BSPP & BSM

Fungal Control & Exploitation

University of Nottingham

11-13 September 2017

Contents

Page

2……..President's welcome

4……Programme

10……..Awards

12…….. Opening plenary talk abstracts

15…….. BMS talk abstracts – Exploitation 1 (Tuesday 12th)

19……..BMS talk abstracts – Control 1 (Tuesday 12th)

23……BMS talk abstracts – Exploitation 2 (Wednesday 13th)

26……BMS talk abstracts – Control 2 (Wednesday 13th)

28……BSPP talk abstracts (Tuesday 12th)

38……BSPP talk abstracts (Wednesday 13th)

44……Closing plenary talk abstracts

46……Poster abstracts

82……Date of next BSPP presidential meeting

British Mycological Society and British Society for Plant Pathology Joint Presidential Meeting 2017

Fungal Exploitation and Fungal Control

University of Nottingham

11th–13th September 2017

Presidents' Welcome

On behalf of the British Society for Plant Pathology and the British Mycological Society, it is our pleasure to welcome you to this joint annual meeting in Nottingham University.
Despite the fact that the two societies share a long history and have much in common, as far as the Organising Committee is aware, this is the first time a joint meeting has been held.

The themes of this conference are the control of plant pathogens (mainly fungi) and the exploitation of fungal species. Plant pathogens continue to cause substantial losses to farmers in all parts of the world. The main methods used to control diseases are genetic disease resistance in crop cultivars, fungicides, biological control agents and cultural practices. This conference will highlight significant advances in the first two methods. Cultivars with improved disease resistance, which combines effectiveness with longevity, are being released through partnerships between academic scientists and plant breeding entities. New fungicides are being developed and better methods to delay and reduce the impact of fungicide resistance are being deployed. A particularly pleasing aspect of this work is that it is now becoming clear that the longevity of fungicides is extended by good genetic disease resistance and vice versa; fungicides protect the longevity of genetic disease genes. A remaining frustration is that GM disease resistance has not been widely deployed even as we pass 20 years since the first cloning of R-genes.
Some fungi and oomycetes can also infect animals and create serious problems, often resulting in fatalities. New methods to detect such pathogens at an early stage would result in better treatment practises and better survival rates. In many animal–fungal interactions, basic biology questions such as "how does the pathogen infect?" and "what does the host to defend itself?" cannot be answered at present. For some animal diseases, control options are very limited and alternative methods are urgently needed.
Besides being pests, fungi can also be very beneficial to us. Obviously, we like to eat some of them, but we can also manipulate them and use them for biotechnological purposes to develop medicines, for example; or alternatively, use them for biomass degradation, insect or fungal biocontrol and enhancing productivity through better understanding of symbiosis in mycorrhizal interactions.

It promises to be an exciting annual meeting and we would like to thank the organising team from both societies: Paul Dyer, Simon Avery, Matthias Brock and Elizabeth Orton, for putting together such a stimulating programme. We also thank Alison Tor for the abstract booklet and Moira Hart who assisted with the organisation.

We wish you a fruitful and enjoyable time in Nottingham.
Richard Oliver, Pieter van West and Nick Read (former BMS president)

The BSPP and BMS are grateful for the support from Apollo Scientific, CABI, Elsevier and Wiley.

Monday 11th September

13.00 Lunch and registration
14.00 Welcome
14.15 Plenary - BSPP President Richard Oliver
Translating understanding of plant pathogen control genetics into grower profits

15.00 Plenary - BMS President Pieter van West
Invasive oomycetes are a serious threat to our natural and man-made aquatic ecosystems

15.45 *Coffee*

16.15 Plenary - Richard Michelmore
The impact of new technologies on strategies to increase the durability of disease resistance

17.00 Plenary - Mark Lynas
Anti-science and post-truth: why we got it so wrong about GMOs

17.45 **Poster session and drinks reception** (sponsored by Elsevier)
18.15 BSPP AGM Small lecture theatre
19.30 **Dinner**

Tuesday 12th September

	Concurrent BMS (Exploitation 1)	**Concurrent BSPP**
9.00	BMS Invited - Vera Meyer Moving from descriptive to predictive biology: Rational rewiring of the cell factory *Aspergillus niger*	BSPP Invited -Bruce McDonald Using population genomics to determine the genetic basis of pathogen adaptation in agro-ecosystems
9.30	BMS Invited - Scott Baker Fungi for biotechnology	BSPP Invited -Lise Nistrup Jorgensen Why does IPM not lead to more reductions in fungicides?
10.00	Muhammad Akbar: A novel inoculation method of mycorrhizae in wheat fields has remarkable effects on crop yield and soil properties	Vanessa McMillan: Identifying root resistance in take-all disease
10.20	Elena Geib: *Aspergillus niger* versus *Aspergillus oryzae*: Expression platforms for heterologous secondary metabolite production	Yongju Huang: Ignored until recently- the story of the fungal pathogen *Leptosphaeria biglobosa*
10.40	*Coffee*	*Coffee*
11.10	BMS Invited - Eckhard Thines Unravelling pyriculol biosynthesis and its regulation in the rice blast fungus *Magnaporthe oryzae*	**PH Gregory Student Competition Talks** 1)Corinne Arnold (JIC): Powdery Mildew: a tale of two fungicides 2) Shaoli Das Gupta(RHUL): *Blumeria* metallo-protease like effector; A zincophore acting as a universal virulence factor in fungi?
11.40	Kristiina Hildén: Biochemical characterization of mannan-acting	3) Kathryn Hales (Warwick): Understanding the ecology and

	enzymes from the white-rot fungus *Dichomitus squalens*	epidemiology of *Pythium violae* to enable disease management in carrot crops. 4) Sascha Jenkins (Warwick): Examining identity, phylogeny and pathogenicity factors in Fusarium species affecting pea.
12.00	Michaela Novodvorska: Pigment biosynthesis in *Penicillium roqueforti*, why be blue?	5) Chinthani Karandeni Dewage (Hertfordshire): Fine mapping of a major gene locus for resistance against *Pyrenopeziza brassicae* (light leaf spot) in *Brassica napus* and identification of candidate resistance genes 6) Joseph Moughan (Rothamsted): From the field to the lab and back again: exploring legacy effects of root genetics and fungicide for take-all root disease control in wheat rotations.
12.20	**Lunch**	
13.30	Elsevier author workshop	
	Concurrent BMS (Control 1)	**Concurrent BSPP**
14.30	BMS Invited - Axel Brakhage Melanin-dependent pathogenicity of *Aspergillus fumigatus*	BSPP Invited - Vivianne Vleeshouwers Exploiting effectors in breeding and deployment of *R* genes in potato
15.00	Janet Quinn: Fungal SAPK pathways as mediators of virulence	Franziska Trusch: The RxLR Motif of the Host Targeting effector AVR3a of *Phytophthora infestan*s is cleaved before secretion
15.20	Cindy Vallieres: Synergistic combinations targeting translation fidelity and diverse fungal pathogens.	Sarah Schmidt: Know your enemy-engineering resistance against Fusarium wilt in banana.

15.40	*Coffee*	*Coffee*
16.10	BMS Invited - Al Brown *Candida albicans* is a moving target for the immune system as it adapts to host signals	BSPP Invited -Karl-Heinz Kogel The Agronomic Potential of Gene Silencing Application
16.40	Rhys Farrer: Targeting infection mechanisms for two amphibian-infecting chytrid species.	Laurence Bindschedler: Combining proteomics of haustoria and gene silencing to discover key players of virulence during barley powdery mildew infection
17.00	**Posters session and drinks reception** (sponsored by Wiley)	
	BMS AGM Small lecture theatre	
19.30	**Conference dinner**	

Wednesday 13th September

	Concurrent BMS (Exploitation 2)	**Concurrent BSPP**
9.00	BMS Invited - Ronald de Vries Diversity in plant biomass degradation approaches of fungi	BSPP Invited - Sander Schouten Direct and indirect effects of fungal endophytes on plant-parasitic nematodes
9.30	Ioly Kotta-Loizou: Mycoviruses as prospective enhancers of biocontrol agents	Christian Voigt: Improving plant resistance to fungal pathogens by callose modification
9.50	Stefany Solano: Genomic and genetic analysis of a basidiomycete yeast focused on the characterisation of MEL biosynthesis	Andrew Taylor: Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in Onion

10.10	Sara Casado López: Intraspecies diversity and induction of the wood decay by the white rot fungus Dichomitus squalens	Ryan Ames: Understanding appressorium development in *Magnaporthe oryzae*
10.30	*Coffee*	*Coffee*
	Concurrent. BMS (Control 2)	**Concurrent BSPP**
11.00	BMS invited - Alan Gange Exploiting unspecialised endophytes for biological control of insects and plants	BSPP Invited - Frank Van den Bosch Integrated deployment of host resistance and fungicides: managing evolution of virulence and insensitivity
11.30	BMS Invited - Mike Bromley Regulation of Drug Resistance in *Aspergillus fumigatus*	BSPP Invited - Neil Paveley Integrated deployment of host resistance and fungicides: economics, efficacy and evolution
12.00	Takanori Furukawa: Systematic functional annotation of transcription factors in the major mould pathogen Aspergillus fumigatus	Andrew Leader: Fenpicoxamid (Inateq ™, active fungicide), a new fungicide with utility in cereals for control of *Zymoseptoria tritici* (Septoria leaf blotch) and other diseases
12.20	End of session	Avice Hall: Controlling Strawberry powdery mildews requires all year-round vigilance
12.40	**Lunch**	

Plenary Session and Close

13.30 Plenary - Rob Johnson
The Science of Quorn

14.00 Plenary - Natalia Requena
Fungal weapons to manipulate plants

14.45 Prizes and Close

Awards

The Boards of the BSPP and BMS wish to encourage young plant pathologists and mycologists to present their work. There are two BSPP prizes and one BMS prize 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, 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, leading to a definitive text (Muskett A. E. & Colhoun J. (1947): The Diseases of the Flax Plant). He became reader at Queen's 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).

The BMS Student Poster Prize: Awarded to the best mycology student poster at the meeting.

TALKS

*PH Gregory Competition entrant

Opening Plenary Talks

Translating understanding of plant pathogen control genetics into grower profits

Richard P. Oliver
Centre for Crop and Disease Management, Curtin University, Perth, WA, Australia
Email: Richard.oliver@curtin.edu.au

Plant fungal pathogens cause major avoidable losses to crop in all parts of the world. Reliable data on the degree of losses is rare. That Australia, with its low inputs and frequent droughts, is the country with the highest documented overall percentage losses in broad acre agriculture is certainly due to the paucity of data from other regions. The major diseases of grain crops in Australia that the CCDM works on are caused by a range of non-obligate fungi; the wheat diseases tan spot caused by *Pyrenophora tritici-repentis,* and septoria nodorum blotch (SNB) caused by *Parastagonospora nodorum,* the barley net blotch diseases caused by *Pyrenophora teres,* the legume leaf blight diseases caused by various *Ascochyta spp.* and the canola disease caused *Sclerotinia sclerotiorum*. Diseases caused by rusts have been reasonably well controlled by the deployment of major R-genes. Such major genes were not available for these blight diseases. The strategy deployed to control them had to await the development of some understanding about how the pathogens interact with the host. A collaborative effort has shown that at least for tan spot and SNB, the key was to determine that the diseases were controlled by the interaction of pathogen gene products named necrotrophic effectors (NEs). NEs interact with corresponding host sensitivity genes. The key to improving disease resistance was to transfer this knowledge to breeders so that they could eliminate germplasm carrying relevant NE sensitivity genes.
Fungicides remain the backstop of fungal disease control even in low intensity countries like Australia and especially since 2005 when prices dropped. Fungicide resistance was soon discovered in many pathogens in Australia. The pattern of fungicide resistance confirmed the developing understanding that fungicide resistance can be delayed by application of core evolutionary principles that guide us to use minimum doses, minimum numbers of applications and mixtures or alternations of modes of action. It is also becoming clear prudent use of fungicides extends the life of genetic disease resistance and vice versa.

Invasive oomycetes are a serious threat to our natural and man-made aquatic ecosystems

Pieter van West
Aberdeen Oomycete Laboratory, International Centre for Aquaculture Research and Development at the University of Aberdeen, College of Life Sciences and Medicine, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK.
Email: *p.vanwest@abdn.ac.uk*

Oomycetes are fungal-like organisms that are classified as Chromalveolates and phylogenetically grouped with diatoms and brown algae. They are among the most important groups of disease-causing organisms in both agriculture and aquaculture and thus represent a huge threat for global food security.
Some aquatic species can cause serious environmental disasters, wiping out our native aquatic animals, for example European crayfish and also several amphibians have been severely affected or have become extinct. One particular pathogen, *Aphanomyces invadans,* is now approaching European waters and represents a devastating pathogen. It is a tremendous problem in countries where it has already arrived and has established (*e.g*. Bangladesh, India and parts of Africa). There are also other aquatic oomycete pathogens known including *Haliphthoros, Halioticida, Lagenidium, Atkinsiella* spp. that infect marine and brackish animals including lobsters, langoustines, abalone and prawns. *Saprolegnia parasitica* is one of the most destructive fish pathogens and is found in most fresh water environments around the world. Remarkably, very little is actually known about the biology of these aquatic pathogens. However, what we do know is that they are: 1) potential invaders of marine & fresh water habitats, 2) they can cause serious economic or environmental damage and most importantly, 3) they are all uncontrollable at present. An overview of these various animal pathogenic oomycetes is presented.
Furthermore, our current knowledge about the cellular and molecular infection strategies of *S. parasitica* and *A. invadans* is being discussed. In order to successfully infect their hosts these parasites rely on their effector repertoires. Effectors are secreted proteins of the pathogen that can alter the host and which advance the infection process. A large number of effectors is located at the interface between pathogen and its host and fulfil a function on the outside of the host cell. Such effectors are often classified as extracellular effectors. While several other oomycete effectors are able to translocate into their host cells where they can, for example, interfere with defence responses of the host. These effectors are commonly referred to as intracellular effectors. The mechanism of translocation or delivery of the intracellular effectors is under intense investigation and several routes of entry have been proposed. Here we will discuss our recent advances in understanding secretion, delivery and functions of effectors from animal pathogenic oomycetes.

The impact of new technologies on strategies to increase the durability of disease resistance.

Richard W. Michelmore
The Genome Center & Department of Plant Sciences, University of California, Davis, Californa, 95616, USA.
Email: rwmichelmore@ucdavis.edu

Great advances have recently been made in the understanding of the molecular and genetic basis of disease resistance in plants. It is now time to deploy this knowledge to provide more durable disease resistance. Many advances have been enabled by technological improvements. In particular, high throughput DNA sequencing enables detailed analysis of crops and their pathogens. It is now possible to characterize variable pathogen populations and use this information for the rational deployment of resistance genes so as to maximize the evolutionary hurdle for the pathogen to become virulent.

Much of our work over the past thirty years has focused on resistance to downy mildew in lettuce. Lettuce (*Lactuca sativa*) is one of the most valuable vegetable crops and downy mildew, caused by *Bremia lactucae*, is its most important foliar disease worldwide. The use of resistant varieties is the most effective method for controlling this disease; however, pathogen variability has led to the rapid defeat of individual resistance genes. Over 50 resistance genes have been identified and lettuce downy mildew is one of the best genetically characterized plant diseases. Whole genome sequencing of multiple genotypes has allowed the identification of candidate resistance genes in the host and virulence factors in the pathogen. Knowledge-driven deployment of effective resistance genes as gene pyramids provides the opportunity for more durable resistance. Gene stacking using genome editing has the potential making this process more efficient. In addition, host-induced gene silencing of vital pathogen genes presents potentially insurmountable evolutionary hurdles for the pathogen to overcome.

Anti-science and post-truth: why we got it so wrong with GMOs

Mark Lynas
@mark_lynas
Email: marklynas36@gmail.com

With “post-truth” the Oxford English Dictionary word of the year for 2016, it seems as if a new zeitgeist is challenging the whole basis of evidence-based thinking and the scientific method. But how new is this really? Mark Lynas, a reformed former anti-GMO activist, traces some of the deeper roots of today’s “post-truth” attitudes in the environmental movement’s rejection of genetically modified crops and other technologies - like nuclear power - that were seen as being ‘unnatural’.

BMS Talks

Moving from descriptive to predictive biology: Rational rewiring of the cell factory *Aspergillus niger*

Vera Meyer
Department Applied and Molecular Microbiology, Technische Universität Berlin, Berlin, 13355, Germany
Email: vera.meyer@tu-berlin.de

Fungal biotechnology is currently undergoing a renaissance with important implications for its role as platform technology for the sustainable production of products, goods and drugs. Allied to this are the recent advances in fungal Systems and Synthetic Biology which can be seen as two complementary approaches to investigate the complexity of biological systems including fungi. Whereas Systems Biology analyzes cellular systems in an iterative cycle of high-throughput generation of 'omics' data and modeling, Synthetic Biology takes a constructive approach to reengineer biological networks and to design novel biological parts and circuits with non-natural function. Both disciplines – systems analysis and design – ideally complement each other and, not surprisingly, aim to discover the design principles of (fungal) Life.
Our goal is to understand and rationally rewire the industrial cell factory *Aspergillus niger*. On the one hand, we generate and evaluate different omics data types for *A. niger* and use these Big Data to predict gene function as well as gene regulatory networks. On the other hand, we engineer titratable and tightly regulated conditional mono- and polycistronic gene switches and target them to specific genomic loci. By combining these two activities, we not only successfully improve *A. niger* as producer for proteins, new natural products or even new-to-nature compounds, we are also entering a new era in *Aspergillus niger* biology: we are moving from descriptive to predictive biology.

Fungi for biotechnology

Scott E. Baker[1,2]
[1]Joint BioEnergy Institute, Emeryville, California; [2]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington, USA
Email: scott.baker@pnnl.gov

Fungi have been long been exploited for production of small molecules and enzymes for centuries. The underlying biology of fungal bioprocesses, however, is not well characterized. Using a variety of cell biological, genetic and genomic methods we have assessed the biological pathways underlying both enzyme and small molecule production. We have explored enzyme production in three phylogenetically diverse

filamentous ascomycetes finding shared genetic determinants of high enzyme production. In addition, we have used a variety of cell biological and genetic methods to better understand factors important for lipid production in the oleaginous yeast, *Yarrowia lipolytica*.

A novel inoculation method of mycorrhizae in wheat fields has remarkable effects on crop yield and soil properties

Muhammad Akbar[1], Safeer A. Chohan[1], Muhammad S. Iqbal[1], Nazir Aslam[1], Aqeel Ahmad[2] and Tayyaba Khalil[1]
[1]Department of Botany, University of Gujrat, Gujrat, 50700, Pakistan; [2]Institute of Agricultural Sciences, University of the Punjab, Lahore, 54000, Pakistan
email: makbarpu@gmail.com

In recent years there is growing trend towards organic farming to meet food security and food safety. Inorganic fertilizers are available to boost yield but there are environmental and public health concerns. Amongst various organic alternatives, using mycorrhizal species is one of the most promising options. Therefore, the present research was conducted to evaluate the influence of indigenous mycorrhizal species on the growth of wheat and soil properties under field conditions. In total 11 species of mycorrhizae were identified from the experimental areas, the most prominent genera being, *Claroideoglomus, Rhizophagus and Funneliformis*. For inoculation of mycorrhizae, their native density was maintained with a novel idea that these species work better in consortia when their native population density was maintained. There were eight different treatments having plot sizes as 6 meters x 2 meters. The whole experiment was repeated at two different sites. Bio-inoculation of consortia of different mycorrhizal species showed a significant increase in all growth parameters studied e.g., number of tiller per plant (up to 39.53 %), plant height (up to 13.66 %), dry biomass (up to 15.84%), grain yield (up to 18.69%) and hay weight (up to 15.69%). Moreover, remarkable effects were encountered on soil fertility such as soil organic matter, available phosphorus and potassium were increased up to 92.30%, 12.24% and 8.83%, respectively. The present study concludes that increasing the mycorrhizal inoculum in crop fields by keeping their density as native has tremendous effects on crop productivity and soil fertility status.

***Aspergillus niger versus Aspergillus oryzae*: Expression platforms for heterologous secondary metabolite production**

Elena Geib and Matthias Brock
Fungal Genetics and Biology, University of Nottingham, Nottingham, NG72RD, UK
Email: mbxeg3@nottingham.ac.uk

To exploit fungal secondary metabolite production a strong and specific expression system is required. We developed such a novel expression system in *Aspergillus niger*,

which bases on regulatory elements from the *Aspergillus terreus* terrein biosynthetic gene cluster. We confirmed its suitability by heterologous production of polyketides (e.g. lecanoric acid), non-ribosomal peptide synthetase-like products (e.g. aspulvinone E) and used it to characterise the Asp-melanin biosynthesis pathway from *A. terreus*.
The latter studies led to an interest in the understanding of the chemistry of NRPS-like enzymes that produce metabolites with antifungal, cytotoxic, antitumorigenic and antiviral activity. While different NRPS-like enzymes may accept the same substrate the resulting product depends on the biochemistry of the thioesterase domain. To study these domains, we compared the aspulvinone E synthetase MelA from *A. terreus* with the atromentin synthetase InvA5 from *Paxillus involutus*. While recombinant expression of *melA* in *A. niger* resulted in aspulvinone E production, expression of *invA5* led to a range of products that were all distinct from atromentin. We therefore speculated that the physiology of *A. niger* might lead to a modification of the InvA5-derived metabolite. Consequently, *invA5* expression was studied in the alternative host *Aspergillus oryzae* and, indeed, atromentin was produced.
In conclusion, our recombinant expression system is perfectly suited for heterologous production of a wide range of different secondary metabolites. However, as the metabolic physiology of fungi differs, at least two different expression platforms should be selected when aiming in the characterisation of novel secondary metabolite biosynthesis genes.

Unravelling pyriculol biosynthesis and its regulation in the rice blast fungus *Magnaporthe oryzae*

Eckhard Thines[1,2] and Stefan Jacob[2]
[1]Institute of Molecular Physiology, University of Mainz, D55128 Mainz, Germany; [2]Instítute of Biotechnology and Drug Research (IBWF), D-67663 Kaiserslautern, Germany
Email: thines@uni-mainz.de

Fungal secondary metabolites have in the past been very successful lead structures for pharmaceutical or agrochemical applications. Many of those compounds have been discovered in axenic cultures in artificial media, which are poor surrogates for mimicking an organism's natural habitat. As a consequence only a small number of genes potentially involved in the biosynthesis of secondary metabolites are expressed under these conditions.
Magnaporthe oryzae is the causal agent of blast, the most devastating fungal disease on cultivated rice. The fungus is known to produce secondary metabolites involved in plant/pathogen interaction, such as pyriculol/pyriculariol. Within the genome 20 genes encoding polyketide synthases have been identified, whereas qPCR experiments revealed that only four of them are transcribed in axenic culture. Apart from cultivation variation approaches molecular based techniques have become suitable methods in order to explore the products of silent secondary metabolite pathways.

In order to investigate regulatory elements of secondary metabolism and to identify new natural products many technologies can be applied. For those studies gene deletion/gene disruption strains were generated, promoters were changed and transcription factors or other pleiotropic regulators were over- expressed. Furthermore epigenetic strategies were successfully applied in order to elucidate new secondary metabolites. The application of demethylase inhibitors led to the identification of novel polyketides from *M. oryzae* that have not been isolated from extracts of axenic cultures before.
Apart from the identification of new secondary metabolites and their potential role in pathogenicity the studies will provide insights into the regulation of phytotoxin biosynthesis in plant/pathogen-interactions.

Biochemical characterization of mannan-acting enzymes from the white-rot fungus *Dichomitus squalens*

Angel Zhang[1], Jaana Kuuskeri[1], Johanna Rytioja[2], Ronald de Vries[3], Miia R. Mäkelä[1] and Kristiina Hildén[1]
[1]Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland; [2]Department of Chemical Technology and Biotechnology, Aalto University, Espoo, Finland; [3]Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Utrecht University, Utrecht, The Netherlands
Email: kristiina.s.hilden@helsinki.fi

White-rot fungi have ability to decompose all components of plant cell wall due to their wide enzyme repertoire. These enzymes could be used for bioconversion of various lignocelluloses in industrial applications. As a typical white-rot fungus, *Dichomitus squalens* has an extensive set of genes encoding enzymes for plant polysaccharide degradation. The previous study [1] indicated that *D. squalens* expresses and secretes variety of mannanolytic genes/enzymes when growing on different plant biomasses.
In the current study, we characterized four mannan-acting enzymes derived from *D. squalens*. In particular, two β-1,4-endomannanases (GH5_7), one β-1,4-mannosidase (GH2) and one α-1,4-galactosidase (GH27) were cloned and expressed in *Pichia pastoris*. The optimal temperatures of the expressed recombinant enzymes were in the range of 50 to 60 °C and optimal pH from 3 to 5. The molecular mass of the characterized proteins ranged from 47 to 110 kDa. The highest thermal tolerance was observed from β-1,4-endomannanase (MAN1). MAN1 retained over 50% of its enzyme activity after 30 minutes of incubation at 70 °C. The measured enzyme activities from two of the characterized enzymes, namely MAN1 and α-1,4-galactosidase, exhibited high enzymatic activities towards the tested substrates. Overall, the results suggest the high potential of *D. squalens* as a source of industrial enzymes.

[1] J. Rytioja, K. Hildén, M. Di Falco, M. Zhou, M.V. Aguilar-Pontes, O.-M. Sietiö, A. Tsang, R.P. de Vries, M.R. Mäkelä, Environ. Microbiol. (2017) doi: 10.1111/1462-2920.13652

Pigment biosynthesis in *Penicillium roqueforti*, why be blue?

Michaela Novodvorska, Matthew Cleere, Jack Whittaker, Paul S. Dyer
School of Life Sciences, Nottingham University, University Park, Nottingham, NG7 2RD, UK
Email: michaela.novodvorska@nottingham.ac.uk

Penicillium roqueforti is an industrially important fungus that is used as a starter culture for the production of blue-veined cheese. The fungus produces volatile compounds that are responsible for the unique blue cheese aroma, these compounds being generated from the extensive catabolism of milk lipids and proteins. The blue colour comes from melanin, and possibly other, pigments covering the surface of spores formed during growth in the cheese. It is known that a DHN-melanin biosynthetic pathway is responsible for conidial melanin pigment biosynthesis in closely related species such as *Aspergillus fumigatus* and *Penicillium marneffei*. In this study we investigated whether the same pathway is present in *P. roqueforti* and assessed the impact of the pathway on resistance against environmental factors, mycotoxin production and volatile profile. We identified six homologous genes of a canonical DHN-melanin biosynthetic pathway, developed a novel transformation protocol for *P. roqueforti*, and generated deletion strains. Deletion of each of these genes had an impact on pigment biosynthesis, with deletion mutants producing conidia exhibiting colour differences to the wild-type parental strain. Deletion mutants showed decreased viability when exposed to UV light and elevated temperatures, indicating the importance of pigment production in spore survival under varying environmental conditions. Production of mycotoxins such as mycophenolic acid and roqueforine C was greatly reduced when the pigment biosynthesis pathway was not fully functional. Deletion of each of the pathway genes also resulted in a change in production of flavour volatiles. These findings are of relevance for cheese manufacture.

Melanin-dependent pathogenicity of *Aspergillus fumigatus*

Axel A. Brakhage[1, 2]
[1]Department, Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany and [2]Institute for Microbiology, University of Jena, Germany
Email: axel.brakhage@leibniz-hki.de

The filamentous fungus *Aspergillus fumigatus* is the most important air-borne fungal pathogen causing 90 % of all systemic *Aspergillus* infections in humans. Previously, we

showed that the conidial pigment consisting of dihydroxynaphthalene (DHN) melanin represents a virulence determinant. Conidia lacking DHN-melanin show reduced virulence in a mouse infection model. We found that the presence of DHN-melanin on the conidial surface influences the response of immune cells at various levels. For example, DHN-melanin inhibits phagolysosomal acidification and causes increased damage to macrophages compared to non-pigmented *pksP* mutant conidia. We discovered a novel molecular mechanism by which DHN-melanin leads to the disturbance of lipid raft microdomains in the membrane of phagolysosomes, thereby preventing the assembly and activity of vATPase and thus inhibiting the acidification of the phagolysosome. Also, assembly of NADPH oxidase complex was lipid raft-dependent and reduced by melanized conidia. Further classification of the lipid raft microdomains showed that flotillin-enriched lipid rafts co-localized with sphingolipids on phagolysosomal membranes containing *pksP* conidia but not wild-type conidia.

Fungal SAPK pathways as mediators of virulence

Alison M. Day[1], Carmen M. Herrero-de-Dios[2], Alistair J. P. Brown[2] and Janet Quinn[1]
[1]Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK; [2]Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, UK.
Email: janet.quinn@ncl.ac.uk

Robust stress responses are common virulence attributes within a broad range of fungal pathogens, including the human fungal pathogen *Candida albicans*. Central to such stress responses are the stress activated protein kinases (SAPK), which are evolutionarily conserved eukaryotic signalling modules that are essential for the virulence of human pathogenic fungi. The Hog1 SAPK is essential for *C. albicans* virulence, however its structural and functional conservation with analogous human SAPKs (p38/JNK) makes the development of antifungal drugs that will specifically target fungal, and not human, SAPKs challenging. Because of this, there is much interest in identifying fungal-specific regulators of SAPKs as potential drug targets. Such targets include two-component related phosphorelay systems, which are used by fungi, but not mammals, to sense and relay stress signals to SAPK modules. Such phosphorelay systems comprise of a stress-sensing histidine kinase, an intermediary phosphorelay protein, and a terminal response regulator. Here I will present recent findings from my group, which have dissected the role of key phosphorelay proteins in regulating Hog1, and explored their potential as antifungal drug targets.

Synergistic combinations targeting translation fidelity and diverse fungal pathogens

Cindy Vallieres, Elena Moreno-Martinez, Ashleigh Gilliot, Sara L. Holland and Simon V. Avery.
School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK.
Email: C.Vallieres@nottingham.ac.uk

A wide range of fungicides (or antifungals) are used in agriculture and medicine. Unfortunately, the growing resistance to current antifungals has become a major issue, and therefore new agents with novel modes of action are urgently needed. Combination treatments offer an efficient method for managing fungal growth by enhancing efficacy compared to individual compounds, decreasing the dose of single agent usage, and subsequently lowering the drug toxicity and cost. Moreover, combinatorial inhibition can slow the evolution of resistance. A study conducted in our group showed that different aminoglycoside antibiotics combined with different sulphate-transport inhibitors produced strong, synergistic growth-inhibition of several human and plant pathogenic fungi mainly by causing, via different mechanisms, errors in mRNA translation. The results indicate that translation fidelity is a promising new target for combinatorial treatment of undesirable fungi. In line with this study, we screened a wider range of compounds known to affect the availability of functional amino acids in combination with either i) another agent that affects the availability of functional amino acids or ii) an aminoglycoside antibiotic to uncover new synergistic drug combinations targeting translation fidelity. Screens were performed in three fungal phytopathogens (i.e., *Rhizoctonia solani*, *Septoria tritici* and *Botrytis cinerea*) and three fungal pathogens of humans (i.e., *Cryptococcus neoformans*, *Candida albicans* and *Aspergillus fumigatus*). 43 combinations out of 172 were able to abrogate fungal growth with 9 combinations being represented in at least three pathogenic fungi. Thus, this study unveils a target process and chemical combinations with therapeutic potential against diverse fungal pathogens.

***Candida albicans* is a moving target for the immune system as it adapts to host signals**

Gabriela M. Avelar, Delma S. Childers, Judith Bain, Arnab Pradhan, Daniel Larcombe, Lars Erwig, Elizabeth R. Ballou, Neil A. R. Gow and Alistair J. P. Brown
Aberdeen Fungal Group, MRC Centre for Medical Mycology, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, UK
Email: al.brown@abdn.ac.uk

The potency of the immune system plays a key role in fungal colonisation and infection. Most studies of *Candida* immunology have been performed on fungal cells grown under standardised conditions *in vitro.* By examining the impact of key host signals upon *Candida albicans in vitro* (such as changes in carbon sources, pH, temperature, oxygenation, stresses and micronutrients), we find that, in addition to altering

resistance to host stresses and antifungal drugs, some host signals trigger changes in the fungal cell wall. This leads to significant modulation of key Pathogen Associated Molecular Patterns (PAMPs) on the fungal cell surface, turning *C. albicans* into a moving target for the immune system. In particular we have found that physiological levels of L-lactate trigger β-glucan masking via a specific lactate signalling pathway that includes the receptor Gpr1 and the transcription factor Crz1. Lactate-induced β-glucan masking leads to a reduction in phagocytic recruitment. This correlates with an increase in the virulence of *C. albicans*. Therefore, we argue that the adaptation of *C. albicans* to host microenvironments can affect its susceptibility to antifungal therapy, its detection by the immune system, and its pathogenicity. These findings may be relevant to other pathogenic *Candida* species.

Targeting infection mechanisms for two amphibian-infecting chytrid species

Rhys A. Farrer[1,2], Anthony Nash[3], An Martel[4], Elin Verbrugghe[4], Amr Abouelleil[1], Richard Ducatelle[4], Joyce E. Longcore[5], Timothy Y. James[6], Frank Pasmans[4], Matthew C. Fisher[2] and Christina A. Cuomo[1]

[1] Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA; [2] Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, London W2 1PG, UK; [3] Department of Chemistry, University College London, London WC1H 0AJ, UK; [4] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium; [5] School of Biology and Ecology, University of Maine, Orono, Maine 04469, USA; [6] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan 48109, USA

Email: rfarrer@broadinstitute.org

Nearly half of all amphibian species are declining globally due to factors such as habitat loss and disease. Two related fungal pathogens (*Batrachochytrium dendrobatidis; Bd* and the recently identified *B. salamandrivorans; Bsal*) have been attributed to these global declines and extinctions. By comparing the genome content and gene expression during infection, we identified fundamental differences in the molecular basis of pathogenesis. Notably, we identified a new group of secreted genes in *Bsal* that have no sequence similarity to previously characterized genes, but are highly expressed in the host. Using structural prediction tools, we are starting to gain clues to its possible functions. Additionally, we identified a large expansion of metalloproteases in *Bsal* compared to *Bd*, genes which have been also been reported in a variety of dermatophytes. Preliminary work to silence these genes using RNAi is being investigated. We found that both of these candidate pathogenicity factors were differentially expressed in the presence of the host, and that the host response is highly distinct to the two pathogens. Together, these analyses demonstrate the divergent

infection strategies and immune response to within this amphibian-killing genus, and two possible routes to further understand their functional mechanisms.

Diversity in plant biomass degradation approaches of fungi

Ronald P. de Vries
Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
Email: r.devries@westerdijkinstitute.nl

There are few aspects of fungal biology that are as diverse as their strategies to obtain carbon and energy from their natural substrates. Plant biomass, and in particular lignocellulose, is a major substrate for many fungi due to its abundance in nature and its high content of monosaccharides. The availability of (post-) genomic datasets have provided an unprecedented view on the strategies of fungi to depolymerize and convert plant-based polysaccharides. Comparative genomics revealed a huge variation in genome (CAZome) content with respect to lignocellulose active enzymes and in many cases these variations could be linked to the natural biotopes of the species.
However, the difference in strategy goes beyond the genomic variations. Recent studies in our lab revealed that even closely related species with similar CAZomes produce significantly different enzyme sets when exposed to the same substrate. This suggests significant differences in the regulation of the genes encoding these enzymes as well as a preference for different components of lignocellulose by the different species.
In this presentation, major differences observed across the fungal kingdom will be presented and placed into context of biotope and life style. Secondly, differences between species of the genus *Aspergillus* will be presented as a showcase for post-genomic variation. Finally, I will address how these insights can be used to improve commercial enzyme cocktails.

Mycoviruses as prospective enhancers of biocontrol agents

Ioly Kotta-Loizou [1] and Robert H.A. Coutts[2]
[1]Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK; [2]Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK.
Email: i.kotta-loizou13@imperial.ac.uk

The current world population is *ca.* 7.5 billion and approximately 2 billion tons/year of crops are needed to cover our nutritional requirements. Pesticides are used to protect the crops from damage and increase agricultural output; however, they are considered one of the top six global threats greatly impacting the biodiversity of the environment and putting 7 million people at risk. Environmentally friendly alternatives to pesticides

are biological control agents, including commercially available entomopathogenic fungi whose growth and pathogenicity may be enhanced by mycoviruses. The ultimate aim of the project is to investigate mycovirus-induced hypervirulence in entomopathogenic fungi. To this end panels of *Beauveria bassiana*, *Metarizhium anisopliae* and *Lecanicillium muscarium* were screened for the presence of double-stranded (ds) RNA elements. Among other mycoviruses discovered, members of the novel virus family Polymycoviridae were identified as the most promising enhancers, since they naturally confer mild hypervirulence to their hosts, are not conventionally encapsidated and are infectious as naked dsRNA facilitating genetic manipulations. The mechanisms controlling mycovirus-induced hypervirulence are still unclear but such knowledge would facilitate genetic engineering of known mycoviruses to increase hypervirulence. Off-target RNA silencing is expected to play a role given the dsRNA nature of these viruses that act as both triggers and targets of the antiviral defence system. Additionally, host-virus protein-protein interactions *via* Eukaryotic Linear Motifs leading to disruption of host pathways may also be implicated in accordance with other host-pathogen systems. In conclusion, this study discusses the potential of mycoviruses as enhancers of biocontrol agents.

Genomic and genetic analysis of a basidiomycete yeast focused on the characterisation of MEL biosynthesis

Stefany Solano[1,2], Anna Sobolewska[3], Doug Cossar[3], Mark Caddick[1] & Alistair Darby[1]
[1]Institute of Integrative Biology, University of Liverpool, UK; [2]Escuela de Ciencias Biologicas, Universidad Nacional, Costa Rica; [3]Croda Europe, Ditton, Widnes, UK
Email: S.Solano-Gonzalez@liverpool.ac.uk

This work focuses on a basidiomycete yeast known to be a grass endophyte. Of specific interest is the strain's ability to produce Mannosylerithritol lipids (MELs). These are of interest to industry as biosurfactants and are classified into four groups (A – D) according to their structure. The basidiomycete in this study is primarily a MEL C producer. The regulation and production of these secondary metabolites and the underpinning biology remain unclear, despite the many studies carried out with basidiomycota, *Ustilago maydis*, and *Pseudozyma aphidis.* Currently there are few annotated genomes for MEL producing fungi. We therefore undertook the genome sequencing, assembly and annotation of our isolate, as a first step to investigating its potential for industrial exploitation. Using the PacBio sequencing platform we have produced a genome assembly, composed of 34 contigs. Transcriptome sequencing has been utilised to facilitate gene calling, with a total of 6603 genes being identified, of which 4877 have been automatically annotated using primary databases. Amongst these are the five genes which form the putative MEL biosynthetic cluster. Phylogenetic analysis shows that amongst sequenced fungi, our strain is most closely related to *S. reilianum* and *U. maydis* (both plant pathogens) and surprisingly not *Pseudozyma* species, as had been expected. We have undertaken transcriptome analysis to

investigate gene expression associated with MEL production over a five day time course and we are currently using directed gene replacement to investigate the function of specific genes in the biosynthetic cluster and the role of putative regulatory proteins in MEL production.

Intraspecies diversity and induction of the wood decay by the white rot fungus *Dichomitus squalens*

Sara Casado López[1], William B. Chrisler[2], Samuel Purvino[2], Alice Dohnalkova[2], Galya Orr[2], Errol Robinson[2], Erika Zink[2], Scott Baker[2], Kristiina Hildén[3], Miia R. Mäkelä[1,3] and Ronald P. de Vries[1,3]

[1] Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University, Utrecht, 3584CT, The Netherlands; [2] Environmental Molecular Sciences Laboratory, Department of Energy, Richland, WA 99354, USA; Department of Food and Environmental Sciences, University of Helsinki, Helsinki, FI-00014, Finland.

Email: s.casado@westerdijkinstitute.nl

Dichomitus squalens is a white rot fungus that preferably grows and colonizes dead softwood, but is also able to utilize hardwood. It is commonly found in the northern parts of Europe, North America and Asia. As a result of sexual crossing of two compatible monokaryons, a fertile dikaryon is formed and fruiting bodies can be produced.

In this study we aimed to dissect the differences in wood colonization by a set of mono- and dikaryotic strains of *D. squalens*, to analyze how varied this process can be within one species. For this purpose, we integrated a combination of transcriptomics, proteomics, metabolomics and high resolution microscopy enabled by the combined facilities of the JGI and EMSL institutes.

All strains were grown on solid spruce sticks, and sampling was performed after two and four weeks to evaluate the variation between the strains during the wood decay process. Moreover, we performed transcriptomics of two of the strains (mono- and dikaryon) on plant polysaccharide derived sugars to start to unravel the induction mechanism of carbohydrate active enzymes (CAZymes) encoding genes in *D. squalens*.

Exploiting unspecialised endophytes for biological control of insects and plants

Alan C. Gange, Amanda F. Currie, Nadia Ab Razak and Ruth Chitty
School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX UK
Email: a.gange@rhul.ac.uk

Herbaceous plants harbour diverse communities of endophytic fungi in their roots and shoots. However, compared to endophytes in grasses or woody plants, almost nothing is known about the ecology of these fungi. It has been suggested that as these endophyte are unspecialised and ubiquitous, then the relation with their hosts may be a loose one, with little effects on the plant itself, or other organisms that attack the host, such as insects. One particular group of fungi that can occur as endophytes in plants are the entomopathogens and there is increasing interest in the use of these fungi as internal plant protectants.
Here we report the results of a meta-analysis, to examine the effects of unspecialised endophytes, including entomopathogens, on insect herbivores. We will combine this with some of our own experimental data to show that these fungi may have great potential in biological control of insect pests. Contrary to expectations, some unspecialised endophytes can also have direct effects on the growth of their hosts in the absence of antagonists, and we will explore how such effects may be exploited in the biological control of weeds.

Regulation of Drug Resistance in *Aspergillus fumigatus*

Michael J. Bromley
Manchester Fungal Infection Group, School of Biological Sciences, University of Manchester, Manchester, United Kingdom.
Email: Mike.bromley@manchester.ac.uk

Aspergillus fumigatus is the most important airborne mould pathogen and allergen worldwide. Estimates suggest that over 3 million people have invasive or chronic infections that lead to in excess of 600,000 deaths every year. Very few drugs are available to treat the various forms of aspergillosis and we rely predominantly on the azole class of agents (Itraconazole, Voriconazole, Posoconazole and the recently licensed Isavuconazole). Resistance to the azoles is emerging. For individuals that are infected with a resistant isolate the mortality rate exceeds 88%. Therapy failure is in part attributed to delays in administering alternative therapies, so methods to rapidly detect resistance are critical. While resistance in around 50% of clinical isolates has been linked to modification of the gene encoding the target of the azoles, *cyp51A*, our understanding of what leads to resistance in the remaining strains is lacking.
We have undertaken a programme of work to identify transcriptional and post-transcriptional regulators of azole resistance. We have discovered a cohort of transcription factors, including the CCAAT-binding complex and Negative Cofactor 2,

which modulate azole tolerability. With a view to understanding which genes are directly regulated by these transcription factors, we have performed genome-wide protein-DNA interaction analysis using ChIP-seq. We have identified that these transcription factors bind the promoters of genes known to be associated with azole tolerance including *cyp51A* the drug transporter *cdr1B*. Our Chip-seq data provides evidence to suggest that these regulators bind the promoters of many genes however do not always modulate their expression.

Systematic functional annotation of transcription factors in the major mould pathogen *Aspergillus fumigatus*

Takanori Furukawa, Norman van Rhijn, Panos Papastamoulis, Sayema Khan, Lea Gregson, Paul Bowyer, Magnus Rattray, Michael Bromley, Elaine Bignell
Manchester Fungal Infection Group, Division of Infection, Immunity, and Respiratory Medicine, University of Manchester, Manchester, M13 9NT, UK
Email: takanori.furukawa@manchester.ac.uk

Aspergillus fumigatus is, globally, the most important airborne fungal pathogen of humans that causes over 90% of all *Aspergillus*-related infections. It has been demonstrated that the virulence of this fungus is multifactorial and several virulence-determining factors have been identified. In particular, the ability of the fungus to respond to various host-imposed stresses has repeatedly been proven to be essential for pathogenicity, and therefore transcriptional regulation plays a critical role in establishing infection. However, we only have a very limited understanding of the molecular basis of *A. fumigatus* pathogenicity.
In this study, we carried out a systematic phenotyping of a transcription factor knockout (TFKO) collection of *A. fumigatus* mutants to define the genomic cohort of transcription factors required for pathogenicity. A high-throughput analytical pipeline was developed in a 96-well plate based liquid culture format to analyze relative fitness of 401 *A. fumigatus* TFKO strains. Growth traits of the TFKO strains were then analyzed in parallel under each of the following host-imposed stress conditions: iron starvation, temperature stress, and pH tolerance. Data analysis was automated to convert optical density readings collected directly from the spectrophotometer into fitness indices. Our study identifies, for the first time, the full cohort of *A. fumigatus* transcription factors that govern responses to significant host-imposed stresses and provides a first genome-scale functional annotation of transcription factors in *A. fumigatus*.

BSPP Talks

Using population genomics to determine the genetic basis of pathogen adaptation in agro-ecosystems

Bruce A. McDonald
Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland
Email: bruce.mcdonald@usys.ethz.ch

Septoria tritici blotch (STB) is the most damaging wheat disease in Europe. The pathogen causing STB, *Zymoseptoria tritici*, is globally distributed and has evolved to become resistant to fungicides and virulent on resistant wheat cultivars. We coupled QTL mapping based on 700 progeny from two crosses with analyses of genome-wide associations (GWAS) based on 150 strains drawn from four global field populations to elucidate the genetic architecture of several traits, including host specialization, virulence and fungicide resistance. Finished genome sequences and extensive RNA-seq profiles obtained for all four parents enabled identification of candidate genes affecting each trait. Several of these genes were functionally validated. While genotyping has become relatively easy, phenotyping remains difficult and limits progress in most marker-trait association studies. We developed high-throughput phenotyping methods to accelerate breeding for STB resistance. Automated analysis of digital images generated 2.7 million phenotypic measurements associated with melanization, virulence, host specialization, fungicide sensitivity and thermal adaptation in the QTL mapping populations. This approach was extended to a naturally infected field planted to 335 elite European wheat cultivars, yielding >15 million phenotypic measurements associated with host damage (percent leaf area covered by STB lesions) and pathogen reproduction (number of STB pycnidia per cm2 of leaf lesion) that ranked the relative STB resistance among these wheat cultivars. A GWAS using these datasets allowed us to genetically separate resistance affecting host damage from resistance affecting pathogen reproduction. Our epidemiological analyses indicated that resistance affecting pathogen reproduction offers the greatest potential for limiting damage due to STB.

Why does IPM not lead to more reductions in fungicides?

Lise Nistrup Jørgensen
Department of Agroecology, Aarhus University - Flakkebjerg, DK-4200 Slagelse, Denmark
Email: lisen.jorgensen@agro.au.dk

Cereal crops account for the major use of fungicides in Europe. Despite the politically driven desire to reduce dependence on pesticides in general, it has proved difficult to reduce the consumption of fungicides significantly. Several non-chemical methods are

known to help reduce diseases in cereal crops. However, farmers' willingness to generate major changes in their cropping systems such as including new crops or adopting new tillage methods is steered by economic rationale and farmers' ability to provide flexible management. Cropping varieties with good resistance are known as the most widely adopted and advocated method of reducing pathogen attack, and variety resistance has in several cases provided good possibilities of reducing the reliance on fungicides. Even so, in cereal crops, no cultivars with complete resistance to all relevant diseases exist and fungicides are commonly used to ensure against major crop losses from diseases. Changes in pathogen virulence have in many cases led to loss of effective cultivar resistance, which again required adjusted input of fungicides. As an element of IPM, farmers in many countries often apply reduced doses, although they most likely still apply higher doses than the optimum to ensure against losses in seasons with a high disease level. Unfortunately, reliable tools, which can improve prediction of future disease damage and account for the combined effect of integrated control measures are still lacking. Ideally, reliable models should predict the optimum number of treatments and the optimum dose needed to minimize both economic costs and environmental impact in a given season.

Identifying root resistance to take-all disease

Vanessa E. McMillan, Gail Canning, Richard J. Gutteridge and Kim Hammond-Kosack
Department of Biointeractions and Crop Protection, Rothamsted Research, UK
Email: vanessa.mcmillan@rothamsted.ac.uk

Take-all (*Gaeumannomyces tritici*) is a major root disease of wheat which is a serious constraint in short wheat rotations, reducing yields of 2nd and 3rd wheat crops by 1 to 3 tonnes/ha. Distinctive patches of disease develop in the field with stunted and prematurely ripening plants where the root system is severely infected. Moderate and severe disease outbreaks also put fields at risk of nitrate leaching as excess unused nitrogen fertiliser is left behind in the soil.
As part of the Wheat Genetic Improvement Network (WGIN, www.wgin.org.uk) and Wheat Improvement Strategic Programme (WISP, www.wheatisp.org) protocols have been developed to phenotype a range of diverse germplasm collections, including elite wheat varieties, landraces and wheat relatives for root resistance to take-all and/or their effect on fungal inoculum build-up in the soil. Phenotyping was primarily carried out under field conditions with the aim of identifying resistance that was consistently expressed across multiple field seasons and sites.
Since 2008 over 700 Watkins hexaploid wheat landraces have been phenotyped for take-all resistance. Moderate resistance to take-all disease was identified for a subset of genotypes within the collection. A Paragon (moderately susceptible) x Watkins 777 (moderately resistant) mapping population is currently being phenotyped to dissect the genetic basis of resistance. Results from the first year of field phenotyping reveal two QTLs conferring resistance in a low disease pressure situation. Work is now underway to

confirm and validate these genomic locations across further field seasons and to explore the root response in more detail.

Ignored until recently – the story of the fungal pathogen *Leptosphaeria biglobosa*

Yongju Huang, Asna Javaid, Lakshmi H. Gajula, Chinthani S. Karandeni-Dewage, Georgia Mitrousia, Bruce D.L. Fitt
School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Hertfordshire, AL10 9AB, UK
Email: y.huang8@herts.ac.uk

Many plant diseases are caused by more than one pathogen and there are many factors affecting their co-existence on their host that lead to changes in their predominance. Phoma stem canker is a globally important disease of oilseed rape, caused by two related pathogens, *Leptosphaeria maculans* and *L. biglobosa.* Since *L. maculans* is often associated with stem base canker while *L. biglobosa* is associated with superficial upper stem lesions, *L. maculans* is considered more damaging than *L. biglobosa*. Although *L. maculans* and *L. biglobosa* co-exist on their host, previous work has mainly focused on *L. maculans* and the importance of *L. biglobosa* in phoma stem canker epidemics has been ignored. Results of recent work show that *L. biglobosa* has increased in importance in the UK, causing both damaging upper stem lesions and stem base cankers. Furthermore, *L. biglobosa* is less sensitive to some triazole fungicides than *L. maculans*. Previous control of phoma stem canker in the UK by cultivar resistance or fungicides has targeted only *L. maculans*, disregarding *L. biglobosa*. Recent studies have shown that cultivars resistant against *L. maculans* are often more susceptible to *L. biglobosa*. For effective control of phoma stem canker, there is a need to target both *L. maculans* and *L. biglobosa.* In this talk, the reasons why *L. biglobosa* has recently increased in importance in phoma stem canker epidemics in the UK will be discussed, based on results from field experiments over three seasons and controlled environment experiments.

***Powdery Mildew: A Tale of Two Fungicides**

Corinne J. Arnold, Diane G. O. Saunders and James K. M. Brown
Crop Genetics, John Innes Centre, Norwich, NR4 7UH, UK
Email: Corinne.Arnold@jic.ac.uk

Blumeria graminis is a powdery mildew pathogen that infects many wild grasses and cereals across the world. If left uncontrolled, it can cause significant yield losses in cereals. One main method for control is the application of fungicides but resistance to strobilurin and older triazole fungicides already occurs in *B. graminis*, and few other fungicides are available for mildew control. I have characterised isolates from an

outbreak of *B. graminis* f.sp. *tritici (Bgt)* on wheat where two of the major fungicides used against *Bgt* were ineffective: cyflufenamid (Cyflamid), an eradicant fungicide with an unknown mode of action, and fenpropimorph (Corbel), a morpholine fungicide targeting a sterol reductase. All *Bgt* isolates from the study site were completely resistant to Cyflamid sprayed at the recommended field rate. However, over the following two years, the *Bgt* population recovered sensitivity to Cyflamid, suggesting that resistance may have a significant fitness penalty. Additionally, following two sprays of Corbel, these *Bgt* isolates had significantly lower sensitivity to fenpropimorph than field isolates. Overuse of fenpropimorph would almost certainly lead to strong resistance in mildew, so it should be used as part of a rotation of fungicides with different modes of action. Likewise, Cyflamid can continue to be used as part of an integrated disease control programme. Genetic and genomic analysis is in progress, with the aim of identifying candidate genes for resistance to cyflufenamid and to fenpropimorph.

******Blumeria* **metallo-protease-like effector; a zincophore acting as a universal virulence factor in fungi?**

Shaoli Das Gupta[1], Kate Orman[1], Martin Urban[2], Gabriel Scalliet[3], Kim Hammond-Kosack[2] and Laurence Bindschedler[1]

[1]School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK; [2]Department of Plant Biology and Crop Science, Rothamstead Research, Harpenden, Hertfordshire AL5 2JQ, UK;[3]Syngenta Crop Protection, Research Biology, Schaffhauserstrasse, Stein, Switzerland

Email: Shaoli.DasGupta.2015@live.rhul.ac.uk

Blumeria effector candidate 19 (BEC1019) is a virulence factor of the biotrophic pathogen *Blumeria graminis* f.sp. *hordei* (*Bgh*), a causal agent of barley powdery mildew. BEC1019 silencing led to reduced *Bgh* growth and disease dispersion. BEC1019 has homologues in one third of the sequenced fungal genomes, including the zinc scavenging virulence factors PRA1 (*Candida albicans*) and Aspf2 (*Aspergillus fumigatus*). BEC1019 homologues lack protease activity but share a conserved zinc binding HRXXH domain, related to the HEXXH-containing M35 zinc metallo-protease superfamily. Therefore, BEC1019 homologues are potential fungal "zincophores" with a universal virulence role.

This project is investigating the potential involvement of BEC1019 homologues in zinc sequestration for the virulence of economically important wheat fungal pathogens.

We will show that BEC1019 silencing in *B. graminis* f.sp. *tritici* (*Bgt)* led to reduced infection of wheat powdery mildew, thus indicating its role in virulence. *Fusarium graminearum (Fg)* and *Zymoseptoria tritici* (*Zt*) BEC1019 knockouts are being produced to estimate the role of BEC1019 during Fusarium head blight and Septoria tritici blotch infection. Since BEC1019 was shown to be highly induced during *Zt* growth under zinc deficiency, the role of BEC1019 and other factors involved in zinc sequestration for pathogen success and countering plant

nutritional immunity will be further investigated, in the quest of finding novel targets for crop protection.

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

Kathryn R. Hales[1], Gary D Bending[2], Tim R Pettitt[3] and John P. Clarkson[1]
[1]Warwick Crop Centre, University of Warwick, Wellesbourne, CV359EF, UK; [2] School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK; [3]National Pollen and Aerobiology Research Unit, University of Worcester, Worcester, WR2 6AJ, UK
Email: k.r.hales@warwick.ac.uk

Cavity spot is a major disease of carrot primarily thought to be caused by the soilborne oomycete pathogen *Pythium violae,* although other *Pythium* species may also be associated with the lesions. Disease management is challenging, as fungicide efficacy is variable and long rotations not always possible. New approaches to control have been hampered by a lack of fundamental knowledge concerning the epidemiology of *P. violae* and basic tools for research. This PhD project aims to address some of these issues by developing molecular detection techniques and artificial inoculation methods.
To determine the range of *Pythium* spp. associated with cavity spot symptoms, a collection of 150 isolates was assembled from diseased carrots. Following DNA extraction and sequencing, 61% were identified as *P. violae* with *P. sulcatum* and *P. intermedium* both accounting for 14%. The remainder comprised other *Pythium* species.
To detect *P. violae* in relatively large samples of soil, a sucrose flotation/filtration method was developed to capture oospores from 10 g soil. When combined with a specific PCR test, initial results suggested that detection of less than 10 oospores is possible. This approach is being developed further for RT-PCR application to enable accurate quantification of *P. violae* and monitoring of pathogen dynamics in soil and on roots.
A method of inoculum production was developed for *P. violae* and used to inoculate pot-grown carrots in the glasshouse and small field 'macrocosms'. Initial results revealed effects on carrot growth and the development of typical cavity spot lesions on roots.

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

Sascha Jenkins[1], Richard J. Harrison[2] and John P. Clarkson[1]
[1]Warwick Crop Centre, University of Warwick, Wellesbourne, CV35 9EF, UK; [2] Genetics, Genomics & Breeding, NIAB-EMR, East Malling, ME19 6BJ, UK
Email: sascha.jenkins@warwick.ac.uk

Pea is an important legume crop for the UK, worth an estimated £50 million per year. The UK is also the largest producer of frozen peas in Europe, due to ideal conditions along the Eastern seaboard. Fields are located close to processing factories, which reduces the potential land available to grow peas.
Soilborne plant pathogens in the 'footrot' complex are one of the main causes of crop loss in peas, resulting in wilting, reductions in pod numbers and plant death. Several *Fusarium* species are major components of this complex with *F. oxysporum* f.sp. *pisi* (FOP) being amongst the most important worldwide. Races of FOP are difficult to distinguish with the current method of pathogenicity tests using pea differential lines. Pathogenicity factors including effector genes have been reported in other pathogenic *F. oxysporum* with Secreted in Xylem (SIX) genes widely studied.
The main aims of this PhD project are to identify the range of *Fusarium* species affecting pea in the UK and identify effector genes associated with pathogenicity. A survey of UK pea fields identified foot rot causing species including *F. oxysporum*, *F. solani* and *F. redolens,* with the most common being *F. oxysporum*. These isolates collected from UK fields and FOP race-typed isolates were used in two pathogenicity tests to distinguish wilt causing and foot rot causing isolates. Initial results from whole genome sequencing and PCR have revealed the presence of 7 SIX genes in FOP races 1,2 and 5, and further tests will study their expression levels post inoculation.

***Fine mapping of a major gene locus for resistance against *Pyrenopeziza brassicae* (light leaf spot) in *Brassica napus* and identification of candidate resistance genes**

Chinthani S. Karandeni Dewage, Henrik U. Stotz, Bruce D. L. Fitt
School of Life & Medical Sciences, University of Hertfordshire, Hatfield, Herts, AL10 9AB, UK
Email: c.s.karandeni-dewage@herts.ac.uk

Plant major gene-mediated resistance is important for management of disease threats to crops. Fine mapping and cloning of these genes can provide insights into host-pathogen interactions to improve disease control strategies. This work on the *Brassica napus – Pyrenopeziza brassicae* pathosystem aims to improve understanding of the operation of major gene-mediated resistance against hemibiotrophic plant pathogens. *Pyrenopeziza brassicae* causes light leaf spot disease on winter oilseed rape in the UK as well as disease on vegetable brassicas, including Brussels sprouts. A major gene locus maps to the bottom end of *Brassica napus* chromosome A01. Using novel genomic information and molecular marker data on *B. napus*, improved marker resolution and understanding of the genetic basis of this resistance can be obtained. We identified the physical location of the closest flanking marker to the resistance locus on *B. napus* chrA01 using marker sequence and the synteny between *B. rapa* and *B. napus* genomes. Single nucleotide polymorphism (SNP) marker information on the corresponding genomic region was obtained and *c.* 400 candidate SNPs specific to chrA01 were identified. Initially, 38 SNPs were selected for development of KASP markers, which

were tested on parental lines of the DH population that segregate for major gene resistance against *P. brassicae*. Polymorphic markers between parental lines were analysed in the segregating population to improve the genetic map resolution. We also analysed three different *Brassica napus* genome sequences to identify gene content in this genomic region and gene predictions will be used to identify candidate resistance genes against *P. brassicae.*

***From the field to the lab and back again: exploring legacy effects of root genetics and fungicide for take-all root disease control in wheat rotations**

Joseph Moughan[1], Vanessa McMillan[1], Rodger White[2], James Melichar[3], David Ranner[3] and Kim Hammond-Kosack[1]

[1]Biointeractions and Crop Protection Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; [2] Computational and Analytical Sciences Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; [3]Syngenta UK Limited, Fulbourn, Cambridge, CB21 5XE, UK

Email: joseph.moughan@rothamsted.ac.uk

Gaeumannomyces tritici is a soil-borne, root pathogen of wheat, causing take-all disease. Some modern, elite, winter wheat cultivars possess a genetic trait that promotes low take-all inoculum build-up (LowTAB) in first wheat (McMillan *et al*., (2011), Plant Pathology, 60: 200-206). This leads to reduced disease if wheat is grown in the same field the next year and has given two tonnes/ha yield advantages in severe disease years (McMillan, submitted).

Using field trials, this research investigated if enhanced or synergistic reduction of take-all inoculum build-up, could be achieved with foliar applications of take-all active fungicide, azoxystrobin, in combination with LowTAB genetics. Subsequent disease was measured in second wheat oversow experiments. To complement this, laboratory screens were performed to check for common target site mutations to the azoxystrobin fungicide, in new and historic *G. tritici* isolates.

Reduced second wheat take-all disease was found resulting from legacy effects in the soil from the previous year's first wheat treatments. Individual effects of first wheat root genetics (LowTAB) ($P<0.001$) and azoxystrobin application ($P=0.009$) were observed, but no synergy was identified. First and second wheat chemistry effects did not correlate. Mid-season first wheat sprays lowered inoculum build-up ($P=0.014$), but only early first wheat sprays effectively reduced second wheat disease. These inconsistent fungicide effects could not be linked to target site resistance, but may be due to low levels of fungicide insensitivity resulting from activity of an alternative respiration pathway in *G. tritici.* We conclude that in the field, azoxystrobin altered the normal take-all disease progression cycle.

Exploiting effectors in breeding and deployment of *R* genes in potato

Vivianne G.A.A. Vleeshouwers[1] , Geert Kessel[2], Theo van der Lee[2], and Richard G.F. Visser[1]

[1]Plant Breeding, Wageningen University & Research, Wageningen, P.O. Box 386, 6700 AJ, The Netherlands; [2]Biointeractions, Wageningen University & Research, Wageningen, P.O. Box 16, 6700 AA, The Netherlands

Email: vivianne.vleeshouwers@wur.nl

The Irish famine pathogen *Phytophthora infestans* causes the destructive late blight disease on potato (*Solanum tuberosum*), a major food crop world-wide. Potato plants attain resistance due to disease resistance (*R*) genes that have co-evolved with effectors, or so-called avirulence (*Avr*) genes of the oomycete pathogen. Since the genomics era, such effectors have emerged as tools to accelerate and improve the identification, functional characterisation and deployment of *R* genes. Upon the availability of the reference genome of *P. infestans,* we have generated a genome-wide infection-ready library of candidate *Avr* genes. These are used in functional assays for detecting activity of *R* genes. This effectoromics strategy has accelerated cloning and specificity profiling of *R* genes from wild potato species and proven effective and complementary to classical resistance breeding. For various R-AVR pairs, we are studying the effector diversity and activity to get insight in the mechanisms that *P. infestans* employs for evading R protein recognition. We have designed a PCR-based monitoring for characterized *Avr* genes to detect virulent isolates among large sets of samples in field trails. These effector profiles are implemented in an integrated pest management (IPM) strategy. With these experiments, we show that a high-throughput spatio-temporal monitoring of effector allelic diversity in *P. infestans* populations can enable a more educated deployment of *R* genes in potato.

The RxLR Motif of the Host Targeting Effector AVR3a of *Phytophthora infestans* Is Cleaved Before Secretion

Stephan Wawra[1,2,], Franziska Trusch[1,], Anja Matena[3], Kostis Apostolakis[1], Uwe Linne[4], Igor Zhukov[5,6], Jan Stanek[7], Wiktor Koźmiński[7], Ian Davidson[8], Chris J. Secombes[9], Peter Bayer[3] and Pieter van West[1]

[1]Aberdeen Oomycete Laboratory, Institute of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, UK; [2]Botanical Institute, Genetical Institute, University of Cologne, 50674, Cologne, Germany; [3]Structural and Medicinal Biochemistry, Centre of Medicinal Biotechnology, University of Duisburg-Essen, 45141 Essen, Germany; [4]Core Facility for Mass Spectrometry, Chemistry, Philipps-Universität Marburg, D-35032, Marburg, Germany; [5]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland; [6]NanoBioMedical Centre, Adam Mickiewicz University, 61-614 Poznań, Poland; [7]Biological and Chemical Research Centre (CENT III), Faculty of Chemistry, University of Warsaw, 02-089 Warsaw, Poland; [8]Proteomics Facility, Institute

of Medical Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, UK; [9] Scottish Fish Immunology Research Centre, Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, AB24 2TZ, UK
Email: franziska.trusch@abdn.ac.uk

When plant-pathogenic oomycetes infect their hosts, they employ a large arsenal of effector proteins to establish a successful infection. Some effector proteins are secreted and are destined to be translocated and function inside host cells. The largest group of translocated proteins from oomycetes are the RxLR effectors, defined by their conserved N-terminal Arg-Xaa-Leu-Arg (RxLR) motif. However, the precise role of this motif in the host cell translocation process is unclear. Here detailed biochemical studies of the RxLR effector AVR3a from the potato pathogen *Phytophthora infestans* are presented. Mass spectrometric analysis revealed that the RxLR sequence of native AVR3a is cleaved off prior to secretion by the pathogen and the N-terminus of the mature effector was found likely to be acetylated. High-resolution NMR structure analysis of AVR3a indicates that the RxLR motif is well accessible to potential processing enzymes. Processing and modification of AVR3a is to some extent similar to events occurring with the export element (PEXEL) found in malaria effector proteins from Plasmodium falciparum.
These findings imply a role for the RxLR motif in the secretion of AVR3a by the pathogen, rather than a direct role in the host cell entry process itself.

Know your enemy – engineering resistance against Fusarium wilt in banana

Lola Penadez-Gonzales, Rafal Zdrzalek, Christopher Stevens, Peter van Esse, Sarah M. Schmidt
The Sainsbury Laboratory, Norwich, NR4 7UH, UK
Email: sarah.schmidt@tsl.ac.uk

Bananas are not just delicious fruits, but are the 4th most important food crop in the world. In many parts of the world bananas are staple crops that are important for food security. Currently, the biggest threat to worldwide banana production is Fusarium wilt disease, a vascular disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). In the 1990s a new *Foc* race called Tropical Race 4 (TR4) emerged in South-East Asia that is spreading around the globe. Fusarium wilt cannot be controlled chemically and breeding bananas is difficult because edible cultivars are sterile and do not produce seeds. In the 2Blades group, I am exploring new routes of engineering resistance in bananas through a better understanding of the pathogen focusing on the following questions; Which virulence genes does it need to infect bananas? Are there resistance genes known against Fusarium wilt disease in other plant species that we can introduce into banana? And finally, can we use antagonistic microbes to control Fusarium wilt disease of bananas?

The agronomic potential of gene silencing application

Aline Koch[1], Dagmar Biedenkopf[1], Elke Stein[1], Lukas Jelonek[2], Alexander Goesmann[2] and Karl-Heinz Kogel[1]

[1]Department of Phytopathology, Justus Liebig University, D-35390 Giessen, Germany; [2]Department for Bioinformatics and Systems Biology, Justus Liebig University, D-35392 Giessen, Germany

Email: karl-heinz.kogel@agrar.uni-giessen.de

Non-coding RNAs have emerged as a powerful genetic tool in research and practical use in medicine and agriculture. The demonstration that agricultural pests/pathogens, such as insects, nematodes, and fungi, are killed by exogenously supplied RNA targeting their essential genes has raised the possibility that non-coding RNA can be developed into a novel class of pesticides. Recently, we provided proof-of-concept that delivery of dsRNA targeting the three cytochrome P450 lanosterol C-14α-demethylases (CYP51) genes of the necrotrophic fungal pathogen *Fusarium graminearum* effectively controls Fusarium Head Blight and Root Rot in barley.[1,2] Here, we will discuss the mode of action of dsRNA-mediated *Fusarium* growth inhibition in different experimental set-ups including in vitro liquid fungal culture, in situ leaf spray application, and transgenic delivery.
Other labs also recently showed that spray applications of RNA to plants, including Arabidopsis, tomato and tobacco, is rather effective in controlling plant diseases caused by microbial pathogens and viruses.[3,4] Moreover, optimization of RNA delivery by certain nanostructure-based formulations was demonstrated.4 Based on those findings and new unpublished data from our lab, we will report on advances and setbacks in controlling other fungal pathogens by dsRNA application. Altogether we will provide information on a fundamentally new plant protection strategy, thereby opening novel avenues for improving crop yields in an environmentally friendly and sustainable manner.

1. Koch et al. (2013) PNAS 110:19324-19329
2. Koch et al. (2016) PLOS Path. DOI:10.1371/ journal.ppat.1005901 :1be8a6b457c73d5c
3. Wang et al. (2016) Nature Plants, doi:10.1038/nplants.2016.15
4. Mitter et al. (2016) Nature Plants, doi: 10.1038/nplants.2016.207

Combining proteomics of haustoria and gene silencing to discover key players of virulence and susceptibility during barley powdery mildew infection.

Kate Orman, Sebastien Lambertucci-Bonnet, Shaoli Das Gupta, Laurence V. Bindschedler[1]

School of Biological Sciences, Royal Holloway University of London, Egham, TW20 0EX, UK

Email:Laurence.bindschedler@RHUL.ac.uk

Cereal powdery mildews, *Blumeria graminis*, are recognised as major contributors affecting yield and grain quality of wheat and barley. Using a proteomics approach, *Blumeria* effector candidates (BECs), some of which are also defined as Candidate secreted effector proteins (CSEPs), were specifically associated with haustoria (Bindschedler et al 2009, 2011, Spanu 2010, Pliego et al, 2013). Haustoria are at the centre of the biotrophic interaction, developing in the plant epidermis without destroying the invaded cells. Haustoria are surrounded by an extra-haustorial membrane (EHM), which is in continuum but distinct from the host plasma membrane. Because little is known about the EHM composition, we investigated the EHM proteome to unravel putative key players of susceptibility and resistance.
Since *Blumeria* is an obligate pathogen, we had to devise a new convenient, cloning- and GM-free RNAi alternative method that delivers small, modified oligodeoxynucleotides to whole leaves, to validate effector function by transient host induced gene silencing. The method was equally suitable for the validation of known susceptibility genes. Using this silencing approach, we challenged the role of a PR5 thaumatin-like protein isoform which interacts with the RNAse-like effector, BEC1054 (Pennington et al 2016) and which is likely associated with the EHM. To our surprise, we established that this PR5 acts as a susceptibility factor, since its silencing led to decreased *Blumeria* infection. This increase in host resistance by targeting PR5 was associated with a decreased amount of the cognate mRNA and an increased percentage of infected epidermal cells producing hydrogen peroxide.

Direct and indirect effects of fungal endophytes on plant-parasitic nematodes

Alexander Schouten
Laboratory of Nematology, Experimental Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
Email: sander.schouten@wur.nl

Although notorious for their pathogenicity, it is becoming evident that most *Fusarium oxysporum* isolates encountered in nature are harmless to plants, some of which can even play a beneficial role as true endophytes, meaning that they can colonise plants without ever provoking a disease phenotype, by antagonizing plant-parasitic nematodes. The involved mechanisms in this quantitative antagonism are only superficially understood. There is evidence that the presence of the endophyte increases plant vigour and induces plant defence responses toward the nematode. Direct negative effects of the endophyte on nematode performance and behaviour cannot be ruled out as well.
Metabolites, produced by the fungal nematode control endophyte, *F. oxysporum* 162 (Fo162), were isolated and fully characterized. Of the eleven resolved compounds, six were isolated from a *Fusarium* spp. for the first time. Toxicity assays showed that, 4-hydroxybenzoic acid (4-HBA), indole-3-acetic acid (IAA) and gibepyrone D were the most potent toward nematodes, being almost as good as the best commercial nematocide.

IAA is a well-known phytohormone and known to play a role in plant development and triggering defences. 4-HBA is reported to increase abiotic stress tolerance. This finding thus suggests that individual metabolites, produced by Fo162, can have a dual activity, i.e. inducing defence mechanisms and/or tolerance toward nematodes in plants and directly killing or compromising nematodes. Such compounds may serve as important leads in the development of sustainable and environmental friendly nematocides.

Improving Plant Resistance to Fungal Pathogens by Callose Modification

Tobias Hanak[1] and Christian A. Voigt[2]
[1]Molecular Phytopathology, Biocentre Klein Flottbek, University of Hamburg, Hamburg, 22609, Germany; [2]Department of Animal and Plant Sciences, P3 Centre for Plant Production and Protection, University of Sheffield, Sheffield, S10 2TN, United Kingdom
Email: c.a.voigt@sheffield.ac.uk

The plant cell wall is complex polymer network that forms the outer barrier of plant tissues to the surrounding environment. Its architecture is of great importance to confer resistance to invading pathogens, which are a major threat for crop yield and food security. Plant defence responses include cell wall adaption at sites of pathogen interaction with a decisive role of the cell wall polymer callose, a (1,3)-β-glucan. Our recent results have proven that modification and optimization of callose biosynthesis provides resistance to invading pathogens through network formation with the preexisting cellulosic cell wall, not in the model plant *Arabidopsis thaliana* but also in *Brachypodium distachyon* and wheat. The application of localization microscopy, the fastest evolving technique in super-resolution fluorescence microscopy, allowed visualization of polymer rearrangements in nanoscale 3D networks and facilitated in situ structural analysis of callose synthase complexes in live cell imaging. With this, in situ live cell super-resolution microscopy represents a new tool in bridging the gap between the molecular and systems level in plant pathology. Understanding the subcellular and molecular mechanisms of plant cell wall biosynthesis and architecture also on a structural level will lead to the identification of key modulators and factors for cell wall engineering to successfully design crops with improved pathogen resistance.

Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion

A. Taylor[1], A.C. Jackson[1], G. Teakle[1], A.D. Armitage[2], R. de Heer[3], N. Zhang[3], W. van Leeuwen[3], S. Ott[4], L. Baxter[4], B. Greenfield[2], R.J. Harrison[2] and J.P. Clarkson[1]
[1] Warwick Crop Centre, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK; [2] NIAB-EMR, New Road, East Malling, Kent, ME19 6BJ, UK; [3] Hazera Seeds B.V., Schanseind 27, 4921 PM MADE, The Netherlands; [4] University of Warwick, Coventry, CV4 7AL, UK.

Email: andrew.taylor@warwick.ac.uk

Bulb onion (*Allium cepa* L.) is a globally important crop but the lack of a genome sequence has hampered genetic research. *Fusarium oxysporum* is an important soilborne pathogen of many crops and comprises different *formae speciales* (f.spp.) that infect particular hosts. In onion, *F. oxysporum* f.sp. *cepae* (FOC) is a major constraint to worldwide production, principally causing a basal rot of bulbs with estimated losses of £11M per annum in the UK. As FOC produces chlamydospores that survive in soil for many years, disease management is challenging and therefore developing resistant cultivars is highly desirable. Initially, onion lines with high levels of basal rot resistance were identified within a unique diversity set. New onion populations segregating for FOC resistance were then developed, genotyped using KASP markers and QTLs identified linked to resistance. Associated genetic markers are now being developed to accelerate breeding of basal rot resistant varieties. In parallel research, the genetic basis for pathogenicity in FOC was investigated and the presence of putative effectors identified by genome sequencing and PCR using a range of pathogenic and non-pathogenic *F. oxysporum* isolates from onion. The presence of 'secreted in xylem' (SIX) effector genes, which have homologs in other *F. oxysporum* f.spp., was clearly associated with pathogenicity and these genes were strongly upregulated during infection. Effector gene complements and sequences may also provide the basis for a diagnostic test for FOC. Knock-out mutant strains have now been developed for several effector genes as a first step toward functional analysis.

Understanding appressorium development in *Magnaporthe oryzae* using analysis of network-extracted ontologies and pathogen transcriptomics.

Ryan M Ames
Wellcome Trust Centre for Biomedical Modelling and Analysis, Living Systems Institute, University of Exeter, Stocker Road, Exeter, EX4 4QD, UK
Email: r.ames@exeter.ac.uk

Magnaporthe oryzae is the causal agent of rice blast disease, the most important infection of rice worldwide. Half the world's population depends on rice for its primary caloric intake and, as such, rice blast poses a serious threat to food security. The stages of *M. oryzae* infection are well defined, with the formation of an appressorium, a cell type that allows penetration of the plant cuticle, particularly well studied. However, many of the key pathways and genes involved in this disease stage are yet to be identified. In this work, I have used network-extracted ontologies (NeXOs), hierarchical structures inferred from RNA-Seq data, to identify pathways involved in appressorium development, which in turn highlights novel genes with potential roles in this process. This study illustrates the use of NeXOs for pathway identification from large-scale genomics data and also identifies novel genes and pathways with potential roles in disease. The methods presented here will be useful to study disease processes in other

pathogenic species and these data represent predictions of novel targets for intervention in *M. oryzae*.

Integrated deployment of host resistance and fungicides: managing evolution of virulence and insensitivity

Frank van den Bosch
Department of crop protection, Rothamsted Research, Harpenden, AL5 2JQ, United Kingdom
Email: Frank.vandenbosch@rothamsted.ac.uk

Sustainable control of plant pathogens is maintained by two highly dynamic interactions: firstly between the introduction of new host resistance genes and pathogen populations overcoming them; secondly between the introduction of new fungicide modes of action and pathogen populations becoming insensitive to them. Virtually all studies on sustainable disease control study one of the control options exclusively. This is surprising, given the widely held belief that integrated control will lead to more durable control than reliance on one control option. In practice both disease control options are integrated, but the effects of integration on the sustainability of disease control are not sufficiently understood to predict which integration strategies will be most effective.
We introduce a governing principle that predicts the direction of selection for fungicide insensitivity and the direction of selection for pathogen virulence under a range of disease management options. The predictions are compared to exiting evidence. This leads us to formulate two strategies improving durable disease control:

1. Deployment of fungicides reduces selection for virulence.
2. Deployment of crop resistance reduces the selection for fungicide insensitivity.

Using this mechanistic understanding about the effect of integration then leads us to hypothesise that it is more sustainable to integrate and balance chemical and genetic crop protection, than to attempt to replace chemical with genetic control or rely entirely on chemical control. Results of modelling studies on two contrasting patho-systems will be used here (and in the paper by Paveley) to explore the practical impact of exploiting the mutual protection mechanism.

Integrated deployment of host resistance and fungicides: economics, efficacy and evolution

Neil D. Paveley
ADAS, High Mowthorpe, Duggleby, Malton, North Yorkshire, YO17 8BP, UK
Email: neil.paveley@adas.co.uk

The aim of disease control is to protect marketable yield in order to minimise the unit cost of crop production. But control by host resistance and fungicides creates selection pressure for pathogen evolution towards virulence and fungicide insensitivity. The commercial and societal need is to balance between achieving cost-effective control in the short term and managing pathogen evolution to ensure control is durable in the long term. The ability to control diseases in some major European crops is tenuously balanced currently. Frank van den Bosch (these proceedings) describes why integrating genetic and chemical control is likely to be more durable than either alone.
Farm uptake of integrated control in major crops depends largely on the short-term economics (yield value minus fungicide costs) of growing disease resistant or susceptible, but sometimes higher yielding, cultivars. The challenge for pathologists, crop physiologists and plant breeders is to minimise the 'yield drag', which can be associated with breeding for disease resistance, so that resistant varieties are more economically attractive. Data from field experiments and analysis by mathematical modelling show that: (i) the combined efficacy of host resistance and fungicides is predictable using a simple multiplicative survival approach, enabling the design of optimal integrated control programmes, (ii) disease forecasting provides greater economic benefits on partially disease resistant, than susceptible, varieties, and (iii) the combination of variety resistance and forecasting to guide fungicide treatment, can reduce the selection pressure for evolution of fungicide insensitive strains, thus increasing long term durability of control.

Fenpicoxamid (Inatreq™ active fungicide), a new fungicide with utility in cereals for control of *Zymoseptoria tritici* (Septoria leaf blotch) and other diseases

Andrew J. Leader[1] and Gregory M Kemmitt[2]
[1]Dow AgroSciences, CPC2 Capital Park, Cambridge, United Kingdom; [2] Dow AgroSciences, 3B Park Square, Milton Park, Abingdon, Oxfordshire, United Kingdom
Email: aleader@dow.com

Fenpicoxamid (Inatreq™ Active) is the first molecule from a new class of fungicides (picolinamides). Fenpicoxamid is derived from a natural compound UK-2A which is produced by fermentation of an Actinomycete (*Streptomyces spp.*). UK-2A is subject to minor alteration post fermentation to produce Fenpicoxamid and impart additional stability to the molecule. Inatreq inhibits fungal respiration in the mitochondria at the Qi ubiquinone reductase binding site in complex III, which is a new target site within the cereal fungicide segment. Most importantly, Inatreq shows no cross-resistance to any existing cereal fungicide chemistries, including triazoles, strobilurins or SDHIs and will be an important new tool in the armoury of cereal growers.
In the European cereals market, *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*, Septoria leaf blotch), the pathogen of greatest concern in terms of yield losses in winter wheat production, has developed widespread resistance to strobilurins class and significant sensitivity loss to the triazoles. Recently isolates with reduced

sensitivity to SDHI's have been detected in the UK and Ireland. An additional concern is the possibility that some molecules that are widely used today will be under regulatory pressure and may exit the market thus reducing the number of solutions available to growers. Fenpicoxamid will be an innovative and effective tool in helping farmers who face increased resistance threats from *Z.tritici* with existing technologies. However, as fenpicoxamid is a single site inhibitor, the product should only be used in combination with other actives to minimize the risk of resistance development.

Controlling Strawberry Powdery Mildew requires all year round vigilance

Hall, A.M., Jin, X., Liu, B.
Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB
Email: a.m.hall@herts.ac.uk

Strawberry Powdery Mildew caused by *Podospheara aphanis* is the most feared disease of protected strawberries in the UK, causing losses of 20% to 70% of the crop per annum, which results in the loss of £56.8 million with only 20% loss. Work at the University of Hertfordshire since 2003/4 has resulted in the development of an all year round control strategy, which, if followed by growers, can result in satisfactory disease control. Commercially, there are many different growing systems for strawberries; in some cases the plants are 'perennial', planted for 3 winters and 4 harvests, in other cases they are planted in February/ March and grown until September / October. In the case of overwintered crops, the disease can overwinter as chasmothecia, whereas plants arriving from the propagators are rarely clean. Controlling this initial inoculum is vital. There is heavy disease pressure in July, August and September, but the epidemics can be reduced by a) the use of a silicon nutrient and b) the use of a prediction system based on disease-conducive conditions of temperature and humidity. Chasmothecia start forming on leaves in August and September and give rise to the overwintering inoculum on the perennial crops. These can be controlled by using a fungicide after harvest has finished, and again in the spring, as the crop is covered with fleece. The research reported here has resulted in an updated fact sheet for growers, which includes an integrated programme for all year round disease control.

Closing Plenary Talks

The Science of Quorn

Rob Johnson
Science Manager- Fermentation Specialist, Quorn, Station Road, Stokesley, North Yorks, TS9 7BR, UK
Email: rob.johnson@quornfoods.com

Quorn has a mere 50 year history and is the first new food type to enter the market since the potato. This talk will give an over view of the history of Quorn, its relevance to the modern world and move on to the science behind the product, including how it is made, where the texture comes from, and what challenges are being addressed for the future. Covering genetics, fermentation, environmental impact and health benefits an overview of one of the fastest growing food products in the world will be outlined.

Fungal weapons to manipulate plants

Natalia Requena, Meike Hartmann, Ruben Betz, Sven Heidt and Carolin Heck
Molecular Phytopathology department, Karlsruhe Institute of Technology, Karlsruhe, 76228, Germany
Email: Natalia.requena@kit.edu

The arbuscular mycorrhiza (AM) symbiosis between Glomeromycotina fungi and most plant roots is one of the most ancient and widespread symbioses on earth. AM fungi provide phosphate to their hosts in exchange for carbon and the establishment and maintenance of the symbiosis requires a complex communication exchange between symbionts and with the environment, because high concentrations of Pi inhibit the symbiosis. Interestingly, AM fungi are able to colonize the majority of land plants in contrast to most pathogenic fungi that have a quite narrow host range. We hypothesize that AM fungi employ conserved effector proteins to rewire the plant physiology. We previously identified the effector protein SP7 and a group of related proteins (SP2, SP5 and SP31) from *Rhizophagus irregularis*. All members of this family show the same basic protein structure with tandem hydrophilic and imperfect repeats and a nuclear localization signal. Here we show that all three effectors redundantly modify the plant phosphate starvation response (PSR). Expression in roots of any of them leads to accumulation of pre-miRNA399s in leaves that ultimately downregulates ubiquitin E2 conjugase PHO2 in roots causing increased PSR. We propose that this mechanism allows the fungus to colonize the root system further despite a net-increase in phosphate. Interaction and localization assays suggest that these effectors modulate the PSR by intersecting with the plant mRNA translation machinery. These results highlight the finesse of symbiotic arbuscular mycorrhizal fungi to manipulate the plant cell program,

guaranteeing their permanence in the root while contributing to plant health and nutrition.

POSTERS

*J Colhoun Competition entrant
**BMS Student Poster Competition entrant

Effects of Leaf Extracts of *Ocimum gratissimum* L. on Quality of Fresh Cut *Cucumis sativus* L.

Oluwagbenga O. Adeogun, Alfred Maroyi and Anthony J. Afolayan
Department of Botany, University of Fort Hare, Alice, Eastern Cape, South Africa
Email: adeogunoluwagbenga@gmail.com; oadeogun@ufh.ac.za

High moisture content and minimal processing of fruits and vegetables encourage microbial incursion and these raised the need for preservation. *Ocimum gratissimum* leaves were extracted with ethanol and hexane separately. The extracts were used to enhance the quality of fresh cut *Cucumis sativus* stored for 9 days using dipping method. Qualitative and quantitative phytochemical analyses of the extracts were determined and essential oil constituents were established using GC-MS. The results showed that the moisture contents of *C. sativus* with extracts were lower compared to stored *C. sativus* without extracts. The acidity of *C. sativus* with extracts was lower in relation to the test sample without extracts. The increase in total soluble solid was higher in *C. sativus* without the extracts. The ascorbic acid, carotenoids and phenolic contents of stored *C. sativus* with extracts had lower range of reduction compared to *C. sativus* without the extracts. The differences in potential browning and turbidity of stored *C. sativus* with extracts during storage were not obvious. There were higher loads of microbes in *C. sativus* without extracts compared to *C. sativus* stored with extracts. The result of the phytochemical screening showed the presence of flavonoid, alkaloid, cardiac glycoside, phenol, tannin, steroid, anthraquinone and saponin in the leaf. Some of the essential oil constituents are γ-Terpinene, Caryophyllene, Oleic acid and (E) - 9-Octadecenoic acid. This study was able to establish that the leaf extracts of *O. gratissimum* have bio-preservation potential that can be used to enhance the shelf of *C. sativus*.

Integrating fungicides with varietal resistance against Septoria tritici blotch in wheat

Olubukola. O. Ajigboye, Dimitra J. Angelopoulou, John Foulkes, Rumiana. V. Ray
School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom
Email: bukky.ajigboye@nottingham.ac.uk

Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici*, is the most important foliar disease of wheat, reducing grain yield by up to 50%. Symptoms often occur following a latent phase between 5-10 days beyond which fungicide effectiveness is impaired. Varietal resistance is not yet fully utilised in field although there are number of commercially available wheat cultivars with moderate to high field resistance. Integrating preventative fungicide applications with varietal resistance is essential to optimize control of STB. The objectives of the present study were to determine the best timing and fungicide against STB for two different wheat varieties, one susceptible cv. Dickens and the resistant cv. Cougar. Field experiments were

conducted during two seasons (2016/17) using natural infection by *Z. tritici*. Combinations of fungicides including multi-site, demethylation and succinate dehydrogenase inhibitors were applied at different crop growth stages (GS) 30, 31/32, 33, 37/39 or 59/61.
The effect of timing and fungicide treatment on the progress and severity of STB in the canopy were assessed at GS 31, 61 and 75 while fungal biomass of *Z. tritici* was quantified in asymptomatic flag leaves at GS 61. These parameters along with the crop photosynthetic efficiency, biomass and final grain yield were used to identify the best strategies for integrating fungicide timing, mode of action and varietal resistance.

Acknowledgments
This study is part of collaboration work between the University of Nottingham, Agrii and RAGT Seeds, co-funded by Innovate UK.

***Powdery Mildew: A Tale of Two Fungicides**

Corinne J. Arnold, Diane G. O. Saunders and James K. M. Brown.
Crop Genetics, John Innes Centre, Norwich, NR4 7UH, UK
Email: Corinne.Arnold@jic.ac.uk

Blumeria graminis is a powdery mildew pathogen that infects many wild grasses and cereals across the world. If left uncontrolled, it can cause significant yield losses in cereals. One main method for control is the application of fungicides but resistance to strobilurin and older triazole fungicides already occurs in *B. graminis*, and few other fungicides are available for mildew control. I have characterised isolates from an outbreak of *B. graminis* f.sp. *tritici (Bgt)* on wheat where two of the major fungicides used against *Bgt* were ineffective: cyflufenamid (Cyflamid), an eradicant fungicide with an unknown mode of action, and fenpropimorph (Corbel), a morpholine fungicide targeting a sterol reductase. All *Bgt* isolates from the study site were completely resistant to Cyflamid sprayed at the recommended field rate. However, over the following two years, the *Bgt* population recovered sensitivity to Cyflamid, suggesting that resistance may have a significant fitness penalty. Additionally, following two sprays of Corbel, these *Bgt* isolates had significantly lower sensitivity to fenpropimorph than field isolates. Overuse of fenpropimorph would almost certainly lead to strong resistance in mildew, so it should be used as part of a rotation of fungicides with different modes of action. Likewise, Cyflamid can continue to be used as part of an integrated disease control programme. Genetic and genomic analysis is in progress, with the aim of identifying candidate genes for resistance to cyflufenamid and to fenpropimorph.

****Use of bulk segregant analysis to analyse azole drug resistance in *Aspergillus fumigatus***

George D. Ashton[1], Céline M. O'Gorman[1], Daniel Zadik[2], Martin Blythe[2], Victoria Wright[2], Simon V. Avery[1] and Paul S. Dyer[1]

[1]School of Life Sciences, University of Nottingham, Nottingham, NG7 2UH, United Kingdom; [2]DEEPSeq facility, University of Nottingham, Nottingham, NG7 2UH, United Kingdom

Email: stxgdas@nottingham.ac.uk

Aspergillus fumigatus is an opportunistic human fungal pathogen causing aspergilliosis. Infections are controlled through the use of azole antifungal drugs. Over the past 20 years there has been an increase in occurrence of azole resistant strains. Resistance is due to a variety of different genetic mechanisms, some of which have been characterized in clinical and environmental isolates. *A. fumigatus* was once thought to reproduce entirely asexually. However, a sexual cycle was discovered in 2009. The fungal sexual cycle is a powerful tool and can be exploited in a number of different areas. It was hypothesized the sexual cycle in *A. fumigatus* might be used to identify azole resistance genes using the technique 'bulk segregant analysis' (BSA) in cases where the mechanism of resistance is unknown. The process of BSA involves a sexual cross being performed between an azole resistant and azole sensitive strain. Following this, a series of backcrosses between the resistant progeny and sensitive parent are performed to minimize genetic differences between them, with the azole resistance gene(s) and any linked genes being the only differing regions. Genome sequencing is then performed on two pools of DNA: one of progeny exhibiting azole resistance; and one showing azole sensitivity, as well as genome sequencing of the sensitive parent. From this, the aim is to identify key areas and genes that may be causing azole resistance in these isolates. This may lead to the development of useful diagnostics to monitor azole resistance and improve the treatment of azole-resistant *A. fumigatus* infections.

An investigation into silicon localization in strawberry plants linked to reduction in disease susceptibility to *Podosphaera aphanis

C. I. Asiana, A. M. Hall and K. Davies

Department of Biological and Environmental Sciences, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

Email: i.asiana@herts.ac.uk

The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by *Podosphaera aphanis,* which has to be controlled by frequent use of fungicides. Silicon is not considered as essential for the plant; however, work carried out at the University of Hertfordshire has shown that weekly use of a silicon nutrient in fertigation tubes at a commercial strawberry farm results in reduced susceptibility to this disease. Silicon can only be taken up in a bioavailable form and the nutrient used in

this experiment is a bioavailable form of silicon. A silicon localization experiment was conducted. Silicon was applied via the soil in the glasshouse, the fertigation tubes at the commercial farm and was sprayed on in a field plot.
In this study, cross sections of leaves, petioles and roots were stained with a fluorescence dye, "lyso tracker yellow HCK-123". Samples were examined using a confocal microscope at x400 and a wavelength 450nm. So far, results showed in the leaf, silicon was found in the cuticle, epidermis, palisade layer, stomata and vascular tissue. In the petiole, silicon was found in the epidermis and xylem and in the roots, the silicon was found in the epidermis, endodermis and vascular tissue. The fluorescence intensity of the cross sections was quantified and this shows that the silicon was 5 times higher in the treated plants than the untreated. In addition, the silicon fertigation field trial has shown that plants with higher levels of silicon are less susceptible to the disease.

Chrysanthemum Chlorotic Mottle Viroid mediated Trafficking of Foreign mRNA into Chloroplasts

Eseul Baek[1], Minju Park[1], Ju-Yeon Yoon[2] and Peter Palukaitis[1]
[1]Dept. of Horticultural Sciences, Seoul Women's University, Seoul, 01797, Republic of Korea; [2]National Institute of Horticultural & Herbal Science, RDA, Wanju, 55365, Republic of Korea
Email: eseul@swu.ac.kr

Chrysanthemum chlorotic mottle viroid (CChMVd), a member of the family *Avsunviroidae*, was identified in chrysanthemum plants causing chlorotic leaf symptoms. To show that CChMVd can traffic into chloroplasts, which is thought to be the site of its replication, CChMVd was fused to the leader sequence of a reporter gene encoding monomer red fluorescence protein (mRFP) and expressed transiently in agroinfiltrated *Nicotiana benthamiana*. Fluorescence from mRFP was observed in chloroplasts by confocal microscopy, but only if the viroid transcription fusions were present. The viroid components of the fusions were full-length 400-nt CChMVd, or one of two partial fragments (nucleotides 125 to 2 and 231 to 372). The mRFP and its mRNA were detected by western blotting and RT-PCR, respectively, in whole tissue extracts of plants infiltrated by each fusion construct. Isolated chloroplasts were shown by RT-PCR to contain the RNA sequences of both CChMVd and mRFP, if both were present, but not the mRFP sequence in the absence of the viroid sequences. Eggplant latent viroid (ELVd), in the same family *Avsunviroidae*, has been shown to traffic mRNA into chloroplasts for expression of a fluorescent marker protein in previous studies (1-3). Since there are differences in sequence between ELVd and CChMVd, it suggests that the ability of both RNAs to traffic RNAs into chloroplasts was probably due to an RNA structure, and not a particular sequence.

1. Gómez, G., Pallás, V. (2010). PLoS One 5 (8): e12269.
2. Gómez, G., Pallás, V. (2010). Plant Signl. Behav. 5 (11): 1517-1519.
3. Gómez, G., Pallás, V. (2012). Plant Physiol. 159: 558-564.

Work was funded by the Rural Development Agency of the Republic of Korea.

Generation of wheat plasma membrane reporter lines to further understand fungal infection mechanisms.

Laura Baggaley[1], Alison Huttly[1], Caroline Sparks[1], Smita Kurup[1], Christine Faulkner[2], Michael Deeks[3] and Kim Hammond-Kosack[1]
[1] Rothamsted Research, Harpenden, AL5 2JQ, UK; [2] John Innes Centre, Norwich, NR4 7UH, UK; [3] University of Exeter, Exeter, EX4 4QD, UK
Email: laura.baggaley@rothamsted.ac.uk

The fungus, *Fusarium graminearum,* is the primary cause of floral cereal infections worldwide, resulting in reductions in wheat grain quality, quantity and adversely affecting human, animal and ecosystem health. How *F. graminearum* establishes infection within wheat has been well studied, however what causes the transition between the asymptomatic and symptomatic phases remains unclear. This transition in infection corresponds with a shift from primarily inter- to intracellular fungal growth, leading to extensive cell death. It has been hypothesised that this change in fungal growth is due to the utilisation of plasmodesmata – thin streams of cytoplasm between adjacent plant cell walls vital for cell-to-cell communication.
Identification of the cellular and molecular mechanisms involved in the *F. graminearum*-wheat floral interaction has previously relied upon histological staining and fungal reporter strains [1]. We have attempted to create two wheat plasma membrane reporter lines, using the genes ROP7 [2] and LTI6b, to allow for a more efficient, detailed, spatio-temporal study, of the infection process. Due to the autofluorescent nature of wheat tissues, tissue specific autofluorescent profiles have also been established using confocal microscopy, to aid image capture and analysis. This new information will be critical for the evaluation of the wheat plasma membrane reporter lines and fungal reporter strains.
Together these new tools and datasets will allow for the identification and characterisation of infection mechanisms in wheat, allowing for a greater understanding of multiple crop-yield limiting pathogens.

[1] Brown *et al.* (2010) *Fungal Biology* 114: 555-571
[2] Kirienko *et al.* (2012) *Plant Physiology* 159: 1309-1318

How to control leaf diseases of faba beans?

Biruta Bankina, Gunita Bimšteine and Arturs Katamadze
Institute of Soil and Plant Sciences, Latvia University of Agriculture, Jelgava, LV 3001, Latvia
Email: Biruta.Bankina@llu.lv

Faba bean (*Vicia faba* L. var *minor*) has become an important field crop in Latvia during the last five years. Four *Botrytis* species as the causal agents of bean diseases have been determined: *B*. *fabae*, *B*. *fabiopsis*, *B*. *cinerea*, and *B*. *pseudocinerea*. Also, several species of *Alternaria* and *Stemphylium* have been found. The aim of the present study was to clarify the efficacy of fungicide application. Trials were conducted at the Research and Study farm "Peterlauki" of the Latvia University of Agriculture in 2015–2016. A three-factor four-replicate trial was established: factor A – variety ('Laura', 'Boxer', 'Isabell'); factor B – sowing rate (30, 40, 50 germinating seeds per m^2); and factor C – fungicide (boskalid 267.0 g kg^{-1} and pyraclostrobin 67.0 g kg^{-1}) 0.75 kg ha^{-1} at the beginning of flowering. Disease assessment was started when the first symptoms occurred: 20 random plants were chosen in each plot, and disease severity was noted (0–9 points scale). Total impact of leaf diseases during vegetation was evaluated by calculating the AUDPC (area under disease progress curve). Fungicide application significantly (P<0.0001) decreased the disease level: efficacy of chocolate spot control was 21.3% in 2015 and 31.7% in 2016, and efficacy of Alternaria leaf blotch control was 40.7% and 35.3% respectively. Further investigations are necessary to essentially improve the efficacy of fungicide application.

Supported by the State Research Programme "Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia": project "Sustainable use of soil resources and abatement of fertilisation risks".

Host and nonhosts interactions of powdery mildews with various crop plants

Balázs Barna, Csilla Juhász, Adél Németh and Gábor Gullner
Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, P. O. box 102, Hungary
Email: barna.balazs@agrar.mta.hu

Powdery mildews are one of the most devastating pathogens of crops all over the world. Triticale, an artificial hybrid of wheat and rye, was resistant to powdery mildew until 2001, but since then this fungus become a serious triticale disease in Europe. Recently it was found that *Blumeria graminis* f. sp. *triticale* is a hybrid between wheat and rye powdery mildews. None of 11 tested triticale genotypes showed susceptibility to our wheat powdery mildew isolate. In order to get information on the role of protein kinases and phosphatases on mildew development the effect of pre-treatment of host plants with the inhibitors staurosporine or okadaic acid was evaluated. Furthermore, the

electrophoretic ribonuclease (RNase) enzyme patterns of barley and wheat were very different from each other and from *Brachypodium distachyon* plant extracts that showed high RNase activity. All investigated host and non-host plant species reacted with an increase of RNase activity to powdery mildew or rust infections. We found that heat pre-pretreatment increased both the susceptibility of barley to powdery mildew and its RNase activity, which confirms the possible role of RNases in susceptibility. Interactions of *Golovinomyces orontii* with various tobacco lines induced a strong increase of RNase enzyme activity, and a new RNase band appeared in the extracts from all infected tobaccos following PAGE and specific staining. Interestingly, powdery mildew infections up-regulated the expression of *PR-1b*, *PR-1c* and *WRKY12* genes in all susceptible tobacco lines to various extents. The possible role of the above changes in plant-powdery mildew interaction is discussed.

Phenotypic, comparative genomic and transcriptomic analysis of recently collected European *Zymoseptoria tritici* strains.

Hongxin Chen[1], Carlos Bayon[1], Robert King[2], Stefano Torriani[3], Stephane Bieri[3], Kostya Kanyuka[1], Kim E. Hammond-Kosack[1] and Jason J. Rudd[1]

[1]Department of Biointeractions and Crop Protection and [2]Department of Computational and Analytical Sciences, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom; [3]Syngenta Crop Protection AG, Schaffhauserstrasse, CH-4332 Stein, Switzerland

Email: hongxin.chen@rothamsted.ac.uk

Septoria leaf blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici*, is one of the most important foliar diseases of wheat, especially in Europe and USA. Due to the poor genetic based resistance of wheat to this pathogen, extensive application of fungicides is currently the most common way to control STB. This has led to rapid evolution of fungicide resistance in many *Z. tritici* populations. To ascertain the variability in virulence profiles of a collection of recent isolates of *Z. tritici* collected from European field sites, 42 strains were inoculated onto 21 wheat cultivars, along with the reference strain IPO323 as a comparison. Disease progress was monitored daily and dates were recorded when 1st disease symptom appeared and full necrosis developed. Fungal sporulation was also measured for each interaction. The results show diverse virulence profiles of different isolates and a range of interaction outcomes. Many of the tested isolates displayed enhanced virulence relative to IPO323. No obvious correlation was found between virulence and sporulation, indicating these two traits might be under the control of different genetic loci. To better understand the genetic basis of differential virulence, genomic DNA of the top 12 most virulent isolates will be subjected to full genome sequencing, and RNAseq based gene expression analysis of *in vitro* cultures, and *in planta* samples, will be performed.

Defining the impact of autophagy on the host-pathogen interaction of wheat and the fungus *Zymoseptoria tritici*

Harry T. Child[1], Steven Bates[1], Jason J. Rudd[2] and Ken Haynes[1]
[1]Biosciences, University of Exeter, Exeter, EX4 4QD, United Kingdom; [2]Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, United Kingdom
Email: hc515@exeter.ac.uk

The fungal wheat pathogen *Zymoseptoria tritici* causes major crop losses and currently demands €1 billion worth of fungicide to control in Europe annually. The infection cycle of *Z. tritici* displays two distinct phases, beginning with an extended symptomless phase of at least a week, before the fungus induces host cell death and tissue collapse in the leaf. Recent evidence suggests that the fungus uses little host-derived nutrition during the early stages of infection, instead relying on macromolecules within the fungal spore. We hypothesise that *Z. tritici* utilises the self-degradative process of autophagy to recycle stored cellular resources to support establishment of leaf colonisation. Using molecular techniques, this research will assess the importance of the autophagy pathway during *Z. tritici* cellular differentiation and infection of wheat. Manipulating the genes involved in autophagy will reveal its importance to fungal virulence, while fluorescently tagging key autophagy proteins will visualise the spatial and temporal occurrence of autophagic processes in vivo. This research will provide fundamental knowledge of the molecular mechanisms of *Z. tritici* infection as well as identifying potential new targets for control of this devastating disease.

***Resistance to Rhynchosporium: Characterisation and identification of candidate genes**

Max Coulter[1], Mark Looseley[1], Robbie Waugh[2] and Anna Avrova[1]
[1]The James Hutton Institute, Invergowrie, Dundee, UK; [2]University of Dundee, Dundee, UK
Email: Max.Coulter@hutton.ac.uk

Rhynchosporium commune, causing leaf scald, is one of the most destructive pathogens of barley worldwide, leading to yield losses of up to 30-40%. Despite advances in molecular marker technology and the sequencing of the barley genome, understanding of barley resistance to *Rhynchosporium* at the molecular level is limited.
The Spanish landrace CIho3515 is highly resistant to scald, containing resistance genes *Rrs1* and *Rrs18*. *Rrs1* was the first R gene against *Rhynchosporium* to be identified, while *Rrs18* has only recently been characterised. *Rrs18* has also been identified in a Steptoe x Morex DH population, and a large Steptoe x Morex BC1 population has been used to fine map the gene to a 650Kb interval on 6HS, identifying 17 candidate genes. To identify a candidate for *Rrs1*, Association mapping using elite spring barley cultivars and Syrian and Jordanian landraces has narrowed the *Rrs1* mapping interval to a single gene

on 3H, a Wall associated Kinase (WAK). Analysis of exome capture data for multiple cultivars carrying *Rrs1* showed evidence of a WAK paralogue not present in the susceptible Morex reference sequence. PCR and Sanger sequencing was carried out to decipher the sequence of the WAK paralogue with the aim of cloning *Rrs1*. Cloning of both *Rrs18* and *Rrs1* will further our understanding of *Rhynchosporium* resistance and enable diagnostic markers to be developed helping breeders to pyramid both genes to create durable resistance to *Rhynchosporium* in barley.

***Isolation and characterisation of a chrysovirus from *Dothistroma septosporum*, the causative agent of pine needle blight**

John Daudu[1], Unnati Shah[1], Kotta-Loizou Ioly[2] and Robert H.A. Coutts[1]
[1]Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK; [2]Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK
Email: j.o.daudu@herts.ac.uk

Dothistroma needle blight caused by *Dothistroma septosporum* has emerged in the British Isles as a major threat to Corsican pine, lodgepole pine and Scots pine. There is increasing evidence that mycoviruses can reduce the growth and pathogenicity of fungal plant pathogens. For instance, a virulence-attenuating RNA molecule, *Cryphonectria hypovirus 1* (CHV1) has been described and successfully used as a biological control agent of *Cryphonectria parasitica*. The aim of the present study is to isolate and characterise double-stranded (ds) RNA viruses present in a collection of *D. septosporum* isolates. To this end 22 *D. septosporum* isolates were screened using a rapid small-scale dsRNA extraction process and only one was found to harbour dsRNA elements. Subsequently these elements were cloned and sequenced, using targeted sequencing and RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE). Computational analysis of the sequences obtained revealed their similarity to members of the established family *Chrysoviridae*, which have 4 genomic segments encapsidated in isometric particles and are known to elicit hypovirulence in their hosts. Generation of isogenic lines of virus-free and virus-infected isolates is in progress to determine any effects of the mycovirus on fungal fitness and pathogenicity; the pathogenicity of the virus-infected isolate will be assessed in comparison to the virus-free one, in order to detect putative hypovirulence, a common feature noted for mycoviruses, which might be used for biological control to invasion by more aggressive strains of *D. septosporum*. In conclusion, this study reports the first mycovirus ever found in *D. septosporum*.

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

Judith Sheldon, Jill Foundling, Mathias Blum, Sian Deller, David Beattie and Mike Csukai
Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire. RG42 6EY. UK
Email: sian.deller@syngenta.com

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

Anti-protozoal agents identified in the Pathogen Box can inhibit *Aspergillus fumigatus* growth

Thaila Fernanda dos Reis and Gustavo H. Goldman
Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil.
E-mail: thailaf@hotmail.com

Aspergillus fumigatus is an opportunistic human fungal pathogen and allergen responsible for invasive aspergillosis, an important infection which causes a high level of mortality in immunocompromised patients. Although the aspergillosis is mainly treated by administration of triazoles, epidemiological studies have indicated that the emergence of azole-resistant *A. fumigatus* isolates has increased significantly over the last decade. Despite the growing needs, treatments for invasive fungal infections remain unsatisfactory and the discovery of novel antifungals drugs is highly desirable. The Pathogen Box (https://www.pathogenbox.org/) is a library composed by 400 synthetic chemicals molecules with activity against different pathogens responsible for important diseases such as malaria, tuberculosis, toxoplasmosis, and others. Aiming to discover new potential agents against aspergillosis we screened the Pathogen Box chemical compounds for antifungal activity against *A. fumigatus.* We identified miltefosine (MMV688990) as an antifungicidal agent against aspergillosis. Using *in vitro* assays, we demonstrated that this compound has a MIC (Minimal Inhibitory Concentration) of 10

μM. Interestingly, a null mutant in the cAMP dependent protein kinase Scha^{SCH9} has a MIC of 20uM. This protein kinase influences sphingolipids biosynthesis, suggesting that miltefosine could act inhibiting the fungal growth via targeting the lipids/sphingolipids synthesis. Additionally, iodoquinol (MMV002817) showed an antifungistatic action with a MIC of 2 μM. Based on our results, we propose that these two compounds could represent potential candidates for further antifungal drug development against invasive aspergillosis.
Financial support: FAPESP and CNPq, Brazil

Functional characterization of the *Aspergillus nidulans* non-essential protein kinases involved in the regulation of sorbic acid antifungal properties

Thaila Fernanda dos Reis[1], Ivey Geoghegan[2], Simon Avery[2] and Gustavo H. Goldman[1]
[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil; [2]School of Life Sciences, University of Nottingham University Park, Nottingham NG7 2RD, UK.
E-mail: thailaf@hotmail.com

Due to a constantly increasing of the world population, the demand for food is also on the rise. However, a high percentage of this food is lost due, partially, to foodstuff spoilage by microorganisms, including filamentous fungi. Weak organic acids such as sorbic, benzoic, propionic and acetic acid are used as antimicrobial agents in order to prevent food and beverage spoilage caused by microorganisms. Unfortunately, the microorganisms show a great resistance to weak acid preservatives and the emergence of resistant population is often reported. The exact mechanism behind the antifungal properties of these compounds is still unknown. Aiming to better understand the mechanisms that control the sorbic acid antifungal properties and identify potential targets of this weak acid, we screened an *A. nidulans* null mutant library composed of 103 non-essential protein kinases. A total of 16 null mutants had altered growth in presence of different sorbic acid concentrations. Interestingly, 03 of them that correspond to genes encoding proteins involved in the High Osmolarity Glycerol (HOG) pathway were sensitive to sorbic acid. We observed that *A. nidulans* HogA phosphorylation was modulated in the presence of sorbic acid suggesting the involvement of this pathway in this process. The acquisition of more details about the mechanisms involved in the antifungal properties of sorbic acid as well the involvement of the HOG pathway in this process will clearly help on the devastating impact of fungal food spoilage on global food security.
Financial support: BBSRC (United Kingdom), FAPESP and CNPq (Brazil)

Frontiers in the understanding and management of *Armillaria* in UK gardens

Jassy Drakulic, Matthew Cromey, Liz Beal[1], Gerard Clover
Plant Health, Royal Horticultural Society, RHS Garden Wisley, Woking, GU23 6QB, UK;
Email: jassydrakulic@rhs.org.uk

Honey fungus root rot, caused by *Armillaria* spp., is the disease most frequently enquired about by UK gardeners to the Royal Horticultural Society (RHS). *Armillaria* epidemiology has been studied within forestry and agriculture, but data is lacking within gardens, which have greater host plant diversity than orchards and vineyards and greater disturbance than woodlands. Surveys of material sent to the RHS by members has helped answer questions about which *Armillaria* species are responsible for the disease in gardens, and how plants from the broad range of susceptible ornamentals are differentially affected by the disease. Further research directions within the RHS focus on evaluation of the principles of management practices such as studies determining the lifespan of rhizomorphs of different size when removed from a food source, screening of *Trichoderma* endophytes for use as biocontrol, and elucidating the nature of the interactions between different *Armillaria* species of differing aggressiveness to host plants. Full results from the survey are presented alongside preliminary findings from the current research projects listed above.

Aphid infestation increases susceptibility of *B. napus* to *Rhizoctonia solani* AG 2-1

Fryni Drizou[1], Toby JA Bruce[2] and Neil S Graham[1]
[1]Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, UK; [2] School of Life Sciences, Keele University, Keele, Staffordshire, ST5 5BG**,** UK
Email: Fryni.Drizou@nottingham.ac.uk

Oilseed rape (OSR), *Brassica napus*, is the primary host of *Rhizoctonia solani* AG 2-1, a soil-borne pathogen that infects young seedlings causing damping-off disease and impaired crop establishment. Currently there is no available resistant germplasm and it is unknown how the pathogen overcomes plant defences. Additionally, OSR is one of the secondary hosts of *Myzus persicae*, an aphid known for its ability to adapt host plant defences.

The aim of the present work was to identify if herbivory by *M. persicae* affects OSR responses to AG 2-1.

We examined how aphid infestation affects response of cultivars 'Canard' and 'Temple' to AG 2-1 by visually assessing disease symptoms 13 days post inoculation on plant stems and by quantifying the fungal DNA. Additionally we examined if there was an alteration in the expression of the defence related genes *LOX3*, *MYC2*, *NPR1*, *PR1* and *WRKY38*.

Our results show that aphid infestation resulted in increased susceptibility of 'Canard' plants to AG 2-1: plants with aphids had more severe disease symptoms but the amount

of fungal DNA was the same between treatments. Gene expression analysis revealed that *M. persicae* infestation decreased the expression of *LOX3* and *MYC2* in plants infected with AG 2-1, hence possible alternations in the jasmonate signalling pathway caused the increased susceptibility to the pathogen. Also, the elevation of *PR1* in AG 2-1 inoculated plants and *NPR1* under dual attack, implies that salicylic acid may play a role in defence against the necrotrophic fungi *R. solani* AG 2-1.

Antifungal and Phytochemical Evaluation of *Annona muricata* plant

Margaret[1] A. Ebabhi., Kanife U. Claris[2], Julius S. Omogbai[3], Nnabugwu E. Chinalurum[2] and Raheem L. Omobolaji[2].

[1]Department of Education Science, Distance Learning Institute, University of Lagos, Akoka, Lagos Nigeria; [2]Department of Biological Science, Yaba College of Technology, PMB 2011, Lagos, Nigeria; [3]Department of Pharmacy, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria

Email: ebabhi.meg@gmail.com

The incidence of fungal infection is on the increase as pathogenic fungi are becoming resistant to the conventional drugs. This research examined the antifungal properties of the aqueous, N-hexane and ethanol extracts of the leaves, fruits, stem, bark and roots of *Annona muricata* using the disc diffusion agar assay. The zone of inhibition varied for the tested dermatophytic organisms- *Candida albicans*, *Trichoderma* sp. and *Trichophyton mentagrophytes*, with respect to the plant parts and type of extract. The aqueous extracts of the test plant parts showed more potency on the test fungal species. Preliminary phytochemical studies of the extracts of the plant parts showed that Alkaloid, Cardiac glycosides and phenol were highly present in all parts while steroid and phobatanin are less present. The quantitative results of various parts of the plant reviewed that Phenol from leaf extracts are highly quantifiable than other phytochemical components. In all, Alkaloids, Cardiac Glycoside and phenol were highly quantifiable, while Tannin, Steroid and Phobatanin were less quantifiable. The quantitative analysis was done using ANOVA method at 0.05 level of significance. These findings give credence to the traditional use of *Annona muricata* for the treatment of fungal infections.

Characteristics and control of *Alternaria alternata* affecting sweet pepper in Egypt

Abdulmageed M. Kamara[1], Ahmed. E. Elkorany[2], Eman E. Ramadan[2], and Ghonem M. Amer[2]

[1] Department of Plant Pathology, University of Alexandria, Alexandria 21561, Egypt; [2]Department of Plant Pathology, University of Damanhour, Damanhour 22511, Egypt.

Email: drelkorany125@yahoo.com

Thirty-nine isolates of *Alternaria alternata* were recovered from sweet pepper fruits showed black mould symptoms collected from different regions in El-Behera governorate, Egypt. The isolates were characterized for their colony phenotypes and virulence where no correlation was detected. The RAPD analysis revealed 100% polymorphism using the OP A-03 primer among six *A. alternate* isolates analyzed and the developed dendogram showed partial clustering for regions and virulence. All tested cultivars, *i.e.* Emberu, Trezaa, Mazurka, and Taranto were susceptible to the artificial infection with *A. alternata* to different degrees. Treatment of pepper fruits with resistance inducers, *i.e.* salicylic acid (SA), abscisic acid (ABA), methyl jasmonate (MJ), and calcium chloride (CaCl2) significantly induced resistance and decreased the developed black mould where calcium chloride (1.5 g/L) and SA (1mM) were the most effective. Induction of resistance in pepper fruits with CaCl2 and SA as well as the tolerance revealed in cv. Mazurka was found to be due to high induction in enzyme activity of polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine (PAL) as well as an increase in the total phenolics in the treated pepper fruits.

***Powdery Mildew Identification: Improving accuracy using previously untapped DNA regions**

Oliver Ellingham[1], John David[2], and Alastair Culham[1]
[1]School of Biological Sciences, University of Reading, Reading, RG6 6AS, UK; [2]Department of Plant Pathology, The Royal Horticultural Society, Wisley, GU23 6QB, UK
Email: o.h.ellingham@pgr.reading.ac.uk

The powdery mildews (Ascomycota, Erysiphales) are a group of obligate biotrophic Fungi found on nearly 10,000 angiosperm plant hosts globally, many of which are important horticultural and agricultural plants. Infection can reduce attractiveness and yields significantly. A reliable method is required for unambiguous identification of these often cryptic species such that spread to new areas and/or new hosts can be detected rapidly and controlled early. This project takes a unique approach to sampling; using a citizen science scheme launched in 2014 to assess the presence of the 876 global powdery mildew species in the UK and the efficacy of techniques currently used for their identification – host identification, fungal morphological analysis, sequencing of the fungal rDNA ITS region. The survey was promoted via an array of online and printed media and over 500 infected plant samples were received – most were powdery mildews. Of these 94% were identified to genus level and 79% to species level. This shows the scope for improvements to these techniques through analysis of supplementary DNA regions. 90 different powdery mildew species were found over the 2-year period; 60% of the 200 species previously recorded in the UK. During the survey period two new hosts were found for species known to occur in the UK. These data provide the most comprehensive survey of powdery mildew species within the UK. This

knowledge is critical for early detection of newly arrived species of powdery mildew and of host shifts or novel hosts.

****Phenotypic heterogeneity as an adaptation of fungi to environmental stress**

David S. Foster[1], Steve P. McGrath[2], Paul S. Dyer[1] and Simon V. Avery[1]
[1]School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK; [2]Sustainable Agriculture Sciences, Rothamsted Research, Harpenden, AL5 2JQ, UK
Email: mbxdf@nottingham.ac.uk

Phenotypic heterogeneity describes variation in a given characteristic that is evident between individual cells of an isogenic population. The extent of heterogeneity in a particular phenotype may be determined by epigenetic or genetic factors, e.g. specific gene-promoter sequences producing noisier gene expression. A number of characteristics displaying phenotypic heterogeneity are known to influence competitive fitness under stress, therefore heterogeneity likely has a bearing on survival under fluctuating-stress conditions. Recent evidence from our laboratory suggests that environmental stress selects for increased levels of heterogeneity in resistance to certain stressors. Wild yeast isolates from polluted sites were found to show elevated heterogeneity in resistance to pollutants, in comparison to isolates from nearby control sites. However, the influence of environmental stability on selection for heterogeneity is not understood, nor its long-term stability. Yeast and filamentous fungal isolates from long-term stressed experimental sites have been studied to provide insight. Dose-response gradients, amongst other measures of cell-cell variation, indicate that a long-term, stable level of stress may select for a decrease in heterogeneity of resistance. The degree of resistance heterogeneity may have the potential to evolve in different directions depending on the stability of the stressor dose in the environment.

Development of CRISPR/Cas9 gene editing tools for the rice blast fungus

Andrew J. Foster[1], Magdalena Martin Urdiroz[1], Nicholas J. Talbot[1]
[1]School of Biosciences, University of Exeter, Exeter, Devon, United Kingdom.
Email: A.J.Foster@exeter.ac.uk

CRISPR/Cas9 gene editing is a rapid developing field which holds great promise for accelerating the pace of targeted mutagenesis in fungi. Currently, although very many fungal genes are predicted based on genome sequencing, the process of specifically mutating selected genes, even in the best studied fungi, requires months rather than weeks. The rice blast fungus *Magnaporthe oryzae* is the most serious pathogen of rice. To accelerate the process of targeted mutation and specific gene edits in this important species we are working towards the development of a rapid Crispr/Cas9 based gene editing technology. Technologies allowing precise edits to coding sequences, gene

tagging as well as deletions of single or multiple genes are being optimised. This research will open the door to analysis of gene families or subsets of target genes. Latest results will be discussed.

Tackling resistance to food preservatives: heterogeneity in fungal spore populations

Ivey A. Geoghegan, Malcolm Stratford, David B. Archer and Simon V. Avery
School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK
Email: mbzig@exmail.nottingham.ac.uk

Food spoilage by fungi is a global issue with far-reaching economic, environmental and health-related consequences. Spoilage is particularly severe within the soft-drinks industry, where even a handful of spoiled bottles can result in the recall of an entire batch, costing millions of pounds. Such spoilage occurs despite the presence of food preservatives in soft drinks, which are generally effective at restricting growth of yeasts and moulds. However, certain species of fungi are highly resistant to these preservatives, and are associated with spoilage incidents worldwide. Thus, effective control of these fungi is an important avenue of research. We are characterizing mechanisms of resistance to sorbic acid, a widely used preservative in soft drinks. The spores of the mould *Aspergillus niger* are highly resistant to sorbic acid. In addition, *A.niger* spore populations display a high degree of heterogeneity in resistance to this particular stress. By combining analysis of single spore phenotypes with cutting-edge molecular indexing technology as well as classical molecular genetics techniques, we have begun to uncover the inextricable links between population heterogeneity and preservative resistance in this spoilage fungus.

***Xanthomonas citri* pv. *citri* pathotypes: Lipopolysaccharide (LPS) structure and function as microbe-associated molecular patterns (MAMPs)**

Lotte Bettina Andersen Gersby[1], Olivier Pruvost[2], Antonio Molinaro[3] and Mari-Anne Newman[1]
[1]PLEN, University of Copenhagen, Thorvaldsensvej 40, 1871 Denmark; [2]CIRAD, PVBMT, Saint Pierre, La Réunion, France; [3]Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
Email: lba@plen.ku.dk

Citrus bacterial canker (CBC) is a disease of citrus plants with remarkable socioeconomic impact on the citrus industry. The causal agent of Asiatic CBC is the Gram-negative bacterium *Xanthomonas citri* pv. *citri* (Xac). Xac strains are classified into three pathotypes. Pathotype A has the broadest host range, infecting almost all *Citrus*, pathotypes A* and A^W show a limited host specificity, only affecting Mexican lime.

LPS is known to act as a MAMP and elicits immune responses in eukaryotic organisms. MAMP-induced responses include production of reactive oxygen and nitrogen species, alterations in the plant cell wall, induction of antimicrobial compounds and expression of pathogenesis-related genes.
LPS is composed of a polysaccharide region (O-chain), a core oligosaccharide and a glycolipid region termed lipid A. A genomic region involved in LPS biosynthesis that is highly variable among the Xac pathotypes was recently identified. Furthermore, partial structure elucidation of Xac LPS revealed the presence of a rhamnose homo-polymer as the O-chain moiety. A full elucidation of the structures of LPS from several Xac strains might clarify the differences in defence responses in different host plants, as well as the host specialization process of the Xac pathotypes. Here, we present the structures of LPS from two Xac strains of pathotypes A and A* and the ability of the whole LPS and lipid A of both strains to act as MAMPs in the model plant *Arabidopsis thaliana* and in the two hosts, Mexican lime (*Citrus aurantifolia*, pathotype A* host) and lemon (*Citrus lemon*, pathotype A host).

****Fungal and Oomycete Biodiversity in the Falkland Islands and South Georgia**

Jacob Hargreaves[1], Jim I. Prosser[2], Paul Brickle[3] and Pieter van West[1]
[1]Aberdeen Oomycete Laboratory, College of Life Sciences and Medicine, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK; [2]Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, Aberdeen, UK; [3]South Atlantic Environmental Research Institute, Stanley, Falkland Islands
Email: j.hargreaves@abdn.ac.uk

Soil microbial communities are extremely complex and heterogeneous entities, which are not fully understood at most levels, from soil pore structural microclimates to global diversity and dispersion. This is despite their crucial involvement in biogeochemical cycles, which affects agricultural and natural ecosystems. A major component of the microbial community are fungi, which have critical roles in breaking down organic matter. A subgroup can also form symbiotic relationships with plants, known as mycorrhizae, which can increase plant productivity. The Falkland Islands and South Georgia represent an isolated natural ecosystem with introduced herbivores such as sheep and reindeer. To investigate fungal diversity in these circumstances, soil samples from West Falkland and South Georgia were taken in the 2017 southern summer to carry out high throughput sequencing of barcode regions. These samples will give a snapshot of the natural eukaryotic microflora of the regions, with areas of relatively little and areas of a lot of grazing pressure. The data will allow fungal community studies to be carried out, looking at the impact of vegetation and reindeer grazing. The subsequent analyses will allow inference of the fungal community function to be determined, with applications towards agriculture and bioremediation. Here we present our latest findings.

Mapping the Adaptive Landscape of *Zymoseptoria tritici CYP51* under Azole Fungicide Selection

Nichola J. Hawkins and Bart A. Fraaije
Biointeractions and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, UK
Email: nichola.hawkins@rothamsted.ac.uk

Fungicides are a key component of control programmes for many plant pathogens, but their use exerts a strong selective pressure for resistance. For some site-specific fungicides, the evolution of resistance is simple and rapid, with a single major target-site mutation resulting in effectively complete resistance. However, for the azole fungicides, the picture is more complex, with multiple target-site mutations and other, over-expression based mechanisms accumulating to produce quantitative, step-wise reductions in sensitivity. The effect of multiple mutations on resistance is often referred to as "additive", but in fact the situation is further complicated by epistatic interactions, both in terms of fungicide resistance, and of impacts on enzyme function and stability of the target protein. This results in a rugged adaptive landscape. Consequently, a detailed understanding of the effect of each mutation, individually and in different genetic backgrounds, on resistance and fitness, is necessary in order to attempt to predict evolutionary trajectories under different fungicide selection scenarios.
We will present an updated fitness landscape of *Z. tritici CYP51* haplotypes reported in field isolates to date, discussing the implications for the predictability of future evolution and experimental approaches for testing this.

Genetic diversity of Fusarium spp. on cereals in Russia

Aleksandr Ignatov[1], Tatiana Suprunova[2] and Natalia Zhemchuzhina[3]
[1]Russian University of People's Friendship, Moscow, 117198, Russia and R&D Center "PhytoEngineering" LLC, Rogachevo, 141880, Russia; [2]"Doka-Gene Technology" LLC, Rogachevo, 141880, Russia; [3] Russian Research Institute of Phytopathology, Bolshie Vyazemy, 143050, Russia.
Email: an.ignatov@gmail.com

The root rot, snow mold, and fusarium ear blight caused by *Fusarium* spp. and *Microdochium* spp. -related fungi damage a wide range of cereal crops in Russia. The disease agents are characteristic to certain ecological and geographical areas. 96 strains of *Fusarium* spp. and *Microdochium* spp. from the State Collection of Plant Pathogenic Microorganisms were used for Multi Locus Sequence Typing (MLST) analysis for genes of translation elongation factor-1 alpha (TEF1), β-tubulin, ITS rRNA (with 3 different primer sets), and RNA polymerase II (RPB2) with primers reported elsewhere (O'Donnell et al., 2000; Debourgogn et al. 2010; CBS-KNAW Fungal Biodiversity Centre's Fusarium MLST website). Results of previous phenotypic identification, PCR Fingerprint (IRAP and ISSR), and MLST analysis were compared. Strains of *F. culmorum* formed the most polymorphic group according to PCR fingerprint and MLST, whereas the most

homogeneous group included *F. sporotrichioides*. Strains of *M. nivale, F. avenaceum, F. gibbosum* were not significantly differentiated statistically from other species. Some strains of *M. nivale, F. sporotrichoides*, and *F. oxysporum* could not be correctly identified by either phenotype or PCR fingerprinting. The species of the pathogens widespread in Russia had various pathogenic and toxic properties, indicating their strong intraspecific variability. Rare fungal species possessed toxicity to test-plant seedlings, but were not pathogenic. The genetic similarity between newly sequences MLST loci and closest Genebank (NCBI) accessions ranged from 81 to 99% indicating presence of both previously characterized and unique genetic linages among strains of *Fusarium* spp. and *Microdochium* spp. infecting cereals in Russian Federation.

Biological management of southern blight of chili by Penicillium oxalicum and leaves of Eucalyptus citriodora

Arshad Javaid, Rabia Afzal and Amna Shoaib
Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.
Email: arshadjpk@yahoo.com, arshad.iags@pu.edu.pk

In the present study, leaf dry biomass of *Eucalyptus citriodora* and a biological control *agent Penicillium oxalicum* were evaluated for management of southern blight disease of chili caused by *Sclerotium rolfsii.* In a pot trial, soil was made sick with inoculum of *S. rolfsii.* Soil was amended with dry leaf biomass of *E. citriodora* (1, 2, 3%) and a biological control agent *Pacicillium oxalicum* alone or in combination. Soil amendment with 3% leaf biomass of *E. citriodora* significantly reduced plant mortality by 65% over positive control. Consequently, root and shoot growth, fruit yield as well as chlorophyll content were significantly enhanced. *P. oxalicum* in combination with 3% leaf biomass also significantly reduced plant mortality and enhanced crop growth and yield. Protein content in *P. oxalicum* inoculated treatments was also significantly higher than control. *S. rolfsii* inoculation significantly enhanced peroxidase (PO), phenylalanine ammonia-lyase (PAL), catalase and polyphenol oxidase (PPO) activities in leaves of chili while these parameters were drastically reduced by application of *P. oxalicum* and leaf biomass of *E. citriodora*. In laboratory bioassay, different concentrations of methanolic leaf extract of *E. citriodora* viz. 1, 2, …, 5% reduced fungal growth by 3 to 51%. In methanolic leaf extract, compounds namely 2-Furancarboxaldehyde, 5-(hydroxymethyl), Cyclohexanol, 2-(2-hydroxy-2-propyl)-5-methyl, Pentadecanoic acid, 14-methyl, methyl ester, and 14, 17-Octadecadienoic acid, methyl ester were identified through GC-MS analysis. The present study concludes that 3% leaf biomass of *E. citriodora* can significantly reduce plant mortality due to *S. rolfsii* and enhance plant growth and yield in chili.

Tracing the transduction of a phenylpyrrole fungicide reveals its effect on cAMP signalling in *Botrytis cinerea*

Jaafar Kilani[1,2], Monica Miazzi[3], Colette Audéon[1] and Sabine Fillinger[1]
[1]UMR BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France; [2]Univ. Paris-Sud, Université Paris-Saclay, France; [3]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy
Email: jaafar.kilani@inra.fr

Fungi rapidly adapt to their environment involving to signalling pathways like those of mitogen activated protein kinases (MAPKs). In *Botrytis cinerea*, the phenylpyrrole fungicide fludioxonil activates the Sak1 and Bmp3 MAPKs, which are involved in osmoregulation, cell wall integrity, development and pathogenicity. In order to trace the transduction of fludioxonil and to identify the signalling pathways involved, we have performed comparative phosphoproteomics of the wild-type strain subjected or not to fludioxonil. This experiment led to the identification of "fludioxonil" candidate signalling proteins such as the phosducin-like protein PhnA. In eukaryotes, phosducin is involved in the G-protein signalling and hence the cAMP pathway. In the phytopathogenic fungi *Cryphonectria parasitica* and *Fusarium graminearum*, phosducin is involved in pigmentation, sporulation, and pathogenicity.

We performed the functional analysis of phnA in *B. cinerea*. Its deletion revealed that phnA is involved in development, pathogenicity and adaptation to cell wall stress. The role of phsoducin in G-protein signalling pushed us to study the impact of fludioxonil on the cAMP signalling pathway. In the wild-type we observed a steady decrease of intracellular cAMP accumulation during exposure to fludioxonil. We also studied the connection between PhnA and Sak1 and we observed no influence of PhnA on Sak1 phosphorylation.

Our analyses revealed that the phenylpyrrole fludioxonil acts on cAMP dependent signal transduction in addition to its activating effect on the osmosensing MAPK pathway. The role of PhnA and the osmotic MAPK pathway in cAMP synthesis under fludioxonil treatment is currently under investigation.

***The impact of microbial communities on the incidence of foliar fungal disease symptoms in wheat**

George Lund[1,2], Matthew Dickinson[3], Tim Mauchline[2] and Jason Rudd[1]
[1] Bio-interactions and Crop Protection Department, Rothamsted Research, Harpenden, AL5 2JQ, UK; [2] Sustainable Agricultural Science Department, Rothamsted Research, Harpenden, AL5 2JQ, UK; [3] School of Biosciences, University of Nottingham, Loughborough, LE12 5RD, UK
Email: george.lund@rothamsted.ac.uk

Septoria tritici blotch disease (STB) caused by the fungus *Zymoseptoria tritici*, is the most economically important wheat disease in Western Europe. With resistance to

conventional fungicides becoming an increasing problem, alternative solutions are required. Primary stages of infection occur in the extracellular spaces of the wheat leaf, where bacteria such as *Pseudomonas fluorescens* can also be found. In our first experiment a Pseudomonad library was screened for direct inhibition of *Z. tritici* on agar plates, to determine if any exhibit antifungal activity. Using a high-throughput screening methodology developed in this project which tested 460 bacterial endophytes and rhizosphere associated Pseudomonads, 40 isolates showed an ability to directly inhibit the growth of *Z. tritici*. Our results raise the possibility that bacteria present on wheat leaves may affect the incidence and/or severity of STB via direct inhibition of fungal growth, through secreted fungal inhibitors. In a second experiment, we plan to grow surface sterilised wheat seeds in sterile growth medium amended with synthetic microbial communities before infecting the plants with *Z. tritici*. This will allow us to monitor the impact of the below ground microbiome on the plant's response to foliar disease, through priming or other mechanisms. These combined approaches may lead to novel mechanisms, which could be exploited for chemical and/or biological control of *Z. tritici*.

Exploring NGS, HIGS and si-RNA technologies for the control of Fusarium ear blight in wheat

Ana K. Machado[1], Robert King[2], Wing Sham Lee[1], Caroline Sparks[1], Neil Brown[1], Kostya Kanyuka[1], Martin Urban[1], Jon West[1], Elene Yamazaki-Lau[3], Casiane S. Tibola[3], Maria I. P. M. Lima[3], Roberto Togawa[4], Natalia Martins[4], Francisco Aragão[4], Camila P. Nicolli[5], Emerson M. Del Ponte[5], Dauri J. Tessmann[6], José Mauricio C. Fernandes[3] and Kim E. Hammond-Kosack[1]

[1] Biointeractions and Crop Protection Department and [2] Computational and Systems Biology Department, Rothamsted Research, Harpenden, UK, [3] Embrapa Trigo, Passo Fundo, Rio Grande do Sul, Brazil [4] Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil, [5] Universidade Federal de Viçosa, MG, Brazil, and [6] Universidade Estadual de Maringá, PR, Brazil.

Email: ana.machado@rothamsted.ac.uk

Fusarium ear blight (FEB) is a major problem in most small-grain cereal growing regions and now threatens global food security. Currently strategies to control FEB are not very effective, fungicides give partial protection and development of genetic-based resistant cultivars has proven to be difficult. Therefore, new ways to control FEB are urgently required. Here, we describe the framework and initial results from a bilateral UK-Brazil project that is using a bespoke whole genome sequencing, *in planta* transcriptome and reverse genetics guided approach to understand and pinpoint the *Fusarium* genes and pathways required to cause disease in wheat heads. Our intention is to identify a new suite of *Fusarium* genes for intervention that can simultaneously be targeted via host induced gene silencing (HIGS). HIGS constructs are being tested for efficacy in Arabidopsis, lettuce and wheat, and promising HIGS constructs have been stably

transformed into a commercial moderately-FEB resistant Brazilian wheat in preparation for field testing in southern Brazil during 2018 and 2019. By taking a *Fusarium* genome/reverse genetics guided approach, this is enabling the development of flexible new ways to control FEB disease in wheat crops grown in Brazil. The main prerequisites needed to apply this approach in other wheat growing regions will be addressed, as well as additional scientific, societal and industry benefits that could potentially emerge when using HIGS technologies for plant disease control.
This research is sponsored by the BBSRC, The Newton Fund and EMBRAPA.

Heterologous expression of feruloyl esterase of *Aspergillus terreus*

Miia R. Mäkelä[1], Adiphol Dilokpimol[2], Salla Koskela[1], Ronald P. de Vries[2], Kristiina Hildén[1]
[1]Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland; [2]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; [3]Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands
Email: miia.r.makela@helsinki.fi

In the cell walls of gramineous plants, hemicelluloses are crosslinked to the aromatic lignin polymer via hydroxycinnamic acids (ferulic acid and *p*-coumaric acid). Feruloyl esterases (ferulic acid esterases, EC 3.1.1.73), present in CAZy family CE1 (www.cazy.org), are enzymes that catalyse the cleavage of covalent ester bonds between carbohydrate and lignin moieties in plant cell walls. Due to the ability to specifically cleave ester linkages, feruloyl esterases are promising biocatalysts for a broad range of biotechnological applications. These include e.g. pharmaceutical, agricultural and food industries, as well as the production of biofuel.
Analysis of the CAZomes of eight *Aspergillus* species revealed a high variability in the gene sets related to plant biomass degradation (1). One of these variations was in the putative FAEs of CAZy family CE1, where all species contain one conserved *fae* gene, but some species possess additional candidate genes for this activity. *Aspergillus terreus* contains two additional putative FAE encoding genes, one of which has orthologs in *Aspergillus oryzae* and *Aspergillus flavus*, but not in any of the other tested species (*Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus clavatus*, *Aspergillus fumigatus* and *Aspergillus fisheri*). One of these putative *A. terreus* FAE encoding genes was chosen to be heterologously expressed in a methylotrophic yeast *Pichia pastoris*. The recombinant enzyme was successfully produced and further studied for its biochemical characteristics.

(1) Closely related fungi employ diverse enzymatic strategies to degrade plant biomass. Biotechnol. Biofuels 8, 107.

Measuring resistance to Septoria tritici blotch in elite wheat cultivars using automated image analysis

Alexey Mikaberidze
Plant Pathology,Institute of Integrative Biology,ETH Zürich, Universitaetstr. 2,ETH Zentrum, LFW B28, CH-8092 Zurich, Switzerland
Email:

Quantitative resistance is likely to be more durable than major gene resistance for controlling Septoria tritici blotch (STB) on wheat. Earlier studies hypothesized that resistance affecting the degree of host damage, as measured by the percentage of leaf area covered by STB lesions, is distinct from resistance that affects pathogen reproduction, as measured by the density of pycnidia produced within lesions. We tested this hypothesis using a collection of 335 elite European winter wheat cultivars that was naturally infected by a diverse population of Zymoseptoria tritici in a replicated field experiment. We used automated analysis of 21214 scanned wheat leaves to obtain quantitative measures of STB conditional severity that were precise, objective, and reproducible. These measures allowed us to explicitly separate resistance affecting host damage from resistance affecting pathogen reproduction, enabling us to confirm that these resistance traits are largely independent. The cultivar rankings based on host damage were different from the rankings based on pathogen reproduction, indicating that the two forms of resistance should be considered separately in breeding programs aiming to increase STB resistance. We hypothesize that these different forms of resistance are under separate genetic control, enabling them to be recombined to form new cultivars that are highly resistant to STB. These data can already be used by breeding programs aiming to increase STB resistance to choose wheat cultivars that are broadly resistant to naturally diverse Z. tritici populations according to the different classes of resistance.

In-Vitro Antifungal Screening of Radish (*Raphanus sativus* L.) Extracts as Safe Botanical Fungicides against *Alternaria alternata and ***Sclerotium rolfsii***

Hassan B. Mohamed and Rashid M. Mamunur
Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, 5200, Bangladesh
Email: medbarkad1991@gmail.com

Two lab experiments were conducted on radish (*Raphanus sativus* var. BARI Mula-1) extractions to quantitatively investigate phytochemicals in methanolic radish root extract (MRRE) and screen antifungal activity of methanolic and aqueous radish extracts against *Alternaria alternata* and *Sclerotium rolfsii* in Agricultural Chemistry and Plant Pathology Laboratories, Hajee Mohammad Danesh Science and Technology University Dinajpur, during September, 2015 – September, 2016. Methanolic solvent was used

using maceration method, where 15.12 g (30.24%) brown pungent greaseless jelly-like crude phytochemical substance was extracted from 50 g radish root powder dissolved with 200ml methanol. TLC analysis of MRRE showed at 1:10 ethyl acetate hexane 6 spots recorded as Rf values, where highest Rf value was 0.91 cm and lowest Rf value 0.29 cm. In-vitro inhibitory activity of the radish extracts against mycelial growth of the test fungi was screened using poisoned food technique. Three poisoned media viz. methanolic radish root extract media (MRREM, T_1), aqueous radish root extract media (ARREM, T_2) and aqueous radish leaf extract media (ARLEM, T_3), and non-poisoned PDA media (T_c) were used as treatments. There were highly significance differences among the poisoned media in reduction of the mycelial growth over the non-poisoned control PDA media. MRREM inhibited the growth of *A. alternata and S. rolfsii* 94 % and 58.1 %, followed by ARREM 93 % and 40 % and by ARLEM 90 % and 20 %, respectively, over the control. It may be concluded that radish extracts can be used as safe botanical fungicides to control *A. alternata* and *S. rolfsii*.

****Resistance of *Saccharomyces cerevisiae* to lignocellulose-derived inhibitors**

Elena Moreno-Martínez and Simon V. Avery
School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK
Email: msxem3@nottingham.ac.uk

Production of bioethanol from lignocellulosic hydrolysates is a promising biofuel technology but is subject to inhibition by chemicals released during the pretreatment of biomass. Weak acids, furans and phenols impair yeast growth and fermentation, thus reducing efficiency of the process and increasing the cost. Some of the molecular mechanisms of action and response to these stressors are characterized, but little is known about how uniform such effects are across individual cells within an isogenic cell population. Non-genotypic heterogeneity refers to the expression of different phenotypes within a clonal cell population, despite all individuals being genetically identical. Understanding individual-cell effects of hydrolysate inhibitors could help engineering of population-level resistance. The degree of heterogeneity in resistance traits was specific to particular inhibitors in the fermenting yeast *S. cerevisiae*. Inclusion of a second inhibitor could alter heterogeneity relative to either inhibitor alone. Short pretreatments with sub-inhibitory concentrations of the inhibitors indicated adaptive resistance and adaptive heterogeneity responses. RNAi assisted genome evolution was also used to reveal combinations of gene knockdowns that can synergistically increase resistance towards these inhibitors. Findings derived from this research will provide better understanding of the behaviour of yeast in lignocellulosic ethanol fermentations, and might help improve production.

Carbohydrate arrays coupled with mass spectrometry to detect activity of plant polysaccharide degradative enzymes from *Aspergillus niger*

Jolanda van Munster[1,2], Baptiste Thomas[1], Michel Riese[1], Adrienne Davis[3], Christopher Gray[1], David Archer[2], Sabine Flitsch[1]

[1] Chemical Biology, Manchester Institute for Biotechnology, University of Manchester, Manchester, M1 7DN UK; [2] Fungal Biology and Genetics, School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK; [3] School of Chemistry, University of Nottingham, Nottingham, NG7 2RD, UK

email: Jolanda.vanMunster@Manchester.ac.uk

Renewables-based biotechnology depends on enzymes to degrade plant lignocellulose to simple sugars that are converted to fuels or high-value products. Identification and characterization of such lignocellulose degradative enzymes could be fast-tracked by availability of an enzyme activity measurement method that is fast, label-free, uses minimal resources and allows direct identification of generated products.

The objective of this work was to develop such a method by applying carbohydrate arrays coupled with MALDI-ToF mass spectrometry to identify reaction products of carbohydrate active enzymes (CAZymes) of the filamentous fungus *Aspergillus niger*. We describe the production and characterization of plant polysaccharide-derived oligosaccharides and their attachment to hydrophobic self-assembling monolayers on a gold target. We verified effectiveness of this array for detecting exo- and endo-acting glycoside hydrolase activity using commercial enzymes, and demonstrate how this platform is suitable for detection of enzyme activity in relevant biological samples, the culture filtrate of *A. niger* grown on wheat straw. We will now apply this method to characterise the degradative potential of the broad set of *A. niger* CAZymes that are secreted in response to plant lignocellulose.

In conclusion, we describe a versatile method that is broadly applicable in screening and characterisation of activity of CAZymes, such as fungal enzymes for plant lignocellulose degradation with relevance to biotechnological applications as biofuel production, the food and animal feed industry.

***Detection and Distribution of *Aphanomyces* Root Rot in Peas within the United Kingdom**

Brian Ó Loinsigh[1], Lea Herold[2], Debbie Sparkes[1] and Matthew Dickinson[1]

[1]Plant and Crop Sciences Division, University of Nottingham, LE12 5RD, UK; [2]Processors and Growers Research Organisation, Peterborough, PE8 6HJ, UK

Email: stxbb13@nottingham.ac.uk

Root rot, caused by *Aphanomyces euteiches*, a strict soil-borne oomycete, is one of the most damaging diseases of pea (*Pisum sativum*) and significantly reduces pea production globally. Since the inoculum persists within the soil, and no resistant cultivars or means of chemical control exists, the disease is difficult to manage.

Moreover, the UK vining pea areas are fixed with no room to move further afield. For these reasons growers find it useful to obtain information on the degree of infestation within their fields to avoid those at high risk. However, the spread and severity of *A. euteiches* across the UK growing regions is currently unknown. In this project, soils from 38 sites were collected spanning the vining pea areas and tested for the presence of *Aphanomyces* root rot through baiting experiments. A Loop Mediated Isothermal Amplification (LAMP) assay for the specific detection of *A. euteiches* from pea plate baiting experiments was developed. The results suggest that this LAMP assay can be used to detect *A. euteiches* DNA on the roots of infected seedlings that has been baited with soil within days of receiving the samples. Combining both visual disease scores and LAMP results, a distribution map of the disease was developed. We show that *A. euteiches* is found across all areas of the UK growing region and is more widespread than previously known, with some areas having a higher burden than others. The LAMP assay allows for fast and specific detection of the pathogen. This information can now be used to further understand the disease in the UK and help slow the spread of the disease.

****Optimisation of sexual development in *Pyrenopeziza brassicae* to allow study of hormonal triggered morphogenesis**

Tom Pearson[1], Alison A. Ashby[2], Paul S. Dyer[1]
[1]School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK;
[2] Croggan Ltd, The Coach House, Long Lane Cambridgeshire, CB24 5LD, UK
Email: Stxtp4@nottingham.ac.uk

Fungicide resistance is a major problem within agriculture, causing millions of pounds' worth of crop loss and threatening food security. Several economically important fungal crop pathogens have developed some level of resistance to common fungicides, one example being *Pyrenopeziza brassicae*, the cause of light leaf spot on oil-seed rape and other *Brassica* species. It is clear that new methods to control disease need to be developed. There is preliminary evidence that *P. brassicae* produces sex hormones (known as Sex Factor) during mating which alter development. These could be utilised as novel fungicides to inhibit asexual reproduction, therefore controlling the spread of disease. To identify model systems for hormone study, sexual crosses are being set up between *P. brassicae* isolates of opposing mating (*MAT*) types. Current work is focused on optimizing production of fertile apothecia in laboratory crosses, using a variety of crossing methods and growth media. Ongoing results will be presented. Future work will focus on further purification and characterisation of Sex Factor, which previous studies indicate is lipoidal in nature and can be extracted using ethyl acetate or methyl chloroform. Bioassays will look for suppression of asexual sporulation and induction of sex.

***Virus- and salicylic acid-induced changes in plant-insect and plant-virus interactions**

Anna Platoni[1,2], Sanjie Jiang[1], Alex M. Murphy[1], Adrienne E. Pate[1], Ju-Yeon Yoon[1,3], Beverley J. Glover[1], John P. Carr[1] and Gerard R.G. Clover[2]

[1] Plant Sciences Department, University of Cambridge, Cambridge, CB2 3EA, UK; [2] Plant Health, Royal Horticultural Society, RHS Garden Wisley, Woking, Surrey GU23 6QB; [3] Virology Unit, Department of Horticultural Environment, National Institute of Horticultural and Herbal Science, RDA, Wanju, 55365, South Korea

Email: amp93@cam.ac.uk

Interactions between plants and insects are complex and mediated by plant-derived signal molecules, including volatile organic compounds. Infection by viruses alters plant volatile emission and consequently affects plant interactions with insects that vector viruses, as well as with beneficial insects. For example, infection with cucumber mosaic virus (CMV) affects interactions with aphids (1), while in other work we found that bumblebees (*Bombus terrestris*) are attracted to CMV-infected tomato (*Solanum lycopersicum*) plants (2). Salicylic acid (SA) and jasmonic acid (JA) are defensive phytohormones that influence interactions of plants with viruses, microbes and insects. In tomato and pepper (*Capsicum annuum*) we are investigating the roles of SA and JA in regulating resistance to CMV infection, and in controlling emission of volatiles that influence interactions of plants with aphids (*Myzus persicae*) or with bumblebees. In particular, we are investigating the effects of exogenous application of SA and JA on emission of insect-perceivable volatiles, and the effects of SA on CMV replication and movement. The wider aim of this work is to further understand the effects of the SA- and JA-dependent defensive signalling pathways on interactions between plants, viruses and harmful as well as beneficial insects to provide amateur and professional horticulturalists with better options for environmentally friendly integrated pest and disease management.

1. Westwood et al. 2013 PLoS ONE 8(12): e83066.
2. Groen et al. 2016 PLOS Pathogens 12(9): e1005906.

Work funded by the Royal Horticultural Society, BBSRC, Leverhulme Trust, and the Rural Development Agency of the Republic of Korea.

Mechanistic basis of epithelial damage in response to *Aspergillus fumigatus* infection

**Norman van Rhijn[1], Takanori Furukawa[1], Panagiotis Papastamoulis[2], Sayema Khan[1], Mike Bromley[1], Magnus Rattray[2] and Elaine Bignell[1]

[1]Manchester Fungal Infection Group, University of Manchester, Manchester, United Kingdom; [2]Division of Informatics, Imaging and Data Sciences, University of Manchester, Manchester, United Kingdom.

Email: norman.vanrhijn@manchester.ac.uk

The human lung is continually exposed to spores of the airborne mould *Aspergillus fumigatus*. Inhaled spores are small enough to bypass mucociliary clearance

mechanisms and reach the alveoli of the lung where interaction between host and pathogen cells can lead to fungal clearance, or to development of inflammatory or invasive fungal diseases. Fuelled by the results of extensive fungal transcriptome research we have developed, in recent years, a programme of research that seeks the mechanistic basis of lung damage during *A. fumigatus* infection. In this study we introduce a new high throughout study addressing the *A. fumigatus* regulatory network driving epithelial damage in cultured human lung tissue and examine how this knowledge might lead to novel therapeutic interventions.

Investigating effector delivery by the rice blast fungus *Magnaporthe oryzae

Clara Rodriguez-Herrero, Magdalena Martin-Urdiroz, George Littlejohn, Miriam Oses-Ruiz, Wasin Sakulkoo, Xia Yan, Michael J. Kershaw, Darren M. Soanes and Nicholas J. Talbot
School of Biosciences, University of Exeter, EX4 4QD, United Kingdom
Email: cr425@exeter.ac.uk

Magnaporthe oryzae is the causal agent of rice blast disease. The life cycle of *M. oryzae* starts when a spore lands on the surface of a leaf. The spore germinates to form a germ tube, which generates the appressorium. The appressorium generates enormous turgor pressure that is translated into physical force that ruptures the rice leaf. The fungus then develops invasive hyphae (IH), which colonizes plant tissue. *M. oryzae* secretes effectors to facilitate host colonization by suppressing plant immunity responses. Effectors in *M. oryzae* can be classified as cytoplasmic, or apoplastic, depending on where they are delivered. Cytoplasmic effectors can be seen to accumulate in a membrane-rich plant structure called the biotrophic interfacial complex (BIC), while apoplastic effectors are at the extra-invasive hyphal membrane surrounding the IH. The BIC is thought to be involved in translocation of effectors into the rice cytoplasm. Cytoplasmic and apoplastic effectors follow two different secretion pathways. Apoplastic effectors are secreted by the conventional ER-Golgi secretory pathway, while cytoplasmic effectors are secreted via an unconventional route. Little is known about how secreted effectors are delivered to the correct domains. To understand this process, we are trying to identify a specific host targeting sequence by generating a library of chimeric effector-encoding gene fusions. We are analysing the chimeras for translocation potential. In addition, we are generating stable transgenic rice plants in which we have GFP-tagged both early and late endosomal compartments to determine the potential role of plant endocytosis in effector uptake into plant cells.

Azole sensitivity in Leptosphaeria pathogens of oilseed rape: the role of lanosterol 14α-demethylase.

Thomas R Sewell[1,2], Nichola J Hawkins[3], YongJu Huang[2], Henrik U Stotz[2], Steven L Kelly[4], Diane E Kelly[4], Bart Fraaije[3], Bruce DL Fitt[2]

[1]Department of Infectious Disease Epidemiology, Imperial College London, UK; [2]Crop Protection Group, Centre for Agriculture, Food and Environmental Management, University of Hertfordshire, Hatfield, UK; [3]Biointeractions and Crop Protection, Rothamsted Research, Harpenden, Hertfordshire, UK; [4]Centre for Cytochrome P450 Biodiversity, Institute of Life Science, School of Medicine, Swansea University, Swansea, UK

Email: t.sewell@imperial.ac.uk

Lanosterol 14-α demethylase (erg11, CYP51) is a key enzyme in the fungal ergosterol biosynthetic pathway, and is the target of azole fungicides. Prior studies have shown that *Leptosphaeria maculans* and *L. biglobosa*, the causal pathogens of phoma stem canker on oilseed rape (*Brassica napus*), differ in their sensitivity to some azoles. Here we used heterologous expression and protein modelling to determine whether there are inter-specific sensitivity differences at the target-site level. Moreover, we determined the practical significance of fungicide sensitivity using a new collection of isolates from the south east of the UK. Heterologous yeast expression of LmCYP51B and LbCYP51B with fungicide sensitivity testing of the transformants suggests that the two proteins are similarly sensitive to azole fungicides flusilazole, prothioconazole-desthio and tebuconazole. These findings are supported by homology protein modelling, which predicts that LmCYP51B and LbCYP51B are structurally very similar, specifically at the azole-binding site. Fungicide sensitivity testing on the new collection of isolates shows that they sometimes have a minor difference in sensitivity *in vitro* and *in planta* but at present both species remain very sensitive to some azole fungicides. These results show that azole fungicides remain a useful component of the integrated management of phoma stem canker in the UK due to their effectiveness at controlling *Leptosphaeria* spp. growth, and suggest that other factors, such as varietal resistance or climate, may be driving frequency changes in the two species.

Bioactive compounds with antimicrobial activity extracted from mushrooms grown in La Rioja

Vanessa Grifoll[1], Ana Gonzalo[1], Maria Luisa Tello[1],Margarita Pérez[1], Carmen Lozano[2], Beatriz Rojo[2], Yolanda Sáenz[2]

[1]Centro Tecnológico de Investigación del Champiñón en La Rioja (CTICH). Ctra. Calahorra, KM 4, 26560 Autol, La Rioja, Spain; [2]Centro de Investigación Biomédica de La Rioja (CIBIR). Calle Piqueras, 98 3º, 26006 Logroño, La Rioja, Spain.

Email: biotecnologia@ctich.com

The prolonged use of antibiotics, in human medicine, veterinary and agriculture, has caused an alarming increase of antimicrobial resistant bacteria worldwide. Concerning to this serious threat, the World Health Organization (WHO) signed in 2016 an overall agreement to address the problem of antimicrobial resistance, and in 2017 published a list of priority pathogens that require the development of new antimicrobial agents. In that line, the aim of this work was to search new antimicrobial alternatives obtained from mushrooms.
Sixteen mushrooms (*Pleurotus eryngii, Agaricus bisporus, Pleurotus ostreatus, Pleurotus salmoneo stramineus, Hypsizygus ulmarius, Pleurotus citrinopileatus, Agrocybe aegerita, Hericium erinaceus, Lentinula edodes, Agaricus brunnescens, Agaricus brazei, Hypsizygus tessulatus gris, Hypsizygus tessulatus blanca, Grifola frondosa, Ganoderma lucidum* and *Pholiota nameko*) were grown on sterilized substrates in La Rioja (Spain) and subsequently freeze-dried to obtain a mushroom powder. Diverse methodologies were used to obtain the lyophilised powders and the different aqueous and alcoholic extracts. Agar diffusion methods were used to test the *in vitro* antimicrobial activity against reference strains of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Micrococcus luteus* and *Staphylococcus aureus* species.
Pleurotus citrinopileatus and *Lentinula edodes* lyophilised powder were active against all studied bacteria except to *P. aeruginosa*. The powder of *Pleurotus ostreatus*, *Hypsizygus tessulatus gris* and *Pholiota nameko* suppressed the growth of *P. aeruginosa* or *S. aureus*.
The first results are encouraging, and raise a possible development of new extracts with antimicrobial properties that may be applicable in different industries.

Methodologies development for the cultivation of new species of fungi in La Rioja (Spain)

Maria Luisa Tello, Margarita Pérez
Centro Tecnológico de Investigación del Champiñón en La Rioja (CTICH). Ctra. Calahorra, KM 4, 26560 Autol, La Rioja, Spain
Email: biotecnologia@ctich.com

The fungal kingdom is characterized by its high biodiversity. However only about 100 species of fungi have been cultivated. In the last years the number of cultivated mushrooms has been increased gradually, optimizing techniques used for growing traditional species to cultivate new varieties. In addition, the mushroom market in Europe has grown and consumers search for new healthy products with high quality and the best price.
Thus, the main purpose of this work is to develop the cultivation of new mushroom species endemic to La Rioja. To achieve this, CTICH have selected 12 strains from the species conserved in the Mushrooms Biobank at CTICH. This biobank is a fungal genetic resource collection that enables the conservation of wild fungal biodiversity in the region of La Rioja (Spain). The species selected were those whose cultivation seemed

more feasible bearing in mind several factors, such as: cost of raw materials and production, simplicity of cultivation and collection, marketing or acceptance by potential consumers. Among the selected species there are species with medicinal properties like *Ganoderma lucidum*, protected species such as *Rhodocybe gemina* and edible mushrooms like *Calocybe gambosa.*
For the cultivation of the selected species it was necessary to develop techniques for conservation of the mycelium and spawn proliferation and new substrate formulae, in addition to designing and optimizing the conditions for incubation, culture and crop management.

Does nitric oxide regulate crop pathogen resistance?

Anna M. M. Tiley and Angela Feechan
School of Agriculture and Food Science, University College Dublin, Dublin, Dublin 4, Ireland
Email: anna.tiley@ucd.ie

Zymoseptoria tritici is a pathogenic fungus that causes one of the worst diseases of wheat worldwide, Septoria Tritici Blotch. The aim of our project is to investigate the importance of nitric oxide (NO) during *Z. tritici* infection.
NO is produced as a defence response by the plant, and can act directly as an antimicrobial or as a signalling molecule. We firstly aim to assess whether NO might regulate the effector proteins released by *Z. tritici* during infection. Candidate effectors have been identified in *Z. tritici,* and are being studied to assess whether they are NO-regulated during plant infection.
This project also aims to identify whether *Z. tritici* employs mechanisms to counter the plant's NO burst. The enzyme GSNO reductase (GSNOR) controls the NO pool in other species, and so we are investigating the effect of disrupting the *gsnor* gene in *Z. tritici*. Bioinformatics studies have identified a potential *gsnor* gene homolog in *Z. tritici, ztgsnor*. Knock-out plasmids have been constructed and transformed into *Z. tritici* to create *ztgsnor* knock-out mutants. The results from these experiments are ongoing, and the most recent findings from these studies will be presented.

Evidence for involvement of an RLXR effector protein in *Phytophthora infestans* sexual reproduction

Georgios Tzelepis, Anna Åsman, Ramesh Vetukuri, Johan Fogelqvist, and Christina Dixelius
Department of Plant Biology, Uppsala Biocenter, Swedish University of Agricultural Sciences, Linnean Center for Plant Biology, P.O. Box 7080, S-75007, Uppsala, Sweden
Email: Georgios.tzelepis@slu.se

Phytophthora infestans is a notorious fungal-like plant pathogen, responsible for major economic losses in potato and tomato cultivations worldwide. In order to establish a successful infection, this pathogen deploys an arsenal of small-secreted proteins, termed effectors. Among them, effectors harbouring the RXLR motif are the best-studied ones. They are highly induced during early biotrophic stage of infection and are able to manipulate plant defence mechanisms operating inside the host cell. In this study we investigated the transcription profiles of different *P. infestans* isolates (originated from different parts in Europe) during the mating process, since mating frequencies are known to differ among European countries. Our results revealed that an RXLR effector-encoding gene is highly induced during mating in most of our crossings. Further analysis showed that oospores production is significantly reduced when this effector has been silenced. Moreover, we observed that this protein is accumulated in hyphal tips during mating, while infection of *N. bethamiana* plants with the GFP-tagged strain and transient expression in potato leaves confirmed the cytoplasmic localization of this effector upon infection. In conclusion, this study revealed a potential multifunctional role of an RXLR effector. Interestingly, the presence of this effector only in heterothallic closely related to *P. infestans* species, supports its potential role in sexual reproduction. We can speculate that certain effectors might be involved in other aspects of *P. infestans* biology than in oomycete-plant interactions. If this function reflects evolutionary adaptation remains to be clarified.

PHI-base: Harnessing community expertise to fight plant disease

Martin Urban[1], Alayne Cuzick[1], Kim Rutherford[2], Helder Pedro[3], Nishadi De Silva[3], Paul Kersey[3] and Kim E. Hammond-Kosack[1]

[1] Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK; [2] Cambridge Systems Biology and Department of Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge, Cambridgeshire CB2 1GA, UK; [3] The European Molecular Biology Laboratory, The European Bioinformatics Institute, Hinxton, Cambridgeshire, CB10 1SD, UK

Email: Martin.Urban@rothamsted.ac.uk

The pathogen-host interactions database PHI-base (www.phi-base.org[1]) is a gold-standard database storing phenotypes on genes implicated in virulence. It is a primary information source for researchers studying plant-pathogen interactions as well as human fungal pathogens. More than 2300 research articles are made accessible for researchers to easily familiarise themselves with relevant information on their target genes of interest. PHI-base provides both manually curated molecular and biological phenotype information on pathogen genes. The multi-species database summarises information from 264 pathogens tested on 173 hosts to allow comparative genomics approaches to facilitate novel fungicide target discovery and identify critically important genes for biotech/breeding control strategies. All major plant pathogens from the bacterial, fungal and oomycete kingdoms are included.

Here we describe our new PHI-base Version 4.3 release (May 2017). Our current data platform includes improved search, filtering and extended data display functions. A protein-to-phenotype BLAST search function is included. Development of a common language (ontology) to describe comparable phenotypes in a wide range of pathogen-host interactions is discussed. Exemplar use cases exploring the vast information in PHI-base for fungal disease control are presented.

[1] Urban, M., Cuzick, A., Rutherford, K., Irvine, A., Pedro, H., Pant, R., Sadanadan, V., Khamari, L., Billal, S., Mohanty, S., et al. (2017). PHI-base: a new interface and further additions for the multi-species pathogen-host interactions database. Nucleic Acids Res, doi: 10.1093/nar/gkw1089.

This work is supported by the UK Biotechnology and Biological Sciences Research Council (BBSRC) (BB/I/001077/1, BB/K020056/1). PHI-base receives additional support from the BBSRC as a National Capability (BB/J/004383/1).

***Effector discovery and characterisation in the *Fusarium graminearum*-wheat interaction**

Catherine Walker[1,2], Wing-Sham Lee[1], Neil Brown[1], Martin Urban[1] and Kim-Hammond Kosack[1]
[1]Biointeractions and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, UK;
[2] School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, UK
Email: catherine.walker@rothamsted.ac.uk

Fusarium graminearum (*Fg*), the causal pathogen of Fusarium Head Blight, exhibits an unusual hemibiotrophic lifestyle characterised by spatially and temporally distinct symptomless and symptomatic phases of infection. These distinct phases are facilitated by the biphasic secretion of an arsenal of proteins, including small, cysteine-rich effectors, which modify living host plants, aiding colonisation and infection. It is hypothesised that the secretion of effectors acts to subvert recognition by the host and/or suppress host defences during the symptomless phase of infection.
To identify candidate effectors, a species-specific microarray analysis was used to interrogate the *in vitro* and *in planta* expression patterns of genes belonging to the *Fg* secretome. Twenty putative Fusarium effectors were identified and have begun to be characterised using the *Barley-Stripe Mosaic Virus*-mediated overexpression system (BSMV-VOX), stable Arabidopsis transformation and via the generation of *Fg* gene deletion mutants.
Initial data from the BSMV-VOX experiments suggests that several candidate effector proteins are biologically active in the *Fg*-wheat interaction. Data from these experiments indicates that viral titre is critical to protein expression in the VOX experiments and that an interaction between the virus and some proteins of interest may be occurring. Preliminary experiments suggest that overexpression of these

proteins in the non-host *Nicotiana benthamiana* results in the differential expression of plant defence-related genes; if so, this could offer a high-throughput method for effector screening. Further work is required to determine whether this is a viable route for effector characterisation and to make efforts to decipher the fungal-viral-plant interaction occurring during BSMV-VOX experiments.

Analysis of pathogenicity shift of *Cucumber mosaic virus* isolates in *Capsicum annuum* in South Korea

Ju-Yeon Yoon[1,3], Bong Nam Chung[1], Seung-Kook Choi[2] and John P. Carr[3]
[1] Virology Unit, Horticultural and Herbal Crop Environment Division, NIHHS, RDA, Wanju 55365 South Korea; [2] Vegetable Research Division, Department of Horticultural Crop Research, NIHHS, RDA, Wanju 55365, South Korea; [3] Department of Plant Science, University of Cambridge, Cambridge, CB2 3EA, United Kingdom
Email: juyeon74@gmail.com

Cucumber mosaic virus (CMV), is an aphid-borne virus that infects a wide range of plant species including vegetable crops and weeds. CMV is one of the most important viral diseases in pepper (*Capsicum annuum*) in South Korea. Since 1990s, commercial chilli pepper cultivars have been bred for resistance against CMV isolates (pathotype-0, CMV-P0) that belong to subgroup IA (S-IA) because the S-IA isolates were dominant. Recently, two CMV isolates (CMV-CaP1 and CMV-GTN), which systemically infect the chilli pepper cultivars resistant to CMV-P0, were reported and belong to CMV subgroup IB; these were designated as pathotype-P1 (CMV-P1). Since 2009, nationwide annual surveys of peppers fields showed the most dominant CMV population (ca. 90%) in pepper and weeds was subgroup IB. In pathogenicity assays, CMV-GTN induced severe mosaic and yellowing on chilli pepper (cultivar Cheong-Yang) 21 days post-inoculation (dpi), while CMV-CaP1 induced mild or occasional mosaic. Detached leaf and DAS-ELISA assays clearly showed that CMV-GTN and CMV-Fny (S-IA) spread faster than CMV-CaP1 in the cultivar Cheong-Yang, resulting in more severe systemic symptoms. Pepper cultivars Manitta, Baerotta and LAM32 contain a resistance gene(s) against CMV-P1. Systemic infection by CMV-CaP1 and CMV-GTN was verified on upper leaves of both 'Manitta' and 'Baerotta' but not on 'LAM32' using ELISA analysis. Interestingly, CMV-GTN induced more severe symptoms on upper leaves and had faster systemic movement than CMV-CaP1 in Baerotta. These results suggest a pathogenicity shift in CMV isolates in pepper plants in South Korea.

Funded by Rural Development Agency (PJ012426) of the Republic of Korea.

Identification of QTLs underlying resistance to *Cucumber mosaic virus* in *Capsicum annuum*

Ju-Yeon Yoon[1,3], Su Kim[2], Seung-Kook Choi[2], Eun Young Yang[2], Bong Nam Chung[1], and John P. Carr[3]

[1] Virology Unit, Horticultural and Herbal Crop Environment Division, NIHHS, RDA, Wanju 55365, South Korea; [2] Vegetable Research Division, Department of Horticultural Crop Research, NIHHS, RDA, Wanju 55365, South Korea; [3] Plant Sciences Department, University of Cambridge, Cambridge, CB2 3EA, UK

Email: juyeon74@gmail.com

Cucumber mosaic virus (CMV) is one of the most important viruses infecting chili pepper (*Capsicum annuum*). Recently CMV isolates that belong to subgroup IB (named CMV-P1 pathotype) overcome CMV resistance of pepper in Korea. Resistance evaluation of pepper genetic resources identified that *Capsicum annuum* 'LAM32' showed novel tolerance (hereafter resistance) against CMV isolates CMV-P1 pathotype. To identify a candidate resistance gene of LAM32 for CMV resistance QTL, *C. annuum* 'Subi' as a susceptible parent were crossed and progenies of F1, F2 and backcross1 populations were produced. Parents and their progenies were inoculated with CMV-GTN isolate that belongs to subgroup IB and were evaluated for resistance using lateral flow immunoassay and DAS-ELISA. The results showed that the resistance of *C. annuum* 'LAM 32' was controlled by a single recessive gene. A subset of 119 putatively molecular markers, well distributed over the pepper genome, was selected for QTL mapping using the composite interval mapping method. Six genomic regions, including two major effects and four minor effect QTLs, may be associated with resistance against CMV. Subsequent mapping analysis, based on a pepper linkage map, revealed that genes controlled CMV resistance might be located at chromosome 4 & 5. Further fine genetic mapping for CMV resistance is in progress.

Funded by Rural Development Agency (PJ012426) of the Republic of Korea.

BSPP Presidential Meeting 2018

10–11th December 2018

@ University of Warwick

Prof. Murray Grant

BSPP President Elect