What Lies Within
imaging plant-microbe interactions

Programme and Abstracts

10 - 11 December 2018 | University of Warwick | UK
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Welcome from the BSSP President

I am delighted to welcome delegates to the British Society of Plant Pathology’s Annual Meeting here at Scarman House and very much hope you enjoy the event. BSPP Presidential meetings are somewhat unique in that the out-going President can choose a topic for the meeting which therein creates a dilemma. A topic too broad makes it challenging to develop a theme, whereas a topic too specific may not address the interest of our members. The idea to run a meeting on imaging plant pathogen interactions evolved in line with how my recent research has transitioned.

It is nearly three score years since the advent of molecular biology, one score since microarrays burst on the scene and a decade since next generation sequencing became democratized. Combined with proteomics and metabolomics we can generate large datasets of plant-microbe interactions. We can unravel the genetic blueprint of any chosen organism and measure how our favourite pathogen can elicit changes in host gene expression, or alter the abundance and/or post-translationally modify proteins.

However, putting these data into a temporal-spatial context that helps mechanistically understand plant-microbe interactions is challenging. There is a growing realisation that many cellular processes are impacted in both host and pathogen. Thus, research towards understanding the dynamics, complexities and contributions of inter-cellular and inter-organellar communication is the core theme of this meeting and - given the remarkable level of interest the meeting has generated - there is clearly a demand for such.

In addition to hosting the traditional PH Gregory offered paper and the John Colhoun postgraduate poster competitions, we also honour RKS Wood, with the inaugural RKS Wood Lecture. It is also with sadness that we note the passing of Richard Cooper, an Honorary Member and past President.

I sign off wishing everyone an enjoyable meeting and hope you take back fond memories.

Murray Grant

University of Warwick
Programme | What Lies Within

10 December, Monday

All talks will take place in the tiered lecture theatre. Refreshments are provided in the bar/lounge area and meals in the adjacent restaurant. Posters are in Space 41. All rooms are located within the same building.

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<th>Time</th>
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<tr>
<td>0815 – 0900</td>
<td>Registration in Scarman Conference centre, next to reception.</td>
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| 0900 – 0910 | President’s welcome  
Murray Grant (University of Warwick, UK) |

**Session 1: Infection and Pathogen Proliferation** *(moderator Murray Grant)*

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<th>Time</th>
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| 0910 – 0940 | Investigating the biology of plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*  
Nicholas Talbot (The Sainsbury Lab, UK) |
| 0940 – 0950 | Exploration of the biotroph to necrotroph transition in the ash dieback fungus *Hymenoscyphus fraxineus*  
John Mansfield (Imperial College, UK) |
| 0950 – 1015 | Studies on *Zymoseptoria tritici*: the dimorphic switch and hyphal growth in wheat infection  
Gero Steinberg (University of Exeter, UK) |
| 1015 – 1030 | Potato virus Y was detected outside the cell death zone in the hypersensitive response-conferred resistance  
Tjaša Lukan (NIB, Slovenia) |
| 1030 – 1050 | **BREAK**                                                                                   |

**Session 2: Cellular Communication and Subcellular Imaging** *(moderator Petra Boevnik)*

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| 1050 – 1120 | Inter-organellar communication during innate immunity  
Savithramma Dinesh-Kumar (UC Davis, US) |
| 1120 – 1140 | Molecular arms race across the plant-pathogen interface: how the Irish potato famine pathogen subverts plant focal immunity  
Tolgar Bozkurt (Imperial College, UK) |
| 1140 – 1200 | Specialised receptor signalling in plasmodesmal membranes  
Christine Faulkner (John Innes Centre, UK) |
| 1200 – 1220 | Intercellular communication in systemic acquired resistance  
Pradeep Kachroo (University of Kentucky, US) |
| 1220 – 1230 | *NbMORF8* encodes a protein localized in mitochondria and chloroplasts and negatively regulates plant immunity to *Phytophthora* pathogens  
Yang Yang (Northwest A&F University, China) |
| 1230 – 1400 | BSPP AGM (1230-1300, members only) AND LUNCH                                              |
Session 3: Emerging Technologies (moderator Darrell Desvaux)

1400 – 1425 Uncovering the dynamics of plant-microbe interactions by imaging effector delivery, using the GFP-strand system
Signe Lolle (UC Davis, US)

1425 – 1455 Fluorescent sensing of subcellular plant physiology and pathology in vivo
Markus Schwarzländer (University of Munster, Germany)

1455 – 1525 Studying the evolution of macromolecular machines using high-throughput electron cryo-tomography
Morgan Beeby (Imperial College, UK)

1525 – 1535 Using Raman micro-spectroscopy to monitor the metabolic activity of plant pathogenic bacteria Pseudomonas syringae
Nattapong Sanguankiattichai (University of Oxford, UK)

1535 – 1600 Break

Session 4: PH Gregory Award (moderator Dawn Arnold)

1600 – 1745 Laura Baggaley; Tom Chaloner; Cian Duggan; Jessica Erikson; Trupti Gaikwad; Anna Gonzalez-Gil; Guiyan Huang

Poster Session and Reception

1800 – 1930 Posters will be displayed in Space 41, accompanied by drinks and canapés

CONFERENCE DINNER begins at 1930. PH Gregory and JH Colhoun award winners will be announced. After dinner speech by Nicola Spence (Chief Plant Health Officer, DEFRA).

Tuesday, 11 December

All talks will take place in the tiered lecture theatre. Refreshments are provided in the bar/lounge area and lunch in the adjacent restaurant. Career Chat sessions (30 mins) will be held in Space 41 (poster room) during lunch time and are aimed at postgraduates and early career researchers.

Session 5: Large Scale Imaging for Plant Health (moderator Mike Csukai)

0900 – 0930 Seeing is believing: imaging disease progression during plant-pathogen interactions
Darrell Desveaux (University of Toronto, Canada)

0930 – 0940 Targeted detection of Zymoseptoria tritici in wheat
Christopher Adams (Imperial College, UK)

0940 – 0950 Benchmarking plant defence priming agents by hyperspectral imaging
Mustafa Yassin (University of Sheffield, UK)

0950 – 1005 Use of chlorophyll fluorescence Imaging to phenotype type 3 effectors impacts of Xanthomonas on leaves
Valerian Méline (INRA, France)

Presidential address

1005 – 1040 A botanist turned molecular biologist masquerading as a plant pathologist
Murray Grant (University of Warwick, UK)

1040 – 1105 Break
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| 1105 – 1135 | Targeting to a non-conventional secretory pathway by the conserved RXLR motif is essential for translocation of *Phytophthora* RXLR effectors  
*Petra Boevink (James Hutton Institute, UK)* |
| 1135 – 1155 | Coupling of the plant cytoskeleton to trafficking at sites of immune response  
*Mike Deeks (University of Exeter, UK)* |
| 1155 – 1210 | Visualizing *Pseudomonas syringae*-host interactions in the anthosphere and the plant-vascular system  
*Riccardo Soldan (University of Oxford, UK)* |
| 1210 – 1230 | Immunity-induced root growth inhibition is mediated by cell cycle arrest  
*Patrick Schäfer (University of Warwick, UK)* |
| 1230 – 1345 | **Career Chats and Lunch**                                                   |

**Session 6: cont. (moderator Ralph Dean)**

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| 1345 – 1400 | Effect of the *Leptosphaeria maculans* effector gene *AvrLm1* on *Rlm7*-mediated defence responses in oilseed rape  
*Henrik Stotz (University of Hertfordshire, UK)* |
| 1400 – 1410 | New insights into the infection process of *Fusarium fujikuroi* in rice using a GFP expressing isolate  
*Maria Aragona (Council for Agricultural Research and Economics, Italy)* |
| 1410 – 1420 | *Fusarium graminearum* effectors: identification and characterisation of three small secreted proteins which contribute towards fungal virulence  
*Catherine Walker (Rothamsted Research, UK)* |

**Session 7: RKS Wood award**

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| 1420 – 1430 | Introduction to 2018 Winner  
*Sarah Gurr (University of Exeter, UK)* |
| 1430 – 1500 | Standing on the shoulders of RKS Wood: from physiological to molecular plant pathology  
*Bart Thomma (Wageningen University, Netherlands)* |
<p>| 1500       | <strong>END</strong>                                                                     |</p>
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Summary of participants by country and organisations

100 participants from 28 organisation in the UK, including 16 universities and 8 research centres.
19 Participants from 15 organisations in 10 other countries.

Australia (1)
Canada (1)
China (3)
Denmark (2)
France (2)
Germany (2)
Italy (1)
Netherlands (2)
Slovenia (1)
USA (4)
BSPP Meetings 2018

The society has an active and varied programme. We are always on the lookout for new meetings to support and welcome ideas and requests for funding. If you’re not a member, please join!

Eric Boa, Programme Secretary [meetings@bspp.org.uk]

April 2018: Applications of plant pathology: field to clinic (held jointly with Society for Applied Microbiology, London)

August 2018: Grand Challenges Plant Pathology Study Group, Chicheley Hall

November 2018: Practical Diagnostics: from symptoms to sequence. (NIAB - East Malling Research station)

And in 2019 ...

Make a note of next year’s Presidential Meeting, which will be held in Bristol. President-elect is Dawn Arnold.

University of West England | 2-3 September 2019
Talks and Posters

abstracts arranged alphabetically by presenter
Targeted detection of *Zymoseptoria tritici* in wheat

Christopher ADAMS

*Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot SL5 7PY, UK*

Global population is expected to increase to 11.2 billion by 2100, increasing food demand by 60%. Additionally, climatic variability is expected to heavily decrease dominant crop production. Overall, highlighting the need to increase current crop yield. 520 million tonnes of wheat are produced annually with 361 million tonnes of that being produced in Europe alone, making wheat a major staple crop globally and in Europe. Pathogens cause substantial yield loss in wheat with the most damaging being *Zymoseptoria tritici* causing an estimated loss of up to 5 tonnes per hectare. Current treatment methods for *Z. tritici* include blanket application of broad spectrum fungicides at key growth stages that is both expensive (costing $1.2 billion annually in Europe) and damaging to the environment. *Z. tritici* is a hemibiotrophic pathogen usually identified through the presence of necrotrophic lesions on leaves however, before visible damage occurs there is a latent asymptomatic phase that lasts 9 - 16 days. If *Z. tritici* could be detected early before necrotrophic damage has occurred a reactive targeted fungicide application could be used which would increase yield. I have developed a remote sensing technique based on hyperspectral sensing to detect presence and stage of lifecycle of *Z. tritici*, as well as infer multispectral image data to capture. The multispectral images are processed and analysed with a semi-supervised AI machine learning approach which can detect *Z. tritici* infection with high accuracy. Use of this method in the field could result in reactive targeted application of fungicide which would 1) increase yield through reduced crop loss to infection, 2) reduce the cost of fungicide application 3) reduce damage to the environment through reduced fungicide application and 4) increase profit for a farmer. There is also potential in the future to apply the method to other diseases and crops.

New insights into the infection process of *Fusarium fujikuroi* in rice using a GFP expressing isolate

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*Fusarium fujikuroi* (teleomorph: *Gibberella fujikuroi*) is the main seed-borne pathogen of rice, causal agent of bakanae, a disease that in the last years has become of increasing economical concern in many Italian rice-growing areas. A virulent *F. fujikuroi* isolate was tagged with the green fluorescent protein (GFP) gene, using *Agrobacterium tumefaciens*-mediated transformation, and the virulence of the GFP isolate has been confirmed. By using the GFP isolate, fungal development during the *F. fujikuroi/rice* interaction was analysed by LASER scanning confocal microscopy (LSCM). The infection of rice roots was investigated from 24 h to 12 days post-inoculation both in resistant and susceptible cultivars. Roots of resistant genotype seem to trigger a hypersensitive response at the infection site and LSCM analysis of root sections allowed the visualisation of fungal growth within host tissues. Gene expression analysis of genes involved in pathogenesis and hypersensitive response is currently under way, by qPCR on the *F. fujikuroi*-infected rice roots. Analysed genes include chitinases, peroxidases and genes involved in gibberellin synthesis. The knowledge of plant infection and colonization mechanisms, together with the host response, will provide useful information for developing better control strategies of the pathogen and for improving breeding programmes for bakanae resistance.
Silicon enhances the constitutive defence pathway in strawberries against strawberry powdery mildew

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The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by the fungus *Podosphaera aphanis*, which has to be controlled by the frequent use of fungicides. There are two defence mechanisms in all plants, constitutive and active. They ensure that most plant species are not attacked by most of the potential pathogens on the planet. Work carried out at the University of Hertfordshire has shown that the constitutive defence pathway is enhanced by the weekly use of a silicon nutrient in the fertigation tubes at a commercial strawberry farm which results in reduced susceptibility to this disease. The work reported here aims to quantify the effect and benefits of silicon nutrient applied through the roots of strawberries in enhancing the constitutive defence pathway against strawberry powdery mildew. The defence mechanism induces wax, epidermis and cuticle thickness. Silicon is not considered as essential for the plant however it is found in all plants, particularly in the leaf hairs. The effect of a bioavailable silicon nutrient in reducing susceptibility to strawberry powdery mildew was monitored fortnightly at Maltmas farm, Wisbech. The six treatments were Sirius (concentration 0.017%) delivered through fertigation tubes once a week with and without commercial fungicides, Sirius twice a week with and without commercial fungicides and an untreated. Samples collected and assessed for mycelium coverage per leaf. The epidemic build-up recorded was for 12 weeks, which started in June and monitoring stopped in September when harvest ended. The results showed that the untreated (no silicon no fungicides) leaves had an average of 61% disease per leaf, silicon plus fungicides had 43% disease per leaf, silicon plus no fungicides had 21% disease per leaf, silicon twice plus fungicides had 6% disease per leaf and silicon twice with no fungicides had 5% disease per leaf. Results also showed a link between disease reduction as higher levels of silicon were found in silicon treated plants and where it had been deposited.

This relates with previous work done which showed the addition of leaf hairs in plants treated with silicon. Silicon deposition was examined in plants in a glasshouse experiment where 0.017% was added through the roots. Treatment lasted for 8 weeks and plants sampled at the end of the experiment. Cross sections of leaves, petioles and roots were cut and stained with a fluorescence dye, the basic amine lyso tracker yellow HCK-123, final concentration 1µM. Samples were examined using a fluorescence microscope at x400 magnification and a wavelength set at 450nm. Results showed that plants treated with silicon contained higher levels of silicon than the untreated and measurements of fluorescence intensity confirmed this. In the glasshouse experiment, treated plants showed that the silicon was found in the vascular tissue throughout the plant, in the leaf deposited in the epidermis, palisade layer and stomata, and in the xylem of the petiole and roots compared to untreated plants. In the silicon field trial, there was more silicon in the treated than the untreated plants and level of silicon correlates with reduced disease susceptibility. This suggests that the addition of silicon nutrient through the fertigation fields enhanced the constitutive defence pathway in strawberries against strawberry powdery mildew.

Are strawberries ever deficient in silicon?

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Work at the University of Hertfordshire has shown that a bio-available silicon nutrient delivered via fertigation tubes to strawberries in the field gives considerable benefits, including reduced susceptibility to strawberry powdery mildew. However, all the literature suggests that silicon is not an essential element for plant growth, and so silicon is often a neglected nutrient. The aim of this experiment was to compare strawberry plants grown in a silicon-free environment with those grown in a similar environment which
including the bio-available silicon nutrient. Dry root strawberry plants from the propagators were grown hydroponically in Hoagland’s solution in plastic, aerated, darkened containers. Half the plants were given no silicon nutrient at all and half the plants were given 50 ml silicon nutrient a week. All containers were topped up with Hoagland solution every week. The experiment ran for 26 weeks in the glasshouse at Bayfordbury. Plants were assessed on leaf size and number, leaf chlorophyll content, number of runners produced, BRIX and strawberry yield. Whilst the plants without any silicon did not show any conventional deficiency symptoms they were smaller than the plants given silicon nutrient. The plants with the weekly input of silicon nutrient showed significant increases on all parameters measured. Results show that the silicon treated plants performed significantly better than those without the silicon nutrient. On average the treated plants had 23 leaves compared with 19 on untreated plants, 12 runners per plant compared with 10, a fruit Brix of 16 compared with 10 and the leaves contained more chlorophyll. In summary, though no conventional deficiency symptoms were seen in the plants that had no Si, plants grown with Si treatment were enhanced for many growth parameters.

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Tracking a cereal killer: how does Fusarium graminearum utilise the plasmodesmata to further infection?

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Fusarium graminearum, a fungal ascomycete, is the primary causal agent of Fusarium Ear Blight (FEB), a devastating disease of small grain cereals worldwide. Economic losses are accounted for mainly by reductions in grain quality – through the production of mycotoxins which make the grain unsuitable for human and animal consumption – but are also a result of reductions in grain yield and ecosystem health. How F. graminearum establishes infection within wheat has been well characterised, but what causes the transition from an asymptomatic phase to a symptomatic one remains unclear. This transition in infection corresponds with one from intercellular to intracellular hyphal growth, resulting in rapid tissue colonisation and cell death. We therefore hypothesise that successful intracellular colonisation relies upon thin streams of cytoplasm between adjacent cells, vital for cell-to-cell communication, namely plasmodesmata. Detailed microscopic studies have demonstrated F. graminearum operates a similar mechanism to that employed by the rice pathogen, Magnaporthe oryzae, during which the intracellular hyphae constrict to pass through the plasmodesmata before diameter enlargement in the adjacent cell.

Utilising a range of imaging techniques, this research aims to discover what happens at the F. graminearum-plasmodesmata interface. Transgenic wheat plasma membrane reporter lines have been produced, via particle bombardment, to ultimately reveal, at both the cellular and molecular level, the dynamics of this interaction. A range of microscopic studies have been trialled to characterise and investigate the cytology of the wheat floral tissue. Alongside this, quantitative data collected from in vitro studies involving different F. graminearum strains, have been used as an initial starting point to understand hyphal behaviour. More recently the establishment of a wheat coleoptile infection assay, has created a high-throughput system useful to image this interaction. Genes associated with plasmodesmatal function and callose deposition have been identified, and in future work will be silenced to test their effect on F. graminearum virulence. Eventually all data will be collated to form a model of the wheat-F. graminearum pathosystem.

Monday | PH Gregory competition laura.baggaley@rothamsted.ac.uk
Cell specific immune responses

Cantug BAR, Miriam L. Gifford, Vardis Ntoukakis
Warwick Integrative Synthetic Biology (WISB) Centre, University of Warwick

Stochastic gene expression has been previously reported in the model plant Arabidopsis thaliana in the absence of external stimuli, however, variation of responses upon stress induction has not been studied extensively. The consequence of variation in responses to pathogens between different cells in plants is likely to have massive implications in controlling the level of plant resistance to microbes. It also provides an excellent system to investigate the consequence of variation in multicellular organisms. To this end, Arabidopsis thaliana reporter lines expressing the promoter of a well-studied Microbe Associated Molecular Pattern (MAMP)-triggered immunity (MTI) gene, WRKY11, fused to a fluorescent protein with a nuclear localization signal was utilized. Mature leaves of the two reporter lines were treated with a synthetic MAMP peptide derived from the Pseudomonas syringae flagellum, flg22. Within cells of the spongy mesophyll layer of each flg22-treated leaf, vast cell-to-cell variation in marker gene expression was observed following activation of MTI. The responsiveness of cells had a slight negative correlation with their initial marker fluorescence levels, suggesting that cells with less MTI expression prior to flg22 treatment showed the largest change in WRKY11 expression. Protoplasts were then generated from leaf tissue of reporter lines, induced with flg22 and protoplasts with highest and lowest marker gene expression were isolated using fluorescence activated cell sorting (FACS). Total RNA was extracted from sorted protoplasts and used for quantitative PCR of MTI marker genes. As anticipated, protoplasts with high marker gene expression as quantified by fluorescence levels also had higher gene expression based on qPCRs, further supporting the observation that genetically identical cells have differential immune responses. The mechanism underlying the variation is now under investigation to determine the contribution of factors such as resource constraints and epigenetic state to cell-to-cell variation in plant immunity.

Studying the evolution of macromolecular machines using high-throughput electron cryo-tomography

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This talk outlines the power of electron cryo-tomography, illustrated by studies on how molecular machines gain complexity in the course of evolution. Electron cryo-tomography involves imaging flash-frozen specimens in an electron microscope over a range of angles; the resultant images can subsequently be used to calculate a 3D model of the specimen to “macromolecular” (<10nm) resolution. My lab uses electron cryo-tomography to image bacterial flagellar motors as a platform for understanding molecular evolution. Although it is known that diverse bacterial flagellar motors produce different torques, the mechanism underlying torque variation is unknown. To understand this difference better, we combined genetic analyses with high-throughput electron cryo-tomography and subtomogram averaging to determine in situ structures of flagellar motors that produce different torques, from Campylobacter and Vibrio species. Our results unambiguously locate the torque-generating stator complexes and show that diverse high-torque motors use variants of an ancestrally related family of structures to scaffold incorporation of additional stator complexes at wider radii from the axial driveshaft than in the model enteric motor.

We identify the protein components of these additional scaffold structures and elucidate their sequential assembly, demonstrating that they are required for stator-complex incorporation. These proteins are widespread, suggesting that different bacteria have tailored torques to specific environments by scaffolding alternative stator placement and number. Our results quantitatively account for different motor torques, complete the assignment of the locations of the major flagellar components, and provide crucial constraints for understanding mechanisms of torque generation and the evolution of multiprotein complexes. I will
conclude by discussing possible pathways to evolve this diversity in the flagellar motors highlighted by subsequent cryo-tomographic imaging.

**Monday | Session 3**  
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**Targeting to a non-conventional secretory pathway by the conserved RXLR motif is essential for translocation of *Phytophthora* RXLR effectors**

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The study of the RxLR class of effectors from oomycete species has provided great insights into the mechanisms of both pathogenicity and plant defence systems. These studies have been conducted on the assumption that RxLR effectors are translocated into host cells. We showed over a decade ago using a GUS reporter fusion that an RxLR effector was translocated and that the RxLR motif was required. However, there has been intense debate and controversy about whether there was sufficient proof that the RXLR motif is a translocation signal and exactly what its function is during effector delivery. Recently we published images showing translocation of mRFP-tagged RxLR effectors from *Phytophthora infestans* into host cells in living tissue. This breakthrough was enabled by our characterisation of effector localisations and interactions and increased sensitivity of confocal microscope detectors. It paves the way for direct investigation of the requirements for translocation in vivo. We have shown that apoplastic effectors, like RxLR effectors, are predominantly secreted from haustoria during infection but that the secretory pathways of the two effector classes are different. Apoplastic effectors and other extracellular pathogenicity proteins are secreted conventionally and are thus sensitive to the inhibitor Brefeldin A (BFA). The secretion of wild-type RxLR effectors is BFA insensitive but mutation of the motif renders them sensitive to BFA, demonstrating that the motif targets the effectors to a non-conventional secretory route. I will present our latest research on *P. infestans* effector delivery.

**Tuesday | Session 6**  
**petra.boevink@hutton.ac.uk**

**Molecular arms race across the plant-pathogen interface: how the Irish potato famine pathogen subverts plant focal immunity**

**Toiga BOZKURT**  
*Imperial College London, Life Sciences, UK / www.imperial.ac.uk/people/o.bozkurt*

During plant invasion, the Irish potato famine pathogen *Phytophthora infestans* penetrates host cells through hyphal extensions known as haustoria. Haustoria are enveloped by a host-derived membrane known as the extra-haustorial membrane (EHM) whose functions and biogenesis are poorly understood. Through this interface, the pathogen secretes effector proteins which comprehensively reprogram cellular trafficking to neutralize host immune response. An interesting group of effectors particularly target the EHM, likely to counteract focal immune responses and remodel the host-microbe interface for safe and efficient nutrient absorption. Using perihastorial effectors as molecular probes, we identified a series of plant defence components deployed to the EHM, and pathogen strategies that antagonize these focal immune responses. I will summarize some of these findings including; (i) modulation of plant autophagy at the haustorial interface; (ii) chloroplast recruitment towards the pathogen interface; and (iii) trafficking of immune receptors towards the EHM.

**Monday | Session 2**  
**o.bozkurt@imperial.ac.uk**
Chloroplasts, a major hub of immune signalling

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Besides photosynthesis, chloroplasts perform many other functions critical for plant development and physiology, including synthesis of phytohormone precursors, amino acids, fatty acids, nucleotides and a range of secondary metabolites. It is increasingly recognised that chloroplasts play pivotal roles in perception, decoding and fine-tuning of responses to constantly varying environmental signals and metabolic demands. Recently chloroplasts have emerged as unexpected, yet key players in plant immune responses - Microbe Associated Molecular Pattern (MAMP) triggered immunity (MTI)1, effector triggered immunity (ETI)2 and systemic immunity3, with effectors specifically targeting the chloroplast or chloroplast components. Using Pseudomonas syringae as a tool, we have been investigating the role of chloroplasts in MTI. MAMPs down-regulate photosynthetic genes early in the infection process4 while effectors secreted later in infection actually modify chloroplast physiology1. Using suppression of photosystem II (Fv/Fm measurements) as a readout, we show that pre-treatment with MAMPs protects chloroplasts from effectors whereas MTI mutants are compromised in their ability to attenuate effector driven suppression of Fv/Fm1. Notably, ABA, which is rapidly induced de novo by P. syringae can suppress chlorophyll fluorescence and basal defence. This appears to attenuate a chloroplastic ROS burst to uncouple PTI response. We are using hormone signalling mutants and genetically encoded ROS reporters targeted to different sub-cellular locations to understand the interplay of hormone signalling and ROS in defence and disease. This data and much more from the field show the importance of this organelle in regulating immunity. Identifying how this complex interplay of MAMPs, effectors and hormones mediate immune signals from the chloroplast will aid future development of resistant plants.

References: (1) de Torres-Zabala et al., 2015 Nature Plants; (2) Caplan et al., 2015 Dev. Cell (3) Cecchini et al. 2015 Nature Comm; (4) Lewis et al., 2015 Plant Cell

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Targeting the arms factory: manipulation of the plant endoplasmic reticulum to enhance plant defence

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As the gateway to the cell’s secretory pathway, the endoplasmic reticulum (ER) provides the critical environment for lipid biosynthesis and protein production, folding and quality control. The ER is a highly dynamic interconnected network of tubules and cisternae (sheets) that extends throughout the cytoplasm and across cellular boundaries, associating with other organelles and the plasma membrane. Hence, the ER is central to the maintenance of cellular homeostasis. During pathogen infection the demand for de novo protein and lipid biosynthesis increases significantly, necessitating rapid, but highly regulated, ER expansion and remodelling as part of a successful defence response. The ER is, therefore, critical to the perception and regulation of adaptive host responses to biotic stress, and, as such, is also a prime target for manipulation by the pathogen as part of its virulence strategy orchestrated through the secretion of small effector molecules. We are currently investigating how the active remodelling of the ER architecture during PTI and ETI (pathogen-/ effector- triggered immunity), both locally and in systemic leaves as priming for systemic acquired resistance, impacts upon the plant’s ability to invoke an appropriate and timely defence response (‘defensive strategies’). Conversely, we are also interested in how effectors from a variety of pathogen
species including several oomycetes and *Pseudomonas syringae*, directly and indirectly manipulate ER morphology and/or activity to cause disease (‘offensive tactics’).

**A systems view of microbial establishment on growing root surfaces**

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The rhizosphere is a region of soil holding incredible biological complexity and diversity with microbial activity contributing to processes such as nutrient cycling and gas exchange. The root surface, or rhizoplane, is often the first area of interaction between plants and soil microbes. Understanding the dynamics of how either beneficial bacteria, or potential plant pathogens, colonise the root surface would be a valuable tool for studying plant-microbe interactions. Despite this our understanding of rhizoplane colonisation is limited. Previous studies of soil microbial dynamics have viewed the rhizoplane as static, failing to explain the large shifts in microbial composition taking place as roots colonise new regions of soil. This project aims to identify and quantify factors contributing to early stage root colonisation, when dynamic processes, such as root elongation, exudation and bacterial motility, are important. To achieve this, colonisation has been broken down into key processes that can be quantified experimentally for a model system of *Lactuca sativa* and *Pseudomonas fluorescens*. Through a combination of microbiological assays and confocal imaging of fluorescently tagged bacteria microbial attachment to, and growth on, roots has been quantified. The localisation of bacteria to different root regions during colonisation has also been described. Bacterial chemotaxis towards lettuce root exudate has been investigated through a series of assays employing transparent soil and live imaging. This data has been fed into a model of bacterial colonisation of root surfaces during the early stages of plant/bacteria interaction. Going forward; the challenge will be to expand this model to include other important aspects of microbial establishment on root surfaces. This model will be valuable in enabling predictions about root colonisation.

**Investigating the circadian clock of *Verticillium dahliae* and its influence on pathogenicity**

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The circadian clock is an internal timekeeper mechanism that allows organisms to anticipate and synchronize to daily environmental changes. The circadian clock is present in most branches of life; in plants, it modulates defence mechanisms impacting plant-pathogen interactions. This suggests that pathogens may also time their biological events in order to optimize infection. A broad knowledge of the molecular mechanism of circadian clocks has been gained through the fungal model *Neurospora crassa*. Nevertheless, little is known about circadian clocks in other fungi. We sought to characterize, therefore, the presence of a circadian clock in the fungal plant-pathogen *Verticillium dahliae*, and assess its influence on pathogenicity. Through comparative genetics, we identified homologs of all the *N. crassa* circadian clock genes in *V. dahliae*. High conservation of clock protein domains between *N. crassa* and *V. dahliae* was observed. We studied whether the daily formation of conidia and microsclerotia (infective propagules) were regulated in a circadian manner. However, no evidence for an entrainable, free-running rhythm was observed. Moreover, temporal gene expression profiling using RNA-Seq over a 24 h time-course showed a lack of rhythmic gene expression. Thus, in order to assess the role of the putative clock genes in *V. dahliae*, gene deletion mutants were produced.

Deleting the core clock gene *frq* did not have an obvious effect on fungal morphology. However, the deletion of the transcription factor and photoreceptor *wc-1* resulted in the abolishment of microsclerotia production, and stopped the light-regulated spore production, confirming that *wc-1* is involved in the production of the daily developmental rhythm by mediating transcriptional responses to white light. RNA-Seq carried out in *wc-1*
knockout mutants demonstrated a widespread effect of wc-1 on metabolism, redox processes and pathogenicity. Finally, frq and wc-1 were found to be required for full pathogenicity of V. dahliae on Arabidopsis thaliana and strawberry plants under light/dark conditions. In conclusion, V. dahliae presents all the necessary genetic loci for a functional clock, but there is no evidence of rhythmicity in either morphological traits or in gene expression. However, putative clock genes play an important role in the overall fitness of the fungus, suggesting that they have additional non-circadian roles.

The “temperature niche” of plant pathogens

Thomas CHALONER, Sarah Gurr, Dan Bebber

Crop plants are continuously threatened by the emergence of previously unencountered pathogens. However, the ability of a pathogen to successfully establish in an area, as well as disperse into virgin territory, is determined by many complex and interacting processes. These include host availability; accessible migratory routes; and abiotic factors directly affecting pathogen physiology, such as moisture availability and temperature. Throughout a pathogens life cycle, it is subject to diurnal temperature variations, seasonal temperature changes, as well as (possibly dramatic) day-to-day temperature fluctuations. However, each life cycle stage (i.e. infection, disease development, and sporulation) can only occur within a thermal limit, whereby optimum temperature is bounded by a minimum and maximum temperature. As temperature deviates from optimum, the rate of a biological process declines from maximum towards zero. Hence, optimum, minimum and maximum temperature - collectively referred to as cardinal temperature (CT) - define a pathogens temperature response, for a given stage of its life cycle. CT in-part shapes pathogen biogeography and resultant crop risk, both in space and time, by restricting pathogens to areas of climatic suitability. Here, published CT estimates were collated for 254 plant pathogenic fungi and oomycetes, recorded within the CABI Plantwise database as posing risk to agricultural and/or natural landscapes. This dataset is utilised to investigate a number of hypotheses. First, I test the hypothesis that temperature has an asymmetric effect on biological processes i.e. increases in temperature from optimum have a greater inhibitory effect than decreases in temperature. Second, I test the hypothesis that temperature-specialist pathogens also specialise in other axes of their niche. Third, I test the hypothesis that the evolutionary capacity of pathogens to alter their CT is limited. Finally, I investigate how climatic change may alter pathogen biogeography on a global scale. My research aims to improve our fundamental understanding of the “temperature niche” of species and how temperature influences the ecology and evolution of plant pathogens, as well as improve disease risk modelling in a warming world.

A new model of weather-dependent Septoria tritici blotch disease risk

Thomas M. CHALONER1, Helen N. Fones1, Varun Varma1, Daniel P Bebber1, Sarah J Gurr1,2

Pathogens destroy around one-quarter of food production, significantly threatening our ability of achieving food security for an ever-growing global population. Septoria tritici Blotch (STB) is a disease of wheat that reduces yields by up to 20%. Here, we created a new, mechanistic model for STB disease risk, parameterised with experimentally-derived data for temperature- and wetness-dependent germination, growth and death of the causal agent, Zymoseptoria tritici. The output of this model (A) was compared to observed disease data for UK wheat over the period 2002-2015. In addition, we compared the output of a second model (B), in which experimentally-derived parameters were replaced by a modified version of a published Z. tritici thermal performance equation [1], to the same observed disease data. Neither model predicted observed annual
disease, but model A differentiated between areas of the UK with differing average disease risks over the entire period, while B could not. The greatest limitations of both models are the broad spatial resolution of the climate data and the lack of host parameters. Model B is additionally limited by its lack of scope for incorporation of pathogen death, leading to a cumulative overestimation of disease over the course of the growing season. It is clear from the model outputs that full, predictive modelling of STB requires the pathogen parameters of model A, combined with explicit, experimentally-derived parameterisation of host and environmental factors, to cover the complete disease triangle.


Coupling of the plant cytoskeleton to trafficking at sites of immune response

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Filamentous actin plays a key role in the assembly of host Cell Wall Appositions (CWAs) that are produced reactively by the plant immune system. CWA construction is achieved with consistent precision at sites of assault to arrest microbial invasion and requires the controlled invagination of the plasma membrane (PM) in co-ordination with the export of barrier material to the volume between the plant PM and cell wall. Molecular, genetic or chemical interference of actin dynamics during this process lowers penetration defence, but it remains unclear how exocytosis and the cytoskeleton are linked in space and time to form functional CWAs. To gain mechanistic insight into this process we searched for Arabidopsis thaliana genes encoding known and predicted actin-binding proteins (ABPs) that responded at the transcriptional and translational level to microbial assault. We have found that membrane-integrated ABP FORMIN4 is delivered to immune response sites and contributes to local cytoskeletal dynamics at CWAs.

Total internal reflection fluorescence (TIRF) microscopy combined with controlled induction of FORMIN4-GFP expression reveals a dynamic population of vesicles that accumulate to form clusters at the PM through an actin-dependent process. Deactivation of FORMIN4 and its close homologues compromises subsequent defence and alters filamentous actin (F-actin) distribution at mature CWAs. Moreover, the tessellation of FORMIN4 at the PM with meso-domains of PEN3 reveals a fine spatial segregation of destinations for actin-dependent immunity cargo. This supports a model in which FORMIN4 is a spatial feedback element in a multi-layered, temporally defined sequence of cytoskeletal response.

Seeing is believing: imaging disease progression during plant-pathogen interactions

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Plant diseases caused by microbial pathogens cause devastating crop losses that continuously threaten global agriculture. A critical component of plant pathology research and crop breeding is measuring the extent and severity of disease symptoms. Unfortunately, most existing visual scoring systems are qualitative and subjective, while quantitative methods tend to be quite laborious. We recently developed Plant Immunity and Disease Image-based Quantification (PIDIQ); a quantitative imaging system to rapidly and objectively measure disease symptoms in a biologically relevant context1. We used PIDIQ to monitor enhanced plant health associated with effector-triggered immunity, as well as elevated disease symptoms associated with effector-triggered susceptibility in the Arabidopsis/ Pseudomonas syringae pathosystem. More recently, we have applied PIDIQ to study the natural variation of immune responses in Arabidopsis as well as high-
throughput screening for immune elicitors in *Arabidopsis* and tomato, demonstrating the power of PIDIQ to non-destructively quantify disease in model and crop plants.

**References:**


https://desveaux.csb.utoronto.ca

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**Inter-organellar communication during innate immunity**

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The innate immune system of both plants and animals employs cell-surface and intracellular receptors to detect pathogens and trigger defenses. Emerging evidence suggests that chloroplasts play an important function during innate immunity and they also have a central role in the production of immune signals. Our recent findings demonstrated that chloroplasts dynamically change their morphology by sending out stroma-filled tubular projections known as stromules during immune responses. We will discuss these results and our recent findings on the dynamics of stromule formation and stromule-driven movement of chloroplasts to nuclei during plant innate immunity.

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**A helper and sensor NLR pair focally accumulate at the host-pathogen interface to provide resistance to the Irish potato famine pathogen**

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In asterid plants, a complex signalling network of nucleotide-binding leucine-rich repeat (NLR) proteins mediates resistance to viruses, nematodes, bacteria, aphids and oomycetes. In this network, the helper NLR NRC4 pairs with multiple sensor NLRs, including agronomically important resistance proteins Rpi-blb2, Mi-1.2 and Rx, to mediate resistance against *Phytophthora infestans*, nematodes/aphids, and Potato virus X (PVX), respectively. Rpi-blb2 provides broad-spectrum resistance to *P. infestans* in both potato and the model plant *Nicotiana benthamiana* by detecting the core pathogen effector protein AVRblb2. However, the molecular basis of resistance mediated through the NRC helper-sensor network is unknown. Moreover, due to the strong cell death response activated following pathogen recognition, the cellular processes triggered upon NLR activation during infection remains poorly understood.

*N. benthamiana* is an excellent model plant enabling fast-forward cell biology of *P. infestans* host colonization. Because *N. benthamiana* naturally lacks sensor NLRs that respond to *P. infestans*, we were able to study the subcellular trafficking of the NRC4 helper during infection. By coexpressing a functional NRC4-GFP construct with a variety of established subcellular markers, we discovered that NRC4 accumulates at the peri-microbial host membrane which engulfs the *P. infestans* haustorium, known as the extra haustorial membrane (EHM). Interestingly, NRC4 localized to EHM micro-domains that are also positively labelled by AVRblb2 and its sensor Rpi-blb2. Remarkably, Rpi-blb2 accumulation around haustoria is dependent on NRC4, indicating that NRC4 functions include the recruitment of sensor NLR to effector delivery sites. In contrast, an NRC4 truncate lacking its extended C terminal domain failed to accumulate around haustoria or rescue Rpi-blb2 mediated resistance in NRC4-silenced plants. These findings support a model in which NRC4 mediates resistance by recruiting Rpi-blb2 to pathogen interface where its cognate effector AVRblb2 accumulates, possibly to maximise immune recognition.
This study represents an unprecedented observation in plant or animal systems of an NLR focally accumulating at pathogen penetration sites for defence. Decoding the signal for focal accumulation should enable improved disease resistance by using this pathway to deploy synthetic NLRs and antimicrobial compounds to the host-pathogen interface.

**Imaging immunity-induced root growth inhibition**

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Plants have efficient surveillance mechanisms to detect and fend-off potentially harmful microbes. The plasma membrane receptor FLAGELLIN-SENSING 2 (FLS2), for example, sets off a number of cellular defence-related signalling processes once it recognizes a 22 amino acid peptide from bacterial flagella. In roots, this leads to an increased level of immunity against invading pathogens but inhibits its growth at the same time. As these growth-immunity trade-offs may inherently limit breeding efforts for crops with higher resistance levels, we need a better understanding of the underlying signalling mechanisms. Here, we used different imaging techniques to visualize how immune activation affects important determinants of root growth. We show that immune activation shortens the length of the root meristem and the number of cells therein. While the root stem cell niche remains largely unaffected by immune elicitor treatment, we see a significant effect on protein abundance of important cell cytoplasm regulators and a reduced level of cell division in the meristem. By providing these functional insights, our findings may help to develop crops with undisturbed growth upon activation of immunity.

**The Xanthomonas effector XopL affects stromules in Nicotiana benthamiana**

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Stroma-filled tubules (stromules) emanate from the surface of all plastid types, in species throughout the plant kingdom. Stromule morphology is flexible, and tubules may extend, branch, and retract within minutes or even seconds. Stromules are frequently observed when plants experience stress. Recently, stromules have been suggested to act as a route for pro-defence signal transfer between plastids and the nucleus during effector-triggered immunity (ETI) culminating in hypersensitive response (HR). However, evaluating the relevance of stromules to cell/plant viability during pathogen attack or under abiotic stress is difficult since there is currently no known mutation that prevents stromule formation. As an alternative to mutational and gene silencing approaches we probed for stromule-relevant processes using type-III secreted effector proteins (T3Es) from *Xanthomonas campestris pv. vesicatoria*. During infection, T3Es are translocated directly into the plant cell where they specifically target host cellular components to provide benefit to the pathogen. Transient expression of 21 effectors in *N. benthamiana* lower epidermis revealed that XopL, an E3 ubiquitin ligase, almost completely abolished stromules.
Specialised receptor signalling in plasmodesmal membranes

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The plant immune system is broadly characterised as a cell autonomous response system; in general, all plant cells are capable of pathogen perception and response. However, plasma membrane-lined pores that connect neighbouring cells called plasmodesmata are regulated during immune responses, identifying that cell-to-cell connectivity and communication is a component of immune signalling. Plasmodesmata close in response to a range of microbe associated molecular patterns (MAMPs), mediated by specialised immune signalling components located at plasmodesmata. For chitin, the LysM domain, GPI-anchored receptor protein LYM2 mediates plasmodesmal closure via complex formation with two additional LysM domain receptor kinases. Upon chitin binding, this receptor complex signals via calcium and reactive oxygen species signalling that ultimately triggers callose deposition and plasmodesmal closure. This is a rapid signalling event that occurs independently of other chitin-triggered responses that occur in the plasma membrane, identifying spatial resolution in receptor signalling cascades in plant cells. We have been using bioimaging approaches to determine the dynamics of the formation of the plasmodesmata-located receptor complex; FLIM-FRET suggests that a specific LYM2 receptor complex forms at plasmodesmata in response to chitin and live-imaging indicates that LYM2 accumulates at plasmodesmata in response to chitin. We are currently using a range of imaging and biochemical approaches to determine receptor complex dynamics in the plasmodesmal plasma membrane and how this underpins both defence and infection.

Monday | Session 2  christine.faulkner@jic.ac.uk

Interactions between Zymoseptoria tritici and ice-nucleating Pseudomonas syringae

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Zymoseptoria tritici is one of the most important fungal pathogens affecting wheat crops in temperate regions, causing yield losses which cost the UK and Germany around €180M and €375M, respectively, each year, despite the use of resistant wheat varieties and fungicide spraying. Z. tritici infections begin with an approximately 10 day symptomless period. Our recently published work shows that, during this time, the fungus may remain primarily epiphytic - on the leaf surface - rather than inside the leaf, as is the case later in infection. My previous work supported the idea that epiphytic growth is random with respect to stomata, through which the fungus enters the leaf. One line of evidence for this was that wounded leaves, which have more openings, develop disease faster than controls. In current work, I am investigating the possibility of a mutualistic relationship between Z. tritici and ice-nucleating (INA+) Pseudomonas epiphytes. INA+ bacteria initiate frost damage at higher temperatures than it would otherwise occur, including temperatures commonly occurring during the wheat growing season. This damage may both promote fungal ingress and increase the leakage of nutrients onto the leaf surface, thus benefitting both organisms. I will present data showing that the combination of freezing temperatures and inoculation with INA+ bacteria renders wheat leaves more susceptible to Z. tritici, and that the bacteria may also benefit from the presence of the fungus. However, data gathered so far indicate that the interaction is more complex than initially hypothesised.

POSTER  hnfones@gmail.com
Development of inoculation methods to understand interactions of phoma stem canker and light leaf spot causal pathogens during leaf infection

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UK oilseed yields have not increased in the last 10 years, in part due to yield losses from diseases, such as phoma stem canker and light leaf spot caused by Leptosphaeria maculans and Pyrenopeziza brassicae, respectively. It is known that some cultivars resistant to L. maculans are susceptible to P. brassicae. This investigation aims to understand interactions between these pathogens during leaf infection. A robust inoculation method is needed so symptoms can develop reliably after inoculation. Conidial suspensions of L. maculans (10⁷ spores/ml) or P. brassicae (10⁸ spores/ml) were prepared with 0.05% Silwet wetting agent. Two oilseed rape cultivars, each with resistance to one of the pathogens, were inoculated using the different methods. Percentage of plants affected were 10%, 67% or 0% and for L. maculans and 15%, 17% or 56% for P. brassicae when plants were inoculated with filter paper discs soaked with conidial suspension, 10 µl drops of conidial suspension or sprayed with conidial suspension, respectively. Disease incidence was low and there is a need to improve inoculation methods. Different resistance responses were observed for L. maculans and P. brassicae. L. maculans induced a resistance response at the inoculation site and P. brassicae induced a resistance response at the inoculation site and on the main vein. However, to do so efficiently a more robust and targeted inoculation method for P. brassicae needed to be developed. This was done by investigating the effect of inoculum concentration (3×10⁵ or 1.5×10⁶ conidia ml⁻¹) and the addition of a wetting agent (Tween 20) on disease incidence using an oilseed rape cultivar susceptible to P. brassicae. The disease incidence and severity were greater when a greater conidial inoculum concentration was used, and the use of a wetting agent increased the disease severity. There is a need to further investigate the resistance response to inoculation for both pathogens alone or together using different inoculation methods.

Investigating induction of SAR during gene-for-gene interactions between Arabidopsis thaliana and Pseudomonas syringae

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Plants have evolved two types of defense mechanisms against 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. In the SAR state, plants are primed to more effectively activate defense responses to the subsequent pathogen attack. 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 associated with ETI. We describe the transcriptional dynamics of A70 encoding a protein 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 related signal that is rapidly generated following ETI recognition. We expand A70::LUC reporter studies showing histological expression of a JA repressor reporter (JAZ10::GUS) and A70::GFP reporter (both validating microarray data, Truman et al. 2007) in systemically responding leaves following avirulent pathogen challenges. Finally, we examine changes in electrophysiological signals following ETI in local and systemic leaves. Our study provides new insight into the integrated signalling mechanisms, dynamics and connectivity underpinning systemic immune responses. We conclude that there are core multicomponent signals generated by activation of ETI. These include the...
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Intimately linked propagation of slow electrical variation potentials with COI1 receptor dependent transcriptional waves that travel sympathetically in specific cell types to initiate a rapid temporal spatial activation of a core systemic immune response in systemic responding leaves. A notable feature is the rapid early induction of jasmonate responsive genes.


Monday: PH Gregory Competition also as a POSTER t.gaikwad@warwick.ac.uk

Identification of new virulent races in Leptosphaeria maculans populations on oilseed rape in the UK

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Phoma stem canker, caused by the fungal pathogen Leptosphaeria maculans, is a damaging disease on oilseed rape in the UK and can cause yield losses up to 50% if the disease is not controlled. Currently, this disease causes UK annual yield losses >£100M despite use of fungicides. With recent loss of the most effective fungicides through EU legislation, potential yield losses will increase. Use of host resistance to control this disease is becoming ever more important. However, new sources of resistance are often rendered ineffective due to pathogen population changes from avirulent to virulent. L. maculans develops gene-for-gene interactions with its host plant resistance (R) genes. A given host R gene is effective only when the protein coded by the R gene recognises an effector produced by the corresponding L. maculans effector gene. With both sexual and asexual reproduction, L. maculans has a high potential for mutation to overcome recognition by host R genes. For effective use of host R genes, there is a need to monitor emergence of new virulent races of L. maculans and prevent them from spreading into new regions. Field experiments were set up at different sites in the UK; from leaf spot lesions on Drakkar (susceptible cultivar, trap crop) and other cultivars (with Rlm7 resistance gene), 64, 88 and 111 L. maculans isolates, were obtained in the 2015/2016, 2016/2017 and 2017/2018 cropping seasons, respectively. Ninety-two single ascospore isolates were also obtained from stem samples from two sites from the 2016/2017 cropping season. Changes in frequencies of avirulent alleles of AvrLm1, AvrLm4 or AvrLm7 effector genes were investigated by testing isolates on cotyledons of a differential set of cultivars. Isolates virulent towards resistance genes Rlm1, Rlm4 or Rlm7 were investigated for molecular events leading to virulence.

There were variations in the frequencies of avirulent alleles of AvrLm1 and AvrLm4 between sites and cropping seasons. All the isolates from different sites were avirulent against Rlm7 in the 2015/2016 season. In the 2016/2017 season, 6.8% of isolates were virulent towards Rlm7, whereas the frequency of isolates virulent towards Rlm7 had increased to 16.3% in the 2017/2018 season. For single ascospore isolates from the 2016/2017 season, 25% of them were virulent towards Rlm7. Forty important isolates were selected on the basis of their race structure for whole genome sequencing to investigate molecular mechanisms of mutations to virulence.

Chromatin remodelling and its conserved role in plant biotic and abiotic stress responses

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The fast expanding human population, together with the global demand for natural resources are creating food insecurity. Plant diseases and water scarcity are causing crop losses exceeding $150 billion worldwide and are among the main causes of severe food shortage in developing countries. A sustainable strategy to prevent these losses is to create plants with enhanced tolerance to biotic and abiotic stresses. Unfortunately, enhanced stress tolerance often comes with severe developmental penalties such as reduced
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We have discovered a Histone Acetyl Transferase as a major regulatory hub controlling plant homeostasis in a manner that simultaneously regulates stress responses and growth. The Arabidopsis mutant of this enzyme (hag5) has enhanced leaf area, longer roots and increased resistance to bacterial pathogens. In addition, the mutant has enhanced drought tolerance and is less sensitive to abscisic acid, a phytohormone known to regulate plant growth and stress responses. We plan to exploit the high level of conservation of HAG5 across plant lineages in an effort to engineer high performing plants with enhanced disease resistance and drought tolerance, yet minimal impact upon growth. We are working in two main lines of research; gene editing of HAG5 using CRISPR technology and developing chemical inhibitors of the enzyme for foliar applications.

Monday | PH Gregory competition  a.gonzalez-gil@warwick.ac.uk

A botanist turned molecular biologist masquerading as a plant pathologist

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As is traditional, I will present a brief history of how I got to be giving this talk and hopefully through this provide some insight as to the theme of this years meeting. I have been fortunate to travel the world as part of my research, always focusing on plants and invariable on plant microbe interactions, moving into pathogens in the early 1990’s. I will touch on cloning the first “dual specificity” plant disease resistance gene, early engagement with “omics” that led to an interest in how pathogens hijack plant hormones, through to current research trying to understand the molecular mechanisms underpinning the temporal spatial dynamics of plant disease and defence responses.

Tuesday | Presidential talk  m.grant@warwick.ac.uk

Microphenotyping for resistance to Puccinia striiformis f.sp. tritici, causal agent of wheat yellow rust

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Wheat yellow rust, caused by the biotrophic fungus Puccinia striiformis f.sp. tritici (Pst), is one of the most challenging diseases facing growers across the world. Resistant varieties are a valuable tool in the fight against this disease, although their effectiveness is challenged by a constant arms race with the pathogen, governed by a gene-for-gene relationship. In the UK, race changes occur on average every 3 years, with the most recent changes occurring as a result of exotic incursions rather than by asexual mutation, adding further complications to breeding for resistance (Hubbard et al. 2015). Historically, major gene, race-specific resistance has been deployed in UK wheat varieties, however race-non-specific and potentially durable resistance is now an important research and breeding target. Identifying durable resistance using existing phenotyping methods under field conditions is difficult, especially as some varieties with partial resistance have succumbed to new races in a race-specific manner.

Microphenotyping is a technique where the host response is investigated at a microscopic level. As part of two new projects, an EU H2020 project called Rustwatch and a BBSRC-LINK project called Yellowhammer, we will continue work done by others (e.g. Jagger et al. 2011; Melichar et al. 2008; Moldenhauer et al. 2008; Moldenhauer et al. 2006) in applying the microphenotyping methods to characterise the resistance found in current European wheat cultivars. Using trypan blue, 3,3-diaminobenzadine (DAB) and Uvitex B staining in combination with light, fluorescent and confocal microscopy, we hope to classify varieties according to their resistance response.

Previous work has shown that the pathogen may be stopped prior to haustoria formation (Mares and Cousen 1977), contained by a hypersensitive response, indicative of a race-specific reaction (Bozkurt et al. 2010), or by other methods not apparent at the microscopic level (Melichar et al. 2008). By characterising cultivars
from a panel of over 200 and 300 European winter and spring wheats, respectively from each project, it will be possible to inform breeders of useful combinations that could be made in future breeding programmes to provide durable resistance to this disease.


A virulence RXLR effector secreted by *Phytophthora parasitica*

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A large number of researches have been carried out to elucidate virulence function of RXLR effectors secreted by *Phytophthora* species during plant-pathogen interactions. However, understanding of the RXLR effectors from *P. parasitica* species is extremely limited. Here, we identified a *P. parasitica* RXLR effector gene, PPTG00121 (PpE4), which is highly upregulated during the early infection stages. To monitor the secretion of PpE4, we generated *P. parasitica* transformants expressing full-length PpE4 (E4FL)-mCherry via polyethylene glycol (PEG)-CaCl2-mediated transformation. Live-cell imaging showed that the red fluorescence was evenly distributed in mycelia cultured in vitro, while it was highly enriched in haustoria during infection of *Nicotiana benthamiana* leaves. Further detailed observation and the fluorescence intensity analyses showed that mCherry fluorescence signal accumulated outside the haustoria, mainly surrounded the haustorial neck, indicating that E4FL-mCherry is secreted from haustoria and accumulates in the extra-haustorial matrix (EHMx) during plant infection. Transient expression of PpE4 in *N. benthamiana* or induced expression in *Arabidopsis thaliana* consistently promoted *P. parasitica* colonization. Furthermore, stable silencing of PpE4 in *P. parasitica* resulted in significantly attenuated virulence on *N. benthamiana*. Transient expression of PpE4 in *N. benthamiana* in turn restored pathogenicity of the PpE4-silenced lines. In conclusion, PpE4 is an RXLR effector secreted by *P. parasitica* and contributes to pathogen infection.

Evolution of host specificity and virulence of *Pseudomonas syringae* on *Prunus*

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*Pseudomonas syringae* pathovars are highly specialised plant pathogens, with individual strains only infecting one or a few hosts. It is believed this host specificity is due to the repertoire of type III effector proteins involved in both virulence and avirulence in planta. This topic was explored using comparative genomics of three divergent clades that have convergently evolved to cause bacterial canker on cherry (*Prunus avium*).

The clades include *P. syringae* pv. *morsprunorum* (Psm) races 1 and 2 (which are now known to be distantly related) and *P. syringae* pv. *syringae* (Pss). Three reference isolates of Psm R1, R2 and Pss were sequenced with PacBio and the genomes of a diverse set of further strains were sequenced using the Illumina MiSeq. Genomic analysis of the *Prunus* strains has revealed highly divergent effector and toxin repertoires within and between the different clades, indicating they use distinct mechanisms to cause bacterial canker. A Bayesian approach was utilised to statistically predict effectors whose evolution is significantly associated with the evolution of pathogenicity for cherry. Candidate virulence effectors have been gained via horizontal gene transfer which has been both plasmid and phage-mediated.
Comparative genomic analysis of three Bacillus species that have an antagonistic effect on Fusarium graminearum

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The fungal pathogen Fusarium graminearum (Fg) causes head blight (FHB) disease in cereal crops of small grains such as wheat, rye and barley. These crops represent a great portion of the calorific consumption around the world. The pathogen also produces mycotoxins that are harmful to humans and livestock.
Fungicides, integrated pest management, resistant plant varieties and biological control agents have been employed to control FHB. We used three different *Bacillus* strains: QST713, FZB24 and EU07 to investigate their antagonistic effect on *Fg*. Although all three *Bacillus* species suppressed the fungal growth, the EU07 gave the highest suppression. During the studies, we also noticed that these three *Bacillus* species have different grades of plant growth promoting (PGP) effect and all of them enhanced the plant defence. However, EU07 showed the highest PGP-activity. This prompted us to carry out a comparative genomic analysis on these bacterial species to identify what makes EU07 superior to others. Our results showed that they are different species and they differ in genes that are involved in the production of antimicrobial compounds such as chitinases, fengycins, surfactins, lytic enzymes, subtilisin and iturin. Latest results will be presented.

Intercellular communication in systemic acquired resistance

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Systemic acquired resistance (SAR) is a form of broad-spectrum resistance induced in response to local infections that protects uninfected parts against subsequent secondary infections. SAR signaling requires two parallel branches, one regulated by salicylic acid (SA), and the other by pipecolic acid (Pip), azelaic acid (AzA) and glycerol-3-phosphate (G3P). AzA and G3P function downstream of the free radicals nitric oxide (NO) and reactive oxygen species (ROS). During SAR, SA, Pip, AzA, and G3P accumulate in the infected leaves, but only a small portion of these is transported to distal uninfected leaves. SA is preferentially transported via the apoplast, whereas phloem loading of AzA and G3P occurs via the symplast. The symplastic transport of AzA and G3P is regulated by cytoplasmic pores called plasmodesmata (PD). PD permeability is regulated by PD localizing proteins (PDLP) and of these PDLP1 and PDLP5 regulate SAR by regulating transport as well localization of proteins associated with SAR. Imaging and biochemical analysis shows an important role for PD in SAR associated signaling.

Monday | Session 2

Specific host-pathogen interactions in the *Brassica napus*-Pyrenopeziza brassicae pathosystem

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Light leaf spot has become the major disease problem for oilseed rape (*Brassica napus*) cultivation in the UK. In England, annual yield losses caused by light leaf spot have been estimated as £15m to £145m between 2005 and 2014. Frequent epidemic development over the past decade has made this a high priority in many oilseed rape growing areas across the country. There are various constraints associated with fungicide applications for controlling the disease, such as poor timing, development of fungicide insensitivity within pathogen populations and legislative and environmental concerns. Therefore, it is important to improve and maintain host resistance for controlling the light leaf spot epidemics. There is a limited understanding of the genetic basis of resistance in oilseed rape against *P. brassicae*, which hinders use of cultivar resistance for effective management of light leaf spot. This research examines specific interactions involved in the *B. napus*- *P. brassicae* pathosystem in relation to host resistance against *P. brassicae*. Selected oilseed rape cultivars and pre-breeding material were spray inoculated with *P. brassicae* conidial suspensions prepared from single spore isolates. Disease assessment was done using both visual (% leaf area with *P. brassicae* sporulation) and molecular methods (quantitative PCR). Correlation analysis was made between different light leaf spot assessment criteria to identify relationships between them. Line-by-isolate interactions were identified using statistical methods. The results suggested that there are two key phenotypes associated with resistance; limitation of pathogen sporulation and formation of necrotic responses, which appeared to be related most
of the time. There were differences between lines/cvs both in terms of P. brassicae asexual sporulation and in other symptoms associated with light leaf spot disease, such as leaf curling and leaf distortion. Selected lines/cultivars were further investigated to identify the phenotype/s of resistance. Plants were point-inoculated on true leaves and disease progress was monitored from 0-24 days post inoculation (dpi) using quantitative PCR and scanning electron microscopy (SEM). The pathogen was able to infect and colonise in all the lines/cultivars tested. Host resistance appeared to be limiting pathogen colonisation and sporulation rather than completely preventing the infection and/or colonisation by the pathogen.

Understanding and mitigating the causes of yield decline in pea

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The field pea (Pisum sativum) is an important legume grown for human consumption in over 110 countries worldwide. In the UK, growth is limited to areas within close proximity of processing facilities, which are situated across eastern England and Scotland. This has led to intensive production and yield declines of up to 40% in recent years, mostly attributed to a build-up of fungal and oomycete pathogens involved in the pea foot rot complex (PFRC). Important pathogens of the PFRC include Fusarium solani f. sp. pisi, Fusarium oxysporum f. sp. pisi (FOP), Aphanomyces euteiches and Didymella pinodella (DP). Whilst initial research into the PFRC has consisted of field surveys, particularly in North America and Europe, to examine the prevalence, severity and identity of the pathogens involved, very little research has been conducted into the abundance, dynamics and interaction of these species within the complex, and crop rotation is currently the only disease management practice. Therefore, the aims of this project are to understand the components and dynamics of the foot-rot complex as well as associated microbiota in the pea rhizosphere using both conventional and metagenomics approaches and to identify green manure and biofumigant crops that can suppress the PFRC.

Preliminary experiments have begun initially to optimise the growth and sporulation conditions of DP for future inoculation experiments. Four agars as used in the literature were tested to determine which produced the highest concentration of pycnidiospores, as well as the growth rate of the colony. Further experiments have also begun to optimise a pea test tube assay to determine dose responses for each of the four PFRC pathogens in order to examine interactions in future co-inoculation experiments. In the first experiment using FOP, pre-germinated pea seedlings in test tubes containing a vermiculite-perlite mix were inoculated with 1 mL of either 1x10^2, 1x10^3, 1x10^4, 1x10^5, 1x10^6 mL spores, or water (control) and examined for disease development and mortality using a scoring system based on root and stem discolouration after 4 weeks. V8 agar and half strength potato dextrose agar (PDA) resulted in significantly higher mean growth rates of DP compared to Coon’s agar and PDA; however, there were no significant differences between agars in pycnidiospore concentration, although half strength PDA was the most consistent and therefore selected for future experiments. In the first dose response experiment with FOP, whilst there was a general increase in disease severity as the dose increased and no mortality/very low mean disease severity in the control treatment, optimisation of the inoculation method used as well as an introduction of a gravimetric watering system will be considered in subsequent assays. These initial results will allow the interactions of PFRC pathogens to be studied in further work under controlled conditions.

Beneficial effects of the use of a bio-available silicon nutrient on courgette plants

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There are many papers reporting that bio-available silicon is a useful plant nutrient. As well as causing modifications to the wax, cuticle and leaf hairs of many plants, silicon products are reported to reduce plant
susceptibility to plant diseases including powdery mildews. A glasshouse experiment was carried out at the Hatfield campus of the Univ. of Herts. with courgette plants that were exposed to natural infection by the powdery mildew fungus *Podosphaera xanthii*. The bio-available silicon nutrient was applied weekly, one treatment was to the roots (0.017%), the other was a weekly foliar spray (0.25%). The results showed that the silicon treated plants, whether the application was to the root, or to the foliage resulted in the growth of more, larger leaves, and a significant reduction in the level of powdery mildew. These results support the hypotheses that use of a silicon nutrient enhances overall plant growth and also enhances the constitutive defence pathway of the courgettes against powdery mildew.

**POSTER** kishankannaya12@gmail.com

**Exploiting fungal proteins to regulate plant signalling pathways**

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Plant-interacting microbes employ a multitude of effector proteins to reprogram host cells during colonisation. These microbial effectors could be powerful tools to engineer plant responses because they have co-evolved with plant signalling pathways. We have identified a set of 150 putative effector candidates from *Serendipita indica* - a symbiotic fungus that can activate beneficial pathways in plants during environmental stress. To study effector function *in planta*, we have developed a set of promoter-reporter constructs that help analysing phytohormone responses in *Arabidopsis* leaf protoplasts. Using an automated approach we have screened more than 1000 marker-effector combinations for changes in signalling following treatment with auxin, cytokinin, abscisic acid, jasmonic acid or salicylic acid. The most promising effector candidates were selected to generate transgenic *Arabidopsis* plants which are being analysed towards changes in development and stress responses. Several transgenic lines expressing *S. indica* effectors show growth promotion, in some cases this effect is maintained under conditions of salt and osmotic stress. Our ultimate goal is to engineer effector proteins to develop bespoke molecular tools that increase plant resistance towards biotic and abiotic stresses.

**POSTER** s.lehmann@warwick.ac.uk

**Uncovering dynamics of plant-microbe interactions by imaging effector delivery using the GFP-strand system**

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As a primary step in infection of plants, gram-negative bacterial pathogens, such as *Pseudomonas syringae*, use the needle like Type III Secretion System (T3SS) to deliver essential effectors into the host cells. Due to size constraints of the T3SS needle proteins needs to be unfolded prior to secretion and effector fusion with a full-length fluorophore will therefore block delivery into the host. Thus, most investigations of effector localization and function rely upon gross overexpression of single effectors in planta. To enable direct visualization of natural effector delivery in planta, we have adapted a GFP-strand system for expression in plants. Variants of the green fluorescent protein consists of an 11 stranded beta barrel, that can be finely divided and reconstituted to make a functional fluorophore. By constitutively expressin10 of the β-strands in plants, and labeling naturally expressed bacterial effectors with the complementary 11th β-strand epitope, we are able to follow effector delivery in host cells by monitoring the reconstitution of the fluorophore. One major advantage of this system is that tagging effectors with only the small 11th β-strand allows for secretion of the effector though the T3SS needle and enable us to monitor natural effector delivery during infection. By utilizing this system we have monitored the temporal and spatial delivery of GFP11-tagged effectors during infection with the foliar pathogen *P. syringae* and the vascular pathogen *Ralstonia solanacearum*. 
The ability to observe the natural delivery of effectors on a cellular level not only allow us to study effector delivery in depth but also give us the tools to study the molecular differences in host responses to effector delivery on a single cell level. Traditionally, plant immune immunity is studied as whole tissue responses, but by utilizing the GFP-strand system can identify cells with and without effector delivery and thereby get the cell specific responses. Data on the use of the GFP strand system for mapping effector delivery during natural infection and strategies for the use of the system to analyze cell specific host responses will be presented at the meeting.

Monday | Session 3
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**Potato virus Y was detected outside the cell death zone in the hypersensitive response-conferred resistance**

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One of the main types of resistance to potato virus Y (PVY) is hypersensitive response (HR)-conferred resistance, which restricts virus spreading and includes induction of cell death, manifested as necrotic lesions. While it is known that salicylic acid is the key component in the orchestration of the events restricting viral spread, the exact function of cell death in resistance is still unknown. To determine the role of cell death in this pathosystem, we used GFP-tagged PVY to follow the presence of the virus inside and outside the cell death zone by confocal microscopy. Our results show that PVY can be detected outside the cell death zone in Ny-1-mediated HR in potato, observed as individual infected cells or small clusters of infected cells outside the cell death zone. We confirmed that the cells at the border of the cell death zone harbor viable PVY that is able to reinitiate infection by exploiting the features of temperature dependent Ny-1-mediated resistance. We studied the dynamics of both cell death zone expansion and occurrence of viral infected cell islands outside it. We compared the response of Rywal plants to their transgenic counterparts, impaired in SA accumulation, where the lesions occur but the spread of the virus is not restricted. We showed that PVY can be present outside the cell death zone in all developmental stages of lesions. Additionally, we measured the dynamics of lesions expansion in long time period in both genotypes using Dino-Lite digital microscope. We showed that while rapid lesion expansion is observed in SA-depleted plants, virus spread is even faster. On the other hand the majority of lesions slowly expand also in HR-conferred resistance opening the possibility that the infected cells are eventually engulfed by cell death zone. We suggest that the HR cell death is separated from the mechanisms which lead to PVY restriction in Ny-1 genetic background. We suggest that HR should be regarded as a process where the dynamics of events is crucial for effectiveness of viral arrest albeit the exact mechanism conferring the resistance remains unknown.

Monday | Session 1
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**Exploration of the biotroph to necrotroph transition in the ash dieback fungus**

_Hymenoscyphus fraxineus_

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The use of ascospore suspensions as inocula has allowed the demonstration that _Hymenoscyphus fraxineus_ has a significant period of biotrophic growth following penetration into epidermal cells of ash. The ash dieback disease is characterised by extensive necrosis of leaf and stem tissues. The switch from biotrophy to necrophy has been examined following inoculation of extending stem tissues that are very amenable to light and electron microscopy. After formation of an appressorial structure and direct penetration into the epidermis, three distinct phases of infection were identified. Phase I involves biotrophic invasion and is characterised by the formation of vesicular structures and intracellular hyphae in living plant cells. Invading hyphae are found in close contact with the plant plasma membrane and few changes in plant ultrastructure
are observed as the membrane is pushed aside. Phase II includes the biotroph/necrotroph transition during which intracellular hyphae extend beyond the first penetrated cell and some invaded cells die. Plant cells at the edge of the lesion remain viable. Penetration from cell to cell often occurs close to plasmodesmata and is associated with a constriction of intracellular hyphae and no widespread degradation of the plant cell wall. No clear change in hyphal morphology is observed at the end of the fully biotrophic phase. Phase III represents the switch to necrotrophy as multiple branching of hyphae occurs at the centre of lesions which spread rapidly, contain degenerating plant cells at the infection centre and are surrounded by a narrow zone of dead epidermal cells in advance of most invading hyphae. However even during this largely necrotrophic development, live plant cells were sometimes found in contact with brown dead cells containing hyphae. Changes such as increased pigmentation (browning) occur in some cells ahead of invasion, possibly indicating the spread of effectors ahead of the necrotic zone. It is expected that the switch in mode of nutrition and fungal development is under strict transcriptional regulation. Phases I and II would require expression of effector proteins that suppress host defences, but not degradative enzymes or toxins that might disrupt the biotrophic balance. By contrast Phase III should be characterised by the additional secretion of cell wall degrading enzymes and other toxic metabolites. The ash dieback fungus appears highly adapted as a hemibiotrophic pathogen. It is unlikely that it has evolved directly from a saprophytic origin.

Monday | Session 1
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Developing diagnostic tools for the pea (Pisum sativum) foot rot complex
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The domesticated Pea (Pisum sativum) is a cool season legume which is grown as an annual crop for both human and animal consumption. Together with other leguminous species it has a crucial role in increasing agricultural sustainability, through reduced nutrient requirements and increasing species diversity within rotational systems. However, often the yield and quality of vining peas is reduced as a consequence of the foot rot disease complex, which comprises of three key soilborne pathogens; Didymella pinodelea (DP), Fusarium solani f. sp. pisi (FSP) and Aphanomyces euteiches (AE). Individually or in combination, these pathogens form necrotic lesions on the lower stem and root system of peas, leading to early senescence and loss of crop yield and quality. Disease management relies on crop rotation but this is limited by the requirement for peas to be grown close to processors. A pre-plant soil test is available for growers to determine the risk of foot rot prior to planting, which uses an in vitro soil baiting technique alongside plating on selective agar media and morphological identification of pathogens. However, this process is time consuming and labour intensive. Consequently, the aim of this project was to develop a rapid and reliable quantitative real-time PCR (qPCR) diagnostic tool to detect and quantify foot rot pathogen levels directly from soil or from baited plant roots.

A collection of UK isolates was assembled for DP, FSP and AE from commercial samples collected by PGRO and isolate identity confirmed through sequencing of ‘housekeeping’ genes including ITS, TEF and RPB2. A combination of housekeeping gene and whole genome sequencing for DP and FSP, identified genomic regions for the development of qPCR assays while a published assay was used for AE (Gangneux et al. 2014). Assays were optimised and validated for use by comparison to a serial dilution of target DNA (100 fg to 10 ng μL-1) extracted from pure culture, and by specificity testing against DNA from a panel of non-target species consisting of 43 common soil-borne fungi and plant pathogens. All three qPCR assays resulted in good amplification over the range of target DNA concentrations with good repeatability (R2 >0.99) and efficiency (82-101%). Each assay amplified the target species, with only very weak cross reaction (Cq = 31) with the related species D. pinodelea in the DP assay, and F. oxysporum (Cq = 28) in the AE assay. However, this was unlikely to cause false positives from environmental samples, due to high amount of pure DNA added (10 ng) and the late amplification. Additionally, the assay developed for FSP was significantly more sensitive than a
previously published assay (Zitnick-Anderson et al., 2018). Ongoing work is optimising DNA extraction from soil samples and will evaluate the use of these assays in comparison with the current in vitro methods used by PGRO.


Use of chlorophyll fluorescence imaging to phenotype type 3 effectors impacts of Xanthomonas on leaves

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Phenotyping biotic stresses in plant-pathogen interactions studies is often hindered by phenotypes that can hardly be discriminated by visual assessment. Particularly, single gene mutants in virulence factor could lack visible phenotypes. Chlorophyll fluorescence imaging (ChlFI) has been increasingly used to quantify the impact of different pathogens on plants. Many ChlFI parameters such as Fv/Fm and NPQ have been identified that may provide physiological interpretation and may be used to phenotype plant-microbe interactions. However, while numerous chlorophyll fluorescence parameters can be measured, most studies only use a restricted number of parameters. This could result in limited abilities to discriminate visually similar phenotypes. We developed a computation method based on the combination of multiple chlorophyll fluorescence parameters. We assessed its ability to improve the discrimination of visually similar phenotypes induced by different virulence factors of Xanthomonas. The method involves histogram Bhattacharyya distance calculations and hierarchical clustering. Such a method used a normalization approach to take into account the inter-leaves and intra-phenotypes heterogeneities.

To assess the efficiency of the method, two datasets were used. In the first dataset, we monitored the impact on Nicotiana benthamiana leaves of mutants of Xanthomonas in single type 3 secreted proteins. Some of the strains displayed visible phenotypes and these were used as ground truth data to setup the method. A second dataset was composed of transient expression (agrotransformation) of type 3 effectors. This second dataset displayed phenotypes that cannot be discriminated by visual assessment and no prior knowledge can be made on the respective impact of each type 3 effectors on leaf tissues. The efficiency of the computation method was demonstrated to improve the discrimination of the visually similar phenotypes of these biotic stresses.

References: Méline V. et al., 2018. Role of the acquisition of a Type 3 Secretion System in the emergence of novel pathogenic strains of Xanthomonas. Molecular Plant Pathology. doi: 10.1111/mpp.12737

Towards identification of novel sources of resistance to phoma stem canker and light leaf spot pathogens in a Brassica napus diversity panel

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Fungal diseases decrease the yield of the economically important crop oilseed rape (Brassica napus). Two of the most important diseases in the UK are phoma stem canker caused by Leptosphaeria maculans together with L. biglobosa and light leaf spot caused by Pyrenopeziza brassicae. There is a need to identify broad-
Sclerotinia sclerotiorum is a soilborne fungal pathogen that causes stem and crown rots in a wide range of crop plants resulting in extensive economic losses worldwide. The fungus can survive for several years in the soil as sclerotia, which when close to the soil surface germinate carpo genically to produce mushroom-like apothecia. Subsequent release of air-borne ascospores then initiates the infection process. Control of S. sclerotiorum focuses on the prevention of ascospore infection with the use of fungicides, but generally there are no attempts to eradicate sclerotia. This project aims to explore practices that reduce sclerotial survival in order to improve Sclerotinia control in celery and lettuce in Spain. The main objectives are to: a) collect S. sclerotiorum isolates from UK and Spanish lettuce and celery crops and characterise using molecular genetics to identify any differences in population structure. b) determine the temperatures required to kill S. sclerotiorum sclerotia prevent carpogenic germination for UK and Spanish isolates. c) investigate other means of killing sclerotia such as bio-solarisation, biofumigation and anaerobic disinfection. S. sclerotiorum isolates were collected from lettuce and celery plants in the UK (Cambridgeshire, Kent and Hampshire) and Spain (Aguilas, Cartagena and Pozzo). Identity was confirmed by PCR and sequencing of the ITS regions of the rDNA while sequencing of the IGS region had provided an initial examination of genetic differences between isolates. A preliminary controlled laboratory experiment was set up to determine the effect of temperature on the viability of S. sclerotiorum sclerotia. Containers (1L) were filled with pasteurised compost, moisture content adjusted to 43.03% and mesh packets containing 100 sclerotia buried in each with a Thermocron temperature logger button. Containers were incubated at 20, 30, 35, 40, 45 or 50°C and a packet of sclerotia removed at 2, 4 and 6 weeks to assess the viability of sclerotia. This was evaluated by bissecting each sclerotium, placing each half face down onto agar and recording presence / absence of S. sclerotiorum colonies. Results indicated that sclerotia were still viable after 6 weeks at 20-30°C but was reduced after 4 weeks at 35-40°C. No sclerotia were viable at >2 weeks at 50°C. A preliminary field trial was set up in Spain to determine the effect of solarisation using plastic sheeting in combination with a manure on viability of S. sclerotiorum sclerotia. Four treatments were set up in a randomised design: i) untreated, no manure, no solarisation; ii) manure only; iii) solarisation only; iv)
manure and solarisation. A mesh packet containing 100 sclerotia and a Thermocron temperature logger was buried in each plot in July 2018 and retrieved approx. 11 weeks later. The viability of sclerotia is currently being assessed as described above.

Identification and characterisation of microbial effectors interfering with the plant circadian rhythm

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Infection of economically important crops by rapidly evolving pathogenic bacteria poses an on-going threat to food security, often causing substantial loss to crop yields. Such aggressive pathogens rely on the delivery of virulence factors, known as effectors, for the suppression of plant immunity and to ensure successful infection of the host. One posited mediator of the Arabidopsis thaliana defence response is the circadian clock, which regulates the expression levels of immune receptors in a temporal fashion. The clock also rhythmically governs stomatal aperture, allowing the innate immune system to limit bacterial entry in an active way. While this rhythmicity enables the plant to maximise efficiency of its immune system through synchronisation with the environment, it also makes the circadian oscillator a likely target for manipulation by bacterial effectors. By evaluating clock gene expression profiles of effector-transformed Arabidopsis using time lapse bioluminescence detection we have identified multiple Pseudomonas syringae effectors that significantly alter period length of the plant’s circadian clock. One such effector, the tyrosine phosphatase HopAO1, has been found to interact with a NAC transcription factor that binds to the promoter of core clock gene ‘late elongated hypocotyl’ (LHY). We hypothesise that this interaction is required for the effector’s clock disrupting phenotype and thus enhances virulence of Pseudomonas syringae. Future work will focus on characterisation of the molecular mechanism employed by this effector and so permit the engineering of more disease resistant crops.

Characterisation of temperature-sensitivity of Brassica napus resistance against Leptosphaeria maculans

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Each year losses in UK oilseed rape (B. napus) production due to phoma stem canker caused by the fungal pathogen L. maculans, cost c. £80 million. Cultivars with qualitative and/or qualitative resistance are used to control this disease. Qualitative resistance is controlled by single, major resistance (R) genes which are often temperature-sensitive. Differences in pathogenesis-related gene expression of B. napus plants with temperature-resilient and -sensitive R genes are being assessed. A temperature-sensitive gene SNC1 and its temperature-resilient paralog FocBr1 are being investigated for their roles in temperature-sensitivity. AtSNC1 acts as a temperature-sensitive component of plant defence responses. FocBr1 confers resistance against Fusarium oxysporum in B. rapa. The B. oleracea ortholog, FocBo1, operates at temperatures >25°C (Shimizu et al., 2015). Resistance phenotypes of isogenic B. napus lines with different R genes (LepR3, Rlm2, Rlm4 or Rlm7) were analysed using cotyledon assays at 20°C and 25°C to identify temperature-resilient or -sensitive R genes. Differences in temperature-sensitivity of Rlm7 and Rlm4 were observed. These phenotypic differences will be exploited for qPCR and RNAseq analysis. TILLING mutations in the two closely related FocBr1 and BrSNC1 genes in the B. rapa line R-o-18 were obtained from RevGenUK. A homozygous TILLING line with a mutation in the P-loop region of FocBr1 will be assessed for temperature-sensitivity of its defence response against L. maculans and back-crossed to B. rapa R-o-18 to purify the genetic background. KASP marker analysis is being done to confirm mutant
Exploiting effectors of a fungal mutualist to enhance crop production

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Irrespective of lifestyle (pathogenic or mutualistic), plant colonizing microbes secrete modular proteins called effectors into their host to achieve infection. Effectors target specific organelles and pathways to reprogram host signalling (e.g. involved in immunity, nutrient supply) to establish their lifestyle. We aim to investigate the functions of effector proteins secreted by the mutualistic fungus *Serendipita indica*. The fungus confers various beneficial properties to colonized plants and we hypothesise that these benefits are associated with the effectors *S. indica* (SIEs) secretes. The interactions of SIEs with *Arabidopsis* proteins were mapped using Yeast-2-Hybrid to reveal significant targeting of transcription factors with functions in immunity and development. Global network analysis of these interactions showed SIE targets are either directly associated or adjacent to hormone pathways, with ethylene, a hormone being significantly overrepresented in the list of targets. Using further biochemical analyses, we show the subcellular targeting of SIEs in * planta* and confirm several interactions via co-immunoprecipitation. In some cases, these target proteins could explain phenotypes observed in plants stably expressing SIEs, which show growth promotion, stress tolerance, and altered sensitivity to jasmonic and salicylic acid, master regulators of plant immune signalling.

Trichoderma as a potential protective control against Armillaria mellea infection

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*Armillaria* species are one of the most widespread fungal plant pathogens worldwide affecting a range of woody trees and shrubs in gardens, agriculture and forestry. The root disease spreads primarily through an underground network of hyphal root-like structures known as rhizomorphs which can grow at a rate of up to 1m a year. Due to a lack of practical and effective control options, there is a demand for a control which is pesticide-free with no negative cultural or environmental impact. This study focuses on the potential of *Trichoderma* endophytes to show antagonistic properties towards *Armillaria* and offer protection to host plants. Forty host-associated isolates of *Trichoderma* were tested with *Armillaria* in dual culture. It was shown that *Trichoderma* is able to grow over *Armillaria* cultures within four days and significantly reduce the *Armillaria* growth over 1.5 months at which point the *Armillaria* culture was no longer viable. A pilot test looking at interactions between *Trichoderma* and *Armillaria* in woody segments showed very low *Armillaria* viability at 3 weeks and after 1.5 months *Armillaria* was no longer viable in the woody segments. An *in planta* experiment investigating the potential for *Trichoderma* to protect strawberry plants from *Armillaria* infection found variation between *Trichoderma* isolates, but there were eight which have the potential to protect hosts from infection. A disease severity index (DSI) was used to rank plants between 0 – 6 (healthy – dead); one isolate left plants fully healthy with no signs of *Armillaria* infection. The remaining seven isolates had an average DSI ≤ 2 where plants were at most showing some minor signs of disease and no *Armillaria* mycelium visible, but infection was confirmed through isolation. Isolates of *Trichoderma* and *Armillaria* have been transformed with GFP or dsRED to act as selective markers and to enable visualisation of the * in vitro* and *in planta* interactions between the two. Future studies will further investigate the potential of the eight isolates.
identified to offer protection to hosts in strawberry and privet plants, including the colonisation success of *Trichoderma* isolates in privet roots.

**POSTER**

Using Raman micro-spectroscopy to monitor the metabolic activity of plant pathogenic bacteria *Pseudomonas syringae*

**Nattapong SANGUANKIATTICHAI**, Wei E. Huang, Gail M. Preston


Raman micro-spectroscopy is a technique that uses inelastic scattering of an incident laser light on a cell to obtain a molecular vibrational spectrum containing information about its biochemical composition. This technique enables a single-cell level characterization of the metabolic phenotype of the cell as well as tracking of biomolecular markers. The aim of this project is to establish the application of the Raman technique in the study of plant-pathogen interactions using the bacterial pathogen *Pseudomonas syringae* as a model organism. Here, we investigate the use of $^2$H (deuterium) stable isotope labeling to monitor the metabolic activity of the bacteria in the context of the early dynamics of plant infection. The incorporation of deuterium into the cells results in a carbon-deuterium Raman band (~2200 cm$^{-1}$), which is a significant and unique shift from carbon-hydrogen Raman band (~3000 cm$^{-1}$). By pre-labeling all bacterial cells with deuterium and using Raman micro-spectroscopy to track the dynamics of reversion of the labelled signals in the cells over time, we could distinguish actively growing cells from cells suppressed by a bacteriostatic antibiotic *in vitro*. Furthermore, we applied this approach during infection *in planta* to monitor the activity of bacteria during the early stages of infection, and investigate the extent to which bacteria are affected by plant defense responses. This work establishes a proof of concept for the application of Raman microspectroscopy and stable isotope labeling to investigate the metabolic phenotypes of microbial pathogens and their use in the study of plant-pathogen interactions.

**Monday | Session 3**

Immunity-induced root growth inhibition is mediated by cell cycle arrest

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Plant diseases caused by pathogenic infections in agricultural crops is a major thread in global crop production, a situation expected to worsen under changing climates. Efforts to increase immunity in crops against pathogens, however, are hampered by the fact that plant immunity reduces growth and, thus, yield to an extent that we know from diseases itself. It suggests a tight interlacement of immunity and growth signalling. In support of this we recently found that cell identity networks integrate with cell immunity networks to activate cell type-specific immune response. Combining cellular biology approaches with genetic studies, we aim to understand this mechanism of immunity-induced growth inhibition and to consequently generate plants able to sustain growth under immunity. Our studies demonstrate that arrest of the cell cycle in the root apical meristem is responsible for the growth inhibition. Furthermore, screening of cell cycle regulator mutant and overexpressing plants has led us to the discovery of lines in which growth and immunity are uncoupled. Here we present these findings and propose a model to explain how immunity interferes with root growth signalling pathways.

**Tuesday | Session 6**
Fluorescent sensing of subcellular plant physiology and pathology in vivo

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Plant cells are frequently exposed to dramatic changes in their immediate environment, such as illumination changes, suboptimal oxygen availability or exposure to pathogens. For the plant to survive as an organism tailored acclimation responses must be mounted mainly at the level of the individual cells, implying that a sophisticated and flexible response network operates within each cell. Yet, our understanding of the response dynamics specifically in different sub-cellular compartments is limited. We have been using quantitative confocal microscopy and fluorimetry to assess transitions in cell physiology in vivo using a growing set of genetically-encoded fluorescent protein sensors. I would like to introduce both fundamental considerations as well as the recent progress that we have made in the dissection of cellular physiology highlighting ATP dynamics, H$_2$O$_2$, and redox regulation as examples. Specifically, the intracellular impact of elicitor-induced oxidative burst on H$_2$O$_2$ dynamics will be explored. I will further discuss our efforts towards multiparametric monitoring, as an approach towards an integrated picture of subcellular stress physiology, while appraising technical and biological limitations. The impact of transitions in subcellular physiology and their control as a determinant of plant stress resistance and immunity will be discussed.

The dynamics of microbial and environmental factors in shaping root microbiomes

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The root microbiota, consisting of diverse microbial communities in and around plant roots, can significantly influence plant development and stress tolerance. The basic aim of our research is to analyse the communication between microbes in the rhizosphere and between plant roots and microbes. For this purpose, Serendipita indica, a mutualistic fungal root endophyte, which is observed to improve plant performance and disease resistance in host plants, is studied. By analyzing the effect of S. indica on the rhizobiome composition of Arabidopsis thaliana, using a synthetic bacterial community, we aim to reveal plant and/or microbe-derived communication patterns that are involved in shaping root microbiomes. To further test these patterns in a natural environment, a bacterial taxon commonly utilized in agriculture, Rhizobia, is used as a mutualistic root symbiont of the legume Medicago truncatula. Rhizobia strains are tested for their abilities to stimulate plant growth and nutrient supply as well as their competitiveness in representative UK soil types and their different natural microbiomes. These analyses will further help in elucidating the stability of beneficial plant-microbe interactions in natural communities and in different environments. Being part of the Warwick Integrative Synthetic Biology Centre, the long-term aim is to integrate molecular and environmental factors to generate customized beneficial microbiomes that can be applied to sustainably support crop production.

Visualizing Pseudomonas syringae-host interactions in the anthosphere and the plant-vascular system

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Pseudomonas syringae pv. phaseolicola (Pph) is a Gram negative bacterium which causes halo blight of Phaseolus vulgaris (common bean) worldwide. Symptoms include water-soaked lesions on both leaves and pods, chlorosis, reduced photosynthesis and consequently reduced yields. Even though it is a seed-borne disease, little is known about its route of colonization, leading to the infection of seeds. In this research study we use different mutants of Pph expressing fluorescent proteins, representing different degrees of
 compatible and incompatible interactions on planta, for deciphering the colonization behaviour of this pathogen in plant vasculature and flowers (hypothetical routes leading to seed infection) through Confocal Scanner Laser Microscopy (CLSM) and 3D modelling. Among the hypothesis that has been tested by using tagged Pph mutants, are the role of bacterial effectors and type III secretion system in the bacterial colonization of flower gynoecium and plant vascular system. Moreover, imaging of lower spatial-resolution is achieved by Pph bioluminescent mutants, allowing macroscopic visualization of the plant-pathogen interaction. In this research we show that Pph can colonize the plant vasculature and spread within the plant. We observed for the first time a real-time interaction between germinating pollen on the flower stigma and the bacterial pathogen, showing colocalization of the germinating pollen tube as it enters the stigma and Pph. Preliminary evidence suggests that flowers are not passively colonized by Pph and that floral tissues can perceive PAMPs, which leads to downstream responses. This is in contrast with the widely adopted hypothesis that flowers represent a permissive colonizable habitat. Our study highlights the gap of knowledge the scientific community is facing between plant-pathogen interaction in the phyllosphere and in the anthosphere and provide new insights in the colonization behaviour of bacterial pathogens in less-investigated plant habitats.

Tuesday | Session 6

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Studies on *Zymoseptoria tritici*: the dimorphic switch and hyphal growth in wheat infection

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*Zymoseptoria tritici*, the causative agent of *Septoria tritici* wheat blotch, undergoes a morphological transition from a yeast to a hypha before colonising the host plant, followed by the formation of pycnidia and new spores. We have investigated this “dimorphic switch” using fluorescent markers for various organelle s and laser scanning confocal microscopy during the infection cycle. This study questions the concept of a yeast-to-hypha transition and provides unique insight into the behaviour of *Z. tritici* inside plant tissue.

Monday | Session 1

Effect of the *Leptosphaeria maculans* effector gene *AvrLm1* on *Rlm7*-mediated defence responses in oilseed rape

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Pathogen effectors are known to manipulate host processes and suppress immune responses like pattern-triggered immunity. The phoma stem canker pathogen *Leptosphaeria maculans* of oilseed rape (*Brassica napus*) produces various effectors, including *AvrLm4-7* that is recognised by the corresponding host *R* genes *Rlm4* and *Rlm7*. *AvrLm4-7* interferes with resistance responses of *Rlm3*-containing cultivars against isolates with *AvrLm3*. Similarly, *AvrLm4-7* blocks recognition of *AvrLm5-9* by *Rlm9*. We are examining responses of cultivar Excel with *Rlm7* to *L. maculans* isolates with *AvrLm4-7* or *AvrLm1* and *AvrLm7*. Induction of *PR1* and *PDF1.2* expression is reduced in response to the isolate with *AvrLm1* and *AvrLm7* compared to the isolate with *AvrLm4-7*. Similarly, diaminobenzidine staining, indicative of *H2O2* production, is reduced in cv. Excel in response to isolate with *AvrLm1* and *AvrLm7* relative to the *AvrLm4-7* isolate. Phenotypic responses of cv. Excel to both isolates do not significantly differ. However, fungal biomass is being assessed based on pathogen DNA quantification. Additional experiments are under way to determine differences between isogenic cv. Topas and cv. Topas-*Rlm7* genotypes. Suppression of *PR1* and *PDF1.2* expression supports published data on suppression of salicylic acid and jasmonic acid signalling by *AvrLm1* through interaction with MAPK9 (Ma et al., 2018, iScience 3: 177-191). On the contrary, the suppression by *AvrLm1* of *Rlm7*-triggered *H2O2* production we observed differs from the published MAPK9 enhancement of *H2O2* production.
Dramatic changes in genetic diversity of the avirulence effector AvrStb6 in global populations of the wheat pathogen Zymoseptoria tritici

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Septoria tritici blotch (STB), caused by the pathogenic fungus Zymoseptoria tritici, is one of the most commercially important diseases of wheat globally. Recently, both genetic factors of the previously characterised gene-for-gene interaction between Z. tritici and wheat have been identified: the wheat receptor-like kinase Stb6 and the Z. tritici secreted effector protein AvrStb6. Genetic diversity analysis of AvrStb6 in historic collections of Z. tritici from global populations has been carried out and recently published. However, the prevalence of AvrStb6 haplotypes that may allow evasion of Stb6-mediated defence in modern Z. tritici isolates is currently unknown. We therefore analysed genetic diversity of AvrStb6 in 2016-2017 Z. tritici field populations from the UK, Western Europe, USA, South America, and Australia. This study revealed that, whilst AvrStb6 is present in all modern isolates tested, remarkably, a small number of haplotypes - encoding the same protein isoform conditioning virulence on Stb6-containing wheat - currently predominate in different parts of the world. The “original”, avirulence isoform of AvrStb6 was not detected. This contrasts with the data collected in a study focused on the analysis of a largely French Z. tritici population, sampled between 2009-2010, in which this avirulent isoform of AvrStb6 was found to be present in 20% of isolates in the collection (Zhong et al. 2017). Data collected on a global Z. tritici population sampled from 1990-2001 (Brunner, McDonald, 2018) also suggested a higher diversity of AvrStb6 haplotypes than we have found. Therefore, it appears that in recent years there has been a significant shift in the global Z. tritici populations towards haplotypes encoding a single Stb6 resistance-breaking isoform of AvrStb6. On one hand, evolutionary pressure to evade recognition by Stb6 - found to be present in over 50% of current commercial UK wheat cultivars (Saintenac et al., 2018) - may explain the elimination of avirulence isoforms of AvrStb6. On the other hand, emergence of a particular virulence isoform of this effector protein may contribute to fungal pathogenicity, fitness, or other important traits. This hypothesis will be tested by transgenic introduction of different AvrStb6 haplotypes into an AvrStb6 deletion mutant of Z. tritici followed by characterisation of the resulting fungal strains, including pathoassays on wheat plants possessing or lacking the functional resistance allele of Stb6. This knowledge would help build an understanding of the relative importance of Stb6 in conferring genetic resistance to modern Z. tritici strains, as well as help identify the mutations that allow evasion of Stb6 detection, helping to further elucidate the function of AvrStb6 in pathogenicity.


Investigating the biology of plant tissue invasion by the rice blast fungus Magnaporthe oryzae

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice cultivation around the world. The fungus also causes wheat blast, a disease that now threatens wheat production in South America and South Asia. During plant infection, M. oryzae forms a specialised infection structure called an appressorium. The appressorium generates enormous turgor, applied as mechanical force
to breach the rice cuticle. Re-polarisation of the appressorium requires a hetero-oligomeric septin complex to organise a toroidal F-actin network at the base of the infection cell. This facilitates invasion of epidermal cells and development of biotrophic invasive hyphae.

Septin-mediated plant infection is regulated by a turgor sensing protein kinase, which can sense when optimal appressorium turgor is achieved and control the to polarised, invasive fungal growth. A pressure-mediated cell cycle checkpoint is also necessary for initiation of septin activation and re-orientation of the cortical F-actin cytoskeleton. Once tissue is invaded the fungus undergoes differential expression and secretion of a large repertoire of effector proteins that are delivered to the apoplastic space which surrounds invasive hyphae, or are directed into plant cells using a specific secretory pathway. The fungus also undergoes distinct physiological changes, including activation of enzymes associated with utilisation of a broad spectrum of carbon sources, and distinct secondary metabolic pathways. M. oryzae suppresses plasmodesmatal immunity in order to facilitate its spread from cell-to-cell in plant tissue. This is controlled by a specific MAP kinase signalling pathway and requires septin-dependent hyphal constriction to enable the fungus to spread rapidly in rice tissue.

www.tsl.ac.uk/staff/professor-nick-talbot

Standing on the shoulders of RKS Wood: from physiological to molecular plant pathology

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The field of molecular plant pathology is highly indebted to the late professor RKS Wood who was an early advocate of 'looking under the bonnet' of the interactions between plants and their pathogens. With this he referred the to the study of the physiology and biochemistry of those interactions, which was captured by the term ‘Physiological Plant Pathology’. RKS Wood worked on a large diversity of plant-pathogen interactions, among which were vascular wilt diseases caused by Verticillium, and focused on toxins, phytoalexins, cell wall degrading enzymes, mechanisms of (induced) resistance and (bio)control of plant diseases by antagonistic organisms. In this presentation, I will discuss our recent advances in understanding the molecular biology of Verticillium wilt diseases and show how our work builds upon the seminal findings of RKS Wood.

Recruitment of defence-related autophagy machinery towards the pathogen interface

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During plant colonization, the Irish potato famine pathogen penetrates host cells and forms infection structures called haustoria that enable translocation of effector proteins. Invaded plant cells respond with a spatially confined cell-autonomous defense response known as focal immunity, a poorly understood process that is implicated in concentration of plant immune responses around pathogen contact sites. This process includes recruitment of defense related host autophagy machinery, including the plant autophagy cargo receptor Joka2, towards the host-derived extrahaustorial membrane (EHM) that engulfs the haustorium. Phytophthora infestans counteracts this by deploying PexRD54, an effector that antagonizes Joka2 mediated immunity across the EHM. However, how Joka2 contributes to immunity and the molecular mechanisms underlying defence-related selective autophagy remain unknown. In this study, we dissect the role of Joka2 in defence-related autophagy. We first identified candidate Joka2 interactors in infected and uninfected cells through complex purification followed by mass spectrometry analysis. Remarkably, this revealed an intricate set of potential Joka2 interactors in infected tissue, including defence-related enzymes and signalling components that are implicated in both basal plant immunity and effector triggered immunity. By using a
reverse co-immunoprecipitation approach, we validated that two defence-related mitogen-activated protein kinases (MAPKs) interact with Joka2 in vivo. We show that this interaction is independent of ubiquitination of MAPKs and is mediated through a previously uncharacterized domain of Joka2. Consistent with this, suppression of autophagy or down regulation of Joka2 gene expression did not affect the rate of MAPK protein turnover, indicating that MAPKs are not autophagy substrates recruited by Joka2. Furthermore, confocal microscopy of fluorescently tagged MAPKs co-expressed with Joka2 uncovered that MAPKs colocalise to Joka2 labelled puncta accumulating around the *P. infestans* haustoria. Finally, we found that Joka2 loses its ability to contribute to immunity upon removal of the domain responsible for MAPK binding, indicating that Joka2-MAPK interaction is essential for activation of defence-related autophagy. These results implicate selective autophagy in plant focal immunity and suggest more complex functions for autophagy than the widely known degradative roles.


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**POSTER**

**The Pathogen-Host Interactions phenotype database, PHI-base: harnessing community expertise to fight microbial incited diseases**

**Martin URBAN**

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The pathogen-host interactions database PHI-base (www.phi-base.org) is a gold-standard database storing phenotypes on genes implicated in virulence. It is a primary information source for researchers studying plant-pathogen interactions as well as human fungal pathogens. Manually curated information from more than 3,000 research articles are made accessible for researchers to easily familiarise themselves with relevant molecular and biological facts on pathogenicity, virulence and effector genes, on 1st host target and *in vitro / in host* phenotypes. High-level phenotypes are used to describe the overall experimental interaction outcomes enabling comparative analysis of different pathosystems. More detailed phenotypes including microscopic observations are captured using free text manual curation and controlled vocabularies. The multi-species database summarises information from 263 pathogens tested on 194 hosts to allow comparative genomics approaches to facilitate novel fungicide target discovery and identify critically important genes for biotech/breeding control strategies. All major plant pathogens from the bacterial, fungal and protist kingdoms are included. Here we describe our new PHI-base Version 4.6 release (November 2018). Our data platform includes simple and advanced search, filtering and extended data displays and a protein-to-phenotype BLAST capability. We will also demonstrate a new web based curation tool called PHI-Canto for authors to use to capture plant-microbe interaction phenotypes reported in their own peer reviewed articles. Different ways to discover novel candidate virulence genes and explore predicted pathogenicity networks using PHI-base data are discussed.

**Fusarium graminearum** effectors: identification and characterisation of three small secreted proteins which contribute towards fungal virulence

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*Fusarium graminearum* is an ascomycete fungus capable of causing a multitude of diseases in cereal crops. Fusarium head blight (FHB), a wheat disease of pre- eminent economic importance, not only results in crop losses but is also highly hazardous due to mycotoxin contamination. A recent transcriptomic investigation revealed that *F. graminearum* exhibits a biphasic lifestyle; the first phase characterised by asymptomatic growth between live host cells before advancement of the second symptomatic phase. Both phases are characterised by the production of unique sub-sets of small secreted proteins (SSPs), or putative effectors, hypothesised to manipulate host defences. The aim of this project was to identify SSPs that contribute towards fungal pathogenicity. Putative effectors were selected on the basis of expression data from a species-specific Affymetrix-microarray exploring transcriptional changes between the distinct phases of *F. graminearum* infection. The expression profiles of selected candidates were validated by quantitative reverse transcription PCR (qPCR) and closely matched the expression profiles of the Affymetrix data. In order to determine the role that selected candidate effectors play in the development of FHB, FgSSPs were overexpressed in wheat using the Barley stripe mosaic virus-mediated overexpression system (BSMV-VOX). Wheat plants prematurely expressing candidate FgSSPs were subsequently inoculated with *F. graminearum* and progress of the fungus throughout the wheat ear was monitored. Several proteins were identified (FgSSP22, FgSSP32 & FgSSP33) which, when overexpressed, led to a reduction in fungal disease symptoms - all of which are expressed during the symptomatic phase of infection. To further explore the role that FgSSPs play in *Fusarium* infection, effectors were transiently expressed in *Nicotiana benthamiana* via the Cowpea mosaic virus (CMV)-derived pEAQ-HT vector system which facilitates recombinant high-level protein expression. Both FgSSP32 & FgSSP33 were shown to induce cell necrosis - particularly around the host vasculature tissue. This work has identified several cell-death inducing effectors in *F. graminearum* with further work required to uncover the exact function of these FgSSPs. Premature expression of FgSSPs led to reductions in fungal virulence, thus demonstrating the importance of maintaining the integrity of the finely-tuned spatial and temporal coordination of effector secretion.

**Use of a real-time decision support system to give accurate timings for fungicide applications**

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Strawberry powdery mildew is a serious disease of the strawberry crop, caused by the fungus *Podospaera aphanis*, which can result in 20-70% yield losses. To control the disease, regular spray programmes utilise fungicide applications every 7/8 days, regardless of environmental conditions. However, previous research at the University of Hertfordshire has shown that environmental conditions, temperature and humidity, affect the development and growth of the fungus. Therefore, a rule-based decision support system was devised recording the accumulated numbers of hours (144) of conditions necessary for the fungus to germinate, grow and produce spores. For germination: RH >60%, temperature 15.5-30°C; with optimum temperature for growth 18-30°C. These parameters were used to develop an online real-time web-based prediction system, with validation through 2017-18. By using the prediction system to support the grower’s decisions, in 2017, the use of the system at one grower site provided satisfactory control of the fungus when compared to a regular spray programme. By using the prediction system to inform decisions, fungicide applications over the season were reduced to 15 sprays, compared to the 20 sprays of the regular spray programme. By reducing the number of fungicide sprays, this allowed better management of fungicide modes of action. The use of the prediction system gave over £300 per hectare of savings, at this grower’s site. By using the prediction...
system as a decision support aid, the grower was able to use fungicides with precision when needed. In 2018, the real-time web-based prediction system was used by three growers across Great Britain, two in England and one in Scotland. Each grower has used the prediction system to help inform their planning of fungicide applications. The prediction system has continued to successfully provide satisfactory control of strawberry powdery mildew. By the end of harvest in the 2018 season, collected data will be analysed to further validate the rule-based prediction system as an aid in decision support.

POSTER

Pathogen microphotography: a powerful tool for diagnostics
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In a world where innovation and technology lead the way, STC is proud to work in association with The Centre for Crop Health and Protection (CHAP), creating opportunities which unite industry, agriculture, horticulture and academia with a view to changing the way agribusiness deals with crop threats, to sustainably change the way we feed the world. Swift, accurate diagnosis is essential to enable growers to create sustainable integrated crop management plans. Using advanced visual aids and imaging resources, we aid farmers globally in the identification of micro-organisms, parasites and pathogens to diagnose crop problems. At the STC site in N Yorkshire the Science Team works actively with industry, delivering prompt Plant Health Clinic diagnostic services and an extensive research portfolio to directly address the pest and disease issues faced by our UK food producers. The Plant Health Clinic offers a unique package of services to growers. It’s a one stop shop to solve immediate diagnostic issues, with professional reports swiftly delivered for urgent crop issues. Lab resources at Stockbridge Technology Centre, provided in collaboration with CHAP, include advanced visual /microscope and micro-photographic equipment, enabling us to push the barriers of plant diagnostic techniques. All work is carried out under strict protocols, working under GLP, GEP and EPPO guidelines. Alongside the diagnostics and research services provided by Stockbridge Technology Centre for the short and medium term needs of growers, CHAP enables growers to invest in people and technology for longer term success, providing professional development programmes to train and equip the next generation of growers, agronomists and scientists with state of the art skills and techniques.

POSTER
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NbMORF8 encodes a protein localized in mitochondria and chloroplasts and negatively regulates plant immunity to Phytophthora pathogens
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In the process of infecting plants, Phytophthora pathogens secrete a large number of effectors into the host intercellular space or intracellular cells. Screening for the candidate effector targets and identifying host factors that have vital roles in plant immunity are useful approaches to dissect molecular mechanisms underlying plant susceptibility to Phytophthora infection. In this study, we employed VIGS (Virus Induced Gene Silencing) to examine the function of the candidate proteins targeted by the Avr3a family effector Phytophthora sojae effector PsAvr1b, obtained in a previous research by mass spectrometry analysis. This led to the identification of NbMORF8, a MORF (Multiple organellar RNA editing factor) family protein involved in C to U RNA editing. We show that it plays dual roles in plant immunity, promoting plant infections of P. parasitica, P. capsici and P. infestans by negative regulation of NbPR1 and NbPR2 expression as a susceptibility gene, and mediating specific HR immune response induced by specific recognition of effector by R protein. In addition, NbMORF8 was essential for the regulation of plant growth and development.
Further analyses showed that the NbMORF8 encodes a protein located in both mitochondria and chloroplasts, and such subcellular location is crucial for its function.

**Monday | Session 2**

**Benchmarking plant defence priming agents by hyperspectral imaging**

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In economically important crops, pesticide application, along with cultivar resistance where available, are the main protection measures against pathogens. However, cultivar resistance usually has a limited life span before breaking down and widespread use of pesticides has resulted in a growing number of pesticide-resistant pathogen populations, necessitating the development of alternative protection measures. A promising alternative is the use of ‘priming agents’. These are molecules of varied origin that sensitise a plant’s innate immune system, resulting in a faster and/or stronger deployment of inducible defences on pathogen exposure. Priming agents typically provide partial resistance, with larger doses generally providing enhanced disease reduction. High doses, however, are usually phytotoxic due to over-stimulation of the plant immune system. Combining low doses of priming agents that work through different mechanisms for additive or synergistic action can potentially increase efficacy whilst reducing undesirable effects. Here we present work carried out in the University of Sheffield Wolfson Centre for Disease Phenomics, that demonstrates the potential of hyperspectral imaging (HSI) as an efficient phenotyping tool for the assessment of the effectiveness of priming agents. Our results show non-destructive measure of the phytotoxicity of three priming chemicals in lettuce and tomato by measuring changes in planta, their growth rate and shifts in key spectral vegetation indices that are indicative of plant health and chemical composition. Furthermore, we highlight the capability of HSI for detecting the presence and severity of infection by a necrotrophic fungus (*Botrytis cinerea* on tomato) and a biotrophic oomycete (downy mildew on *Arabidopsis*), without the need for laborious manual assessment methods. Together these capabilities make HSI a valuable tool for high throughput screening of combinations of priming chemicals for suitability as a crop protection strategy against economically important crop diseases.

**Tuesday | Session 5**

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