

**BBSRC – Doctoral Training Partnership
Food Security**

Role of the rice blast fungus cell wall in host-fungal interactions and virulence

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Food security is one of the most pressing challenges currently facing humanity. The world population is predicted to reach nine billion by 2050, requiring food production to increase by 50% against a backdrop of climate change and the loss of arable land. Despite agricultural advances plant pathogens still account for losses of 10-16% in the global harvest, for example, the agent that causes rice blast disease, *Magnaporthe oryzae*, leads to global yield losses of 10-30% each year, sufficient rice to feed over 60 million people. The need for new control strategies for this and other plant diseases is therefore urgent. In pathogenic fungi the cell wall is the principle point of contact with the host; as such our central hypothesis is that the *M. oryzae* cell wall plays a vital role in virulence, host recognition and disease. To address this hypothesis we have three specific objectives: [1] to undertake a detailed analysis of *M. oryzae* cell wall structure; [2] to conduct a systematic analysis of the importance of cell wall components in virulence; [3] to understand the relative role of cell wall epitopes in triggering the plant defence response. Through this project we will therefore fully characterise the cell wall in this important plant pathogen, and determine the importance of cell wall epitopes in both plant infection and the triggering of the plant defence response. Understanding how the pathogen is recognised, and an effective defensive response mounted, could ultimately direct the development of novel strategies for providing durable plant resistance.

Climate change impacts on mineralisation of organic nitrogen in contrasting land uses

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Climate change will have both direct and indirect effects on the rates and timing of organic N mineralisation and subsequent plant availability of mineral N forms. Direct effects include changes in fluctuations in air and surface soil temperatures, rainfall distribution and intensity, and wetting and drying episodes. Indirect effects will be through modification of the residue quality (e.g. C:N, lignin content) and changes in microbial community functioning. This studentship will explore the relationships between the direct and indirect controlling factors and the kinetics of mineralisation. The project will explicitly measure the impact of simulated climate change on nitrogen transformations, thus extending ongoing BBSRC and NERC-funded work, on the direct and indirect climatic controls (in particular temperature) on decomposition rates in soils, and recent Defra-funded modelling work to predict impacts of climate change on soil N and C cycling. Such work would be timely, as the effects of temperature on carbon and nitrogen mineralisation have been shown to differ. Thus, our increasing understanding of the responses of total decomposition rates may not provide the information required to predict how climate change will affect nitrogen availability to plants. The project will also determine responses to changes in soil moisture (both constant and fluctuating), in isolation and in combination with the temperature manipulations. Both grassland and tillage land will be included, since some management factors may also interact with the changes in environmental factors as a consequence of climate change, while, a more natural ecosystem (land use) may also be included, e.g. extensive upland moorland, or woodland, to provide an end member with a less anthropogenically-perturbed nitrogen cycle. The student will use both field studies (perhaps using climate analogues via a transect) and controlled laboratory incubations to achieve this greater understanding.

Characterisation of novel small molecules involved in plant disease and defense.

Professor Murray Grant (University of Exeter)

Background: An enhanced knowledge of plant disease resistance provides considerable potential to enhance food security. Global crop losses due to pathogens still exceed 40%, either from field or post harvest disease. Recent genetic and genomic approaches have identified core components of plant defense networks. However, it is the small, disease inducing or defense promoting, bioactive molecules that determine the pathogenic outcome. This project aims to address this knowledge bottleneck.

Underpinning studies: We have used liquid chromatography/mass spectrometry [BBSRC funded] to compare a time-course of metabolite profiles in resistant and susceptible leaf tissue. We identified many hundred significantly differentially abundant molecules. A combination of mass fragmentation data and advanced machine learning techniques has enabled us to infer novel metabolic networks.

PhD Project: This multidisciplinary collaborative [Exeter (Grant, Wood); Rothamsted (Ward)] project, will exploit these unique data via two approaches. One will focus on NMR based structural identification of differentially abundant metabolites whose profiles also change significantly in plant defence mutants. The second will characterise a novel class of di-ribosyl-adenosine derived plant metabolites. We predict these previously unknown defense molecules interfere with signalling and energy balance in plant-pathogen interactions.

Plant pathology, metabolite profiling and bioassays will be undertaken in Exeter. Structural identification by NMR and detailed analytical studies will be undertaken at Rothamsted.

Outcomes: The identification of novel metabolites and their temporal dynamics associated with disease development and characterisation of a totally novel class of pathogen induced signalling molecules.

Exploring the genetic and mechanistic basis of resistance to Take-all disease in wheat

Professor Kim Hammond-Kosack (Rothamsted Research)
Dr Richard Whalley (Rothamsted Research)
Dr Allison van der Meene (Rothamsted Research)
Professor Nicholas Talbot (University of Exeter)

Background: Take-all, caused by the fungus *Gaeumannomyces graminis* var *tritici*, is one of the most root damaging diseases of wheat. In severely infected fields this disease causes grain yield losses of between 1 to 3 tonnes / hectare. In addition, due to compromised root function, considerable residual N left at harvest can leach into the surrounding watercourses.

For many decades wheat breeders searched for resistant germplasm, but were unsuccessful. However, recent research at Rothamsted indicates that a few diploid wheat species *T. monococcum* (*Tm*) and some hexaploid accessions, within the historic AE Watkins collection as well as UK elite varietal pool, may consistently exhibit highly reduced take-all disease (KHK defra WGIN project, Newsletter, Nov 2011+ unpublished). This project seeks to build on this work.

This project has three main research aims:

1. To further characterise the most Take-all resistant lines via field screening and pot tests (~20 lines + controls, to be explored in 5 rep trials over 3 seasons).
2. To explore the genetic basis of resistance in *Tm* and the loci conferring the highest level of resistant in hexaploid wheats, using available mapping populations, high density KASPAR marker maps and refined field phenotyping protocols (including the use of *in situ* soil moisture probes).
3. To character in detail the cellular aspects of the root resistant phenotype in seedling assays using the Take-all fungus and the taxonomically related species *M. oryzae* (available in a GFP expressing form).

Additional DTP training component:

The student will spend in total 3 months at the commercial breeding companies, Limagrain, KWS, RAGT and Syngenta for specific training in the different wheat breeding techniques (Pedigree, Double Haploid and Single Seed Descent), early generation field selection and molecular marker analyses.

Chemical Genomics of the Plant Pathogenic Fungus *Mycosphaerella graminicola*

Professor Ken Haynes (University of Exeter)

Background. Food security is one of the major issues facing humanity. There is a pressing need to increase food production to feed the world's growing population. The fungus *Mycosphaerella graminicola* is the principle pathogen of wheat, responsible for up to 20% yield loss per year. New, durable fungicides that target this species are needed as a matter of urgency. The principle bottleneck in drug discovery programmes is the identification of the molecular targets of a compound (mode of action) and its off-target effects. Chemical genomics offers an alternative to the currently employed methods to define these. It combines the identification of the impact of a library of chemicals on a series of mutants from the same species. These data are then analysed using advanced mathematical and statistical analyses to build a chemical genomics interaction map that can be very revealing for mode of action studies.

Aims & Deliverables. To realise this ambition a collection of mutants is required. This is the principle aim of this PhD project. Specifically the students will: 1) create a library of *M. graminicola* strains over-expressing each of the ~10,000 ORFS (these are currently being cloned in another project); 2) screen these mutants using a Singer RoToR robot for susceptibility to all known fungicides and 3) produce a chemical genomics profile for *M. graminicola*.

Sex, selfish genes, and insecticide resistance

Professor David Hosken (University of Exeter)
Professor Nina Wedell (University of Exeter)

Insecticide resistance is reaching global fixation in many pest populations, yet we know little about how resistance is achieved and spreads. We will examine the mechanism and fitness consequences of resistance in *Drosophila melanogaster*, where resistance is conferred by an *Accord* retrotransposon (TE) inserted into a cytochrome P450 genes (the *Cyp6g1* gene). TE's are jumping genes that selfishly replicate within a host genome. *Accord* up-regulates *Cyp6g1* expression facilitating DDT detoxification and also causes females to pack eggs with more RNA, increasing egg survival. However there are sexually antagonistic effects of the allele because in males, up-regulation of *Cyp6g1* can decrease male mating-success and alter male aggression.

Recently, new TE insertions and duplications of *Accord* have been discovered. These are associated with large sex differences in DDT resistance, but we do not understand why. We have shown that the fitness consequences of resistance depend upon genetic background and fly gender. Understanding how resistance alleles spread requires a complete understanding of these effects. To that end, this project will build on previous results to further investigate the mechanisms underlying these background and sex differences. This is a unique opportunity to examine the impact of multiple jumping genes inserting into the promoter region of a single P450 gene.

This project will be undertaken at the Centre for Ecology & Conservation at the Cornwall Campus (<http://biosciences.exeter.ac.uk/cec/>), and a background in evolutionary or molecular biology would be advantageous.

Evolution-proof combination treatments for plant-pathogenic bacteria

Dr Britt Koskella (University of Exeter)
Professor Angus Buckling (University of Exeter)

Project description:

The evolution of antibiotic resistant bacteria is arguably the major problem facing humanity today. While last-defence antibiotics are still available to treat the majority of clinical infections, these are necessarily unavailable for agricultural use. Bacterial plant pathogens are responsible for significant reductions in UK agricultural output, hence the urgent need to develop novel, "evolution-proof" intervention strategies. Here we propose to apply evolutionary ecological theory to develop novel combination treatments for plant-pathogenic bacteria.

Biological control of plant pathogens using competitors or viruses (bacteriophages) is now commercially available. We have recently shown how precise combinations of phages can minimise long-term resistance evolution in plant-pathogenic *Pseudomonas syringae*¹, as well as identifying the genetic and ecological factors responsible for phages overcoming resistance^{2,3}.

Our project will expand on this research idea by combining evolutionary ecological theory and knowledge of the molecular mechanisms underpinning resistance to predict and test treatment combinations for plant-pathogenic bacteria. The aims are (a) to predict the critical interactions among phages, non-pathogenic bacterial competitors, and chemical control of tomato pathogens, (b) to examine the consequences of combination treatments for bacterial-plant interactions, and ultimately (c) to develop effective combination treatments for crop plants in the UK. The work will involve experimental evolution of *P. syringae* both *in vitro* and *in planta* (tomato hosts), microscopy and bioimaging of plant responses to local pathogenic and non-pathogenic bacteria, and next generation sequencing and bioinformatic analysis of evolved populations to determine the molecular mechanisms underlying resistance, as well how different resistance mutations interact within the genome.

¹ Koskella B., Thompson J.N., Preston G.M., Buckling A. 2011 Local biotic environment shapes the spatial scale of bacteriophage adaptation to bacteria. *American Naturalist* **177**(4), 440-451.

² Gómez, P. and Buckling, A. 2011. Bacteria-Phage Antagonistic Coevolution in Soil. *Science* **332**(6025),106.

³ Paterson, S. et al. 2010. Antagonistic coevolution accelerates molecular evolution. *Nature* **464**(7268), 275-278.

Magic lines

Dr Steven Penfield (University of Exeter)

Producing high quality seeds is crucial for the plant breeding industry, where germination and establishment vigour are often key determinants of final crop yield and the value of new hybrids. The consistent production of high quality seeds is confounded by environmental impacts during seed production which affect the dormancy, germination rate and establishment vigour of seed lots after harvest and processing. Producing seed of uniform high quality requires that we understand the underlying biology through which the seed production environment controls seed traits, whether it is possible to breed for consistent high seed quality in the face of multiple environmental challenges, and helping to design seed production strategies for growers and producers. In this project we will screen an Arabidopsis multiparent recombinant inbred (MAGIC) population comprising variation derived from 19 natural accessions to understand the genetic basis of the effect of the seed production environment on seed quality traits. Lines will be selected in which seed quality is insensitive to the production environment, and used to identify the genes and processes controlling seed traits.

Sub-telomeric activation of novel plant-growth-promoting compounds in the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with rhizosphere pathogens in soil

Dr Chris Thornton (University of Exeter)

Professor Murray Grant (University of Exeter)

Food security has risen to the top of political agendas, and the need to develop sustainable alternatives to environmentally damaging fungicides and costly fossil fuel fertilisers means that beneficial soil fungi such as *Trichoderma* are becoming increasingly attractive as natural biocontrol and biofertiliser agents. Very few *Trichoderma* strains exhibit both properties, biocontrol and plant-growth-promotion, but we have identified a novel isolate of *Trichoderma hamatum* (strain GD12), that displays both traits. Recently, we showed that genetic modification of GD12 significantly increases its plant-growth-promotion activities, with concomitant hyper-secretion of water-soluble compounds that stimulate root growth and canopy development (Ryder et al. [2012] *Microbiology* **158**: 84-97). Furthermore, we showed that antagonistic interactions of GD12 with root pathogens in the plant rhizosphere not only provides complete protection from disease, but also enhances the plant-growth-promotion activities of the fungus. We hypothesise that co-culture of GD12 with rhizosphere pathogens leads to chromatin re-modelling of ordinarily silent telomeric secondary metabolite gene clusters leading to the production of novel bioactive compounds with previously uncharacterised P-G-P activities. To test this hypothesis, single and double knock-out mutants of GD12 will be generated that differ in loss of one or more of a number of genes whose products regulate telomeric gene expression of secondary metabolites. Metabolomic characterisation of the GD12 and mutant secretomes will be used to identify novel bioactive chemistries, and plant bioassays and soil microcosms will be used to establish the effects of gene disruption on P-G-P activities and ecological and biological fitness of the mutants as biocontrol agents.

***Campylobacter jejuni* Niche Adaptation**

Professor Rick Titball (University of Exeter)

Dr Olivia Champion (University of Exeter)

Dr David Studholme (University of Exeter)

Background: *Campylobacter jejuni* (*Cj*) is the leading foodborne bacterial cause of diarrhoeal disease in the world. The burden of human infection due to *Cj* from different reservoirs is poorly understood, severely hampering our ability to control disease. In a preliminary study we have genome sequenced seven clinical *Cj* strains, revealing marked genomic differences in function unknown (FUN), flagellin glycosylation and capsule biosynthesis genes. We have previously correlated flagellin glycosylation with colonization of poultry (Champion *et al*, PNAS 2005). **We hypothesise that genomic differences in *Cj* strains can be attributed to adaptation to their natural environmental niche.**

Aim 1: Genome sequencing of *Cj* strains isolated from environmental sources (poultry, ovine, bovine and environmental).

Deliverable: *de novo* genome sequence assembly and annotation

Aim 2: Construction of core and accessory *Cj* genome phylogenies.

Deliverable: Phylogenies. Identification of source specific genetic markers.

Aim 3: Functional studies of genetic markers, including FUN genes, to understand their role in niche adaptation and virulence. We have recently developed a novel assay for assessing *Cj* virulence (Champion *et al*, JID, 2010) and we are developing a zebrafish embryo infection model to study the interaction of *Cj* with the host. These models along with BIOLOG phenotyping will be used to characterise wild type and mutant *Cj* strains.

Deliverable: Mechanisms of niche adaptation and survival.

A greater understanding of *Cj* niche adaptation and reservoirs of infection that contribute human infection will allow the identification of genetic and phenotypic markers that will inform biocontrol strategies to eliminate *Cj* from the foodchain.

An evolutionary fluke resulting in novel and safe drugs to protect crops and humans

Dr Mark van der Giezen (University of Exeter)

Dr David Studholme (University of Exeter)

We identified a unique and novel mechanism that can be exploited to tackle major plant pathogens such as *Phytophthora*. This proposal addresses the BBSRC Food Security strategic priority and will provide combined training in bioinformatics and biochemistry/cell biology. Both supervisors have produced 4*-publications and will create a high quality student experience.

The aims are:

- Identify the novel stramenopile-specific mitochondrial transport protein by comparative genomics.
- Interfere with this glycolytic transporter using substrate-analogs.

We have demonstrated that in a large group of organisms, which includes major plant pathogens such as *Phytophthora* and the human parasite *Blastocystis*, the second half of glycolysis is localized in mitochondria rather than the cytoplasm. As this is in contrast with our general understanding of glycolysis, we anticipate producing 4*-output.

Mitochondrial localization of the latter half of glycolysis necessitates a novel transporter. As several genomes of related organisms are available, and both supervisors have produced additional related human and plant pathogen genomes (for example the Gatsby/FERA-funded *P. ramorum* sequencing project aimed at understanding the pathogen that destroys our larch population), comparative genomics will allow us to identify this transporter. This prediction will be tested in the lab where natural and synthetic substrates will be employed to characterise and block the function of this novel transporter. Interference with this transporter is expected to be lethal for these organisms opening the way to new and safe herbicides to be developed against a major plant pathogen in line with BBSRC priorities.

From research to behaviour change: contrasting models of farmer engagement in agricultural research

Professor Michael Winter (University of Exeter)
Dr Matt Lobley (University of Exeter)
Dr David Chadwick (Rothamsted – North Wyke)
Dr Phil Murray (Rothamsted – North Wyke)

Ensuring that the findings of agricultural research are developed and applied in the farming industry is a challenge that continues to confront the science policy community, and most new research initiatives make some effort to address the issue. For example, the BBSRC funded North Wyke Farm Platform, with its *in situ* state-of-the-art instrumentation in hydrologically isolated fields and farmlands, seeks to address key issues in sustainable agriculture and to engage and involve farmers in that research. An ESRC Knowledge Exchange grant has been awarded to help that engagement process. The proposed studentship would examine the actual and potential Knowledge Exchange (KE) activities associated with the Farm Platform through the application of research methods on social learning and behaviour change drawn from Sociology and/or Social Psychology. It is expected that the student will undertake an initial analysis of the comparative strengths of contrasting approaches to research and demonstration in the context of food security and climate change, exploring different 'models' currently used in the UK/Europe, Australia, the USA and China. These include direct research-to-farmer Knowledge Transfer (KT) approaches, farmer-to-researcher-to-farmer participative KE approaches, and Agricultural Knowledge System approaches which seek to combine KT and KE. Drawing on perhaps three case studies for in-depth investigation, the PhD student would develop ideas to test with farmers associated with the Farm Platform in order to come up with firm evidence-based proposals for an on-going programme of farmer engagement at North Wyke that would contribute to social learning, behaviour change and sustainable agriculture.