs play important roles in stress responses have been collected stepwise and comprehensive approaches are lacking. Therefore, to gain global perspective into the role of RNA-Binding Proteomes (RBPomes) in plant immunity, we are currently optimizing the cutting-edge technique 'RNA interactome capture' to our model system. By applying 'RNA interactome capture' to *Arabidopsis thaliana* plants infected with *Pseudomonas syringae*, we hope to identity the scope of RBPs involved in the plant defence response against pathogens. Targeted functional analyses will be performed to further validate the identified RBPs and determine their specific role in RBP-mediated plant immunity.

#34

LIGHTING UP PLANT DEFENCES; FROM OUR LAB TO YOUR FRIDGE

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Grey mold, *Botrytis cinerea*, is one of the most important fungal pathogens. It is estimated to cause losses of over £88 million per year in the UK tomato and cut flower industries, due to high fungicide cost, reduced yield and quality of produce. Broad host range, quiescent life cycle and resistance to fungicides are main challenges of *Bortytis* control. An industrial-academic research partnership was therefore formed to develop commercially applicable, non-chemical method to control *Botrytis* in pre-harvest environments.

The method consists of short pulses of extremely high intensity broad spectrum light (UV-VIS-IR) applied to vegetative part of tomato plant. Light treatment elicits plant defenses before inoculation and reduces Botrytis induced disease symptoms. We developed commercially applicable light treatment that elicits maximum resistance and causes minimal or no damage to the plant. We determined the longevity of light induced resistance and tested its applicability in different tomato cultivars. The method is being validated in production tomato greenhouse where *Botrytis* control and effect on yield are tested on a large scale.

To understand the molecular basis of pulsed light induced resistance we have performed whole transcriptome analysis (RNA-seq). Transcriptomic response to light treatments was determined at different time points during and after the treatments. Additionally, response to *Botrytis* inoculation was compared between treated and control plants. Involvement of ROS and ethylene signaling in light induced resistance was highlighted.

#35

INTERPLAY BETWEEN PLANT APOPLASTIC PROTEASE AND INHIBITOR DURING INFECTION

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During infection, plants and pathogens secrete a range of proteins that dynamically interact in the apoplast, the intercellular space of plant tissues. Proteases are among the prominent players in plant defense responses, with members of the subtilase family identified as pathogenesis-related proteins and involved in plant immunity. Using activity-based protein profiling (ABPP) on *Nicotiana benthamiana* plants infected with *Pseudomonas syringae* pv. tomato DC3000, we found that the activity of plant apoplastic subtilases is reduced upon infection. Further experiments hinted at an involvement of an inhibitor whose origin and identity remains unknown. Preliminary data from mass spectrometry experiments and characterisation of the physical properties of the inhibition suggest K2, a plant-derived Kunitz inhibitor, as a potential candidate. This project aims to characterise the role of kunitz inhibitors in suppressing subtilase activity during plant immunity. To test this hypothesis we have produced *Nb*K2 in *N. benthamiana* by agroinfiltration and tested subtilase inhibition *in vitro* using ABPP. Furthermore, we performed virus-induced gene silencing (VIGS) experiments to address the role of kunitz inhibitors in the defence response of *N. benthamiana* to bacterial pathogens.

#36

INVESTIGATING THE ROLE OF NADPH OXIDASES DURING INFECTION RELATED DEVELOPMENT OF THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE.

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Rice blast disease is a major threat to global food security and remains very difficult to control in all rice-growing regions of the world. The rice blast fungus *Magnaporthe oryzae* infects plants with a specialized single-celled infection structure called an appressorium, which develops enormous intracellular turgor to drive a rigid penetration peg through the rice leaf cuticle. NADPH oxidases (Nox) are flavoenzymes that function by transferring electrons across biological membranes to catalyse the reduction of molecular oxygen to superoxide. Nox enzymes found in animal cells are implicated in cell proliferation, cell signaling, and apoptosis, whereas in plants Nox are necessary for programmed cell death, the response to environmental stresses, pathogen infection, and polarized growth of root hairs. In filamentous fungi, Nox enzymes are necessary for cellular differentiation during sexual reproduction and for developmental processes that involve transitions from non-polarized to polarized cell growth, such as tissue invasion by mutualistic and pathogenic fungi, and fungal virulence. Previously, it was shown that Nox are essential for septin-mediated re-orientation of the F-actin cytoskeleton in *M. oryzae* to facilitate cuticle rupture and plant cell invasion. Moreover, the Nox2-NoxR complex, in particular, is essential for spatial organization of a hetero-oligomeric septin ring at the appressorium pore, necessary for assembly of a toroidal F-actin network at the point of penetration peg emergence. We have also demonstrated a direct effect of reactive oxygen species on F-actin polymerisation and appressorium function. However, the mechanism for assembly and activation of the Nox complex at the plasma membrane in *M. oryzae* is still unknown. Here, we have investigated the protein-protein interactions that occur within the Nox1 and Nox2 complexes by yeast two-hybrid analysis. We have defined the interactions between Nox1, Nox2, NoxR and regulators of polarity, such as Cdc42, Cdc24, Bem1, and Rac1. We aim to determine the structural dynamics of the protein machinery required for activation and function of the *M. oryzae* Nox complexes.

#37

EXAMINING IDENTITY, PHYLOGENY AND PATHOGENICITY FACTORS IN FUSARIUM SPECIES AFFECTING PEA.

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Pea is an important legume crop for the UK, worth an estimated £50 million per year. The UK is also the largest producer of frozen peas in Europe, due to ideal conditions along the Eastern seaboard. Fields are located close to processing factories, which reduces the potential land available to grow peas.

Soilborne plant pathogens in the 'footrot' complex are one of the main causes of crop loss in peas, resulting in wilting, reductions in pod numbers and plant death. Several *Fusarium* species are major components of this complex with *F. oxysporum* f.sp. *pisi* (FOP) being amongst the most important worldwide. Pathogenicity factors including effector genes have been have been reported in other pathogenic *F. oxysporum* with Secreted in Xylem (SIX) genes widely studied.

The main aims of this PhD project are to identify the range of *Fusarium* species affecting pea in the UK and identify effector genes associated with pathogenicity. A survey of UK pea fields identified foot rot causing species including *F. oxysporum*, *F. solani* and *F. avenaceum,* which were used to develop inoculation methods to determine pathogenicity. Whole genome sequencing has revealed the presence of 7 SIX genes in FOP races 1 and 2.

#38

UNDERSTANDING PRIMING OF THE JASMONIC ACID DEFENCE PATHWAY: A MULTISPECIES APPROACH

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Plants are sessile organisms, who in order to survive, must be able to endure the stresses present in their environment. In the case of biotic stresses, plants protect themselves with constitutive barriers along with inducible defences. Specific aggressive attackers have evolved to overcome these defences. In response, exposure of plants to certain environmental cues can induce resistance. Defence priming, the environmental cue triggered sensitisation of a plants immune system resulting in a faster, stronger and/or more sustained induction of defence mechanisms, is one explanation for this elevated resistance[1–7]. Our understanding of short-term defence priming, including the mechanisms underpinning the memory of the initial cue, is well established[8–15]. However, if this acquired trait is to provide maximum benefit to agriculture and forestry, we need to increase our understanding of longer-term defence priming in economically important species. Norway spruce (*Picea abies*) is an economically and ecologically important tree species in Scandinavia. It is threatened by outbreaks of the aggressive bark beetle *Ips typographus* and its associated necrotrophic blue stain fungi. Taking an angiosperm centric view, both invaders are conventionally considered to be resisted by defences under the control of the phytohormone Jasmonic Acid (JA)[16]. Understanding long-term priming of the JA defence pathway could help develop strategies to alleviate the devastation caused to Norway spruce. While the majority of previous studies in Norway spruce have focused on priming of anatomical and chemical defences[17–21], mRNA-seq analysis of bark tissue sampled 24 hours after wounding of Methyl Jasmonate (MeJA) pre-treated mature trees, suggests proteinaceous defences, such as pathogenesis-related (PR) genes, are primed. MeJA treated spruce seeds and seedlings will be used to assess the mechanisms underlying the PR gene priming. Due to the abundance of tools and resources available, parallel studies testing initial hypothesises are being carried out in *Arabidopsis thaliana.* The two-species approach will both increase speed of knowledge gain and allow comparisons of the mechanisms underpinning priming of the JA defence pathway in angiosperms and gymnosperms.

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#39

BLUMERIA METALLO-PROTEASE LIKE EFFECTOR: A VIRULENCE FACTOR FOR CEREAL POWDERY MILDEWS OR A UNIVERSAL VIRULENCE FACTOR IN FUNGI?

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Blumeria metallo-protease like effector (BEC1019) is a virulence factor specifically expressed in the haustoria of the obligate biotrophic fungus *Blumeria graminis* f. sp*. hordei* (*Bgh*), the causing agent of barley powdery mildew. BEC1019 silencing led to reduced haustoria formation, hyphal growth and disease dispersion, while BEC1019 protein introduction in barley leaves reduced bacterial pathogen induced hypersensitive reaction (HR). BEC1019 homologues are present in 96 out of 241 sequenced fungal genomes comprising plant and animal pathogens, including known virulence factors in *Candida albicans* (PRA1) and *Aspergillus fumigatus* (Asf2). These proteins share a conserved HRXXH domain, closely related to M35 superfamily of zinc-binding proteases. A conserved ETVIC motif mediates barley HR suppression. Therefore, these findings suggest a universal virulence role for BEC1019 homologues in fungi. Using gene knock-outs and a novel short antisense oligonucleotide based host induced gene silencing (HIGS) mechanism, the role of BEC1019 homologues will be investigated in the virulence of economically important wheat fungal pathogens. In particular, silencing of the BEC1019 homologue in *Blumeria graminis f. sp. tritici* (*Bgt),* the causative agent of wheat powdery mildew, led to reduced secondary hyphae formation and fungal biomass, indicating a role of BEC1019 in *Bgt* virulence. To our knowledge, this is the first convenient HIGS methodology established in the wheat – *Bgt* pathosystem. Following these encouraging results, BEC1019 knock-outs will be produced in *Fusarium graminearum (Fg),* causing Fusarium head blight (FHB) and *Zymoseptoria tritici* (*Zt*), causing Septoria tritici blotch (STB) to evaluate the putative universal virulence role of this metallo-protease protein, with the ultimate goal of exploiting effector biology knowledge to devise new strategies for crop protection.

#40

CF2/RCR3 PATHOGEN PERCEPTION MECHANISM: ITS NATURAL VARIATION AND EVOLUTION IN SOLANACEAE FAMILY

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Plant pathogens secrete inhibitors into the extracellular space of leaves. The Cf2 resistance gene in tomato encodes a transmembrane receptor-like protein that confer recognition of Avr2, a protease inhibitor secreted by the fungus Cladosporium fulvum. Avr2 first interacts and inhibits a secreted papain-like cysteine protease in plants, called Rcr3. Recognition of Avr2-Rcr3 complex triggers a hypersensitive cell death response (HR) in plants, leading to resistance against the fungus. Other inhibitors, for instance, EpiC1 and EpiC2B from Phytopthora infestans and Cip1 from Pseudomonas syringe are also known to inhibit Rcr3, but contrastingly, Cf2 does not confer their recognition. Cf2 and Rcr3 were originally identified in Solanum pimpinellifolium (wild tomato). However, the molecular intricacies underlying perception of pathogens by this system have not been investigated in wild plants. We aim to identify wild species of Solanaceae family that can recognize Avr2/EpiCs/Cip1. To this aim, we have performed heterologous expression of Avr2/EpiCs/Cip1 and their inactive mutants in E. coli. Further, we have screened several populations of Solanum pimpinellifolium for HR upon injection with each purified wild-type and mutant protease inhibitors. PCR-based genotyping of responsive plants showed recognition occurring in the presence or absence of Cf2. Using transient approaches, we will now investigate if Cf2-Rcr3 pathway is involved in mediating recognition in responsive plants. Similar screening of wild tomato germplasms against Avr9 of C. fulvum had uncovered evolution of known resistance genes. Therefore, our study would potentially provide insights into the natural variation and evolution of Cf2-Rcr3 system in Solanaceae.

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#41

STRESSED YET INFECTIOUS: THE BIOSYNTHESIS OF ALPHA-GLUCAN IN PSEUDOMONADS

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Pseudomonas spp. are important pathogens of both humans and plants. It has been shown that the disruption of trehalose biosynthesis in pseudomonads reduces pathogenicity *in planta*, implicating trehalose as an important virulence factor during plant infection. Advances in our understanding of trehalose metabolism in other bacteria has identified an alternative metabolic pathway, also present in pseudomonads, which utilises trehalose to produce branched alphaglucan. Glucan biosynthesis is intrinsically linked to trehalose biosynthesis, meaning previous work must be revisited, this project will discern the role of glucan and its contribution to pathogenicity in the infection process.

#42

CHARACTERISING PHYTOPHTHORA FRAGARIAE, THE CAUSATIVE AGENT OF STRAWBERRY RED CORE DISEASE TO UNDERSTAND THE RESISTANCE RESPONSE

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Strawberries are a valuable fruit crop both for the UK and worldwide, in the UK the berry market was worth £1 billion in 2015. Strawberries made up 70% of this berry market in 2015. However, with the increased threat from unpredictable weather conditions and the withdrawal of many preventative chemical fungicides and soil fumigants; this industry is at risk. One of the major threats to the industry is the water-borne disease strawberry red core, caused by the oomycete pathogen *Phytophthora fragariae*. This work aims to improve the understanding of the nature of the resistance of strawberry plants to the pathogen.

One analysis approach being taken is to identify the genes within the pathogen that encode effectors, which when detected by the corresponding resistance gene in the host, trigger a resistance response. Resistance to *P. fragariae* is known to be based upon a gene-for-gene model, where a resistance protein in the plant detects a single secreted protein from the pathogen and triggers a resistance response.

In order to aid with this investigation, the genomes of ten isolates of *P. fragariae* were sequenced by Illumina short read sequencing and one isolate by single molecule real time sequencing using PacBio. These strains comprised five previously characterised races. The gene complements of these genomes were predicted and analysed to provide a list of predicted proteins containing key features of avirulence genes: secretion signal peptides and the RxLR, EER and WY motifs. The current focus is the characterisation of 'race 2' isolates for which the corresponding resistance locus has been mapped with a 257kb segment.

Another approach is to examine the variation between isolates of different strains, in order to understand the nature of the population structure of this pathogen and the potential step-wise loss of effector genes in this apparently clonally evolving pathogen. An analysis of the SNP distribution between different strains allowed the construction of a phylogenetic tree, identifying a key set of closely related strains.

Further investigation will allow the identification of avirulence genes to aid in the breeding of resistant plant cultivars.

#43

POPULATION ANALYSIS OF THE FINGER MILLET BLAST PATHOGEN MAGNAPORTHE ORYZAE IN EAST AFRICA USING MOLECULAR MARKERS

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Blast caused by the fungus *Magnaporthe oryzae* is a serious disease of finger millet wherever the crop is mainly grown. The disease affects many other cereal crops and it is a major threat to food and nutritional security. Knowledge of the pathogen population structure and evolutionary pattern is a key resource to develop sustainable disease management strategies.

The objective of this study is to investigate the population diversity of the pathogen in relation to time and space using sequence-based molecular markers. Geographic focus of the study is the major cropping districts of Uganda, Kenya, Tanzania and Ethiopia as part of a wider collaboration with partner institutions in East Africa and the USA. Bioinformatic analysis of the genome sequences available for the blast pathogen specifically from rice were used to identify loci that are highly informative in distinguishing pathogenic isolates associated with a single crop. Primers were designed to PCR amplify the variable regions in these loci and sequence analysis was carried out using a set of samples from more than 300 isolates from finger millet and related weed hosts previously collected from Uganda and Kenya.

We have identified a set of markers including a novel locus to assess the pathogen genetic diversity. Current results from more than 70 isolates representing the historical collection suggest the existence of diverse haplotypes. We have also generated a draft genome assembly of a key *M. oryzae* isolate from finger millet utilising NGS technology. These resources along with mating assays to assess the reproductive behaviour will enable comparative analysis of the historical (2000-2004) and contemporary (2016 -2017) populations of the finger millet blast pathogen in East Africa.

#44

A GENOME WIDE ASSOCIATION STUDY OF HOST SPECIFIC ADAPTATION IN *ZYMOSEPTORIA TRITICI*

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In a fungal plant pathogen interaction identifying significant associations with virulence and/or host specific adaptation is a critical step before functional validation of loci potentially involved in the infection process. An ideal tool for this is genome wide association study of polymorphisms identified by re-sequencing; this process is most effective on pathogens undergoing frequent sexual reproduction and rapid linkage-disequilibrium decay. Here we propose use of a modified GWAS on a contemporary population *Zymoseptoria tritici*, sampled from four wheat cultivars with differing levels of host resistance.

A standard GWAS involves univariate testing of each polymorphism association with the trait of interest independently, meaning limited power in identifying associations with combinations of genes, or genes with low or epistatic effect, which have recently been highlighted as a major barrier in our understanding of the *Z.tritici* pathosystem. Our GWAS will therefore make use of a statistical learning method of data analysis capable of resolving associations with combinations of polymorphisms. Following this, phenotypes of these isolates will be quantified by re-inoculation onto each of the four host cultivars in a greenhouse assay, and an association analysis carried out between reproductive success, and genotypes of predictively important polymorphisms using a generalized linear model.

#45

TAXONOMY OF *PSEUDOMONADACEAE* ASSOCIATED WITH AOD

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Oak trees native to Britain, *Quercus robur* and *Quercus petraea*, are under the threat from a disease called Acute Oak Decline (AOD). AOD was first reported in Britain about thirty years ago and is significantly increasing in incidence in the last several years. Affected trees have also been reported in several other countries in Europe such as Spain, France and Germany. The symptoms of infection are necrotic, longitudinal, bleeding cracks in the bark, from which emanates dark fluid. Severe cases are usually lethal within 4 to 5 years. Larval galleries of the buprestid beetle *Agrilus biguttatus* are regularly associated with the necrotic tissue.

It is believed that the syndrome has a polymicrobial origin. Over the past decade, many strains, mainly *Enterobacteraceae* have been isolated and identified. The composition of the bacterial community isolated from the necrotic lesions appear to be consistent amongst different sites. The predominant pathogens belong to the novel species *Gibbsiella quercinecans* and *Brenneria goodwinii* but also undescribed species included in the family *Pseudomonadaceae* have been isolated, although their role in the disease is not yet clear.

Before identification and diagnostic methods for the bacteria isolated from symptomatic oak can be optimised, it is necessary to know in detail the species involved in the infection, and their roles. To determine the phylogenetic position of the undescribed Pseudomonads, 16S rRNA sequencing of the strains was performed on strains from symptomatic tissue. Three protein-encoding genes (*rpoB, rpoD* and *gyrB*) were also sequenced as part of a multilocus sequence analysis (MLSA) study for a more robust taxonomic classification. The phylogenetic analyses indicate that several possible novel species of Pseudomonas are associated with AOD in Britain. Further work, including phenotypic, genotypic and hypersensitivity response assays, will be performed to formally classify these strains in the genus *Pseudomonas*.

#46

Z. TRITICI: LONG TERM SURVIVAL AND SUBSEQUENT PATHOGENICITY OF ASEXUAL SPORES

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Zymoseptoria tritici, the causal agent of Septoria tritici blotch in wheat, has evolved resistance to all major antifungal chemistries. The speed at which this has been achieved, and the associated expense in controlling this fungus, has elevated its status to the most economically important pathogen of wheat in temperate climates, now accounting for up to 70% of EU fungicide spending. The ability of *Z. tritici* to evolve resistance so rapidly, relies heavily on both the plasticity of its genome, and the diversity of genetic resources available for sexual recombination. It is unknown however, how such diversity, which apparently includes non-virulent strains, is maintained in the field as opposed to being outcompeted by strains able to undergo multiple asexual sporulation events during a growing season. Evidence in the literature is beginning to suggest a role for host-independent survival of *Z. tritici* spores, thus proposing an alternative survival strategy for any non-virulent strains present. This study, which assesses the ability for off-host spore survival in nutrient free environments, shows that spore populations can survive autonomously for extended periods, a trait strongly correlated with lipid nutrition. Additionally, somewhat surprisingly, spores from these 'starved' populations suffer no significant loss in virulence, thus potentially allowing for non-virulent strains to infect subsequent crops. Together, these findings suggest a route for the maintenance of high genetic variability and subsequent pathogenicity between crop cycles, as well as the and thus have far reaching consequences for how and when we treat *Z. tritici* in field situations.

#47

EVOLUTION OF SWIMMING MOTILITY IN AFLAGELLATED STRAINS OF *PSEUDOMONAS FLUORESCENS*

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Swimming motility is a vital trait for root colonisation and survival in harsh environments, and the flagellum is the organelle responsible for this type of movement. *Pseudomonas fluorescens* strain SBW25 is a plant-growth-promoting rhizobacterium which is important for maintaining plant health and the uptake of nutrients. In this study, aflagellated strains derived from *P. fluorescens* SBW25, ΔFleQ, AR1ΔFleQ::IS-Ω-Km/hah and AR2ΔFleQ:: IS-Ω-Km/hah, where the master flagella regulator, σ^{54}-dependent FleQ, is absent, were used to conduct experimental evolution experiments. The strain ΔFleQ slides on the surface of soft-agar and has a dendritic colony appearance; it moves slower (velocity 566.340 ± 900.264 mm^2/h) than the wild type strain SBW25 (velocity 670.709 ± 778.886 mm^2/h) because its movement is reliant on cell division. The biosurfactant (viscosin) operon in the other mutant strains is interrupted, and consequently colonies of these sessile strains expand only through accumulative cell growth; velocities for these strains on soft-agar are 0.968 ± 0.370 mm^2/h and 1.080 ± 0.322 mm^2/h for AR1ΔFleQ::IS-Ω-Km/hah and AR2ΔFleQ:: IS-Ω-Km/hah, respectively. Other researchers have found that these sessile bacteria evolve and become swimmers by diverting σ^{54}-NtrC in order to trigger expression of the flagellar regulon. Therefore, it was hypothesised that the physiological status of these sessile strains growing in minimal media under different nitrogen sources (NH_4^+, Gln and Glu) would evolve swimming motility depending on the nitrogen source present due to the evolutionary pressure exerted over the NtrBC system. The evolution of swimming was found to occur earlier for the nitrogen sources Gln and Glu due to the NtrBC system being activated under nitrogen limitation conditions. The evolved mutants showed a single point mutation within the *ntrB* gene, and demonstrated different surface motility profiles and distinct swarming types. Therefore, this study shows that homologous gene cross-talk occurs under specific stress conditions.