

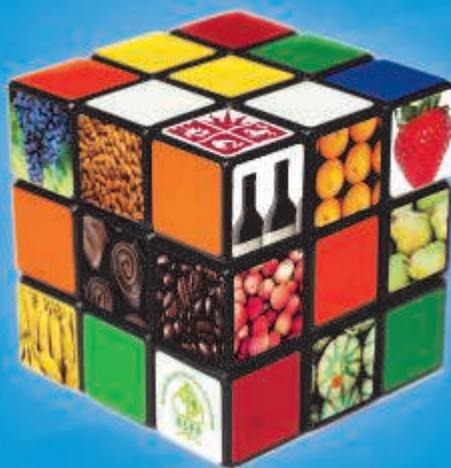


University of
BRISTOL



BSPF 2015 Presidential meeting

The **impact** of
plant pathology
on **everyday lives**



13th - 15th September
2015

Bananageddon: Millions face hunger as deadly fungus Panama disease decimates global banana crop

4 April 2014; The Independent

Coffee in Crisis: The Silent Disaster You've Never Heard Of

21 November 2013; Triple Pundit

The cocoa crisis: why the world's stash of chocolate is melting away

21 November 2014; The Guardian

Prosecco shortage: Producer warns there could be a 'global shortfall'

22 May 2015; The Independent

'Major consequences' if olive disease spreads across EU

9 January 2015; BBC News

Citrus Disease With No Cure Is Ravaging Florida Groves

9 May 2013; New York Times

President's Welcome

The main theme of this year's meeting is to consider the impact of plant pathology on a wide range of plants and situations and not just the usual big crops that normally get attention, although these will be covered in the meeting. We do, however, want to highlight the effects of plant pathogens on the plants we grow in our gardens, to the trees we walk through in the woods, the grass we play on, and very important stuff like the threats to chocolate, juniper berries for gin, hops for beer, olive oil and many other of life's essentials!

It is events like these that have raised the profile of plant pathology onto the national and international agenda, and it is the current ambition of BSPP to launch a series of initiatives to further enhance the importance of this branch of science, so watch this space.

It is a pleasure to be holding the meeting in Bristol and the South West, which has always had a proud tradition of research into plant pathology. Today we can boast of active plant pathology groups at the Universities of Bath, UWE, Bristol and Exeter, with many past and current members of BSPP having strong links to the area.

We sincerely hope you enjoy the meeting, the modern and indeed the historic area that represents the University of Bristol, as well as the local entertainment and nightlife. We hope you find time to take a tour of the new Life Sciences Building, which at £60 million is a major investment by the University into this area, including state of the art facilities for plant and crop research.

As they say in Bristol, we hope the meeting is '**Gert lush**' for you all.

Gary Foster, BSPP President 2015

The President, as many of you will know, lives in a wonderful world of organized chaos. Without the guiding hand of more organized people than him, this meeting would not have taken place, so special thanks to many that have been involved but in particular, the BSPP Programme Secretary Elizabeth Orton, Carol Jenner, Will Kay, Diane Hird, members of the Plant Pathology team at Bristol, and our sponsors:



BSPP Presidential Meeting 2015: The impact of plant pathology on everyday lives

University of Bristol, Life Sciences, Tyndall Avenue, Bristol, BS8 1TL, UK

Sunday 13 September

Atrium, Life Sciences Building

5.30–6.30 pm Registration and welcome drinks

Tyndall Lecture Theatre, HH Wills Physics Laboratory

6.30 pm Guest speaker James Wong

A science geek's guide to growing for flavour

Monday 14 September

Tyndall Lecture Theatre, HH Wills Physics Laboratory

9.00–9.45 Gary Foster

President's address

Cassava session

Chair Andy Bailey

9.45–10.15 James Legg

The cassava brown streak pandemic in Africa: history, development and impacts

10.15–10.45 Stephan Winter

The virus side of cassava brown streak disease

10.45–11.15 *Tea/coffee*

11.15–11.45 Maruthi Gowda

Transcriptional response of virus-infected cassava and identifying virus resistance genes and markers for cassava brown streak disease

11.45–12.15 Nicola Spence

The Plant Health skills pipeline – developing future plant health professionals

12.15–1.15 *Lunch*

1.15–3.30 P H Gregory Prize talks

Barbara Franco

Identification and characterization of a novel fungal PAMP recognised in dicots

Georgia Mitrousia

Phoma stem canker on oilseed rape cultivars with good resistance against *Leptosphaeria maculans*

Sarah Nanyiti

Construction of full-length infectious clone of *Ugandan cassava brown streak virus* (UCBSV) using yeast homologous recombination technique

Fay Newbery

Maintaining oilseed rape yield in a changing climate: modelling for a warmer future

Mojgan Rabiey

Piriformospora indica, a potential biocontrol agent?

Claire Stoker

Molecular mechanisms of rhythmic plant defence against *Botrytis cinerea*

Francis Wamonje

Effect of virus infection on aphid behaviour and VOCs emitted by common bean (*Phaseolus vulgaris* L.)

Huan Wang

Essential virulence related proteins differentially expressed during *Pectobacterium carotovorum* interacting with coloured calla

3.30–4.00 *Tea/coffee*

Late blight session

Chair Ian Toth

4.00–4.30 Bill Fry

Phytophthora infestans: history, human impact and field pathology

4.30–5.00 Sophien Kamoun

Keeping up with THE Plant Destroyer

5.00–5.30 Jonathan Jones
Humbly Ren-seeking after the truth; immune receptor sequence capture
(‘RenSeq’) accelerates resistance gene cloning and reveals novel R
protein domain architectures

Atrium, Life Sciences Building
5.30–7.30 *Posters and drinks*

Sky Lounge, Life Sciences Building
6.30 *BSPP Annual General Meeting*

Victoria Rooms, Queens Road, BS8 1SA
7.45 for 8.00 *Dinner and presentations*

Tuesday 15 September

Tyndall Lecture Theatre, HH Wills Physics Laboratory

Rice blast session

Chair James Fountaine

9.00–9.30 Sarah Gurr

IMPACT: Rice and rice blast disease

9.30–10.00 Lauren Ryder

Investigating the biology of plant infection by the rice blast fungus

Magnaporthe oryzae

10.00–10.30 Thomas Kroj

Knowledge and diversity for sustainable control of rice blast disease

10.30–11.00 *Tea/coffee*

Offered papers

Chair Lauren Ryder

11.00–11.20 Nik Cunniffe

Optimizing control of an established plant disease epidemic using a
landscape-scale mathematical model: sudden oak death in California

11.20–11.40 Kathryn Ford

Investigating the molecular pathogenicity of a tree killer: *Armillaria mellea*

11.40–12.00 John Dempsey

Suppression of *Microdochium nivale* by phosphite in amenity turfgrasses

12.00–12.20 Rebekah Robinson

Emerging pathogens in UK horticulture: a case study on *Puccinia heucherae*

12.20–12.40 Rachel Warmington

The diversity and distribution of *Sclerotinia subarctica* in the UK and Norway

12.40–1.40 *Lunch*

1.40–2.10 Seirian Sumner

Soapbox Science – taking science and gender issues to the streets

Offered papers

Chair Sian Deller

2.10–2.30 Richard Cooper

Fusarium oxysporum: world travels of a systemic killer

2.30–2.50 Katherine Denby

A systems approach to breeding disease resistance in lettuce against necrotrophic fungal pathogens

2.50–3.10 Gail Preston

Bacterial blotch: more than one way to eat a mushroom

3.10–3.30 Ian Toth

Decisions to be made: How will we protect our crops from pests and diseases in the coming years and how is Europe approaching this compared to the rest of the world?

3.30–4.00 Closing remarks

Coffee and departure

Awards

The Board of BSPP wishes to encourage young plant pathologists to talk about their work. There are two prizes awarded at the presidential meeting:

The P H Gregory prize, awarded for the presentation of an oral paper. The competition is open to (a) members of BSPP who have not previously presented a paper to a meeting of a learned society; and (b) all registered postgraduate students, whether or not they are members of the society, or have presented a paper before to a meeting of a learned society. Contestants should not have entered the P H Gregory competition previously. The winner receives a certificate and a cheque for £250.

Philip Gregory (1907–1986) pioneered aerobiology as a topic for research, combining many disciplines to contribute to better understanding of fungal spore dispersal and plant disease epidemiology. He developed theories of spore dispersal, which was published in his classic paper on the dispersion of airborne spores (Gregory PH. (1945) *Trans. Br. Mycol. Soc.* 28: 26–72). He became Head of the Plant Pathology Department at Rothamsted in 1958 where he further developed his research on spore dispersal and sedimentation velocities. In his retirement, Philip Gregory continued work on elucidating the epidemiology of black pod disease of cocoa in Nigeria, and maintained enthusiasm for his wide range of scientific interests. He proudly and enthusiastically showed his garden to visitors and they often regarded this as the highlight of their visit to Harpenden (Source: Lacey *et al.* (1997) *Ann. Rev. Phytopathol.* 35: 1–14).

The John Colhoun Prize, awarded for a poster. The work presented in the poster must form part of a research project conducted by the entrant in support of a PhD or Masters degree, and the entrant must not have been awarded the degree prior to the deadline for abstracts date. Students need not be members of BSPP. The winner receives a certificate and a cheque for £100.

John Colhoun (1913–2002): cryptogamist and plant pathologist. John Colhoun was awarded a MAgr in 1937 at Queens University, Belfast, and then moved to Imperial College, London, working on fungal pathogens of apples for his PhD, awarded in 1940. He returned to Northern Ireland where he worked on flax, an important crop in the province during World War II, leading to a definitive text (Muskett A. E. & Colhoun J. (1947): *The Diseases of the Flax Plant*). He became reader at Queens University in 1954. Subsequently, he took up the Chair of Cryptogamic Botany at the University of Manchester in 1960, where he worked on *Fusaria*, *Phytophthora*, *Septoria* and *Phoma*, with hosts ranging from cereals to chrysanthemum, yam, oil palm, and banana. In 1968 he was elected Chairman of the Federation of British Plant Pathologists, forerunner of the British Society for Plant Pathology. He retired from Manchester University in 1980 as Professor Emeritus, having occupied the Barker Chair of Cryptogamic Botany for 20 years. (Source: Epton H. (2003) *Mycol. Res.* 107: 377–381).

A science geek's guide to growing for flavour

James Wong

@Botanygeek

The single biggest promise of 'growing your own' is better flavour. Yet from a scientific point of view, most domestic gardening practices are either based on pure myth, or worse, actively designed to dilute flavour in the all-consuming pursuit of yield. Based on a 3 year research project, including a review of nearly 3000 peer-reviewed studies and a multi-site UK growing trial, botanist James Wong reveals an evidence-based guide to scoring harvests with measurably improved flavour.

James Wong is a Kew-trained botanist, science writer and broadcaster based in London, UK. Graduating with a Master of Science degree in Ethnobotany in 2006, he has pursued his key research interests of underutilised crop species, ethnopharmacology and traditional food systems through field work in rural Ecuador, Java and China. He is the author of the internationally best-selling books *Grow Your Own Drugs*, *Homegrown Revolution* and *RHS Grow For Flavour*. His presenting work spans a range of BBC programmes, including the award-winning *Grow Your Own Drugs* and *Great British Garden Revival*, as well as Radio 4's *Gardeners' Question Time*. Becoming an RHS Ambassador in 2014, James is passionate about communicating plant science to new audiences in relevant and accessible ways. In 2015 *The Sunday Times* listed him as one of the Top 20 most influential people in horticulture.

The cassava brown streak pandemic in Africa: history, development and impacts

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Cassava is a major food staple, of particular importance in developing countries in the tropics. In Africa, its capacity to yield when grown in poor soils and under conditions of moisture stress means that it provides food security for hundreds of millions of people. The two main biotic threats to cassava production in Africa are the virus diseases: cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). The causal viruses are transmitted by the whitefly *Bemisia tabaci*. Pandemics of both CMD and CBSD have recently had devastating impacts in cassava-growing areas of East and Central Africa. Annual losses for the two diseases exceed US\$ 1 billion. The deployment of highly effective, **conventionally bred host plant resistance effectively ‘stemmed the tide’** of the advancing CMD pandemic in the late 1990s/early 2000s. The absence of similar sources of resistance to CBSD, however, has meant **that the emergence and spread of a ‘new’ pandemic of CBSD, in 2004,** has gone largely unchecked. Whilst CBSD was for many years confined to coastal East Africa, the current pandemic has spread rapidly through Uganda (2004), western Kenya (2006), north-western Tanzania (2006), Burundi (2011), Rwanda (2012) and eastern Democratic Republic of Congo (DRC) (2012). In view of the speed of spread of the disease and the threat posed to major cassava production zones further west in DRC, Cameroon and Nigeria, a Global Alliance was formed in 2013, with the aim of setting out a road-map to tackle this emerging crisis. Experience derived from CMD pandemic mitigation initiatives has played a key function in building a multifaceted programme for CBSD management that has attracted strong support from affected governments and donors. The hope is that this single largest investment into plant virus research in Africa will provide answers to the critical research questions and deliver robust and sustainable management solutions for the millions of farmers affected.

The virus side of cassava brown streak disease

Stephan Winter and Marianne Koerbler

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Since the reconstruction of the first complete *Cassava brown streak virus* (CBSV) ssRNA genome, most research effort has been focused on virus surveys to reveal occurrence and spread of the brown streak viruses, the diversity of isolates and the responses of cassava cultivars to infection with particular isolates. However, the infection process is less well understood. To further our understanding about the virus component of the brown streak disease, we have generated several cDNA clones of complete genomes of *Ugandan cassava brown streak virus* and CBSV and inoculated the clones to infect cassava and the model host *Nicotiana benthamiana*. In both hosts, infections from cloned cDNA resembled wildtype virus infections and resequencing confirmed master sequences of all viruses. Using infectious virus cDNA clones, defined virus infections were introduced in cassava to follow virus movement and replication in particular genotypes and the fate of the virus in tissues and organs. We generated viral chimaeras between virus species and strains and established that, in addition to the gene silencing suppression function of the P1 gene it is also a determinant of virulence. This resides in the N-terminal amino acids of P1 and results in the production of necrosis in *N. benthamiana*. We now use the infectious CBSV cDNA clones for gene function analysis of further viral genes, in particular the gene coding for the Ham1-like protein upstream of the coat protein and VPg, to elucidate biological functions, vector transmission and pathogenesis. The current state of our research will be demonstrated.

Transcriptional response of virus-infected cassava and identifying virus resistance genes and markers for cassava brown streak disease

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Cassava brown streak disease (CBSD) has been a major obstacle for achieving food security in eastern Africa. Growing virus-resistant cassava is the realistic way of controlling the disease. We have initiated research to identify resistant cassava by inoculating 20 cassava lines with *Cassava brown streak virus* (CBSV) and their response to virus infection as well as virus concentrations was determined. The amount of virus in each variety was used for grouping varieties into resistant, tolerant and susceptible categories, and four varieties were found to be resistant. A time-course experiment was conducted on eight varieties inoculated with CBSV, and their transcripts were analysed by Illumina RNA-sequencing to further understand the genetic basis of resistance. RNA-Seq identified over 700 differentially expressed genes unique to the resistant var. Kaleso. Mapping quantitative trait loci to the cassava genome has identified several of them matching to the chromosome V, the importance of this should be further explored. A novel allele-specific expression analysis was also carried out on RNA-Seq data to identify single nucleotide polymorphisms (SNPs) linking to resistance. Our analysis is ongoing, but has identified several thousand SNPs (e.g. 7949 for var. Kiroba). About 1/3 of these were specific to virus-infected plants, which generally expressed 1.7 times more SNPs than mock-inoculated plants. These will be further analysed, and validated for identifying resistant genes and markers in future work. The markers that will be developed will be highly useful in future breeding programmes for the rapid development of farmer-preferred virus-resistant cassava varieties.

The Plant Health skills pipeline – developing future plant health professionals

Charles Lane¹, Celia Knight² and Nicola Spence³

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The BSPP and the Tree Health and Plant Biosecurity expert task force **have identified that 'there is an urgent need to train the next generation of plant pathologists' and 'address key skills shortages'**. More recently the Government Office for Science assessment of future science capability animal and plant health in the UK identified the need to develop a plant health skills pipeline and create a recognised profession for plant health.

Nicola Spence will report on progress towards inspiring the next generation of plant health scientists, inspiring graduate entry into plant health science and identifying and developing formal professional qualifications and continuous professional development for plant health science.

Identification and characterization of a novel fungal PAMP recognised in dicots

Barbara Franco¹, Adokiye Berepiki¹, Kostya Kanyuka², Paul R. J. Birch³ and Anna Avrova¹

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Rhynchosporium commune is the causal agent of scald, one of the most destructive diseases of barley, leading to significant yield losses. Grain quality can also be affected by the disease, resulting in substantial losses for barley producers. *R. commune* populations are highly diverse and can change rapidly, overcoming barley resistance and some fungicides. Plants have evolved defence mechanisms to neutralize pathogen attack. Microbial or pathogen elicitors, also called pathogen-associated molecular patterns (PAMPs), are detected by pattern-recognition receptors (PRRs) and elicit PAMP-triggered immunity (PTI). Sequencing of RNA from epidermal strips of barley leaves three days post inoculation with *R. commune* revealed abundant transcript coding for a novel small secreted fungal protein, Rc1. It is most highly up-regulated early during barley infection with *R. commune*. Rc1 and its homologues from different fungal species, produced using *Pichia pastoris*, exhibit PAMP activity triggering cell death in solanaceous and brassica species, but not in monocots. Using virus-induced gene silencing (VIGS) of known components of PTI in *Nicotiana benthamiana*, Rc1-triggered cell death was shown to be SGT1- and BAK1-dependent. In contrast, CMPG1 was shown not to be involved in response to Rc1 recognition. Transient expression of *truncated* versions of Rc1 *protein* in *N. benthamiana* indicated that cell death is triggered upon recognition of the N-terminal region of the protein. Identification of the plant receptor involved in Rc1 recognition in dicots will provide a valuable resource for engineering non-host resistance in monocots.

Phoma stem canker on oilseed rape cultivars with good resistance against *Leptosphaeria maculans*

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Phoma stem canker, caused by the related pathogens *Leptosphaeria maculans* and *L. biglobosa*, is an economically important disease of oilseed rape (*Brassica napus*) worldwide (Fitt *et al.*, 2006). There is a **'gene-for-gene' interaction between *L. maculans* and *B. napus*** at the leaf spot stage of the disease. It has been suggested that one of the resistance genes (*Rlm7*) is more durable than other commercially available *R* genes (Clarke *et al.*, 2011). *Leptosphaeria* populations on *Rlm7* cultivars in the UK were examined to investigate emergence of isolates virulent against *Rlm7* and to determine the importance of the co-existing pathogen *L. biglobosa* in development of phoma stem canker on cultivars with the *Rlm7* gene. Leaves with phoma leaf spots and stem samples were obtained from cultivars with the *Rlm7* gene and a cultivar with no known *R* genes (Drakkar) in autumn/winter in the UK (2011/12, 2012/13 2013/14 cropping seasons). Phoma leaf spotting and phoma stem canker severity were assessed. Single pycnidial isolates were obtained from leaf lesions and pathogen identification was done by observations on PDA and species specific PCR. Changes in frequencies of the avirulent *AvrLm2*, *AvrLm3*, *AvrLm4* and *AvrLm7* alleles in *L. maculans* populations were investigated at different sites in the UK by inoculation on cotyledons of cultivars with the corresponding *Rlm* genes.

References

Clarke M, *et al.*, 2011. Proceedings of the 13th International Rapeseed Congress, Prague, June 2011, p. 293.

Fitt BDL, *et al.*, 2006. European Journal of Plant Pathology 114: 3–15.

Construction of full-length infectious clone of *Ugandan cassava brown streak virus* (UCBSV) using yeast homologous recombination technique

Sarah Nanyiti¹, Titus Alicai², Andy M. Bailey¹, Sue Seal³ and Gary D. Foster¹

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Cassava brown streak disease (CBSD) caused by *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV), is one of the most damaging diseases in cassava, often causing over 50% yield loss and threatening the livelihoods of farmers in the sub-Saharan region. The virus belongs to the family *Potyviridae* and genus *Ipomovirus*. Many methods have been used to control the spread and damage caused by CBSD, but the only reliable approach is to use resistant varieties. Understanding the population genetic structure of CBSV would be a step to breed for resistance.

Infectious clones of full-length cDNA are important tools for investigating the molecular biology of plant RNA viruses. Construction involves production of a dsDNA copy of the virus cloned inside a suitable vector. Placing the viral sequence under the control of a bacteriophage promoter allows infectious transcripts to be generated *in vitro*.

In this work, an infectious full-length clone of UCBSV was constructed by homologous yeast recombination technique. The complete genome was cloned inside a yeast-adapted plasmid pYES2.1. Restriction analysis was done on the clone to confirm its integrity. An SP6 promoter was introduced and the plasmid used as template in *in vitro* transcription to synthesize run-off transcripts that were inoculated onto indicator plants (*Nicotiana benthamiana* and *N. clevelandii*). The infected plants showed mosaic-like symptoms characteristic of the virus, which confirmed that the infectious clone was infectious. RNA was extracted from the symptomatic plants and used in RT-PCR. PCR and sequencing results confirmed the infection.

Maintaining oilseed rape yield in a changing climate: modelling for a warmer future

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Oilseed rape has become a major crop in arable rotations with UK production now exceeding 700,000 hectares. Rapeseed oil is an established ingredient in food due, in part, to its low saturate fat content. World production is threatened by phoma stem canker, caused by the fungus *Leptosphaeria maculans*, which grows within the stem base and taproot of the plant, restricting uptake of water and nutrients. Disease progress has been predicted using readily obtainable weather variables such as rainfall and air temperature. Yet for pathogens growing within plant tissue, the environmental temperature is that of the infected tissues. These tissue temperatures are more closely linked to soil temperature than air temperature but are modified by the plant and affected by plant growth stage. Experimentation has shown that the response of disease severity to air temperature in controlled environments cannot be predicted from temperature response curves for *in vitro* growth on artificial media. Modelling based on field data may, therefore, continue to be the most useful technique for predicting future impacts of climate warming on oilseed rape yield loss due to phoma stem canker. However, soil temperature may be more appropriate than air temperature as a predictive variable and parameters may require adjustment for plant growth stage. The ability to make predictions of future yield based on reliable models is essential to enable breeders, farmers and governments to develop long-term strategies to maximise food security as our climate continues to warm.

Piriformospora indica, a potential biocontrol agent?

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Piriformospora indica, a root endophyte with a wide host range belonging to the Sebaciales (Basidiomycota), was found in India in 1997. *P. indica* association promotes plant growth, increases root and above ground biomass and final yield of a broad range of host plants. It improves plant mineral nutrient acquisition from the soil and helps plants to grow under temperature, water and physical stresses and also protects plants against pathogens of roots, stems and leaves.

Our previous work has shown that *P. indica* protects wheat from *Fusarium* head blight (FHB) and seedling rot and reduces mycotoxin DON in the grains. However, wheat is subject to many other infections. The effect of *P. indica* on foliar diseases arising from natural infections will be reported.

It is possible that some wheat cultivars benefit more than others from association with *P. indica*. The effect of *P. indica* on FHB on 6 different cultivars of spring wheat will be discussed.

Some root associated fungi including *P. indica* improves nutrient uptake.

This may or may not be the main way *P. indica* improves growth of

wheat. The results of an experiment to examine this will be reported. If *P. indica* was as beneficial to weeds as to wheat, it could make weed control more difficult, or increase the damage done by weeds. This is being checked by planting black-grass, wild-oat, or cleavers each with wheat inoculated $\pm P. indica$; the results will be discussed.

P. indica survival results under UK winter conditions by PCR/RT-PCR will be presented.

Molecular mechanisms of rhythmic plant defence against *Botrytis cinerea*

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The circadian clock is an internal time-keeping mechanism that gives a wide variety of organisms the ability to anticipate daily environmental change. We are investigating how the circadian clock influences defence against the necrotrophic fungal plant pathogen, *Botrytis cinerea*. *B. cinerea* causes huge yield losses of up to 20% per annum in a wide variety of crops, and the cost of control measures against *B. cinerea* are estimated at \$780 million per year. We have demonstrated that under light:dark growth conditions *Arabidopsis* exhibits rhythmic resistance to *B. cinerea*, with maximal resistance observed when leaves are inoculated at dawn. Crucially, this variation in resistance is maintained under constant light conditions indicating it is driven by the circadian clock. Furthermore, arrhythmic clock mutants do not show differences in lesion size following inoculation at dawn or early night.

To understand how the circadian clock was driving an effective defence response, we identified genes that were more rapidly induced or repressed after inoculation at dawn compared to night. This analysis indicated a complex interaction of the clock with the defence regulatory network and that hormone signalling, in particular jasmonic acid and ethylene responses, influences the observed rhythmic variation in resistance. This was confirmed by the identification of a jasmonic acid signalling mutant that displayed no differences in resistance to *B. cinerea* after inoculation at dawn or early night under day:night or constant light conditions. We are currently investigating this mechanism in greater detail and how it is influenced by the clock.

Effect of virus infection on aphid behaviour and VOCs emitted by common bean (*Phaseolus vulgaris* L.)

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The potyviruses *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV), and to a lesser extent *Cucumber mosaic virus* (CMV), (a cucumovirus) are constraints on common bean (*Phaseolus vulgaris* L.) production in Eastern Africa. They are nonpersistently transmitted by aphids. Viruses alter the interactions of plants with insect vectors. Using a popular East African bean variety (cv. Wairimu), we describe changes in aphid–plant interactions caused by BCMV, BCMNV and CMV. Two aphid species, *Aphis fabae* (a bean specialist) and *Myzus persicae* (a generalist), were used. Bean plants were infected with BCMV, BCMNV or CMV and at 10 days post-inoculation exposed to either *A. fabae* or *M. persicae*. Aphid settling was monitored at 1 h and 24 h post-release. For both aphids, virus-infected plants were less attractive than mock-inoculated plants and aphids preferentially settled on uninfected beans. Differences at 24 h post-release were statistically significant ($p < 0.05$). To investigate if the repulsion was due to qualitative or quantitative changes in volatile blends we conducted gas chromatography-mass spectrometry of headspace volatiles from infected and mock-inoculated plants. Virus infection decreased the overall quantity of plant-emitted volatiles. Principal component analysis showed that the volatile blends from uninfected plants were qualitatively different from that of virus infected plants. Direct observation of foraging of aphids showed significant differences in time taken to make first probes on virus-infected plants (ANOVA, $p < 0.05$). Taken together, these three results suggest that virus infection inhibits aphid settling and coupled with ease of probing promotes onward virus transmission.

Essential virulence related proteins differentially expressed during *Pectobacterium carotovorum* interacting with coloured calla

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Pectobacterium carotovorum subsp. *carotovorum* (Pcc) is an important phytopathogen of *Zantedeschia elliotiana* (coloured calla) and other ornamental and crop plants. To better understand the genes responsible for pathogenesis in coloured calla, we investigated the protein expression profiles of strain PccS1 during growth in both plant extract in medium and following recovery from petioles of the host plant. Two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) results showed that 53 proteins were altered in expression across both conditions compared to PccS1 grown in LB medium. We constructed deletion mutants in genes *clpP*, *potG*, *mreB*, *flgK*, *prmA*, *kdgR* and *aceE* by homologous recombination. The pathogenicity assays showed that mutations in *clpP*, *potG*, *mreB*, *flgK* attenuated the virulence of Pcc in both leaves and petioles of coloured calla, while *prmA*, *kdgR* and *aceE* had no significant effect on pathogenicity. This is the first study to show that *potG*, a spermidine /putrescine ABC transporter ATPase, is involved in virulence in this group of bacteria. Our results indicate that the growth conditions used to study pathogenesis are essential for accurate results.

Phytophthora infestans: history, human impact and field pathology

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Phytophthora infestans has interacted with *Solanum* spp. for millennia, but its history with humans is only about 150 years old. During the early part of its interaction with humans it was a fungus, but it has now evolved in our thinking into an oomycete – now considered to be the most important oomycete in molecular plant pathology. Its center of origin has also changed from South America to central Mexico to South America and back again to central Mexico. Its impact on humans is multifaceted, but this talk focuses on the impacts of this organism as the cause of a devastating plant disease. The most famous devastating epidemics led to the Irish potato famine, but there have been many subsequent devastating epidemics of late blight and some of these will be chronicled in this presentation. In the field, populations of this organism can grow rapidly – **seeming to explode ‘in a matter of hours’**. These populations can be very simple and strictly clonal or highly diverse and sexual. Migrations of this pathogen have figured prominently in population structure and there continue to be occurrences of unexpectedly severe late blight due to migrations of the pathogen. While *P. infestans* is the most important oomycete in molecular plant **pathology, it remains much more ‘at home’ as a pathogen living on plants (outdoors?) than as a saprophyte growing in an agar medium in the lab.**

Keeping up with THE Plant Destroyer

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Phytophthora, which stems from Greek words meaning plant-destroyer, is a diverse group of plant pathogens with over 100 species known to science. It includes the infamous Irish potato famine pathogen *Phytophthora infestans*. When this pathogen reached Ireland in the 1840s, it triggered famine and mayhem with one million people dead and another million forced to leave the island. Today, the late blight disease caused by *P. infestans* threatens not only tomatoes and potatoes in your gardens but also commercial and subsistence farming worldwide. But why all the misery? Why are oomycetes the scourge of farmers worldwide? The truth is, although *Phytophthora* are astonishing plant killers that can wipe out crops in days, the secret of their success is their ability to rapidly adapt to resistant plant varieties. Just like the constantly morphing flu virus, the potato blight pathogen and its relatives continuously spawn new races adapted to the resistant varieties released by plant breeders and even occasionally to new host plants.

Like Lewis Carroll's fictional Red Queen, plant breeders and biotechnologists only hope is to strenuously run to keep in the same place. If we could only produce resistant varieties more often then perhaps we'll have a chance to outrace the ever-evolving blight pathogen.

Humbly Ren-seeking after the truth; immune receptor sequence
capture ('RenSeq') accelerates resistance gene cloning and reveals
novel R protein domain architectures

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In plants, *Resistance (R)* genes provide resistance against pathogens and pests such as bacteria, viruses, fungi, oomycetes and insects. *R* genes typically encode nucleotide-binding, leucine-rich repeat (NB-LRR) proteins, also known as NLRs. ***R* gene enrichment sequencing, 'RenSeq'**, involves sequence capture to enrich the putative NB-LRR-encoding complement of plant DNA prior to sequencing (Jupe *et al.*, 2013). We are using RenSeq to identify functional *R* genes in diverse *Solanum* species against *Phytophthora infestans*, and have identified two new genes from a wild diploid *Solanum* species. These will be stacked with the already-defined *Rpi-vnt1* gene to provide a three gene stack that we hope will confer more durable resistance than any single *R* gene. We also use RenSeq to define new *R* genes against various pathogens of the **Brassicaceae, in particular the 'white rust'-causing *Albugo* species**, and to investigate natural diversity in *Arabidopsis thaliana* *R* gene repertoires. NB-LRR gene clusters are complex and difficult to resolve using Illumina reads. We are combining long-read PacBio sequencing with RenSeq to define the diversity of NB-LRRs in *Arabidopsis* and beyond, and are using this information to better understand the evolutionary history of these fascinating genes.

Jupe *et al.* (2013) *Plant Journal* 76, 530–544.

IMPACT: Rice and rice blast disease

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Today, global food supply and demand are roughly in balance. However, hunger and malnutrition prevail because of the inequality between crop production and demographic demand. Our population is predicted to rise to 9.2 billion by 2050. With 86% of these people in underdeveloped countries, it is paramount that we boost crop production.

Wheat, rice and maize dominate ~40% of available global cropland. Less rice is produced than maize, but rice is the more important staple because it feeds half of the world's population. Rice production has almost doubled recently - in 2014, 745 million tonnes of rice were produced in 114 countries, with 90% of the harvest grown and consumed in Asia. Currently, each hectare of agricultural land in Asia produces food for 27 mouths but by 2050 this area will need to feed 43 people. We need to more than double global rice production by 2050, and protecting rice from disease is a key player in the crusade to develop Green Super Rice.

Rice is vulnerable to disease wherever it is grown. Protection strategies safeguard ~38% of attainable rice production from pests. In their absence, annual losses caused by the rice blast fungus, *Magnaporthe oryzae*, vary between 10 and 30% of the harvest. But even 10% is significant, being sufficient to feed 60 million people for one year. Rice blast disease has been found in more than 85 countries. Today, blast-preventive, low-cost measures include the burning of crop residues, such as diseased straw and stubble, planting of disease-free seed, avoiding excess nitrogen-based fertilizer, water-seeding and growth under conditions of continuous flooding. However, these low-impact control measures are rarely efficient under blast-favourable conditions. I shall review how biotechnology could influence future rice blast disease management and hence boost yields via the development of molecular assays to evaluate fungal diversity, coupled with rice breeding, fungicide discovery and rice genetic modification.

Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

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The filamentous fungus *Magnaporthe oryzae* is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, cellular differentiation of *M. oryzae* results in the formation of a specialised infection structure, the appressorium. This infection cell generates enormous cellular turgor that is sufficient to rupture the plant cuticle. We have observed that a single round of mitosis occurs prior to appressorium morphogenesis and precedes autophagic cell death of the three-celled conidium, which is necessary for plant infection. Development and re-polarisation of the appressorium requires a hetero-oligomeric septin GTPase complex for re-organisation of a toroidal F-actin network at the base of the appressorium. This allows the host cuticle to be breached and leads to invasion of epidermal cells by biotrophic invasive hyphae of *M. oryzae*. Septin-mediated plant infection is controlled by NADPH oxidase activity. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. The appressorium pore is also the site of polarised exocytosis during plant infection and the octameric exocyst complex localises to the pore in a septin-dependent manner and is essential for cytoskeletal regulation. Both cell cycle and pressure-mediated checkpoints appear necessary for initiation of septin ring activation and reorientation of the cortical actin cytoskeleton to facilitate plant tissue invasion. *M. oryzae* shares many characteristics associated with other important cereal pathogens, such as appressorium formation and intracellular tissue invasion. This opens up the possibility of finding generic processes and disease determinants that can be targeted for broad-spectrum crop disease intervention.

Knowledge and diversity for sustainable control of rice blast disease

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Blast disease is considered the most dangerous disease of rice. It causes frequent and devastating epidemics in all rice growing areas of the world and is an important limitation to rice production and a threat to global food security. The causal agent is the ascomycete fungus *Magnaporthe oryzae* that has a hemibiotrophic infection strategy relying on growth on living tissue in early infection stages followed by active killing of host tissue at later stages. Efficient control of rice blast requires resistant rice varieties that exploit genetic disease resistance. However, sources of blast resistances in rice germplasm are limited and genetic resistance is constantly challenged by the extreme plasticity of the *M. oryzae* genome that frequently enables the pathogen to overcome host resistance and genetic control strategies. Due to these limitations, genetic resistance must be considered a precious, limited and threatened resource that has to be used in a responsible, knowledge-based manner to maximize its benefit for human societies in a durable fashion.

I will give an overview on current knowledge on the mechanisms governing quantitative and qualitative rice blast resistance that have to be combined to achieve durable rice protection. Particular emphasis will be put on recent data from our group on immune receptors of the class of nucleotide-binding and leucine-rich domain proteins (NLRs) that are central for natural blast resistance and on the interaction between rice resistance and environmental and agronomic parameters such as drought and nitrogen fertilization. In addition, I will present first results of our approach to study rice production in the Yuanyang terraces in the Yunnan province of China. Blast disease is present in this traditional agrosystem but seems controlled in a sustainable manner since it has only very limited impact on productivity which is stable and high.

Optimizing control of an established plant disease epidemic using a landscape-scale mathematical model: sudden oak death in California

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We use mathematical modelling to analyse control of sudden oak death in California. Sudden oak death, caused by the oomycete *Phytophthora ramorum*, has killed millions of oak and tanoak in California since its first detection in 1995. Despite some localised small-scale management, there has been no large-scale control in California to date. However, much information from both basic and applied research has accumulated over the past two decades, and we now have a good understanding of *P. ramorum* epidemiology. Mathematical models have been developed which integrate this knowledge, providing us with the **opportunity to use modelling to test ‘what if’ scenarios concerning management**.

We therefore use the Californian epidemic as a case study to illustrate how modelling can address general issues for invading epidemics. How quickly must control start for it to be effective? When is an epidemic so large that effective control is impossible? How should local treatment be deployed around infected sites? How does this depend on the available budget and level of risk aversion? Which sites should be targeted for control when there is insufficient resource to treat all infected locations? How can expenditure on detection and control be balanced? What is the effect of a budget that changes over time? The epidemiological principles underlying these questions are important for control of all invasive plant pathogens.

Investigating the molecular pathogenicity of a tree killer: *Armillaria mellea*

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Armillaria root disease, caused by many *Armillaria* or ‘honey mushroom’ species, is a devastating disease affecting hundreds of woody species in gardens, forests and agricultural systems worldwide. Disease losses can be substantial: up to \$4 million of losses in peach-producing states in the US, 40% yield reductions in infected Californian vineyards and \$5000 lost per avocado tree in Mexico. In the UK, the majority of plant disease enquiries received by the Royal Horticultural Society over the past 19 years have been concerning *Armillaria*. Several factors impede research into *Armillaria* root disease, including the lack of a reliable *in vitro* fruiting system for heterothallic *A. mellea* that hinders genetic work requiring basidiospores, and laborious inoculation assays that are usually conducted over several years. In order to overcome these difficulties, we have established a reliable and reproducible *in vitro* fruiting method for heterothallic *A. mellea* to obtain basidiospores for use in *Agrobacterium tumefaciens*-mediated transformation. A series of vectors have been constructed using homologous recombination in yeast to establish a genetic toolkit for fluorescent protein expression and RNAi-mediated gene silencing in *A. mellea*. An improved inoculation assay using herbaceous plants has been developed that permits observation of disease symptoms and mortality within three months and has been used with GFP and mRFP-expressing transformants to visualise root infection and facilitate earlier detection of disease. The development of this reliable *in vitro* fruiting method and these genetic tools for *A. mellea*, coupled with the improved inoculation assay, should expedite research into this important disease.

Suppression of *Microdochium nivale* by phosphite in amenity turfgrasses

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Worldwide, amenity turfgrasses provide surfaces for numerous sports and recreational facilities and disease control is vital in their successful maintenance. *Microdochium nivale*, an ascomycete fungus, is a ubiquitous and damaging pathogen of these turfgrasses. Fungicides are the main means of control, making alternative methods desirable. Phosphite, an anion of phosphorus, has proven efficacy in reducing susceptibility to phytopathogens; the aims of this research therefore, were to determine if phosphite suppresses *M. nivale* incidence and to identify the processes involved. Field trials determined significant suppression of *M. nivale* incidence and enhanced fungicide efficacy in phosphite treated turfgrasses. Analyses of treated tissues determined rapid *in planta* accumulations, symplastic mobility and no conversion to phosphate. *In vitro* inhibition of mycelial growth and disruption of hyphal morphology were determined, with phosphite concentrations of 100 $\mu\text{g ml}^{-1}$ fully inhibiting growth. It was also determined phosphite was fungistatic, not fungicidal, and that phosphite in the growth media significantly inhibited conidial germination. Assessment of *M. nivale* turfgrass infection incidences determined hyphae as source of inoculum and that infection was by means of stomatal penetration, conidia via sporodochia are the means of propagation and dispersal. Analyses of infected turfgrasses confirmed that increased generation of phenolic compounds and hydrogen peroxide are components of defence responses and that phosphite pre-treatment enhanced these responses. This work has shown that phosphite suppresses *M. nivale* incidence and increases fungicide efficacy. These results will lead to changes in turfgrass management procedures, benefits being reduced requirements for fungicides, cost savings and a reduced environmental impact.

Emerging pathogens in UK horticulture: a case study on *Puccinia heucherae*

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Horticulture contributes £9 billion to the UK economy every year, employing approximately 300 000 people across a range of disciplines. There are over 20 million gardens in the UK, accounting for 4% of UK surface land area. Gardens play an important role in supporting UK biodiversity and provide numerous socio-economic benefits. New and emerging pathogens in horticulture therefore have the potential for widespread negative impacts on the economy, the environment, and our health and wellbeing.

The Royal Horticultural Society (RHS) provides an advice and diagnostic service to its UK members. In the last year alone, over 60 000 enquiries were received by the RHS with around 5% requiring expertise from pathologists. This puts the RHS in a unique position to detect pathogens new to the UK, to monitor the spread of pathogens and to detect host shifts.

Heuchera rust (*Puccinia heucherae*) was first detected in UK gardens in 2004 on a leaf sent to RHS Gardening Advice. It was subsequently detected in nurseries in 2005, and by 2010 was common across the UK in both gardens and nurseries. *P. heucherae* is common in the USA and East Asia but little is known about the epidemiology of the disease, host susceptibility and chemical control. We will be presenting an ongoing research project investigating the outbreak of rust on *Heuchera* plants in the UK. This work includes development of a molecular test, sampling of *Heuchera* plants throughout the growing season and investigations into the epidemiology of the rust.

The diversity and distribution of *Sclerotinia subarctica* in the UK and Norway

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Sclerotinia sclerotiorum is a soilborne pathogen with a global distribution and a wide host range including many economically important crops, such as oilseed rape, lettuce, carrots and beans. It can cause pre- and post-harvest epidemics, causing substantial reductions in yield. Sclerotia of the pathogen survive in soil, germinating to produce apothecia that release airborne ascospores causing plant infection. Recently, the related species *Sclerotinia subarctica* was identified in England on wild buttercup, having previously only been found in Norway and Alaska. The symptoms caused by *S. subarctica* are very similar to *S. sclerotiorum* and hence the pathogen may be undetected in crops in the UK. Little is known about the epidemiology, pathogenicity and control of this species.

Crop plants and buttercups with sclerotinia disease in England, Scotland, Norway and Sweden were sampled in 2012 and 2013 to establish whether *S. subarctica* was present. The 420 isolates obtained were identified as either *S. sclerotiorum* or *S. subarctica* by amplification of the large subunit ribosomal DNA. *S. subarctica* was found on carrot, swede, potato, lettuce and pea crops as well as buttercup in Scotland, and in several crop species in Norway and Sweden, suggesting it is more common in northern latitudes. Additional work to establish whether colder conditions are more favourable for this species indicated that *S. subarctica* may have a lower tolerance for mycelial growth at high temperatures than *S. sclerotiorum*, and that *S. subarctica* requires longer durations of cold conditioning for apothecial production than *S. sclerotiorum*.

Soapbox Science – taking science and gender issues to the streets

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Sixty percent of biology undergraduates and postgraduates are female, yet less than 18% of biology professors being female. We urgently need to address the gender imbalance in science training and science careers. Challenging social norms on the perception of who a scientist is, what their work–life balance is like, and the culture in which they work is fundamental to achieving parity, and stemming the leaky gender pipe.

Soapbox Science seeks to open dialogue on the issue of women in science. It is a novel public outreach platform for promoting women scientists and the science they do, as a regular annual science communication event across the UK. We place female scientists on soapboxes on urban streets around the UK. Unlike most other science events, a **'Soapbox' audience will not have necessarily planned to come** and learn about science. *Soapbox Science* has been incredibly successful on the Southbank in London over the last four years, achieving high footfall and high-profile national and international press coverage. Thanks to these successes, *Soapbox Science* is becoming a leading advocate for improving the visibility and representation of women in STEM in the UK. 2015 saw our expansion to seven cities, across the four nations of the UK, reaching over 30,000 members of the public.

Seirian Sumner, co-founder of *Soapbox Science*, gives a potted history of the ethos behind this initiative, the mission, and how *Soapbox* has **helped promoted the careers of some of the UK's top female scientists**, including plant biologists.

Fusarium oxysporum: world travels of a systemic killer

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Current cases of world impact caused by this soilborne, vascular pathogen are epitomised by strains (forma specialis, f. sp.) attacking two tropical monocots. *F. sp. cubense* is currently threatening world banana production. Cavendish (47% of crop) is resistant, but a new strain, TR4, is now in SE Asia and spread to E Africa and Jordan in 2014. Central American production is especially vulnerable. Developing countries rely on banana for about one third of calories, plus the resulting economic impacts even affects their banking systems. *F. sp. elaeidis* has devastated oil palm production in Africa (centre of diversity), resulting in SE Asia as the main (86%) producer. Palm oil represents almost 40% of world edible oils, valued \$46 billion p.a., but production supports many smallholders in SE Asia. Where the pathogen is non-endemic, exclusion is crucial as palms are susceptible, so specific detection is required. The vascular location of *F. oxysporum* facilitated finding virulence proteins, 'SIX' (secreted-in-xylem), in the tomato pathotype f. sp. *lycopersici*. Its genome revealed more SIX effectors in this and in other pathotypes. We identified in f. sp. *elaedis* a unique effector, to create a specific, PCR-based detection kit (patented). This test is ideal for quarantine of contaminated African seed, exported to expand genetic diversity in SE Asia and S America. The pathogen can be seed-transmitted and we showed has been accidentally exported to S America. We also developed a simple method to eradicate *F. oxysporum* from *inside* seed by infiltrating a fungicide under reduced pressure, as now used by companies.

A systems approach to breeding disease resistance in lettuce against necrotrophic fungal pathogens

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Botrytis cinerea and *Sclerotinia sclerotiorum* are fungal pathogens of global importance, each causing multi-million pound crop losses pre- and post-harvest on most dicotyledonous crops annually. These pathogens cause substantial losses on field-grown and protected lettuce crops, an industry worth almost £200M/yr in the UK. Chemical control is problematic with restrictions on spraying and fungicides being medium-high risk for development of resistance. Development of host resistance is a more sustainable solution, but has been an intransigent problem for breeders.

We are taking a novel approach to breeding for disease resistance against *B. cinerea* and *S. sclerotiorum* in lettuce combining systems biology and quantitative genetics. Genetic variation for susceptibility to these pathogens was identified in a diversity set of lettuce accessions, based on the size of lesions after leaf inoculations. Furthermore, the susceptibility to both pathogens in different accessions was correlated increasing the potential of identifying alleles conferring broad resistance. Identification of disease resistance QTL is underway.

Using RNAseq, we have generated a time series of gene expression in lettuce leaves over 12 time points, between 9 and 42 hours after inoculation with *S. sclerotiorum* or mock inoculation. We are using this time series to investigate transcriptional reprogramming in lettuce after infection, to infer regulatory networks mediating this response and to determine key regulators within the networks. The RNAseq data is also being used to interrogate transcriptional changes occurring in *S. sclerotiorum* during infection, with the potential for joint network inference to identify points of interaction between pathogen and host.

Bacterial blotch: more than one way to eat a mushroom

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Bacterial blotch is an important disease of cultivated mushrooms that can be endemic on mushroom farms, damaging mushroom quality and causing yield loss. Bacteria in the *Pseudomonas fluorescens* complex are the main cause of blotch symptoms, which manifest as superficial yellow or brown lesions on mushroom tissue. Blotches commonly appear when mushrooms are in the early button stage, but can also occur post-harvest, even on refrigerated mushrooms. Bacterial blotch is most **commonly attributed to ‘*Pseudomonas tolaasii*’** or *Pseudomonas fluorescens* biotype G. However, blotch symptoms can be caused by a wide variety of bacteria within the *P. fluorescens* species complex, a group of bacteria that also includes the causal agents of other mushroom diseases such as drippy gill (*Pseudomonas agarici*) and ginger blotch (*Pseudomonas gingeri*), as well as bacteria that are valued for their antifungal properties and used as biocontrol agents in an agricultural context. The pathogenicity factors responsible for the development of blotch symptoms on mushrooms include pore-forming lipodepsipeptide toxins such as tolaasin, which are produced by strains from both major lineages within the *P. fluorescens* complex. However, the production of these toxins is not a universal feature of blotch-causing pseudomonads. We have identified and characterised a novel group of mushroom pathogenic bacteria, typified by the strain *P. fluorescens* NZ011, which do not produce tolaasin and which use a previously undescribed antifungal mechanism for mushroom pathogenesis. The diversity of bacterial blotch pathogens and pathogenicity mechanisms has significant implications for the detection and control of bacterial blotch disease.

Decisions to be made: How will we protect our crops from pests and diseases in the coming years and how is Europe approaching this compared to the rest of the world?

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Chemical controls are the mainstay of modern agriculture and have allowed us to increase significantly our crop production in Europe over the last decades. However, increasing concerns over the safety of these chemicals, herbicides, pesticides and fungicides, are leading European Union officials to reduce the use of some products while stopping the use of others altogether. If this is to continue, alternative methods of crop protection are essential to prevent reductions in crop products, especially food, and avoid issues of food security, increased imports and potential price rises. There are a number of alternatives methods for crop protection, including organic production, genetically modified crops and biocontrol, all culminating in more Integrated Pest Management (IPM) solutions. **Are any of these methods, alone or in combination, truly a replacement for chemical control, what is Europe's view on this, and how is the rest of the world responding to the challenges of crop protection?**

Posters

(*J Colhoun competition)

*Phylogeny of *Fusarium oxysporum* f. sp. *elaeidis*

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Fusarium wilt caused by the *Fusarium oxysporum* f. sp. *elaeidis* (Foe) is the main constraint to increasing production in the major oil palm-producing countries, including Ghana. The current study investigated the level of variation of Foe in three main locations in Ghana juxtaposing these isolates with Foe from Suriname, DR Congo and Ivory Coast, using a phylogenetic approach to analyse genes including effector protein-genes. The translation elongation factor 1 alpha gene (*TEF-1 α*) and the **effector protein gene called 'Secreted in xylem' (*Six*)** resolved Foe isolates into distinct clades indicating a polyphyletic nature of the pathogen. In addition, AFLP analysis showed a wide variation of Foe isolates representing different countries and serves as a potentially effective molecular tool to investigate diversity in Foe at a genome-wide level.

*Genetic analysis of *Lecanicillium fungicola* as a fungal pathogen of mushrooms

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Lecanicillium fungicola is a pathogenic fungus notable for its virulence against the cultivated mushroom *Agaricus bisporus*, causing dry bubble disease, a serious threat to mushroom production. We aim to understand more about the way that *L. fungicola* causes disease using a combined genomics and gene disruption approach.

We have generated a draft genome sequence of *L. fungicola* using MySeq reads, and assembly gave a genome of 44 Mb, split over 781 contigs with an N₅₀ of 154124.

We have developed an *Agrobacterium tumefaciens*-mediated transformation system for *L. fungicola* using *bar* as a selectable marker giving resistance to Basta. The resistance cassette from pCB1635 with *bar* driven by the *trpC* promoter from *Aspergillus nidulans* was successful in conferring tolerance to the herbicide Basta at 450 µg/ml. A *tumefaciens* strain LBA1126 was most successful giving 200 transformants per plate when using conidial concentrations of 10⁶ conidial/ml and a 2-day co-cultivation.

Several candidate genes have been selected for investigation by targeted disruption based on genes known to be involved in regulating pathogenesis in plant-, fungi- and insect-pathogenic fungi as well as from an *L. fungicola* EST database. The knockout constructs targeting *cag8*, *vta2*, *magB*, *cpc1*, *jen1* and *bos1* have been made in a yeast-adapted binary vector pCAMBIA 0380 YA. Transformation of *L. fungicola* is in progress using ATMT and knockouts will then be tested for altered pathogenicity against *A. bisporus* to determine whether infection-related processes of *L. fungicola* are controlled in the same way as in various plant pathogenic fungi.

Aphids and associated virus species on common beans in Africa

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Aphids are small sap-sucking insects in the family Aphididae. They are polyphagous in nature, attaching several crops including common bean (*Phaseolus vulgaris*), an important subsistence crop for smallholder farmers in Africa that provides a major source of protein and micronutrients such as iron and zinc. On *P. vulgaris* aphids are vectors of viruses that cause severe losses in Africa. In addition to *Bean common mosaic virus* and *Bean common mosaic necrosis virus* (BCMNV) transmitted by the black bean aphid (*Aphis fabae*) and other aphid species, ELISA analysis detected presence of cucumber mosaic virus on *P. vulgaris* in Central Uganda. Analysis of partial sequence of coat protein of BCMNV isolates showed that NL3 strain is the most common in Uganda. Partial cytochrome oxidase subunit I gene sequence of aphid samples on *P. vulgaris* from Uganda, Malawi and Mozambique indicated that the *A. fabae* is the major species found on *P. vulgaris* farms. The *A. fabae* samples from Uganda were phylogenetically homogenous and showed 99% nucleotide identity to those from Malawi and Mozambique. However, one sample from Malawi had by 97% nucleotide identity to *Aphis coreopsidis*. *A. coreopsidis* has hitherto only been reported in North and South America including the island of Hawaii. This is the first report of this aphid on *P. vulgaris* in Africa and outside the Americas. *A. coreopsidis* is known to transmit viruses such as *Sonchus yellow net virus* and *Bidens mosaic virus* but it is unknown if it transmits any virus on *P. vulgaris* in Africa.

*Exploiting next generation sequencing to investigate the genetics of parsnip root disease and develop a marker-assisted breeding strategy

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Parsnip (*Pastinaca sativa*) is a speciality crop within the UK, covering an area of around 4100 ha and a value of £64M annually. Currently the major constraint to production is the losses associated with root canker diseases caused by fungal pathogens such as *Itersonilia* and *Mycocentrospora* spp.

This project aims to address this problem by facilitating breeding for quantitative resistance to these major diseases. Some resistance to parsnip canker exists but is difficult to select for using traditional phenotype screening methods. The main objectives are to further understand the epidemiology of the pathogens involved in parsnip cankers; to develop specific plant bioassays to identify resistant parsnip breeding lines; and to develop markers for mapping quantitative trait loci (QTL) conferring resistance.

Resistance screening assays have been developed using both parsnip seedlings and roots. In a root assay using 96 parsnip breeding lines and 10 parent lines, a large range of resistance responses to *Itersonilia* and *Mycocentrospora* was observed amongst different lines. A further SNP-genotyped parsnip mapping population will be screened to try and determine the QTLs conferring resistance.

The project will have a direct impact upon the industry by enabling improved sustainability of parsnip production in the UK.

Know your onions: resistance to *F. oxysporum* f. sp. *cepae* and its pathogenicity

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Bulb onions are grown throughout the world having a global production of 83 Mt per annum, and are for many people part of their everyday diet, being a staple ingredient for a range of cuisines. Consumption is also associated with a range of human health benefits. Despite this, there has been less research on onion and its pathogens compared to some other crops. One of the major constraints to production is basal rot, caused by *Fusarium oxysporum* f. sp. *cepae* (FOC). This soilborne fungus is part of the *F. oxysporum* species complex, which includes other important *formae speciales* adapted to particular crop plants, as well as non-pathogenic isolates. As FOC produces long-lived chlamydospores, disease management is challenging and hence identifying and developing resistance to FOC is very desirable. Moreover, distinguishing FOC from other members of the species complex would also enable detection of infested fields or onion bulbs with latent infection that may develop in store. Here we report new sources of FOC resistance following screening of an onion diversity set in seedling and bulb assays. Through genotyping and phenotyping onion populations segregating for resistance, we aim to identify molecular markers to enable rapid breeding of FOC resistant cultivars. In addition, whole genome sequencing and PCR approaches using a range of pathogenic and non-pathogenic *F. oxysporum* isolates, has identified a correlation between **pathogenicity and the presence of selected 'secreted in xylem' (SIX)** genes (first reported in *F. oxysporum* f. sp. *lycopersici*) and other novel putative effector genes which may enable specific diagnostics in the future.

Advances in chemical genetic tools and impact on the research and development of novel crop protection products

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There is strong demand within agriculture and horticulture for ever-improving control of crop pests and diseases. In order to support the discovery and development of new active ingredients a wide range of chemical genetic tools are utilised and are under constant development. Historically many of these tools used the model organism *Saccharomyces cerevisiae*, but improvements in genomic information and tools make it possible to perform research directly in field-relevant plant pathogens. Some of the wide range of tools used in support of new fungicide discovery will be outlined, including:

- Forward genetic tools, which have long been the mainstay of mode of action diagnosis, provide an unbiased approach for the identification of the molecular target of a chemical inhibitor. If resistant mutants can be generated and uninucleate cells/spores can be isolated, the latest sequencing technologies can now be applied to any pathogen with a reference genome.
- Libraries of engineered model organism strains. Chemistry with lower resistance risk is often associated with the inability to isolate resistant mutants in the plant pathogen of interest. In this case unbiased genetic tools are still of value and model organisms are employed. Haploinsufficiency or overexpression-induced resistance within a yeast library can provide information on proteins or protein families targeted by novel chemistry. Hypotheses can then be based on the phenotypes seen, and compared to information from biochemical pathway inhibition and microscopic observations.

Pythium as a cause of foliar blight of mature woody plants

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Foliar blights have been observed on evergreen mature shrubs in the UK during 2006 – 2009 via the Royal Horticultural Society (RHS) Advisory Service. *Rhododendron*, *Ilex* and *Osmanthus* plants had dark spotting, often causing a V-shape lesion, similar to those typical for phytophthora blights. Following identification of organisms associated with the lesions through sequencing of the ITS region, over 50% could be ascribed to three species of *Pythium*, namely *Pythium intermedium*, *Pythium attrantheridium* and a third probably undescribed species closely related to the other two. Detached *Rhododendron*, *Ilex* and *Osmanthus* leaves were used in infection assays with all three species, the results indicating varying susceptibility of the host plants and differing aggressiveness of the *Pythium* species. The *Pythium* species were reisolated from the lesions and identification confirmed by morphology or sequencing of the ITS region. We conclude that some *Pythium* species can cause foliar blights that might previously have been ascribed to *Phytophthora*.

*Towards identifying PAMPs from the downy mildew pathogen
Hyaloperonospora arabidopsidis

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Downy mildews are oomycete microbes and obligate parasites, belonging to the Order Peronosporales. They display a variety of hosts, infecting crop plants belonging to different families, such as lettuce, grapevine or tomato, but also ornamental plants. They develop in humid and moist conditions and represent a threat for growers and producers. The system composed by the plant *Arabidopsis thaliana* and its natural oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) has been used as a model to investigate plant–oomycete pathogen interactions. We aimed to discover conserved molecules deriving from the pathogen, called PAMPs (pathogen-associated molecular patterns), which are recognized by the plant immune system and that are able to trigger an immune reaction in the plant. To look for PAMPs originating from *Hpa*, an extract from the spores of the pathogen has been obtained. The extract has been tested using an assay able to highlight one of the first signs of PAMP-triggered immunity (PTI): ROS production. Furthermore, it has been treated with proteinase K to better understand the nature of the active molecules. In addition, various mutants of putative receptors have been tested using the same assay. We demonstrated that the extract contains molecules able to trigger an immune reaction in *Arabidopsis* Col-0 plants and that the proteinase K-treated spore extract lost its immune triggering ability, indicating a proteinaceous nature of the active molecules. Testing the extract on mutant plants showed that the perception of the active molecules might be *Bak1*-dependent. Recent findings will be presented.

Protection of Chinese agriculture by preventing invasion by the damaging oilseed rape pathogen *Leptosphaeria maculans*

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Leptosphaeria maculans causes phoma stem canker disease that results in substantial worldwide losses in brassica crops, including oilseed rape (>£1110 million per year). It has spread globally since the 1970s into areas where the less damaging *L. biglobosa* was present. This pathogen has not yet reached China, which grows >10 million ha of susceptible brassicas (Fitt *et al.*, 2008). Descriptions of observed spread of *L. maculans* into areas previously colonised by *L. biglobosa* across a spring oilseed rape growing region (Alberta, Canada, westwards, 1984–1998) and across a winter oilseed rape growing region (Poland, eastwards, 1984–2004) were used to estimate potential westward spread of *L. maculans* in China across spring oilseed rape (north China) and winter oilseed rape (central China), respectively. Rates of spread were estimated as 47 km/year across spring oilseed rape in north China and 70 km/year across winter oilseed rape in central China (Zhang *et al.*, 2014). In response to our work, the Chinese government had issued a quarantine measure restricting import of oilseed rape seed to Chinese ports in regions without the crop, unless seed was certified free from *L. maculans*. The Chinese decision to protect its crops from this invasive species affected trade with Canada and Australia, worth >\$1 billion/year, and thus led to intergovernmental discussions. Our recommendations to prevent entry of the pathogen that have been implemented by China include testing imported seed, surveying crops and training farmers to recognise disease symptoms, besides initiating work to breed resistance against *L. maculans* into Chinese cultivars.

Evidence for direct and indirect mechanisms in biological control of spot blotch of barley by *Trichoderma harzianum* T-22

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Spot blotch caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is one of the most serious diseases of barley (*Hordeum vulgare*) worldwide. We have previously found that treatment of barley plants with the commercially available biological control agent *Trichoderma harzianum* T-22 can reduce the severity of spot blotch disease. With foliar application, *T. harzianum* T-22 was more effective when applied at the same time as the pathogen than when applied one week before or four days after. When droplets of a suspension of *T. harzianum* T-22 spores and *B. sorokiniana* spores were applied to barley leaves, pathogen spore germination and hyphal growth were inhibited. Germination of *B. sorokiniana* spores and hyphal growth were similarly inhibited when *B. sorokiniana* spores were suspended in the supernatant obtained by centrifuging a *T. harzianum* T-22 spore suspension. When supernatant was applied to barley leaves and the leaves were inoculated with pathogen spores 24 h later, inhibition still occurred. Boiling the supernatant did not significantly reduce its effectiveness, suggesting that the active substance is a heat-stable chemical or combination of chemicals. Therefore, direct chemical inhibition of infection may contribute to biological control of spot blotch by *T. harzianum* T-22. Indirect mechanisms also contribute to control, since soil treatment and seed coating with *T. harzianum* T-22 also reduced spot blotch severity.

*Understanding the ecology and epidemiology of *Pythium violae* to enable disease management in carrot crops

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Cavity spot is a major disease of carrot primarily caused by the soilborne oomycete pathogen *Pythium violae*. Disease management is challenging as fungicide efficacy is variable and long rotations are not always possible. There are a lack of effective *P. violae* research tools such as procedures for detection/quantification, and artificial inoculation techniques. Moreover, there are fundamental knowledge gaps concerning the epidemiology of *P. violae* and how it interacts with its environment and hosts. This PhD project aims to address some of these issues and understand the potential role of soil microbial communities in cavity spot disease development/suppression.

To determine the range of *Pythium* spp. associated with cavity spot, a collection of approximately 80 isolates was assembled from diseased carrots. Following DNA extraction and sequencing, 64% were identified as *P. violae* with 17% and 9% being *P. sulcatum* and *P. intermedium*, respectively. The remainder comprised other *Pythium* species. Although a PCR test for *P. violae* in soil is available, only small samples can be used. A sucrose flotation and filtration method was developed to capture oospores from 10 g soil. When combined with the PCR test, initial results suggest detection of less than 10 oospores is possible. This methodology will now be developed further for RT-PCR to enable accurate quantification of *P. violae* and monitoring dynamics in soil and on roots. A method of inoculum production for *P. violae* has also been developed and experiments are now investigating the effect of oospore numbers on carrot seedling mortality and symptoms on more mature plants.

Effects of quantitative resistance on *R* gene-mediated resistance against *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape)

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Phoma stem canker, caused by *Leptosphaeria maculans*, is a damaging disease on oilseed rape in the UK. Use of durable host resistance to control this disease is becoming more important. Resistance against *L. maculans* may be major resistance (*R*) gene-mediated resistance or quantitative resistance (QR). *R* gene-mediated resistance is race-specific and often rendered ineffective due to pathogen population changes from avirulent to virulent. QR is race non-specific and is considered more durable but it is a partial resistance. To investigate effects of QR on resistance against *L. maculans* mediated by different *R* genes, winter oilseed rape cultivars with different *R* genes in backgrounds with or without QR were used in field experiments at 11 different sites in the 2010/11, 2011/12 and 2012/13 growing seasons. Results showed that there were effects of background quantitative resistance on the effectiveness of an *R* gene. The severity of stem canker on DK Cabernet (*Rlm1* + QR) was less than on Capitol (*Rlm1*), suggesting that *Rlm1* is more effective when it is introduced into a host background with QR than in one without QR. Similarly, less severe stem canker on Adriana (*Rlm4* + QR) than on Bilbao (*Rlm4*) suggested that *Rlm4* is more effective when it is introduced into a host background with QR than in one without QR. Cultivars Roxet and Excel both carried an effective resistance gene *Rlm7*; there were no significant differences between them in severity of stem canker. Results suggested that quantitative resistance increased effectiveness of *R* gene-mediated resistance against *L. maculans* in oilseed rape.

*Examining identity, phylogeny, and pathogenicity factors in *Fusarium* species affecting pea

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Pea (*Pisum sativum* L.) is an important legume grown all over the world, with approximately 17.4 Mt of green pea produced in 2013. In the UK, the frozen pea market is worth approximately £50 million per year, produced from fields clustered around production factories mainly on the East coast. They are also grown as break crops as they leave residual nitrogen in the soil.

Soilborne plant pathogens in the ‘footrot’ complex are one of the main causes of crop loss in peas, resulting in wilting and a reduction in the number of pods produced. Several *Fusarium* species have been reported in peas with *F. oxysporum* f. sp. *pisi* (FOP) being one of the most commonly reported. Pathogenicity factors such as effector genes have been implicated in other pathogenic *F. oxysporum* with Secreted in Xylem (*SIX*) genes widely studied and reported. Pathogenicity (*PEP*) genes have also been identified in isolates affecting pea.

The main aims of this PhD project are to identify the range of *Fusarium* species affecting pea in the UK and characterise effector genes relating to pathogenicity. Within 86 *Fusarium* isolates obtained so far, seven different species of *Fusarium* have been identified, with 33% being *F. oxysporum*, 35% *F. redolens*, and others including *F. culmorum* (13%) and *F. avenaceum* (8%). Four different pathogenicity tests using different methods of inoculation (seed, soil, root dip) have also been developed to determine the relative pathogenicity of these isolates.

*Investigation of genetic resistance operating in oilseed rape against the light leaf spot pathogen, *Pyrenopeziza brassicae*

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Light leaf spot disease, caused by *Pyrenopeziza brassicae*, is currently the most damaging foliar disease on winter oilseed rape in the UK. The severity of epidemics has increased progressively across the UK since 2006 (<http://www.cropmonitor.co.uk/>). Breeding for host resistance can be used as an effective control strategy against this economically damaging pathogen. However, there is little information available about the genes involved in resistance against *P. brassicae*. A major gene has been mapped to the bottom end of chromosome A1 in *Brassica napus* cultivar Imola and the main objective of this study is to fine map this resistance gene.

A flanking marker close to the resistance gene on chromosome A1 was PCR-amplified from the parental lines of a doubled haploid (DH) population segregating for resistance and the PCR product was sequenced. The physical location of this marker on chromosome A1 in *B. napus* was identified by using marker sequence alignment to the *B. rapa* genome and the synteny between *B. rapa* and *B. napus* genomes. All the genes present between the flanking marker and the telomere of chromosome A1 of *B. napus* will be analysed. This information will be used to develop a KASPar marker array for the genomic region between the flanking marker and the telomere of chromosome A1 and will be tested with DNA of the segregating DH population to fine-map disease resistance. Fine-mapping of resistance locus and identification of candidate resistance genes in this region of *B. napus* genome will be used to sequence the resistance locus.

*The root of the problem: fungal pathogens and communities associated with the roots of commercial oilseed rape (*Brassica napus*) crops in England

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Oilseed rape (OSR) is a popular break crop in UK arable production systems, being the third most widely grown crop after wheat and barley. Despite recent advances in genetics and agronomy, on farm yields have failed to rise. One suggestion for this is decreasing rotation length with OSR often grown at the same site with short breaks of 2–3 years.

Previous work has demonstrated how decreasing rotation length can lead to the build-up of soilborne pathogens such as *Olpidium brassicae* and *Pyrenochaeta* sp. This study has used high-throughput sequencing (HTS) methods to examine the fungal communities associated with commercial field grown crops of OSR in England.

Fifty individual OSR field sites were selected from counties across England. Root material from the growing crop was collected and used for DNA extraction. Fungal DNA was amplified using universal fungal PCR primer pairs designed to amplify the ITS1 region of rDNA. The resulting amplicons were sequenced using an Illumina MiSeq platform (2 × 300 bp reads) and compared to published DNA databases for speciation and relative-quantification.

In total over 19 million sequences were attained, with the majority (81.1%) being assignable to either genera or species level. Analysis using ANOSIM showed no significant differences in fungal communities between different sites, cropping frequency or other agronomic factors. The samples were largely dominated by fungi belonging to Ascomycota and Basidiomycota, and in particular *Mortirella* spp. and *Rhizoctonia solani*.

Molecular diversity of *Fusarium verticillioides* isolated from maize in three agroecological zones of southwest Nigeria using AFLP markers

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Ear rots caused by *Fusarium verticillioides* are major economic concern to maize farmers and processing industries due to losses in grain yield and quality. This could be due to variations in disease severity of *F. verticillioides*. This necessitates the study to investigate the molecular diversity of *F. verticillioides* strains in agroecological zones of southwest Nigeria. Three amplified fragment length polymorphism (AFLP) primers were combined. 164 polymorphic bands were detected using one base extension of *EcoR1* and *Mse1* primers in the selective amplification. Primer combination EC + MC gave the highest number of polymorphic bands of 58, while primer combination ET + MC had the highest percentage polymorphism (100%). The dendrogram result grouped *F. verticillioides* isolates into 4 major clusters with 77% similarity and other subgroups. High genetic similarity (67%) was observed between isolates originating from IBD (34,36), IGH (39), EKT (46,47), SAK (52) and IGB (57,58,59 and 60). Isolates 19 and 20 from ILH had the highest pairwise similarity coefficient at 97% and were grouped in cluster II. Most of the *F. verticillioides* isolates contained in group 2 were from Eruwa. AFLP molecular marker provides information to ascertain genetic variations in *F. verticillioides* isolates, and can be fundamental in implementing an effective control strategy.

Population structure of the ash dieback pathogen *Hymenoscyphus fraxineus* in the UK

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Common European ash trees, *Fraxinus excelsior*, have been subject to heavy dieback and mortality in their native range caused by the pathogenic ascomycete fungus *Hymenoscyphus fraxineus*. *H. fraxineus* was first observed in the UK in 2012 in the east of England, although it is likely to have been present for many years before this.

In this study to determine the population structure of *H. fraxineus* in the **UK, three sites were identified as ‘established’ where it was estimated that the pathogen population has been established over an extended period by natural spread from Europe. Three sites were identified as ‘planted’, which were locally isolated from other ash woodlands and were away from the predicted zone for wind dispersal and had been planted with ash saplings from nursery stocks in the previous 10–20 years. It was hypothesised that the genetic differentiation of the isolates collected from ‘planted’ sites would show a less diverse population structure than that of the ‘established’ sites based on the assumption that fewer individual isolates would have started the local epidemic.**

Fungal isolates were collected from lesions on stem samples collected in 2014 and one isolate per individual lesion was used for analysis.

Vegetative compatibility among the isolates was tested to determine the **population structure. A total of 28 SNP markers based on KASP™** genotyping chemistry were developed using the sequence information of 43 *H. fraxineus* isolates from across Europe including the UK to study genetic differentiation among local populations and between planted and established sites.

*Exploring the susceptibility of cereal species to *Phialophora* fungi

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Take-all, caused by the soilborne ascomycete fungus *Gaeumannomyces graminis* var. *tritici*, (*Ggt*), is a root disease that devastates wheat production worldwide. Current control measures consist of partially effective chemical seed dressings and cultural methods such as crop rotation and later sowing of crops at risk. *Phialophora* fungal species, belonging to the genus *Gaeumannomyces*, colonise the roots and stems of cereals and grasses and form the *Gaeumannomyces–Phialophora* complex. Unlike *Ggt*, *Phialophora* fungi do not destroy the vascular tissue of the roots and have previously been found to suppress take-all disease.

This project is exploring the susceptibility of cereal species and modern elite wheat cultivars to *Phialophora* fungi. The aim is to explore the potential of *Phialophora* as a novel way of controlling take-all. *Phialophora* species were isolated from soil collected from the Rothamsted farm to generate a new culture collection for future tests. The DNA for three different *Phialophora* species have now been sent off for full genome sequencing using a next-generation approach to allow species-specific PCR primers to be designed. The objective is to develop a robust diagnostic PCR assay in order to identify agricultural fields naturally infected with single/multiple *Phialophora* species populations. A working seedling pot bioassay method has been devised to screen the colonisation ability of *Phialophora* on different cereal species. Colonisation ability of natural populations of *Phialophora* on AHDB Recommended List winter wheat varieties is also being explored under field conditions.

*Investigation of pathogenicity determinants in *Cassava brown streak virus* (CBSV) with an approach to viral protein functional analysis

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Cassava brown streak virus (CBSV) is a positive-sense ssRNA virus belonging to the family *Potyviridae*, genus *Ipomovirus*. It is one of the main causes of yield reduction and economic losses on cassava crops in Africa. CBSV is mainly transmitted by the whitefly *Bemisia tabaci* and it is distributed along the east coast of Africa.

CBSV encodes for a polyprotein of 2912–2916 aa that later is cleaved into 10 mature proteins. Their functions have not been described; however, in other members of the family, functions can just be speculated. CBSV possess a peculiar genome among the *Potyviridae* family, lacking for a multifunctional protein, the Helper component proteinase (HC-pro), and expressing a Ham1-like protein, a novel protein in the *Ipomovirus* genus, appearing in *Euphorbia ringspot virus* (EuRSV) as well.

The present research is designed to investigate specific functions of CBSV -encoded proteins during infection. Systems that express each individual encoded proteins of CBSV were developed in *N. tabacum*. Each of these proteins was tested for synergistic interaction with TMV, identifying enhancement of the infection, in viral titre and symptom development. Additionally, each encoded protein in CBSV was tested for silencing suppressor activity, performing agroinfiltration assays in *N. benthamiana* line 16c. The information produced during synergisms of CBSV proteins and other viruses including CBSV will help to understand which elements in CBSV are pathogenicity determinants during infection and how the absence of HC-pro and the presence of new features in the genome make CBSV successful.

*Can we use large datasets to investigate fungicide efficacy?

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Long-term datasets of spring barley field trials have been collected by SRUC. The data collected includes disease burden for a number of key pathogens – including mildew, *Rhynchosporium* and *Ramularia* – crop yields, pesticides applied and variety planted since 1986, as well as site-specific climate data since 2005. These trials are run with both fungicide-treated and untreated plots, allowing a direct comparison of disease and yield levels to be made.

This data has the potential to add greatly to our understanding of crop–disease relationships, climate implications for disease epidemics, pesticide impact, and the efficacy of alternative management techniques. Nonetheless, a number of drawbacks to working with data initially collected for other purposes pose difficulties in analysing this information. This work will therefore be used as a case study to highlight the complications and opportunities of analysing information collected from a range of different trial designs, and with variable data recording style/quality. Lack of true replication over time, in particular, will be focused on for key parameters such as variety choice, and possible solutions will be presented.

While ‘big data’ can provide useful insights into complex systems and interactions, the difficulties in preparing and analysing such datasets must be recognised, particularly in project planning.

Understanding effector-triggered defence responses against the phoma stem canker pathogen *Leptosphaeria maculans*

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Effector-triggered defence responses of oilseed rape against the phoma stem canker pathogen *Leptosphaeria maculans* are not well understood. Many aspects remain to be clarified, including variation in host defence responses against *L. maculans* with different effectors or effector combinations. Furthermore, expression of receptor-like proteins (RLPs) and their encoding genes has to be determined to better understand effector-triggered defence responses against this apoplastic fungal pathogen. Towards this end, susceptible and resistant oilseed rape cultivars containing different resistance (R) genes were challenged with *L. maculans* isolates that differed in effector gene composition. The strength of the host defence response was a function of the *L. maculans* effector combinations. Effectors may differ in their ability to suppress host defence responses. Expression of *LepR3* was studied at mRNA and protein levels. *LepR3* mRNA expression was low. Bands of 80 and 140 kDa were recognised by an antibody against the LepR3 protein. The expression of this RLP appeared to decrease in response to infection by an *L. maculans* isolate with the corresponding effector *AvrLm1*. The LepR3 antibody offers a new tool to better understand the fate of RLPs during host defence responses against *L. maculans*. Subcellular localization of the LepR3 protein during the defence responses will be needed to further characterise the process of effector-triggered defence.

*Circadian rhythm regulates growth of a biotrophic pathogen on *Arabidopsis thaliana*

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Most organisms have an internal biological clock, called the circadian rhythm. The clock is synchronized by the day–night cycle, allowing the organism to accommodate the daily cycles of light and dark attributable **to the Earth’s rotation. Circadian clocks have three basic properties**, they: have a period length of about 24 hours, can be reset by environmental factors such as light and temperature, and have at least one internal autonomous circadian oscillator. These oscillators contain positive and negative elements that form autoregulatory feedback loops, and in many cases these loops are used to generate 24-hour timing circuits.

Identification of clock-regulated genes leads to the determination of the common elements that regulate these genes. If these common elements can be identified, it enables the search for the same elements in potential virulence factors. This would give us information on whether the virulence factors are also regulated by the circadian rhythm. Since the link between plant immune system and the circadian clock has been identified, it is imperative that we identify the link between the pathogenicity factors and the circadian rhythm.

Arabidopsis thaliana and its natural biotrophic pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) have been used as a model system for this study. Pathogen growth has been investigated under normal (light–dark cycle) and different light conditions (light–light and dark–dark cycles) to understand whether the circadian rhythm has an effect on pathogenicity. In addition, RNA-seq experiments were performed to elucidate differentially expressed genes in both plant and the pathogen.

*Investigating the sporulation pattern of *Zymoseptoria tritici*, a pathogen of wheat

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Zymoseptoria tritici is an ascomycete fungus which causes Septoria Tritici Blotch, a major disease of wheat. One of the ways in which the pathogen spreads to new hosts is via asexual spores (pycnidiospores) made by the asexual fruiting bodies (pycnidia). The purpose of this research is to identify key genes involved in regulating this process by creating knock-out mutants impaired in asexual sporulation.

The recent sequencing of the *Z. tritici* genome has provided useful insights into the pathogen, and has been used to identify candidate genes for investigation. Key genes likely to be involved in asexual sporulation in *Z. tritici* were identified using literature searches, comparative genomic analyses and molecular genetic analyses. These results, in addition to recent RNAseq data, have been used to select target genes for knock-out experiments.

Knock-out plasmids containing a hygromycin resistance cassette have been constructed using homologous recombination in yeast, and inserted into *Z. tritici* via *Agrobacterium tumefaciens*-mediated transformation. The *Z. tritici* genes *ZTabaA*, *ZTbriA*, *ZTflbB* and *ZTsch1* have already been disrupted, and the others are in progress. The phenotypes of these mutants are currently being characterised and assessed for their ability to sporulate *in planta* and *in vitro*.

Reliable sporulation of *Z. tritici* has been established *in vitro* on wheat leaf extract agar. This will help future research into the fungus by allowing the uncoupling of sporulation from virulence. The most recent finding from this ongoing research will be presented.

*The use of a fungicide prediction system to control strawberry powdery mildew

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Podospheara aphanis is the ascomycete fungus that is responsible for powdery mildew on strawberry plants, a disease of economic significance in protected crops. The disease can reduce yield by half and also requires many fungicide sprays to control it. A prediction system was used that monitored both humidity and temperature to determine the optimal time for growers to spray strawberry crops with fungicides; this prediction system was shown to be significantly more effective than a commercial spray programme (in 2014) at reducing the levels of disease. The prediction system also allowed for precision spraying, and so fewer fungicides sprays were applied to control the disease as compared to the commercial programme. Trial results for 2015 will also be reported. Effective in season disease control results in fewer smaller colonies of strawberry powdery mildew, and this reduces the number of overwintering chasmothecia and thus reduces the initial inoculum for the following season in perennial plantings. Therefore if farmers were to adopt the prediction system it would result in higher yields and therefore higher profit margins.

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- Blue/White/Black
- Green
- Blue
- Grey

