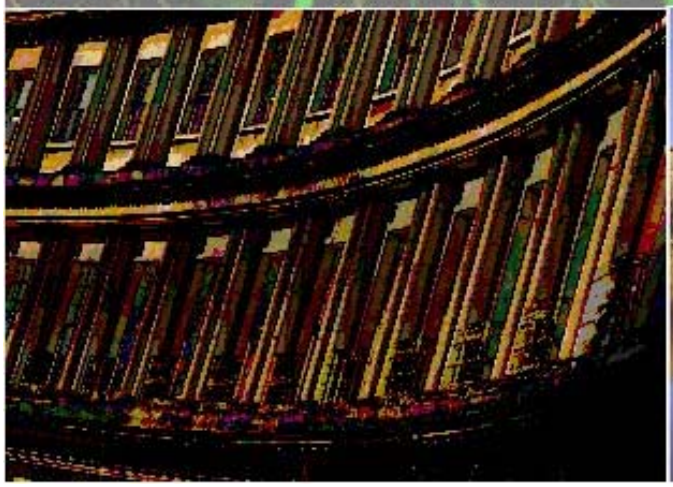
Attack and Defence in Plant Disease

BSPP Presidential Meeting
University of Bath, UK
12 - 14 September 2007



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Foreword from the President for 2007

Welcome to "Attack and Defence" at the University of Bath. The theme of the BSPP Presidential Meeting typically reflects the main research area of the incumbent. I have worked on mechanisms of pathogenicity and defence throughout my career, so the topic to choose was clear. In particular this field is moving at a pace undreamt of but a few years ago and justifies frequent exposure. This progress was made clear by me in a fairly detailed account of plant-pathogen interactions over the last 25 years (to coincide with the anniversary of this Society; see December 2006 BSPP Newsletter available on www.bspp.org.uk). In this article I described what was known then and what we did not know, then why and how progress was made. The main black hole was specificity. Avirulence (*avr*) genes clearly existed as did resistance (*r*) genes but what were they and how did they interact? There was abundant speculation but no molecules. The picture is still incomplete, but this week you will hear remarkable detail on this crucial aspect. What is especially rewarding is that progress has not only come from bacterial-plant models but with fungi, viruses and the once intractable oomycetes, obligately biotrophic fungi and phytoplasmas and how their effectors target host defences. The discovery of gene for gene interactions was made with flax rust back in the 1940s and I am delighted that the Keynote Address given by Jeff Ellis will take this forward to recently discovered protein-protein interactions in this classic disease. Hypersensitivity is the typical outcome of R-AVR interactions and you will gain more than just the biology and ultrastructure of this phenomenon that we once knew, but how cell death is regulated and its diverse effects.

Add to this considerable advances made on basal defence or innate immunity-how pathogens are perceived and the pathways, genes and defence-related products that result. Also plant growth regulators were once considered as merely irrelevant by-products of disease but are now known to play key roles in some cases.

I have also added speakers reflecting some of my specific interests; thus a vascular fungal pathogen had to be there as did the considerable but underestimated influence of sulphur as the fourth macro-nutrient for plants and the myriad S-containing defence compounds that require the element.

Read the abstracts to realise that this should be a feast of good science; delegate numbers and the venue should encourage compatible person-person interactions. The conference dinner may not quite match a feast but the music before and after should amuse then encourage you to shed some calories.

If you are not a member of British Society for Plant Pathology then I hope after all this you will realise what you are missing. It is a very cheap way to join a very generous (travel and fellowship awards) and prestigious international society and receive one of its quality journals (Molecular Plant Pathology or Plant Pathology).

I should add that if you find time, you should try to spend at least a couple of hours in the beautiful, compact city of Bath. All the key sites are within walking distance. Those with transport can easily reach fine old villages and of course the traditional English pub. Ask me or local helpers how and where to go. Enjoy your stay.

Conference Organisers:
Programme Secretary: Gerry Saddler
Scientific Programme: Richard Cooper; Paul Birch; Matt Dickinson; Gary Loake

Richard M. Cooper. Bath, UK

Programme

Wednesday 12th September

11.00-onwards	**Registration**
13.00–14.00	Lunch
	GARRETT MEMORIAL LECTURE
14.00–14.45	**George Salmond - University of Cambridge, UK** "How to have fun with a little rotter: team building for attack and defence in the bacterial world"
14.45–15.20	Tea / Coffee

SESSION 1 - ATTACK (BIOTROPHY THROUGH NECROTROPHY)

15.20–15.45	**Nick Talbot - University of Exeter, UK** "Investigating the biology of plant infection by the rice blast fungus *Magnaporthe grisea*"
15.45–16.10	**Ralf Voegele - University of Konstanz, Germany** "Biotrophy or not to be! The story rust fungi have to tell"
16.10–16.35	**Martijn Rep - University of Amsterdam, The Netherlands** "*Fusarium oxysporum*: from harmless root colonizer to host-specific vascular wilt pathogen"
16.35–17.00	**Max Dow - University College Cork, Ireland** "*Xanthomonas campestris* virulence: regulation and signalling"
17.15 – 19.00	**POSTERS**
19.30	Dinner

Thursday 13th September

SESSION 2 - ATTACK (EFFECTORS FROM PATHOGENS)

09.00–09.25 **Jim Alfano - University of Nebraska, USA**
"The molecular basis of plant immunity suppression by the *Pseudomonas syringae* type III effector HopU1"

09.25–09.50 **Paul Birch - Scottish Crops Research Institute, Dundee, UK**
"*Phytophthora infestans* effectors that are translocated into the host cell"

09.50–10.15 **Chris Ridout - John Innes Centre, Norwich, UK**
"Powdery mildews: secrets of their success"

10.15–10.40 **Joel Milner – University of Glasgow, UK**
"A cauliflower mosaic virus effector protein that manipulates multiple defence pathways in Arabidopsis"

10.40–11.20 Tea / Coffee

SESSION 3 ATTACK (GENOMICS)

11.20–11.45 **Ian Toth - Scottish Crops Research Institute, Dundee, UK**
"The enterobacterial plant pathogen *Pectobacterium atrosepticum*: Genomics and the adaptation to a life on plants"

11.45–12.10 **Jim Beynon - Warwick HRI, UK**
"The effector proteins of *Hyaloperonospora parasitica*"

12.10–12.35 **Ralph Dean - North Carolina State University, US**
"An 'Omics' perspective on fungal pathogenesis"

12.35–13.00 **Saskia Hogenhout - Ohio State University, USA**
"A secreted effector protein of AY-WB phytoplasma accumulates in nuclei and alters gene expression of host plant cells"

13.00–14.00 Lunch

14.00–14.45 **PRESIDENTIAL ADDRESS**
Richard M. Cooper - University of Bath, UK
"Polymers and oligomers in attack and defence"

14.45–15.00 Tea / coffee

Thursday 13th September

PH GREGORY PRIZE

15.00-15.15	**Terry Evans - University of Cambridge, UK** "The role of lipopolysaccharide in the virulence of the phytopathogen *Erwinia carotovora* subsp. *atroseptica"*
15.15-15.30	**Pari Skamnioti - University of Oxford, UK** *"Magnaporthe grisea* cutinase 2 mediates appressorium differentiation and host penetration and is required for full virulence"
15.30-15.45	**Kathy Mitchell - Imperial College London, UK** "Exploring the apoplast – what stops *hrp* mutants in Arabidopsis?"
15.45-16.00	**Michael Ravensdale – Scottish Crops Research Institute, Dundee, UK** "Regulation of phytotoxin production in the plant pathogen *Pectobacterium atrosepticum* and microarray analysis of the effects of these toxins on potato (*Solanum tuberosum*)"
16.00-16.15	**Mary Illes - University of Oxford, UK** "Role of nitric oxide and nitric oxide synthases in *Magnaporthe grisea"*
16.15-16.30	**Kee Hoon Sohn - Sainsbury Laboratory, John Innes Centre, Norwich, UK** "The downy mildew effector protein ATR13 promotes *Pseudomonas syringae* susceptibility in *Arabidopsis thaliana*"
16.30-16.45	**Jennifer Hodgetts - University of Nottingham, UK** "Phylogenetic analysis of phytoplasmas based on sequences derived from the *secA* and 23S rDNA genes"
16.45-17.00	**Henry Dzahini-Obiatey - University of Reading, UK** "Further evidence for high "*Cacao swollen shoot virus"* content in the cotyledons of infected cocoa plants"
19.30-00:00	Presidential Dinner

Friday 14th September

SESSION 4 – DEFENCE (BASAL / NON-HOST)

09.00–09.25 **Jürg Felix - Eberhard-Karls-University, Tübingen, Germany**
"PAMP perception"

09.25–09.50 **David Mackey - Ohio State University, USA**
"Basal defenses underlying bacterial resistance"

09.50–10.15 **Rüdiger Hell - University of Heidleberg, Germany**
"Sulphur nutrition and S defence compounds"

10.15–10.40 **Gary Loake - University of Edinburgh, UK**
"Uncovering the functions of S-nitrosothiols in plant disease resistance"

10.40–11.20 Tea / Coffee

SESSION 5 – DEFENCE (HR)

11.20–11.45 **Ari Sadanandom - University of Glasgow, UK**
"A pair of E3 ubiquitin ligases act antagonistically to regulate plant cell death and defence"

11.45–12.10 **Renier van der Hoorn - Max Planck Institute for Plant Breeding Research, Cologne, Germany**
"Applying Activity-based Proteome Profiling to study the *Pseudomonas-Arabidopsis* Interaction"

12.10–12.35 **Savithramma P. Dinesh-Kumar - Yale University, US**
"Life after death: Role for autophagy during innate immunity"

12.35–13.00 **Dominique Roby – INRA, Toulouse, France**
"Key genes for HR signalling in *Arabidopsis*"

13.00–14.00 Lunch

SESSION 6 – R GENES AND INTERACTIONS

14.00–14.45 **KEYNOTE ADDRESS**

Jeff Ellis Australia - CSIRO, Canberra, Australia
"Flax rust–from gene for gene to protein-protein"

Friday 14th September

SESSION 6 – R GENES AND INTERACTIONS

14.45–15.10	**Murray Grant - University of Exeter, UK** "RPM1 - distal effects"
15.10–15.35	Tea / Coffee
15.35–16.00	**Vivianne Vleeshouwers - Wageningen University & Research Centre, The Netherlands** "Effector genomics: the key to novel R genes and their exploitation"
16.00–16.25	**James Brown, John Innes Centre - Norwich, UK** "Discovering and using resistance genes in improved crop varieties"

Posters

P1 **Characterization and pathogenicity of isolates of *Albonectria rigidiuscula* and *Botryosphaeria rhodina* from dieback lesions of cocoa in Ghana**
R. Adu-Acheampong & S. Archer

P2 **Yeast 2 hybrid screening as a method for the identification of plant targets of oomycete effector protein AVR3a**
M.R. Armstrong, S.C. Whisson & P.R.J. Birch

P3 **Transient gene silencing – a step forward in identifying novel pathogenicity factors in the late blight pathogen, *Phytophthora infestans*.**
A. Avrova, P. Boevink, L. Grenville-Briggs, P. van West, P. Birch & S. Whisson

P4 **Molecular genetics to identify genes involved in the pathogenicity of *Fusarium* spp. on wheat ears**
T. Baldwin, M. Urban, J. Antoniw, F. Trail & K.E. Hammond-Kosack

P5 **PHI-base: A database of experimentally verified pathogenicity, virulence and effector genes in fungal, Oomycete and bacterial pathogens of animals and plants**
T.K. Baldwin, R. Winnenburg, A. Beacham, M. Urban, C. Rawlings, J. Koehler & K.E. Hammond-Kosack

P6 **Identification of pathogenesis-related determinants in the rice blast fungus**
M.I. Besi & A. Sesma

P7 **Functional characterisation of nematode chorismate mutases – a role in suppression of plant defence signalling pathways?**
V. Blok, M. Phillips, L. Pylypenko, J. Manuel Rodriguez, R. Shaw, A. Booth, E. Gilroy, C. Plain & J. Jones

P8 **Defining Downy Mildew (*Hyaloperonospora parasitica*) Avirulence Loci**
M. Coates, W. Badejoko, L. Baxter, P. Bittner-Eddy & J. Beynon

P9 **Functional evaluation of plant defence signalling against Fusarium ear blight disease in Arabidopsis**
A. Cuzick, M. Urban, K. Maguire, S. Lee & K. Hammond-Kosack

P10 **Investigating the roles of reactive oxygen species during *Mycosphaerella graminicola* infection of wheat**
S. Deller, J. Keon, J. Antoniw, K. Hammond-Kosack & J. Rudd

P11 **Innate Immunity: Plant recognition of bacterial PAMPs**
G. Erbs, T. Tandrup Jensen, J. Maxwell Dow, A. Molinaro, M. Parrilli & M.-A. Newman

P12 **Analysis of metabolic profiles associated with basal defence and the establishment of pathogenicity**
S. Forcat, M. Bennett, J. Ward, M. Beale, M. Grant & J. Mansfield

P13 **Identification of environmental factors driving *in-planta* evolution of virulence by genomic island loss in *Pseudomonas syringae* pv. *phaseolicola* 1302a**
S.A.C Godfrey, H.C. Lovell, J.W. Mansfield & D.L. Arnold

P14 **Cellulose synthesis in *Phytophthora infestans* is required for appressorium formation and successful infection of potato.**
L.J.Grenville-Briggs, V.L. Anderson, A.O. Avrova, A.Williams, S.C. Whisson, P.R.J. Birch & P. van West

P15 **A 2-nitropropane dioxygenase from *Botrytis cinerea***
H. B. Hughes, R. K. Poole & J. D. Scholes

P16 **Genomics approaches uncover an alternative life-style of the plant pathogen *Pectobacterium atrosepti*cum**
S. Humphris, G. Waersted Takle, H. Liu, L. Hyman, L. Pritchard, M.-B. Brurberg, P. Birch & I. Toth

P17 **Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable brassica fields in Nepal and identification of a new race**
B. D. Jensen, J. G. Vicente, H. K. Manandhar & S. J. Roberts

P18 **Biocontrol and plant transformation as a new alternative integrated strategy to control *Botrytis cinerea* on lettuce**
R Laboh, M R Davey, J B Power & S Rossall

P19 **Identifying resistance to Fusarium Ear Blight in wheat**
S. Lee, A. Daudi & K. Hammond-Kosack

P20 **A whole genome transcriptomics approach to determine the quorum sensing regulon of *pectobacterium atrosepticum* during potato infection**
H. Liu, S. Humphris, L. Pritchard, P. Hedley, M. Ravensdale, P. Birch & I. Toth

P21 **DON mycotoxin biosynthesis by Fusarium species, a metabolomics perspective.**
R. Lowe, J.W. Allwood, M. Urban, M. Beale, J. Ward & K. Hammond-Kosack

P22 **How and where do plants make salicylic acid?**
K.M. Macaulay, J.P. Carr & A.G. Smith

P23 **Unraveling gene regulatory networks governing the Arabidopsis response to *Botrytis cinerea* infection**
P. Madhou, J. Mulema, O. Windram, N. Adams, Z. Paniwnyk & K. Denby

P24 **Barley non-host and host interactions with *Polymyxa* species elicit a similar basal resistance response**
G.R.D. McGrann, B.J. Townsend, J.F. Antoniw, M.J.C. Asher & E.S. Mutasa-Göttgens

P25 **The Roles of Cathepsin B-like Proteases in Plant Disease Resistance**
H. M^{c}Lellan, E. Gilroy, I. Hein, P. Boevink, R. van der Hoorn, P. Birch & G. Loake

P26 ***Agrobacterium*** **suppresses P.** ***syringae*****-elicited salicylate production in** ***Nicotiana tabacum***
A. Rico, W. Huang, I. Moore & G. Preston

P27 **The effect of mycotoxigenic microorganisms on the quality of palm oil produced at the cottage industry level**
S.M.A. Tagoe & M.J. Dickinson

P28 **Phenotypic and metabolomic analyses of the** ***Fusarium graminearum snf1*** **mutant**
M. Urban, J. Antoniw, W. Allwood, A. Beacham, J. Ward, M. Beale & K. Hammond-Kosack

P29 **NpPDR1, a Pleiotropic Drug Resistance transporter from** ***Nicotiana plumbaginifolia*** **is implicated in plant-pathogen interactions**
T. Trombik, A. Bultreys, E. Peeters, Y. Stukkens & M. Boutry

P30 **New sources of resistance to UK isolates of** ***Hyaloperonospora parasitica*** **in** ***Brassica oleracea*** **L.**
J.G. Vicente, D.A.C. Pink & E.B. Holub

P31 **Investigating the effects of fungicides and biocontrol on take-all of wheat**
M. Yu, S. Rossall & M. Dickinson

How to have fun with a little rotter: team building for attack and defence in the bacterial world

George Salmond

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The enterobacterial genus of plant pathogens, *Erwinia* (now *Pectobacterium),* has been investigated by plant pathologists over many years. The "soft rot" group of erwinias has been of special interest because, even back in the era when real molecular biological analysis was not possible in virtually any phytopathogen, the enterobacterial nature of the erwinias enabled a faster and deeper genetic tractability than in most other phytopathogens.. Also, because of the obvious rotting phenotypes caused by these bacteria, there was a considerable interest in the plant cell wall degrading enzymes (PCWDEs) elaborated by the soft rot erwinias because they could be studied biochemically and phytopathologically. The concentration of interest in the PCWDEs helped to encourage a view that soft rotting erwinias were rather "crude" plant pathogens that simply produced and secreted enzymes that led to the rot. On one level, this was a useful view because the international research community could concentrate for a while on investigating the nature of the enzymes (genes, organisation, catalytic properties, evolutionary families etc) and their regulation and secretion. The mechanisms involved in their regulation and secretion turned out to be extremely interesting in that the research showed that there were strongly convergent themes in the regulation and secretion of bacterial virulence factors of plant and animal pathogens – a view that might have been somewhat heretical in earlier years! However, this improved the intellectual "image" of molecular phytopathology because it helped to put plant pathogenesis research on an equivalent platform to that of animal pathogenesis and showed that plant pathogen research provided excellent models for molecular pathogenesis in general – a view that everyone now accepts as uncontroversial.

In a genomics era there has been an explosion of new information uncovered about the erwinias that has taken the field into diverse protein secretion systems (Types I to VI); antibiotics and other bioactive secondary metabolites; toxins, surface components and other factors that impinge on plant attack or suggest possible roles in the ecological fitness of the pathogen. Insect vector connections and innate immunity stimulation roles have also been uncovered and the area of population biology research, horizontally-acquired gene island transfer and phage-driven adaptive evolution are all now topical in a way that would have been unimaginable only a decade ago.

I will discuss selected aspects of the history of soft rot erwinia research to highlight some issues of defence and attack, and will focus on the key role of intercellular chemical communication ("quorum sensing") in the general biology, physiology and phytopathogenesis of the erwinias.

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

Nicholas J Talbot, Diane O. C. Saunders, Darren M. Soanes, Richard A. Wilson, Michael J. Kershaw, Claire Veneault-Fourrey, Elise Lambeth, Thomas A. Richards, Martin J. Egan, Han-Min Wong & Zaira Caracuel-Rios

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The rice blast fungus *Magnaporthe grisea* causes one of the most serious diseases of cultivated rice. The availability of full genome sequences for *M. grisea* and its host, rice, has allowed the first opportunity to define the gene inventory associated with a fungal crop disease and has provided insights into both the fungal-plant interaction and the evolution of fungal pathogenicity. During plant infection, the rice blast fungus elaborates a specialised infection structure known as an appressorium. This unicellular, dome-shaped structure generates turgor that is translated into mechanical force to allow rupture of the rice cuticle and entry into plant tissue. We set out to explore whether the development of a functional appressorium was linked to the control of cell division. This was based on the observation that following germination of a conidium on the rice leaf surface, a single round of mitosis always occurs during germ tube elongation, prior to the formation of an appressorium. We found that blocking completion of mitosis by generation of a temperature-sensitive *MgnimA* mutant prevented appressorium morphogenesis. Furthermore, we found that following mitosis, conidia always undergo cell collapse and cell death, which appears to be a programmed, autophagic process. Deletion of *MgATG8* prevented autophagy in *M. grisea* and rendered the fungus non-pathogenic. Taken together, our results indicate that appressorium morphogenesis requires genetic control by completion of mitosis and autophagic cell death of the conidium. Once the *M. grisea* appressorium has formed, cellular turgor is generated by accumulation of osmotically-compatible solutes, notably glycerol. We have used genetic, biochemical, proteomic and, most recently, metabolomic analysis to investigate how turgor is generated and to define the key genetic determinants of appressorium function. Of particular interest is the central role of trehalose metabolim to the genetic control of fungal virulence and the role of peroxisomal fatty acid beta-oxidation to appressorium physiology. The appressorium brings about plant infection by elaborating a penetration hypha that differentiates further into invasive hyphae which grow rapidly within the host plant cells. One of the key challenges in understanding rice blast disease is to determine how fungal proteins are delivered to the host during plant infection and to define the mechanisms by which the fungus proliferates biotrophically within the rice leaf. Progress towards determining the secretory processes necessary for *M. grisea* plant tissue colonization will be presented.

Biotrophy or Not to Be! The Story Rust Fungi Have to Tell

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On a global scale, some of the most serious fungal plant pathogens are obligate biotrophic parasites. The term obligate biotrophic characterizes a specific interaction in which the host as a whole suffers only minor damage over an extended period of time. The pathogen in turn is completely dependent on a living host plant to complete its life cycle. In order to clearly distinguish true obligate biotrophic fungi from hemibiotrophs or necrotrophs the following five criteria were suggested: a) highly differentiated infection structures; b) limited secretory activity; c) a narrow contact zone separating fungal and plant plasma membranes; d) long term suppression of host defense responses; e) the formation of haustoria. According to these criteria the range of organisms designated as true obligate biotrophs comprises the Downy Mildews (Oomycota), the Powdery Mildews (Ascomycota), and last but not least, the Rusts (Basidiomycota).

Especially haustoria have stimulated the interest of plant pathologists ever since their discovery more than 150 years ago, for they are one of the hallmarks of the obligate biotrophic lifestyle. Already in naming these structures [fr. L. haurire (haurio, hausi, haustum): to drink, to draw] de Bary proposed one of the possible functions for haustoria - the uptake of nutrients from the host. However, the close interaction of host and parasite and the fact that haustoria cannot be produced *in vitro* have hampered the analysis of the roles haustoria may play in establishing and maintaining the obligate biotrophic lifestyle for decades. Only recently it has become possible to analyze haustorial function at a molecular level.

For the last decade, my group has been busy to apply molecular, biochemical, and cytological tools using the pathosystem *Uromyces fabae* and its host *Vicia faba* in order to elucidate the possible roles haustoria may play in this intricate interaction. Using our "toolbox", we were able to demonstrate that haustoria indeed serve in nutrient uptake - the task already postulated for these structures. However, in addition they also seem to perform enormous biosynthetic duties. Furthermore, there is growing evidence for an engagement of these structures in the suppression of host defense responses and in redirecting and/or reprogramming the host's metabolic flow.

This presentation will recapitulate some of the most important results obtained from our research and will integrate them with results from ongoing projects. Quantification of the pathogen *in planta*, establishment of a reliable transformation system, and the increasing availability of fungal sequence data have opened new vistas in our quest to better understand the function of haustoria.

Fusarium oxysporum: from harmless root colonizer to host-specific vascular wilt pathogen

H. Charlotte van der Does, Petra M. Houterman, Wilfried Jonkers, Caroline B. Michielse, Ringo van Wijk, Ben J.C. Cornelissen & Martijn Rep

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The ascomycetous fungus *Fusarium oxysporum* lives in and among root systems of plants worldwide. It is commonly harmless and can sometimes even suppress diseases caused by root-invading pathogens. However, a great number of host-specific pathogenic forms (*formae speciales*) have emerged in agriculture and horticulture that cause serious diseases such as panama disease of banana, cotton wilt, and bulb rot of tulips. No sexual stage has yet been found, and genetic analyses have revealed that *F. oxysporum* mainly or exclusively propagates asexually. Interestingly, many *formae speciales* consist of multiple clonal lines of independent origin.

Using the interaction between *F. oxysporum* and tomato as a model system, we present evidence that specific virulence genes encoding small secreted proteins lie at the basis of host-specific pathogenicity. These genes are present on a small chromosome in tomato-infecting strains that may be subject to horizontal gene transfer. Monogenic resistance of tomato is based on recognition of some of these *forma specialis*-specific small secreted proteins.

Apart from this specific adaptation to particular plant species, the basic ability of *F. oxysporum* to colonize living plants also relies on a set of conserved genes that we are discovering through insertional mutagenesis. The predicted functions of several of these genes implicate cell wall integrity, peroxisome function and particular metabolic capabilities as being required for pathogenicity.

Xanthomonas campestris virulence: regulation and signalling

Max Dow, Yvonne Fouhy, Belén Fernández García & Robert Ryan

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The full virulence of *Xanthomonas campestris* pv. *campestris* (*Xcc*) to plants depends upon cell-cell signalling mediated by the diffusible signal molecule DSF, which has been characterised as *cis*-11-methyl dodecenoic acid. Synthesis and perception of DSF require protein products of the *rpf* gene cluster. RpfF directs the synthesis of DSF and the RpfC/RpfG two-component system couples DSF perception to metabolism of the second messenger cyclic di-GMP. The *rpf*/DSF system regulates many processes including synthesis of extracellular enzymes, synthesis of the extracellular polysaccharide xanthan, synthesis of cyclic glucan and biofilm formation. Some of these regulated functions have been shown to contribute to virulence. Xanthan appears to have multiple roles in disease; it is an integral component of *Xcc* biofilms and can also act in localised suppression of plant defence responses. Extracellular cyclic glucan also acts in plant defence suppression, but can exert a systemic effect, which is associated with the systemic movement of the molecule within plants. DSF signalling has to be finely balanced for the formation of structured biofilms in vitro and for optimal virulence to plants. Interference with DSF signalling may thus have a role in the control of diseases caused by *Xcc* and perhaps other *Xanthomonas* spp.

The molecular basis of plant immunity suppression by the *Pseudomonas syringae* type III effector HopU1

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The bacterial pathogen *Pseudomonas syringae* is dependent on a type III protein secretion system and the effector proteins it injects into host cells to cause disease. Many *P. syringae* effectors have been shown to suppress plant innate immunity. However, the majority of their enzymatic activities and their plant targets remain unknown. The *P. syringae* effector HopU1 was purified and we demonstrated that it was a mono-ADP-ribosyltransferase (ADP-RT) active on the artificial substrate poly-arginine as well as plant proteins. HopU1 suppressed plant innate immunity in a manner dependent on its ADP-RT active site. Using ADP-RT assays coupled with mass spectrometry we identified the major HopU1 substrates in *Arabidopsis thaliana* extracts to be several RNA-binding proteins that possess RNA-recognition motifs (RRMs). *A. thaliana* knock-out lines defective in the glycine-rich RNA-binding protein *At*GRP7, a HopU1 substrate, were more susceptible than wild type plants to *P. syringae* infection suggesting that this protein plays a role in innate immunity. The ADP-ribosylation of *At*GRP7 by HopU1 required two arginine residues within the RRM of *At*GRP7 suggesting ADP-ribosylation may interfere with the ability of *At*GRP7 to bind RNA. Our results suggest bacterial pathogens can ADP-ribosylate plant RNA-binding proteins to suppress host innate immunity by affecting immunity-related RNA.

Phytophthora infestans effectors that are translocated into the host cell

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Plant pathogens establish disease by suppressing or manipulating host innate immunity. To this end, bacteria, oomycetes and fungi translocate effector proteins into host cells where they may directly interact with plant defences. Translocation of bacterial effectors is through the well-understood type III secretion system (T3SS), but analogous processes for effector delivery are uncharacterised in fungi and oomycetes. Here we report the functional analysis of two motifs, RXLR-EER, present in translocated oomycete effectors. We use the *Phytophthora infestans* RXLR-EER-containing protein AVR3a as a reporter for translocation, as it triggers RXLR-EER-independent hypersensitive cell death following recognition within plant cells containing the R3a resistance protein. AVR3a, with or without the RXLR-EER motif, is secreted from *P. infestans* biotrophic structures called haustoria, demonstrating that the motif is not required for targeting to haustoria or for secretion into the extra-haustorial matrix. However, following replacement of the AVR3a RXLR-EER motifs with alanine residues, singly or in combination, or with residues KMIK-DDK, representing changes that otherwise conserve the properties of the protein, *P. infestans* fails to elicit R3a-mediated cell death, demonstrating that these motifs are required for translocation of the protein into the host cell. We show that 38 expressed RXLR-EER-encoding genes from *P. infestans* are transcriptionally up-regulated immediately prior to and during infection. A combination of two algorithms identifies 425 potential genes encoding secreted RXLR-EER-class proteins in the *P. infestans* genome. Identification of this class of proteins provides an unparalleled opportunity to determine how oomycetes manipulate hosts to establish infection. We show that AVR3a interacts with a number of host proteins and we speculate on the role of this protein in subverting host defences.

Powdery Mildews: secrets of their success

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Powdery mildews are extremely successful parasites, infecting over 9000 dicot and over 650 monocot plant species and causing economic losses to many crops in temperate areas. They are obligate biotrophs, growing and reproducing entirely on living epidermal cells and obtaining nutrients from their host by means of specialised feeding structures known as haustoria. Their obligate lifestyle has resulted in a high degree of specialisation, and many powdery mildews are able to infect only a few closely-related host plant genera.

Blumeria graminis f.sp. *hordei* (*Bgh*) causes one of the most significant diseases of barley in temperate latitudes worldwide and is the most intensively studied of all powdery mildew fungi.
Bgh displays gene-for-gene (GFG) interactions with its host plant, in which the recognition of an avirulence (AVR) molecule by a host resistance (R) protein so triggering a localized cell death known as the hypersensitive response. More than 85 barley R genes, each conferring resistance to specific *Bgh* AVR elicitors have been described, including 28 alleles at the Mla locus. More than 25 independent AVR gene loci have been described in *Bgh* isolates. Mla resistance has been used in agriculture to control *Bgh*, but field control of the disease is short-lived because the fungus rapidly evolves to lose AVR molecules and become virulent.

To understand the recognition function of Mla proteins and to gain insight into the evolution of virulence, AVR genes were isolated from *Bgh* (Ridout et al, 2006, The Plant Cell 18, 2402-2414). AVRk1 and AVRa10 belong to a large gene family in the genome of *Bgh*, and homologous sequences are present in other *formae speciales* of *B. graminis* which infect other grasses. The genes encode proteins that have dual functions, both as 1) elicitors of the resistance response and 2) as effectors, which increase infection success on susceptible hosts. These findings suggest that *Bgh* and other *formae speciales* of *B. graminis* might have a repertoire of related effectors. Redundancy in this gene family would explain why individual AVR elicitors can be lost without apparent loss of fitness. Investigations into the function and localisation of AVRk1 and AVRa10 are currently in progress.

With the imminent release of the *Bgh* genome sequence, the isolation of further AVR genes will be a priority. Initial analysis of the raw sequence data indicates that in the genome of *Bgh* there are about 300 paralogues of AVRk1 which are more distantly related than those identified by hybridisation. Comparison of these sequences will enable further investigation into the evolution of the gene family and on the selection constraints imposed on their diversification. High throughput sequencing of the genomes of the *B. graminis formae speciales* adapted to different grasses will allow investigations into the basis of host specialisation and non-host recognition.

A Cauliflower mosaic virus effector protein that manipulates multiple defence pathways in Arabidopsis.

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CaMV infection promotes strong SA-dependent responses in pre-invasion tissues of Arabidopsis but this is ameliorated as virus titres rise, suggesting that CaMV can suppress defence responses locally. P6, a 520 amino-acid polypeptide, is the main pathogenicity determinant encoded by CaMV, and functions as a suppressor of RNA-silencing. Transgenic plants that express P6 exhibit a symptom-like phenotype and show altered ethylene and auxin responses. We have recently shown that P6 also has a profound effect on SA- and JA- signaling, depressing SA-accumulation, inhibiting the expression of SA-dependent markers, and stimulating JA biosynthesiss. Transgene-mediated expression of P6 leads to greatly enhanced susceptibility to both virulent and avirulent isolates of *Pseudomonas syringae* pv tomato, and delays the onset of HR with avirulent strains. We have used a transgenic line expressing a NPR1:GFP fusion protein to demonstrate that in the presence of P6, fluorescence is always localized to the nucleus even in the absence of SA, and that the NPR1:GFP fusion protein shows an altered electrophoretic mobility. These results suggest that P6 functions as an effector molecule by interfering with the cytoplasmic-nuclear translocation of NPR1. This provides an attractive explanation for the effect of P6 expression of SA and JA signaling.

The enterobacterial plant pathogen *Pectobacterium atrosepticum*: Genomics and the adaptation to a life on plants

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In 2004 SCRI led a joint project with the Sanger Institute to sequence the genome of the enterobacterial potato pathogen *Pectobacterium atrosepticum* (*Pba* - formerly *Erwinia carotovora* subsp. *atroseptica*). The availability of this sequence has led to a series of functional genomics and systems biology approaches to investigate the *Pba*-potato interaction.

Computational analyses are at the heart of our genomics research and to this end we have developed a software package called GenomeDiagram for the visualisation of large genome datasets. This software has been used to compare the *Pba* genome with over 200 other fully sequenced genomes. This includes, for the first time, an investigation into the similarities and differences between *Pba* and the enterobacterial human pathogens (enterics). *Pba* shares a common backbone of around 75% of its genes with the enterics, with the remaining 25% appearing to represent horizontally-acquired islands. These islands have a propensity for genes involved in *Pba*'s plant-associated life-style and several novel targets are being investigated, including putative phytotoxins and Type III effectors. These determinants have been shown to play a role in pathogenicity and in manipulating potato resistance. From this work we have identified a potato defence response gene that appears to be involved in defence against *Pba* and, using transgenic technology, we have manipulated this gene to develop a fully *Pba* resistant plant. Mechanisms involved in a lifestyle away for diseased plants are also being investigated and include root attachment and nitrogen fixation. While many of the genes in *Pba* appear to have been acquired from other plant-associated bacteria, enabling it to live in a plant environment, recent evidence from our comparative analyses shows that the enterics may also have acquired genes involved in survival on plants. Can a better knowledge of such genes be used to help reduce the incidence of human diseases associated with the environment?

Microarrays representing the majority of *Pba* coding sequences are being used to investigate differences in gene content between *Pba* and its closest (unsequenced) relative *Pectobacterium carotovorum* (*Pcc*) to identify key genetic differences between them. We are also using these arrays in combination with bioinformatics and mathematics as part of a systems biology approach to investigate the regulation of pathogenesis in *Pba*, beginning with an investigation into quorum sensing.

The effector proteins of *Hyaloperonospora parasitica*

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Hyaloperonospora parasitica is an obligate oomycete and the causal agent of downy mildew on Arabidopsis. Recently we have cloned two pathogenicity effector genes, *ATR1* and *ATR13*, from the pathogen and shown them to code for unique proteins that are under amazing levels of diversifying selection. This implies that they are locked in an "arms race" with the plant's pathogen detection system and consequently these levels of diversity are mirrored in the host resistance genes, *RPP1* and *RPP13*, associated with recognition of ATR1 and ATR13, respectively. We have used this natural diversity to reveal that this co-evolutionary story is far more complex than immediately apparent. The newly sequenced genome of *H.parasitica* is allowing a more global approach to the identification of effector proteins. These highly diverse effectors will be valuable tools in extending our understanding of the mechanisms underlying pathogenicity and the host plant resistance mechanisms.

An "omics" perspective on fungal pathogenesis

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Magnaporthe grisea is the causal agent of rice blast, the most devastating disease of rice worldwide and is a seminal model to elucidate the basis of pathogen–host interactions. Following the completion of the genome sequence of both the fungus and its host, rice, research is focused on functional and comparative approaches to uncover the molecular and evolutionary foundation of fungal pathogenesis. Whole genome microarray analysis of appressorium initiation, development and maturation induced by hydrophobic surfaces and by cAMP revealed a core set of 357 genes that were differentially expressed when compared to conidial germination under non-inductive conditions. In addition to genes involved in lipid, carbohydrate and secondary metabolism, numerous genes involved in protein turn over and amino acid catabolism were significantly induced. The critical requirement for protein catabolism, including endo-proteases and key enzymes involved in shuttling carbon back into the Kreb cycle, was demonstrated by gene knockout. To examine the evolution of pathogenesis, a semi-automated high throughput computational platform to facilitate large-scale comparative analyses was developed. Comparison of gene sets from 11 fully sequenced fungal pathogens and related non-pathogens revealed evidence for duplication of genes associated with several functional categories including signaling and hydrolytic activities. A database, FEGA (*F*unga*l* *E*volutionary *G*enomic *A*nalyses), was created and provides information about gene copy number in a genome, gene family constituency among genomes, and functional descriptions of gene families, including Gene Ontology (GO)-based functional annotation. Indices of selection (Ka/Ks ratios) and divergence (Ks, synonymous nucleotide substitution) are also included. Other efforts are currently focused on examination of novel non-coding transcripts, transcriptional networks and protein-protein interactions to define the circuitry regulating rice-rice blast interactions.

A secreted effector protein of AY-WB phytoplasma accumulates in nuclei and alters gene expression of host plant cells

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Phytoplasmas are insect-transmitted plant pathogens. They are intriguing bacteria that replicate intracellularly in plant and insect cells, and induce major developmental modifications of plant tissue, including the clustering of branches of developing tissue (Witches' broom) and retrograde metamorphosis of the floral organs to the condition of leaves (phyllody). They reside in the live phloem cells of their plant hosts, and invade and replicate in most organs of their insect vectors, which are mainly phloem-feeding leafhoppers (Hemiptera; Cicadellidae). Phytoplasmas belong to the Class Mollicutes that have diverged from a *Clostridium*-like Gram-positive bacterium through extensive genome reductions and loss of outer cell wall. Phytoplasmas are surrounded by one cell membrane that is in direct contact with cytoplasm of plant and insect host cells. Phytoplasmas cannot yet be cultured in cell-free culture media.

The extent to which phytoplasmas rely on effector proteins to manipulate their host plants and insects can now be studied with the availability of phytoplasma genome sequences. We mined the complete genome sequence of Aster Yellows phytoplasma strain Witches' Broom (AY-WB) (http://www.jic.ac.uk/staff/saskia-hogenhout/genome.htm) for the presence of genes encoding secreted proteins based on the presence of N-terminal signal peptides. We identified 56 secreted AY-WB proteins (SAPs). These SAPs are candidate effector proteins potentially involved in modulating the physiology of plant and insect hosts. One of these, SAP11, carries, besides the signal peptide, a eukaryotic nuclear localization signal. Yellow fluorescence protein-tagged SAP11 targeted plant cell nuclei in an importin α-dependent manner suggesting that SAP11 recruits host factors for nuclear localization. Most interestingly, SAP11 was detected in nuclei of young sink tissues of AY-WB-infected China aster plants confirming that this protein targets nuclei during plant infection. Microarray gene expression profiling experiments indicated that SAP11 alters the expression of plant genes and notably up-regulates host transcription factors.

Immunofluorescence microscopy experiments detected SAP11 beyond the phloem tissue in mesophyll and other cell types, suggesting that SAP11 moves out of the phloem into adjacent tissues. Indeed, the size exclusion limits (SELs) of plasmodesmata change during organ development. The SELs of sink tissue plasmodesmata range between 10 and 40 kDa, whereas those of source tissues are smaller. The majority of secreted AY-WB proteins (51 of 56), including SAP11 and SAP30, are smaller than 40 kDa, indicating that most AY-WB secreted protein of AY-WB may move out of the phloem and target plant developing tissues. This is consistent with the appearance of symptoms predominantly in developing tissues of plants.

In summary, we propose that SAP11 is an effector protein that is secreted directly inside phloem cells and then is transported to mesophyll and other plant cells where it targets nuclei to affect the expression of various genes. This is the first report of an effector protein of a plant-pathogenic bacterium with Gram-positive ancestry. Extending the concept of effectors to the phytoplasmas points to novel research strategies for unraveling pathogenicity mechanisms of these fascinating pathogens.

Polymers and oligomers in attack and defence

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Host and pathogen structural polymers play fundamental protective roles during pathogenesis. In the case of pathogens these highly conserved molecules betray their presence during invasion, and in some cases at nanomolar levels trigger innate immunity responses. These elicitors or MAMPs (*m*icrobial *a*ssociated *m*olecular *p*atterns) may be shed naturally, such as bacterial lipopolysaccharide, peptidoglycan, flagellin along with others including elongation factor (EF-Tu); or they may be released by host glycanases, such as glucan and chitin fragments from fungal cell walls. Our recent work reveals that bacterial polyanionic, extracellular polysaccharides (EPS) from diverse pathogens such as *Xanthomonas campestris, Erwinia amylovora*, *Pseudomonas syringae*, *P. aeruginosa*, *Ralstonia solanacearum*, and mutualistic *Sinorhizobium meliloti*, which are essential for full virulence, have more than just a protective role. They suppress detection of MAMPs and EPS-defective mutants by chelation of apoplastic calcium. Influx of external calcium to the cytosol is a key component of signalling for induction of defence-related genes. Endogenous elicitors can also signal attack; oligogalacturonides are released following host cell death (e.g. HR) or by microbial pectinase action. My early research was on cell wall-degrading enzymes (CWDE) partly in the context of understanding host-pathogen specificity. Oligosaccharides can present enormous variation and were surmised to hold the key to specificity. One hypothesis implied specific regulation of myriad CWDE by complex host wall polysaccharides. Induction was indeed specific, but by simple monomers or dimers (in the absence of catabolite repression), which refuted any role in specific interactions. We showed specificity at a much broader level, with the dependence of cereal pathogens, such as *Stagonospora nodorum*, on (arabino)xylanases; in contrast to most necrotrophic pathogens of dicots which degrade polygalacturonan. This pattern reflects the main matrix polymers of the primary walls of the respective host groups. Biotrophic fungi use developmental rather than biochemical regulation and seem to rely on breakdown of neutral hemicelluloses, thereby avoiding extracellular pectinases, which are (indirectly) toxic. CWDE can also function in xylem colonization by vascular fungi, in mycoparasitism (or competition) by *Trichoderma* against the commercial mushroom, and proteases are crucial in penetration of insect cuticles by entomopathogenic fungi. Plant pathogen proteases can have counter defence functions, but a trypsin SNP1 from *S. nodorum* was the first shown to degrade wall structural protein. Functional redundancy, resulting from multiple or latent genes, has precluded in depth analysis by gene disruption of the role of most CWDE in virulence. One unexpected and unrelated oligomer discovered in my laboratory was that of Man's oldest fungicide, produced as a component of active defence in various plant families. Elemental sulphur exists as cyclo- octosulphur, is highly fungitoxic and its kinetics and location in resistant interactions closely resemble those of organic phytoalexins. The latter was initially discovered in cacao. We have contributed much to the understanding of infection and the nature, screening and deployment of disease resistance in cacao, cassava and oil palm. If time allows I may offer a brief tropical interlude to contrast with the more mechanistic conference theme of attack and defence.

PAMP perception : *PAMP receptors as models for receptor activation and transmembrane signalling*

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Plants and animals can sense the presence of microbial invaders via perception of pathogen-associated molecular patterns (PAMPs). The corresponding pattern recognition receptors (PRRs) include Toll and Toll like receptors of animals and receptor kinases and receptor like proteins in plants. Using *Arabidopsis* as a model we study perception of the bacterial PAMPs flagellin and EF-Tu by their corresponding receptor kinases FLS2 and EFR. Mutants lacking these receptors still respond to bacterial preparations with induction of basal defense responses. This indicates perception systems for further bacterial PAMPs. Attempts to identify additional elements of the PAMP-repertoire are in progress. Some PAMPs like the flg22-epitope of flagellin are recognized by a broad variety of plants while others appear to act on a limited number of species only. For example, perception of bacterial EF-Tu as a PAMP seems restricted to *Arabidopsis* and closely related *Brassicaceae*. Similarly, perception of bacterial cold shock protein is found in species of the *Solanales* only. One can speculate that receptors for these PAMPs are to be found among species-specific members of large and rapidly evolving gene families such as the receptor like kinases (RLKs) and receptor like proteins (RLPs).

The flg22 epitope of bacterial flagellin is perceived by FLS2 in *Arabidopsis* and by close homologs of FLS2 in other plant species. Although detecting the same flg22-epitope these receptors show species-specific characteristics when probed with different flg22-derivatives. Currently we use FLS2 from tomato and *Arabidopsis* as chimeric receptor constructs to identify the sites of ligand-receptor interaction and to study the mechanism of receptor activation. Extending this approach, chimeric receptors with the intracellular output domain of FLS2 and extracellular recognition domains from different RLKs are under investigation.

Flagellin and EF-Tu, like several other PAMPs and wound-related signals, interact with their respective receptors according to the address-message concept with binding and activation as two consecutive steps. FLS2 and EFR are single-span transmembrane receptor kinases and one can hypothesize that transfer of extracellular information input to intracellular signal output requires changes in oligomerization of elements in the receptor complex. A reverse genetics approach showed that responses to flagellin and EF-Tu require an additional RLK for full functionality. Surprisingly, this RLK is BAK1/SERK3 (BRI Associated receptor Kinase1/Somatic Embryogenesis Receptor-like Kinase 3) which has been previously identified as co-receptor for the brassinolide receptor BRI1. Most interestingly, BAK1 and FLS2 form a stable complex within 2 min of treatment with the ligand flg22. This indicates that BAK1 has a role as co-receptor for regulating very different cellular programs.

In plants, single-span transmembrane receptor kinases function as a major class of sensors for a variety of extracellular signals. The well characterized receptors FLS2 and EFR can serve as suitable models to study the molecular mechanisms of receptor activation and transmembrane signaling for this eminent class of receptors.

Basal defenses underlying bacterial resistance

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Plants defend themselves with induced immune responses. One class of response, basal defense, is elicited by microbe-associated molecular patterns (MAMPs) and by pathogen-derived effectors that trigger "weak" R-proteins. A second class of response, cultivar level or "gene-for-gene" resistance, is elicited by effectors that trigger "strong" R-proteins that induce potent defenses including the hypersensitive response (HR). Basal and cultivar level defenses long had been considered independent. However, recent studies, including our studies of *Arabidopsis* RIN4 (RPM1-interacting protein 4), indicate that the two defense classes share molecular components.

RIN4 regulates the function of the "strong" R-proteins RPM1 and RPS2, which induce an HR in response to *P. syringae* effectors AvrRpm1 and AvrRpt2, respectively. RIN4 physically associates with both R-proteins and is targeted by each effector. AvrRpm1 interacts with and induces phosphorylation of RIN4. AvrRpt2 is a protease that degrades RIN4. These effector-induced modifications of RIN4 are thought to elicit activation of the R-proteins. In addition to the "strong" function of RPM1 and RPS2 in response to AvrRpm1 and AvrRpt2, respectively, RPM1 and RPS2 have also been found to function as "weak" R-proteins in response to AvrRpt2 and AvrRpm1, respectively. The weak activation of RPS2 by AvrRpm1 contributes quantitatively to resistance against *P. syringae* expressing AvrRpm1. Thus, RIN4 regulates R-proteins that, depending on the elicitor, induce either "strong" cultivar level or "weak" basal defenses.

In addition to regulating the function of R-proteins, RIN4 also regulates basal defenses induced by MAMP-receptors. Over-expression of RIN4 suppresses cell wall thickening elicited by MAMPs. Palmitoylation of RIN4's C-terminus localizes it to the plasma membrane where it regulates function of RPM1 and RPS2. Surprisingly, derivatives of RIN4 not targeted to the plasma membrane were found to be hyperactive in their ability to suppress MAMP-signaling. Also, studies of deletion derivatives revealed that a domain of RIN4 sufficient to suppress MAMP-signaling is dispensable for function RPM1 and RPS2. Thus, this domain represents a novel regulator of MAMP-signaling. AvrRpm1 and AvrRpt2 also suppress MAMP-signaling (in plants lacking RPM1 and RPS2). Our results lead to testable models for how targeting of RIN4 by these effectors relates to their virulence functions and how the molecular framework of the two defense classes are related.

Sulphur nutrition and S defence compounds

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Defence mechanisms of plants against bacterial and fungal pathogens include preformed as well as inducible mechanisms that result, amongst others, in the formation of a considerable number of sulphur-containing compounds. Examples are sulphur-rich peptides of the thionin and defensin families and several secondary metabolites such as phytoalexins, glucosinolates and elemental sulphur. While not all of the signal transduction and biosynthetic pathways leading to these compounds are fully understood, it is evident that part of the assimilated sulphur in a plant is diverted into defence compounds, in particular in the presence of pathogens. This requirement is reflected in agronomic field experiments where crop yield could be stabilized after fungal infections, provided that the plants were optimally fertilized with sulphate. This sulphate-enhanced defence (SED) has therefore a quantitative character and thus differs from the defined resistances of gene-for-gene interactions. Most likely SED employs at least in part the same signal transduction pathways. The complex process of SED can be dissected using defined bacterial and fungal pathovars and a susceptible Arabidopsis thaliana ecotype. Targeted expression and metabolite profiles revealed a coordinate response of key genes as well as primary metabolites of sulphur metabolism short time after infection. The data reveal a distinct role of sulphur metabolism in response to pathogen infection and an influence of mineral nutrition on the formation of defence-related sulphur compounds.

Uncovering the functions of S-nitrosothiols in plant disease resistance

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Animal S-nitrosoglutathione reductase (GSNOR) governs the extent of cellular S-nitrosylation, a key redox-based post-translational modification. Mutations in *AtGSNOR1*, an *Arabidopsis thaliana GSNOR*, modulate the extent of cellular S-nitrosothiol (SNO) formation in this model plant species. Loss of *AtGSNOR1* function increases SNO levels and compromises multiple modes of plant disease resistance. Furthermore, over-expression of *AtGSNOR1* establishes basal resistance against a broad spectrum of ordinarily virulent microbial pathogens. We are employing directed proteomics, gene expression profiling and forward and reverse genetics to charter the molecular landscape underpinning SNO function during the establishment of plant disease resistance.

The cell death regulator AtPUB17 directly interacts with the BTB/POZ domain transcriptional repressor, AtBTB1 to control disease resistance in plants.

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Programmed cell death or hypersensitive cell death and disease resistance are intimately connected and crucial in plant defence against pathogens. Although resistance proteins are known to be important players in pathogen recognition little is known of the molecular mechanisms downstream of this recognition event leading to hypersensitive cell death. We have established the Ubox protein NtACRE276 and its functional homolog, AtPUB17 as key proteins required for multiple resistance proteins mediated hypersensitive cell death in tobacco and *Arabidopsis*. E3 ligase activity of both NtACRE276 and AtPUB17 is required for ubiquitinating key negative regulators of programmed cell death. Our data identify the presence of a conserved class of U-box ARM repeat E3 ligases across the Solanaceous and Brassica genera that act as positive regulators of cell death and defence.We have identified AtBTB1, a BTB/POZ domain transcriptional repressor, as a direct interactor of AtPUB17. AtBTB1 is located in the nucleus during the hypersensitive response. AtBTB1 knockout plants produce spontaneous cell death in the absence of pathogen infection indicating that AtBTB1 has a critical role in cell death signalling. We detail experiments which show that the BTB/POZ domain of AtBTB1 is required for its interaction with AtPUB17 and how this interaction regulates its transcriptional repression role during defence. We show that AtPUB17 interacts with AtBTB1 to regulate disease resistance in *Arabidopsis* and tobacco. The target genes whose expression is repressed by AtBTB1 are key molecules which regulate defence against pathogenic Pseudomonads. By focussing on the biochemical function of AtBTB1 we reveal specific molecular events which bring about cellular signalling during disease resistance. The data yields new insights into our understanding of ubiquitin mediated signal-transduction mechanisms controlling defence against pathogenic Pseudomonads and its relevance will extend to all biotrophic pathogens.

Activities of Plant Papain-like Cysteine Proteases

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Activity-based protein profiling is a powerful technology to display the activities of classes of enzymes in plants during their infection by pathogens. The technology is based on biotinylated inhibitors that covalently react with the active site residues of enzymes in an activity-dependent manner. The biotinylation is irreversible, which facilitates further analysis by mass spectrometry and protein blots. The activity of papain-like cysteine proteases can be monitored using DCG-04, a biotinylated version of E-64, a mechanism-based inhibitor of papain-like cysteine proteases. This technology was used to show that the Cladosporium Avr2 protein inhibits the tomato RCR3 protease (2), and that the Phytophthora EPIC2 protein inhibits the tomato PIP1 protease (3). When applied on Pseudomonas-infected Arabidopsis cell cultures, we found that the activity of one of the cysteine proteases is upregulated during a defense response, and down regulated during infection with virulent Pseudomonas bacteria. The data indicate that Pseudomonas secretes cysteine protease inhibitors that inhibit secreted host proteases. To further develop tools for *in vivo* profiling, we have established a two-step *in vivo* labeling procedure for papain-like cysteine proteases using membrane-permeable azide-labeled E-64. Azide-labeled proteases are extracted under denaturing conditions and coupled to alkyne-biotin through 'click-chemistry'. The two-step labeling is robust and specific and can be used to display activities of vacuolar proteases that were 'out-of-reach' for DCG-04. We are currently applying this method to investigate plant-pathogen interactions and we are using other probes to expand the range of displayed enzyme activities beyond cysteine proteases.

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Life after death: Role for autophagy during innate immunity

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Plants mount various defense responses against invading microbes. The most extensively studied plant innate immunity involves recognition of pathogen-encoded avirulence (Avr) proteins by plant resistance (R) proteins. Often this R-Avr interaction leads to the hypersensitive response (HR), a form of PCD, at the site of pathogen infection. HR-PCD is often observed as necrotic lesions and the pathogen is restricted to these lesions and cells immediately surrounding it. Therefore, it is thought that the HR-PCD functions to limit the spread of pathogen from infection sites into adjacent healthy tissue. Although HR-PCD is morphologically well characterized, the molecular mechanisms that regulate cell death during an immune response remain obscure. Recently we have shown that autophagy, an evolutionarily conserved process of bulk protein and organelle turnover, play an important role in limiting cell death initiated during plant innate immune responses. Consistent with its role in plants, several studies in animals also demonstrate that the autophagic machinery is involved in innate as well as adaptive immunities. We will discuss our efforts to understand the relationship of HR-PCD and autophagy.

Key genes for Hypersensitive Response (HR) signalling in *Arabidopsis thaliana*

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The Hypersensitive Response (HR), characterized by a rapid and localized cell death at the inoculation site, is one of the most efficient resistance reactions to pathogen attack in plants. Several strategies were developped in order to identify regulators of this plant response : i) identification of genes specifically expressed during the HR, ii) identification of mutants altered in HR, or displaying HR-like lesions in absence of pathogen attack. *AtMYB30,* a transcriptional factor which is specifically, rapidly and transiently expressed during incompatible interactions between *Arabidopsis* and bacterial pathogens, will be described as an example. Its expression was also found to be misregulated in *Arabidopsis* mutants affected in the control of cell death initiation (Daniel et al., 1999). Using transgenic tobacco and *Arabidopsis* lines overexpressing AtMYB30 in sense or antisense orientation, we demonstrated that AtMYB30 is a positive regulator of the hypersensitive cell death (Vailleau *et al*., 2002). AtMYB30 may thus be a component of a cell death pathway conditioning the HR but its mode of action remains unclear.

Identification of interacting partners, analysis of post-translational modifications and subcellular localization of the protein have been undertaken. But an obvious way of understanding the mode of action of this putative transcriptional regulator and, more widely, the molecular mechanisms involved in the regulation of the HR, is to search for downstream target genes of AtMYB30.

Using Affymetrix whole genome array and quantitative RT-PCR analyses of Arabidopsis plants misexpressing *AtMYB30*, putative AtMYB30 target genes were identified which are mainly related to lipid metabolism, and more particularly to the biosynthesis of Very-Long Chain Fatty Acids (VLCFAs). Results will be presented allowing us to propose a model in which AtMYB30 modulates cell death lipid signaling leading to HR, through VLCFAs by themselves, or VLCFA-containing sphingolipids, acting as pro-cell death messengers in plants in response to infection.

Flax rust: from gene-for-gene to protein-for-protein interactions

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Current advances in the study of the flax-flax rust interaction are based on earlier classical genetic studies of H.H. Flor whose simultaneous analysis of genetics of host resistance and rust virulence gave rise to the gene-for-gene relationship. Three sorts of genes were identified that determine whether the interaction between the flax plant and flax rust leads to disease. These are resistance (R) genes in the host and in flax rust, avirulence (Avr) genes and inhibitor of avirulence (I) genes. About 30 host R genes that occur at 5 loci have been identified in flax and a corresponding number of Avr genes, more dispersed in the rust genome, have been identified in flax rust. These genes "interact" in a gene-for-gene fashion such that incompatible interactions (no rust disease) result only when the host and rust carry "corresponding" R and Avr genes, respectively. I genes map to a single locus in rust and for specific R gene-Avr gene interactions, have the effect of changing what is normally an incompatible interaction into a compatible interaction.

The molecular basis of the flax-flax rust gene-for-gene specificity in the flax-flax rust interaction is beginning to be understood with the availability of 18 cloned flax R genes from 4 loci and more than 20 Avr gene variants from 4 loci in the rust. All the host R genes are members of the TIR-NBS-LRR disease resistance gene family whereas the 4 Avr loci each contain unrelated genes that encode small secreted proteins. Gene-for-gene specificity is determined principally by the LRR domain of resistance proteins and for R proteins encoded by the L and M genes, specific recognition is based on direct interaction between the resistance proteins and corresponding avirulence proteins within the host cytoplasm in a receptor-ligand fashion. This implies that Avr proteins, secreted by the rust fungus during infection, are taken up by plant cells. The Inhibitor (I) genes have not yet been cloned and how I proteins function is yet to be determined. As in other host pathogen systems, it is postulated that flax rust Avr proteins normally have a virulence function and that R proteins have evolved to counteract this function. Consequently in an evolutionary arms race scenario, Avr proteins evolve to avoid recognition by the host while retaining virulence function and R proteins have evolved to re-engage these modified Avr proteins. An additional evolutionary strategy for the pathogen is the evolution of I proteins that can 'mask' the recognition of Avr proteins from by R proteins.

So in the flax-flax rust system several important questions remain to be answered. How do Avr proteins enter the host cell and what are their virulence functions? What is the nature and function of I proteins? Do they also function inside the host cell? The crystal structure of one flax rust Avr protein has been determined and the corresponding R protein has been expressed and purified from yeast. Can understanding the R-Avr interaction at the atomic level provide design rules for the synthesis of artificial R genes to target any pathogen virulence factor secreted into host cells?

Molecular basis of systemic immunity in plants

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Despite the absence of an adaptive immune system in plants, an important consequence of a classical gene-for-gene interaction is the establishment of broad spectrum systemic immunity to normally virulent pathogens.
Initiation of systemic immunity requires the generation, translocation and decoding of one or more mobile signals. Identification of the signals, their distal receptors and the activated signalling networks conferring immunity represents a formidable challenge.
Using *Arabidopsis thaliana* carrying the RPM1 resistance protein and *Pseudomonas syringae* carrying the cognate AvrRpm1 effector we have investigated the events involved in establishment of systemic immunity. A systemic signal(s) is rapidly generated in infected leaves and phloem translocated to effect a specific transcriptional reprogramming in distal tissues. This transcriptional reprogramming share many components involved in PAMP triggered immunity (PTI) suggesting similar pattern recognition receptors are engaged in innate and systemic immunity. Additionally, transcriptional reprogramming also induces components associated with induced defences to insects and necrotrophic pathogens (e.g. glucosinolate, jasmonate, alkaloid and terpenoid biosynthetic pathways), consistent with establishment of broad spectrum immunity.
We report here on progress towards identifying the signalling pathways contributing to systemic immunity through integrated genetic and metabolomic approaches.

Effector genomics: the key to novel *R* genes and their exploitation

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Potato is the world's fourth largest food crop yet it continues to endure late blight, a devastating disease caused by the Irish famine pathogen *Phytophthora infestans*. The best strategy to genetically manage this disease requires rapid identification and functional characterisation of resistance (*R*) genes, but current approaches are slow and inefficient. We adopted a functional genomics approach which combines mining effectors from the genome sequence of *Phytophthora infestans* with screening for responses in resistant wild *Solanum* species.

A repertoire of RXLR effector genes were predicted computationally from the *P. infestans* genome. These candidate avirulence (*Avr*) genes were cloned in a *Potato virus X* expression vector and profiled on our collection of resistant wild *Solanum* species for activation of innate immunity. A large variation of responses to various RXLR effectors became evident, and candidate *Avr* genes were identified for almost all tested *Solanum* clones. Sexual crossing of these plants resulted in populations, which were investigated for segregation of resistance to well-characterized *P. infestans* strains and response to the effector. When resistance and effector response are co-segregating, an R-AVR interaction may occur. Indeed, one of the effector families was found to contain the cognate *Avr* gene of a late blight *R* gene which was previously cloned from an unrelated *Solanum*. Co-infiltration of *Agrobacterium tumefaciens* strains containing the candidate *Avr* and the *R* gene in *Nicotiana benthamiana* leaves resulted in a specific hypersensitive response. This prompted us to follow a candidate gene approach and clone specific full-length *R* gene analogues (RGAs) from the selected wild *Solanum* genotypes. An extensive number of RGAs were obtained, yet we could efficiently identify the functional homologues that displayed the specific effector response in transient assays and the functional *R* genes were cloned exceptionally fast.

For identified R-AVR combinations, we extended this effector approach to functional allele-mining and thereby exploit the natural variation of late blight *R* genes in *Solanum*. High throughput profiling of effectors on an extensive series of *Solanum* germplasm already yielded a variation of candidate *R* gene homologues. For one of the *R* genes, we have identified three functionally equivalent *R* homologues derived from different *Solanum* species. This functional allele-mining approach demonstrates how conserved *R* genes with identical recognition specificities can efficiently be identified and cloned from unrelated *Solanum* species. It has already appeared useful for the practical application, as the newly identified genes are more easily crossable with the cultivated potato, and can now be used for cisgenesis as well as traditional introgression breeding.

In summary, our findings indicate that effector genomics enables discovery and functional profiling of late blight R genes at an unprecedented rate and promises to accelerate the engineering of late blight resistant potato varieties through transgenesis, cisgenesis, or traditional breeding.

Discovering and using resistance genes in improved crop varieties

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In laboratory experiments in plant pathology, it is usual to work with one pathogen of one host and to control as many of the variables involved as possible. This approach has led to rapid progress in understanding the biology of pathogen attack and host defence. In particular, it has led to huge advances in understanding the genetics of disease resistance.

It is often difficult, however, to extrapolate from simplified laboratory conditions to the control of disease in crops. Even in intensive agriculture, which is itself a much simpler ecological system than those in nature, many factors affect the performance of crops. A plant breeder must not only try to improve resistance to several diseases (not just one), but must also improve yield, quality and agronomic characters. Not only that, but these traits interact with one another and are influenced by environmental conditions over which a farmer has limited control. When, in that case, can knowledge about the genetics of resistance gained in highly controlled conditions be usefully applied to breeding for resistance in the complex environment of a farm?

I will discuss approaches to using genetics in breeding for resistance using powdery mildew of cereals and septoria tritici blotch of wheat as examples. I will discuss the implications of having to select simultaneously for disease resistance and other traits, and what that implies about the kinds of resistance gene that are most likely to be useful in breeding. I will show how marker-assisted selection can be used to support conventional field selection but is unlikely to replace it. Finally, I will discuss how the experience gained from a century and a half of immense success in breeding for disease resistance can be applied to optimising strategies for the use of GM technology to improving resistance in crops.

The role of lipopolysaccharide in the virulence of the phytopathogen *Erwinia carotovora* subsp. *atroseptica*

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Erwinia carotovora subsp. *atroseptica* (*Eca*) is a member of the soft rot erwinias, which are Gram negative phytopathogens. *Eca* infects potato almost exclusively, and is the etiological agent of black leg and tuber rot. Globally, potato is the fourth most cultivated crop and therefore *Eca* infections, both pre- and post-harvest, are economically important, although no chemical control measures currently exist.

In this study, three bacteriophage that infect *Eca* were isolated from environmental sources. *Eca* strains that are resistant to infection by these bacteriophage were isolated, and the mutations were mapped. In each case, the mutations resulted in altered lipopolysaccharide (LPS) structure. LPS is a branched polymer with a membrane-associated inner core region, an outer core region, and a distal O-antigenic portion. LPS covers ~75% of the bacterial outer membrane, and has been shown to be important for membrane integrity. The mutations in the phage-resistant strains created here mapped to different genes involved in LPS biosynthesis: two distinct mutations (*rfbI* and *rffG*) affects synthesis of the O-antigen, and the third mutation (*waaJ*) is required for synthesis of the core region of LPS. These LPS mutants were compared with the wild type for a variety of pathogenicity-associated phenotypes, including motility, exoenzyme production, and *in planta* virulence. Truncations in the LPS structure were correlated with decreased virulence: the greater the perturbation to the LPS structure, the greater the deviation from the wild type phenotype. Interestingly, the *rfbI* gene is found on a putative horizontally acquired island, encoding a second, structurally distinct O-antigen. Therefore, the LPS population in *Eca* is expected to be heterogenous. This demonstrates that lateral transmission of genetic elements has contributed to the evolution of *Eca*, in this case by making it sensitive to a bacteriophage we isolated, and plays an important role in the ecology of both phage and bacteria.

Magnaporthe grisea cutinase 2 mediates appressorium differentiation and host penetration and is required for full virulence.

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The rice blast fungus *Magnaporthe grisea* infects its host by forming a specialized infection structure, the appressorium, on the plant leaf. The enormous turgor pressure generated within the appressorium drives the emerging penetration peg forceably through the plant cuticle. Hitherto, the involvement of cutinase(s) in this process has remained unproven. We identified a specific *M. grisea* cutinase *CUT2*, whose expression is dramatically upregulated during appressorium maturation and penetration[1]. The *cut2* mutant has reduced extracellular cutin-degrading and serine esterase activity, when grown on cutin as the sole carbon source, compared with the wild-type strain. The *cut2* mutant strain is severely less pathogenic than the wild-type or complemented *cut2*/CUT2 strain on rice and barley[2]. It displays reduced conidiation and anomalous germling morphology, forming multiple elongate germ tubes and aberrant appressoria on inductive surfaces. We show that Cut2 mediates the formation of the penetration peg but does not play a role in spore or appressorium adhesion, or in appressorial turgor generation. Morphological and pathogenicity defects in the *cut2* mutant are fully restored with exogenous application of synthetic cutin monomers, cAMP, IBMX and diacylglycerol (DAG). We propose that Cut2 is an upstream activator of cAMP/PKA and DAG/PKC signaling pathways that direct appressorium formation and infectious growth in *M. grisea*. Cut2 is therefore required for surface sensing leading to correct germling differentiation, penetration and full virulence in this model fungus.

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2. Skamnioti and Gurr. (2007). Plant Cell, *in press.*

Exploring the apoplast – what stops *hrp* mutants in Arabidopsis?

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Basal resistance against non-pathogenic bacteria has been associated with alterations to the plant cell wall and the formation of papillae at reaction sites. Whilst pathogenic bacteria multiply freely in intercellular space, non-pathogenic bacteria are restricted to areas in close proximity to the wall. Using a wide variety of approaches, including histochemistry and immunocytochemistry at the electron microscope level, we have established a time-frame for cell wall modification in relation to bacterial multiplication. *Pseudomonas syringae* pv. tomato (wt) is a pathogen of both tomato and *Arabidopsis* and requires a type III secretion system to deliver effector proteins across the wall into the cell. The system is encoded in plant pathogens by the *hrp* gene cluster; *hrp* mutants are non-pathogenic and produce very little in the way of symptoms.

Analysis of bacterial multiplication showed that *hrp* mutants were restricted to numbers reached by 6h after inoculation whereas wt initiated rapid multiplication from 4h onwards. Conditions in the apoplast therefore became bacteriostatic after inoculation with *hrp* mutant bacteria. Over the same time period, similar papilla development occurred with both the wt and *hrp* mutant interaction, suggesting that papilla development was not responsible for the restriction of bacteria. Significantly, higher levels of localised H_2O_2 accumulation around the *hrp* mutant were noted 4hai along with differences in the redox state of apoplastic ascorbate. Our data suggest that the cause of restriction of the *hrp* mutant is immobilisation due to H_2O_2 induced cross-linking between bacterial and plant cell walls. In consequence, the compromised bacteria fail to divide, but do not die for some days.

Regulation of phytotoxin production in the plant pathogen *Pectobacterium atrosepticum* and microarray analysis of the effects of these toxins on potato (*Solanum tuberosum*)

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Pectobacterium atrosepticum (*Pba* - formerly *Erwinia carotovora* subsp. *atroseptica*), the causal agent of blackleg of potato, is a highly host-specific bacterial pathogen. Sequencing and annotation of the complete *Pba* genome has identified a number of new potential virulence factors, including a cluster of genes similar to that encoding the phytotoxin coronatine (COR). COR has been well characterized in the phytopathogen *Pseudomonas syringae* and is synthesized by the ligation of two sub units: coronafacic acid (CFA) and coronamic acid (CMA) by coronafacate ligase (CFL). Comparative genome analysis has revealed that only the genes encoding the CFA sub-unit and CFL are present in *Pba*; the CMA biosynthesis genes and genes involved in the regulation of the entire cluster in *P. syringae* are absent. *Pba cfl*$^-$ and *cfa*$^-$ mutants that are significantly reduced in virulence on both potato stems and tubers have been selected from a Tn*5*-based mutation grid by PCR. Restoration of virulence has been demonstrated by *cfl*$^-$ mutants expressing *cfl in-trans* from a pGEM-T based plasmid. Gene expression and mutational studies throughout the course of tuber infection and during *in vitro* culture have determined that the regulation of these genes is linked to quorum sensing and pectin catabolism. LC/MS analysis has identified the chemical structure of the phytotoxin isolated from the supernatant of *Pba* cell cultures. Bioassay and microarray analysis of changes in potato gene expression caused by the exogenous application of purified phytotoxins has revealed that both CFA-valine and CFA-isoleucine are biologically active and are capable of inducing chlorosis on potato leaves.

Role of Nitric Oxide and Nitric Oxide Synthases in *Magnaporthe grisea*

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Nitric oxide (NO) is an important signalling molecule with a diverse range of functions. In animals, the free radical acts in neurotransmission and regulation of blood pressure, and is induced during the inflammatory response to microbe invasion. In plants, NO affects development, can protect against abiotic stresses and may contribute to the induction of the hypersensitive response to pathogen challenge. Relatively little is known about the functions of NO in fungi; this work investigates its roles in the rice blast fungus *Magnaporthe grisea*.

BLAST analysis of the *M. grisea* genome identified four genes with high sequence homology and identical domain structure to animal nitric oxide synthases. QrtRT-PCR traced changes in the transcription profiles of these genes during germination, germling differentiation (appressorium formation) and coincident with penetration of host leaves. The transcript profile of one gene, *NOS3*, was upregulated 200-fold at the time of appressorium maturation and penetration, compared with levels seen in the ungerminated spore. A *nos3* knockout mutant was generated in the Δ*ku70* background and is unable to form appressoria on inductive hydrophobic plastic, but is rescued by treatment with the NO donor sodium nitroprusside. Pathogenicity is markedly reduced in the mutant, with disease lesions forming at only 20% of the wild type level. Chemical inhibitors of NOS enzymes delayed the formation of appressoria in wild type. Collectively, these data imply that a NO signal is required for full development of infection structures in the rice blast fungus.

The Downy Mildew Effector Protein ATR13 Promotes *Pseudomonas syringae* Susceptibility in *Arabidopsis thaliana*

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Plant innate immunity provides defense against microbial attack. The plant innate immune system involves at least two components called PAMP (Pathogen Associated Molecular Patterns)-triggered immunity (PTI) and effector triggered immunity (ETI). The activation of PTI or ETI by direct or indirect recognition of extracellular or intracellular pathogen molecules, respectively, enhances plant disease resistance and restricts pathogen proliferation. However, successful pathogens efficiently suppress plant immunity and cause disease on hosts. One of the primary roles of pathogen effectors is to suppress innate immunity. In *Pseudomonas syringae*, the type III secretion system (T3SS) has been found to direct the translocation of virulence effectors to the plant cell cytoplasm. *Hyaloperonospora parasitica* (*H.p*), a filamentous oomycete pathogen of *Arabidopsis thaliana*, is thought to secrete effectors, such as ATR1 or ATR13, to promote host disease susceptibility during pathogenesis. An *H. p* effector protein ATR13 triggers RPP13-Nd-dependent resistance in *A. thaliana*. To better understand the functions of these effectors during compatible and incompatible interactions of *H. p* isolates on *Arabidopsis* ecotypes, we developed a novel delivery system using the N-terminus of a *P. syringae* T3SS effector protein, AvrRPS4. ATR13 triggered hypersensitive response (HR) and resistance to bacterial pathogen in *Arabidopsis* carrying *RPP13-Nd* when delivered from *P. syringae* pv. *tomato* (*Pst*) DC3000. In addition, multiple alleles of ATR13 confer enhanced virulence of DC3000 on susceptible *Arabidopsis* accessions. We conclude that ATR13 positively contributes to the pathogen virulence inside host cells. ATR13 suppressed bacterial PAMP-triggered callose deposition in susceptible *Arabidopsis* leaf cell walls when delivered by *Pst* ΔCEL suggesting that virulence function of ATR13 suppresses plant cell wall defense. Furthermore, some of the N- and C-terminally truncated ATR13 proteins that are impaired in avirulence activity still retained callose suppression function on *Arabidopsis* when delivered by *Pst* DC3000, indicating that avirulence and virulence functions of ATR13 can be genetically uncoupled. $ATR13^{Emco5}$, when delivered by *Pst* DC3000, triggered localized immunity including HR on accession Ws-0 that is highly susceptible to *H. p* isolate Emco5. Based on these results, we propose the presence of unknown suppressor(s) of ATR13-triggered immunity in *H. p* Emco5.

Phylogenetic analysis of phytoplasmas based on sequences derived from the *secA* and 23S rDNA genes

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Phytoplasmas are wall-less bacteria in the class Mollicutes that have a small genome size, cannot be grown in culture and are transmitted between plants by insect vectors. They are phloem-limited and are found at very low concentrations in plants making these pathogens extremely hard to detect and identify. Phytoplasmas infect a wide range of plant species and cause symptoms such as yellowing, virescence, phyllody, witches broom and general decline resulting in diseases such as aster yellows, soybean phyllody and coconut lethal disease. The inability to culture phytoplasmas has led to the identification and classification system being based primarily on sequencing of the 16S rRNA gene, which is highly conserved throughout phytoplasma groups. *Candidatus* categories are then used for nomenclature so that strains within a '*Candidatus* phytoplasma' species share at least 97.5% of their 16S rRNA gene sequence. However it is of interest to determine how a group system based on 16S rDNA similarity compares when constructed with other genes such as the 23S rDNA or less well conserved genes such as *secA*.

To this end, phylogenetic analysis of a 500bp fragment of the *secA* gene has been performed on 36 phytoplasma isolates representing 10 of the 16S rDNA groups. The lineages obtained coincide with those delineated by 16S rDNA phylogenetic analysis but much greater genetic variability was evident. Similarly a 750bp sequence that included the 16S/23S spacer region and approximately 500bp of the 23S rDNA was cloned and sequenced from the same set of phytoplasmas and a phylogenetic tree produced. When combined with T-RFLP (terminal restriction fragment length polymorphism) analysis utilising the same region, both these methods also support the lineages produced with 16S rRNA and *secA* genes. The results clearly indicate a significant delineation between the 16SrI and 16SrXII phytoplasmas and those in other 16Sr groups, and support the proposals that the coconut lethal yellowing type phytoplasmas should be divided into three *Candidatae* species (*Ca.* P. palmae, *Ca.* P. cocosnigeriae and *Ca.* P. cocostanzaniae) as proposed at the X International Congress of the IOM. The sequence data generated is now being used to allow for the development of both generic and group-specific real-time PCR assays, with the aim of improving, and increasing sensitivity, of phytoplasma detection methods.

Further evidence for high "*Cacao swollen shoot virus*" content in the cotyledons of infected cocoa plants.

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Unavailability of rapid screening methods for *Cacao swollen shoot virus* (CSSV) has hampered breeding for resistance against the virus, which causes considerable destruction to cocoa in West Africa. We recently reported on how a new approach of cytological studies to explore resistant markers in CSSV-infected germinating cocoa plants enabled us to determine apoptosis, encasement of viral particles by dense matrix of phenolic substances, and the presence of nucleic acid-rich inclusion bodies in some cells, as structural and functional changes that accompany CSSV infection. We hereby provide further evidence (molecular) that seem to confirm one of the tissues (cotyledon) to be indeed rich in the viral DNA, and to have higher total DNA content that was more readily detected by PCR than in the leaves and the uninfected healthy control. This is interesting since cotyledons have never been considered as tissues for sourcing CSSV to study the virus. The significance of these findings to understanding CSSV-cocoa interactions, and the possibility of using the cotyledons for large scale CSSV-purification are discussed alongside the resistance implications for disease.

Characterization and pathogenicity of isolates of *Albonectria rigidiuscula* and *Botryosphaeria rhodina* from dieback lesions of cocoa in Ghana

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Dieback disease of cocoa (*Theobroma cacao* L.) caused by *Albonectria rigidiuscula* (Berk. & Broome) Rossman & Samuels (formerly *Calonectria rigidiuscula* (Berk. & Broome) and *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx is the second most important fungal disease of cocoa in West Africa, causing up to 30% yield loss of dry cocoa beans. Both fungi are often encountered in their respective anamorph forms as *Fusarium decemcellulare* Brick and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. The aim of this study was to define precisely the pathogens causing dieback of cocoa in Ghana and to assess the diversity of the pathogen populations with respect to their cultural characteristics, growth and conidial morphology. Forty-eight *L. theobromae* isolates and 85 *Fusarium* isolates were isolated from dieback lesions from cocoa stems from Ghana and compared with reference isolates supplied by CABI, UK. All isolates failed to grow at 5 and 10 ˚C and could not withstand temperatures above 35°C, 30°C being the optimum growth temperature. Colonies of *L. theobromae* grown in the dark were black and non-sporulating, but pycnidia, characteristic of *L. theobroma*e, lined internally with a dense layer of conidiophores were formed after two weeks on PCA at 30 ˚C under light. Conidia at first were hyaline, broadly ellipsoidal, smooth, and unicellular, later becoming medially uniseptate, brown, typically 12 x 22 µm. Colony appearance and mycelial growth on PDA or PCA was different for *Fusarium decemcellulare* and other unidentified *Fusarium* spp. The former were pinkish while pigment was absent from many of the other *Fusarium* spp. All *Fusarium* isolates produced conidia whether they were grown under light or in darkness. The sizes of the spores, averaged 9 x 66 and 4 x 42 µm, for *F. decemcellulare* and *Fusarium* spp., respectively, and the former were identical to those previously reported from cocoa. Microconidia of all *Fusarium* spp. were borne on short conidiophores, and were typically 0-1 septate and 4 x 13 µm. Macroconidia of *F. decemcellulare* were fewer than microconidia on PDA but were produced readily by old cultures on PCA or water agar. Sporodochia and sclerotia were absent from all the isolates. In pathogenicity tests on Amelonado cocoa seedlings using spore suspensions and mycelium plugs as inoculum, *F.decemcellulare*, *L. theobromae* and one other *Fusarium* isolate (C136) were highly pathogenic. A few of the *Fusarium* spp. were moderately pathogenic while many were not. Symptoms included leaf wilt, death of the shoot from the crown and browning of the vascular tissue. In seedlings whose leaf, petiole and pulvini were wound-inoculated, no disease was induced.

Yeast 2 hybrid screening as a method for the identification of plant targets of oomycete effector protein AVR3a

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The oomycete *Phytophthora infestans* causes late blight, the potato disease that precipitated the Irish famines of 1846 and 1847. It represents a re-emerging threat to potato production and is one of over 70 oomycete species which are arguably the most devastating pathogens of dicotyledonous plants. Until recently, little was known about the molecular bases of oomycete pathogenicity, especially the avirulence molecules that are perceived by host defences. The cloning of one such avirulence gene (*Avr*3a) offers an opportunity to study pathogenicity at the molecular level with the twin aims of; understanding the process of recognition and defence response initiation; and gaining an insight into what the effector function of an avirulence gene might be. To this end we have screened yeast 2 hybrid libraries for plant proteins which interact with AVR3a. Progress in this area will be reported.

Transient gene silencing – a step forward in identifying novel pathogenicity factors in the late blight pathogen, *Phytophthora infestans.*

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Phytophthora infestans causes late blight, a devastating and re-emerging disease of potato. It produces several different cell types including sporangia, zoospores, germinating cysts, and appressoria prior to penetration of the host plant and during the early stages of infection. Appressoria, structures formed just prior to infection, are likely to contain many transcripts required for successful penetration of the host and establishment of a compatible interaction. Appressoria can be generated in the absence of the host plant and so form the basis for stage-specific gene discovery. Over 200 pre-infection- and *in planta*-induced transcripts identified to date encode proteins potentially involved in adhesion, host cell wall degradation, signalling, virulence, amino acid and protein biosynthesis, stress response, and detoxification. The majority of these proteins are predicted to be secreted. Over 60 percent of gene transcripts do not exhibit homology with sequences held in databases. Real-time RT-PCR confirmed that transcripts identified were all up-regulated prior to host plant penetration, with a smaller subset also up-regulated during host infection.

dsRNA-induced transient gene silencing was used in a high-throughput screen for fifty appressoria- and *in planta*-up-regulated genes to determine their role in the pathogen lifecycle and interaction with the host plant, potato. Thirty of these genes encode novel secreted proteins including RxLR class effectors. The remaining genes encode proteins potentially involved in adhesion, a PAMP, cell wall degrading enzymes, cysteine protease inhibitors, ROS detoxification enzymes, membrane proteins, transporters and enzymes affecting development. Phenotypes identified include compromised host penetration and attenuated pathogenicity. Real-time RT-PCR has been used to confirm the gene silencing associated with the observed phenotypes. Localisation of structural proteins during appressorium formation and biotrophic stage of interaction with the host by translational fusion to fluorescent proteins, and subsequent observation by confocal microscopy is in progress.

Our preliminary results show that transient RNAi is effective for identifying genes with a major role in pathogenicity and can inform hypotheses for downstream analysis of pathogenicity in *P. infestans*. Both formation of functional pre-infection structures and pathogenicity require the action of many genes.

Molecular genetics to identify genes involved in the pathogenicity of *Fusarium* spp. on wheat ears

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Fusarium Ear Blight (FEB) disease is caused by up to seventeen related *Fusarium* species and occurs on all Gramineae species, including the economically important crops, wheat, barley, rice and maize. *F. graminearum*, an important global FEB pathogen, produces trichothecene compounds, including nivalenol (NIV) and deoxynivalenol (DON), which can cause health problems in animals and humans. *F. graminearum* has a haploid genome of 36MB. The genome of the strain PH-1 has been sequenced to 10x coverage and is predicted to contain 14 086 genes [1]. EST collections (containing 40 000 sequences) are available from the COGEME (cogeme.ex.ac.uk) and the Broad Institute (www.broad.mit.edu) databases.

The aim of this project is to identify novel pathogenicity genes in *F. graminearum* by random insertional mutagenesis. A library of PH-1 mutants was created using a protoplast-PEG transformation method and tested for pathogenicity defects on wheat ears. Three mutants with reduced disease causing ability were identified. Plasmid rescue was used to identify the disrupted gene in one of the mutants as coding for the topoisomerase I enzyme (TOP1). Targeted deletion of *TOP1* has verified its role in *F. graminearum* and *F. culmorum* pathogenicity on wheat ears. Plasmid rescue of another reduced virulence mutant (*daf*10) revealed a large deletion had occurred at the end of chromosome 1 resulting in the loss of at least 145 genes. The Pathogen-Host Interactions database (www.phibase.org) was used to analyse these mutants [2,3].

Acknowledgements
Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC). This PhD project was funded with a BBSRC industrial CASE studentship awarded to Syngenta

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PHI-base: A database of experimentally verified pathogenicity, virulence and effector genes in fungal, Oomycete and bacterial pathogens of animals and plants

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Pathogens of plants and animals are a major global problem. In the last 15 years many genes required for pathogenicity and virulence have been determined for different species. In addition, other studies have characterised effector genes required to activate host responses. By studying these types of pathogen genes, novel targets for control can be revealed. In this presentation, we will describe a Pathogen Host Interaction database (PHI-base) which systematically compiles the genes involved in each interaction (Winnenburg et al. 2006; Baldwin et al. 2006). Each PHI-base entry is curated by domain experts and supported by strong experimental evidence as well as literature references in which the experiments are described. Every gene in PHI-base is presented with its nucleotide and deduced amino acid sequence as well as a detailed description of predicted protein's function during the host infection process. To facilitate data interoperability, we have annotated genes using controlled vocabularies (Gene Ontology terms, EC Numbers, etc.), and provide links to other external data sources (for example, NCBI taxonomy and EMBL). We explore the utilisation of PHI-base for the computational identification of pathogenicity genes through comparative genomics. In this context, the importance of standardising pathogenicity assays as well as integrating databases to aid comparative genomics is discussed. PHI-base can be found at http://www.phi-base.org/.

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Baldwin et al. (2006) PHI-base provides insights into generic and novel themes of pathogenicity. *Mol. Plant. Microbe Interact.* **19**, 1451-1462

Identification of pathogenesis-related determinants in the rice blast fungus

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Fungi belonging to the *Magnaporthe grisea* species-complex infect a wide range of cereals and grasses, including economically important crops such as rice, barley, wheat and millet. *Magnaporthe oryzae* is a member of this species-complex and causes rice blast disease which is responsible for the loss of 10-30% of the annual rice harvest worldwide.

M. grisea has been regarded as a typical foliar pathogen, but recently it has been shown that it can also infect cereal roots, undergoing developmental steps typical for root-infecting pathogens. Interestingly, appressorium formation or the CPKA gene, encoding for a catalytic subunit of a cAMP-dependent protein kinase that are essential for aerial infection are not required for root infection by the fungus.

A forward genetics screen using *in vitro* tests on hydrophilic and hydrophobic surfaces as well as *in planta* assays on roots and leaves has enabled the identification of tissue-specific and general determinants involved in pathogenicity of *M. grisea*. A transformant of the fungus identified during the *in vitro* screen on polystyrene hydrophilic surfaces has been found to be compromised in root and leaf pathogenesis. The disrupted gene was identified by recovery of the sequence flanking the T-DNA insertion site and encodes a putative transcription factor, previously identified in *Aspergillus terreus*. Targeted gene replacement mutants of this gene have been generated, via the *Agrobacterium tumefaciens*-mediated transformation.
The coding sequences of other two putative transcription factors, related to the one previously identified in *A. terreus*, have also been selected and disruption mutants have been generated, as part of a reverse genetics approach. The phenotype of all generated mutants is currently being characterised.

Functional characterisation of nematode chorismate mutases – a role in suppression of plant defence signalling pathways?

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Chorismate mutases (CM) have been identified from both cyst forming and root-knot nematodes. The CM proteins are produced in the subventral gland cells in cyst nematodes and are likely to be secreted into host plants. Their precise functional role is currently being investigated in several different laboratories. It has been suggested that CM may deplete precursors of cytoplasmic auxin thus changing auxin levels and triggering the changes associated with early feeding site development (Doyle & Lambert, 2003; MPMI 16,123-131). Some evidence for the association of certain forms of CM with avirulence against resistance sources has also been reported for *Heterodera glycines* (Bekal, Niblack & Lambert, 2003; MPMI 16,439-446).

We are currently investigating the functional role of CM in *Globodera pallida* using a variety of molecular and genetic techniques. Expression of the nematode CM as a fusion with a fluorescent protein from a plant virus showed that the CM is localised to the cytoplasm when expressed in plant cells. Knocking out CM expression using RNAi resulted in reduced levels of infection compared to plants treated with dsRNA from a non-endogenous gene. The effect was most pronounced when looking at adult females and, since sex is determined by food availability in this nematode, this indicates that one role of CM may be to ensure a healthy and fully functional feeding site is induced, either by inducing the formation of the feeding site or by preventing its breakdown by plant defence systems.

Current work is examining a potential role for the nematode CM in suppression of plant basal defences. Preliminary results suggest that expression of the PCN CM in *Pectobacterium atrosepticum* mutants deficient in genes required for normal suppression of basal defences (*hrpW, dspE*) restores the ability of the bacterium to suppress such defence signalling pathways, suggesting that the role of the CM may be to protect the developing feeding site from plant defences rather than inducing the formation of this structure.

As part of the work described above, we have identified a large multigene family of chorismate mutase genes in *G. pallida*. Analysis of single nucleotide polymorphisms (SNPs) in specific alleles has revealed one SNP which is associated with virulence to potato clones that have resistance derived from *Solanum vernei* . We are currently testing this SNP as a molecular marker for virulence in genomic and cDNA prepared from populations that have been biologically characterised.

Defining Downy Mildew (*Hyaloperonospora parasitica*) Avirulence Loci

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Hyaloperonospora parasitica causes downy mildew on Arabidopsis. This obligate biotrophic oomycete has co-evolved with its host and is unable to infect other plant species. Such host-specialisation has resulted in extensive genetic variation between different Arabidopsis accessions and downy mildew isolates. Isolate specific resistance is based on a gene-for-gene relationship, where a host resistance gene product and its corresponding pathogen avirulence gene product must both be present to trigger a resistance response. Two *H. parasitica* avirulence genes have been cloned, *ATR1* and *ATR13*. *ATR1* and *ATR13* encode for small, secreted proteins that have no homology to known proteins and both have an RXLR motif downstream of the signal peptide. The RXLR motif has been identified in a number of oomycete effector proteins and is thought to play a role in transport of these proteins into host cells. SSH and spore cDNA libraries were screened for secreted RXLR proteins along with a bioinformatics approach to mine the *H. parasitica* genome for this class of protein. Allele sequencing was used to determine if these genes are under selective pressure as a result of interacting with host resistance genes. Prior to this study a cross was made between the isolates Emoy2 and Maks9, in which 18 avirulence genes segregate. During this study a second cross was generated between the isolates Cala2 and Noks1, in which eleven avirulence genes segregate. Candidate avirulence genes were mapped onto the segregating pathogen-cross populations to determine if they co-segregated with the identified avirulence genes. Nine showed linkage to seven avirulence loci, five of which were tested in a biolistic assay but failed to trigger a host resistance response. These genes were then used as anchor points to construct BAC contigs spanning the genetic map intervals of four avirulence genes to enable a map based cloning approach.

Functional evaluation of plant defence signalling against Fusarium ear blight disease in Arabidopsis

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Fusarium ear blight (FEB) infections of cereal crops cause considerable losses to grain quality and safety. The two main causative agents of this disease on UK wheat crops are the fungal pathogens *F. culmorum* (*Fc*) and *F. graminearum* (*Fg*) (sexual stage *Gibberella zeae*). Floral infections by *Fc* and *Fg* also cause the developing cereal grains to become contaminated with various fungal mycotoxins, including the highly toxic trichothecene mycotoxin deoxynivalenol (DON). The molecular basis of resistance to FEB in cereal species is poorly understood but it is QTL based and Fusarium species non-specific.

We have previously demonstrated that *Fc* and *Fg* conidia can infect the floral tissues of Arabidopsis to cause disease symptoms on flowers, siliques and upper stem tissue [1]. DON mycotoxin production was detected in infected flowers. This novel Arabidopsis floral model provides a tractable system for elucidating fundamental aspects of this globally important cereal-fungal interaction.

We have undertaken a detailed analysis of the FEB infection phenotype in Arabidopsis genotypes with defined gene mutations. Data will be presented on the effects of genes involved in basal and race-specific defence signalling, including *RAR1, SGT1* and *EDS1*. Infections are quantified by FAD values (Fusarium-Arabidopsis Disease) [1].

A detailed study of Arabidopsis floral tissues inoculated with a *Fg* strain containing a deletion in the *Tri5* gene, revealed that *Fg* disease formation on Arabidopsis floral tissue does not require DON production.

This research is supported by the Biotechnology and Biological Sciences Research Council of the UK.

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Investigating the roles of reactive oxygen species during *Mycosphaerella graminicola* infection of wheat

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Mycosphaerella graminicola (anamorph *Septoria tritici*) is a hemibiotrophic fungal pathogen of wheat leaves. Infection reduces crop yield via the appearance of chlorotic / necrotic lesions, which reduce the photosynthetically active leaf area. In the early stages of infection, following penetration through stomata, the fungus grows in the leaf intercellular space without producing any specialised feeding structures or causing visible disease symptoms. In the later infection stages, fungal biomass increases, hyphal nutrition becomes necrotrophic and localised host programmed cell death occurs (1). Septoria leaf blotch disease is currently regarded as the most economically damaging disease of wheat in the UK and Western Europe.

Microscopy with reactive oxygen species (ROS)-specific stains has shown that hydrogen peroxide and superoxide are present in infected leaves, and that levels of ROS increase with the appearance of disease symptoms (1, 3, 4). In particular ROS are present on and within the asexual fruiting bodies, pycnidia. Microarray expression profiling of *M. graminicola* during infection of susceptible wheat genotypes has shown a number of genes, many of which have functions that are ROS-associated, that have greatly increased expression as disease progresses and symptoms become visible (1,2, 3).

This project aims to better understand the molecular basis of the involvement of ROS in the disease-causing ability of *M. graminicola* on wheat. Candidate genes have been selected for functional analysis based either upon literature or their transcriptional up-regulation *in planta* during disease symptom formation. The recent availability of the sequenced genome of *M. graminicola* has enabled targeted *Agrobacterium*-mediated gene deletion to be used for investigating fungal genes involved in the production of ROS or the oxidative stress response. Results will be presented describing the progress made in the functional characterisation of these genes to evaluate their roles in plant infection

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This project receives financial support from Syngenta and the Biotechnology and Biosciences Research Council (BBSRC) of the UK. Rothamsted Research receives grant aided support from the BBSRC.

Innate Immunity: Plant recognition of bacterial PAMPs

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Innate immune defences of plants include a set of basal responses that can be triggered by the perception of general elicitors that have been termed Pathogen Associated Molecular Patterns (PAMPs). Examples of PAMPs include lipopolysaccharides (LPS) from Gram-negative bacteria, peptidoglycans from Gram-positive bacteria, flagellin, fungal cell wall glucans and chitin. Recognition of PAMPs in both insects and animals leads to activation of defences and is often mediated by LRR (Leucine Rich Repeat) proteins such as Toll in Drosophila and the Toll-like receptors (TLRs) in mammals. LRR proteins also serve as receptors for PAMPs in plants; examples include the flagellin receptor FLS22 and elongation factor Ef-Tu receptor EFR. It is now established that LPS has myriad effects in plants including the ability to prevent the HR induced by avirulent bacteria, priming of some plant defence responses and elicitation of others, induction of the oxidative burst, nitric oxide synthesis, and phosphorylation of mitogen-activated protein (MAP) kinase. However little is known about perception of LPS by plants or the associated signal transduction pathways that trigger LPS-induced plant disease resistance. We have recently addressed this issue by analysing those sub-structures within LPS from the black rot pathogen *Xanthomonas campestris* that are required to trigger immune responses in Arabidopsis. Lipo-oligosaccharide, lipid A and core oligosaccharide from *X. campestris* were active in elicitation of PR1 expression, although differences in the temporal pattern of induction indicated that the plant could recognise lipidA and core oligosaccharide independently. This was the first step in devising a strategy to identify plant receptors for LPS in Arabidopsis and progress towards this goal will be presented.

Reference: Newman et al. (2007). Priming, induction and modulation of plant defence responses by bacterial lipopolysaccharides. J. Endotoxin Research 13:69-84

Analysis of metabolic profiles associated with basal defence and the establishment of pathogenicity

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We are developing metabolomics approaches to analyse changes in secondary metabolites associated with the activation of basal defences and the development of disease. Comparative analysis of tissue challenged with virulent wild type *P.s.* pv *tomato* DC3000 or the *hrpA* mutant which fails to colonise Arabidopsis leaves, has allowed us to discriminate between the activation of basal PAMP-mediated defences and the induction of conditions that favour bacterial multiplication. Intitial comparisons have been based on NMR profiling of extracts without fractionation. Differentiation between responding tissues highlighted changes in sugars and indolic metabolites. More targeted profiling using LSMS has focused on glucosinolates and changes to the plant cell wall. Cross-linking of indole carboxylic acid and several phenylpropanoid derivatives onto plant cell walls was found to occur during the early stages of the response to the *hrp* mutant. Localised changes to the wall appear to be suppressed by wild type DC3000 which hijacks plant hormones to divert plant metabolism to generate favourable conditions in the apoplast.

Identification of environmental factors driving *in-planta* evolution of virulence by genomic island loss in *Pseudomonas syringae* pv. *phaseolicola* 1302a

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Pseudomonas syringae pv. *phaseolicola* (*Pph*) is the seed borne causative agent of halo blight in the common bean, *Phaseolus vulgaris*. Gene for gene interactions underpin varietal resistance and race structure in the *Pph*/bean interaction. *Pph* race 4 strain 1302A contains the avirulence factor *avrPphB*, which matches resistance gene *R3* and causes a rapid hypersensitive reaction (HR) in *P. vulgaris* cultivar Tendergreen. *avrPphB* resides on a 106kb genomic island designated PPHGI-1, which if lost from 1302A, causes change to a virulent phenotype that produces water-soaked lesions typical of *Pph* disease. Evolution of 1302A virulent strains (by loss of PPHGI-1) occur by selective pressure due to exposure to *R3* based HR in *P. vulgaris* Tendergreen leaf tissue.

This study aimed to identify environmental factors responsible for signalling PPHGI-1 island excision during bacterial colonisation and plant-tissue interactions. We describe the development of fluorescent reporter technology and *in vitro* induced counterselectable *sac*B (levansucrase) to determine PPHGI-1 loss, and quantitative PCR to determine PPHGI-1 excision and loss. Results of PPHGI-1 chromosomal excision and cellular loss in response to abiotic and biotic factors are presented.

Cellulose synthesis in *Phytophthora infestans* is required for appressorium formation and successful infection of potato.

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The oomycetes are a diverse group of organisms, containing many devastating papthogens, including the late blight pathogen, *Phytophthora infestans*. Unlike the fungi, which they superficially resemble, the cell wall of the oomycetes is largely composed of cellulose. Upon encystment, *P. infestans* spores produce a cellulosic-cell wall, which is extended and thickened upon germination and appressorium formation. Inhibition of cellulose synthesis, using 2,6,Dichlorobenzonitrile (DCB) leads to a dramatic reduction in the number of normal-looking appressoria, severe disruption of the cell wall, and a complete loss of pathogenicity. Here, we also report the identification and characterization of a family of four *CES* genes within *Phytophthora* species. These genes represent a novel class of cellulose synthases that are phylogenetically distinct from other described cellulose synthases. Silencing of this gene family in *P. infestans* leads to the disruption of the cell wall surrounding appressoria and an inability to form typical functional appressoria. Our *in vitro* and ultrastructural studies show that the production of normal appressoria and specifically, the uniformity and thickness of the cell wall is disturbed in *CES1-4* silenced lines or in the presence of the inhibitor DCB. Remarkably, our data show, for the first time, that infection by a plant pathogen requires the synthesis of cellulose.

A 2-nitropropane dioxygenase from *Botrytis cinerea*

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2-nitropropane dioxygenases are a class of flavoenzyme first identified in filamentous fungi that catalyse the degradation of nitroalkanes. Nitro compounds are generally toxic, for example the antibiotic chloramphenicol and toxic glycoside conjugates of 3-nitro-1-propionic acid found in legumes. A gene with homology to 2-nitropropane dioxygenase was identified in the *Botrytis cinerea* genome sequence. The open reading frame of this gene was cloned into the pET28a expression vector and the protein overexpressed with an *N*-terminal His-tag in *E. coli* for biochemical characterisation. Studies show that the expressed protein possesses 2-nitropropane dioxygenase activity and contains flavin. Further experiments aim to determine the role 2-nitropropane dioxygenase in *Botrytis*.

Genomics approaches uncover an alternative life-style of the plant pathogen *Pectobacterium atrosepti*cum

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The plant pathogen *Pectobacterium atrosepticum* **(***Pba* - formerly *Erwinia carotovora* subsp. *atroseptica)* is a member of the bacterial family Enterobacteriaceae and has a host range limited to potato in temperate regions. In the UK *Pba* is an economically important pathogen that causes blackleg of potato plants in the field and soft rot of tubers in storage. Disease in potatoes can be established by contaminated seed tubers and under the right environmental conditions *Pba* can spread to daughter tubers through the stem and over short distances in the soil. However, very little is known about the life style of *Pba* away from potato. The genome of strain *Pba* SCRI1043 was recently sequenced and annotated revealing many new plant-associated life-style determinants not necessarily related to disease. These include a number of putative agglutinins and genes involved in nitrogen fixation. We examined the ability of *Pba* SCRI1043 to bind to other crops including brassica and weed plants, the results revealed that a mutant in a gene encoding a putative agglutinin showed a decrease in the ability of *Pba* to bind to all plant species tested. Preliminary results also indicate that *Pba* is able to fix nitrogen *in vitro*. These results may indicate an alternative niche for *Pba* as a nitrogen fixer living in the rhizosphere of other plants.

Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable brassica fields in Nepal and identification of a new race

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Black Rot, caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), is one of the most serious diseases of vegetable brassicas worldwide, particularly in warm and humid climates.

Cabbage and cauliflower production increased significantly in Nepal during the 1990's. Cabbage is an important crop, cultivated in approximately 20,000 ha, accounting for 11% of the vegetable area; in some areas, production is almost continuous with up to three crops per year. A survey of cabbage and cauliflower fields was carried out in five major districts of vegetable brassica production in Nepal in 2001. Plants with typical symptoms of Black Rot were observed in all fields. Seventy-seven pathogenic *Xcc* isolates were obtained for further studies.

Forty-nine isolates were inoculated onto a differential series of *Brassica* spp. to determine their race-type. Four of the six previously described races (1, 4, 5, 6) were identified. Race 4 (40%) was the most common followed by races 1 (27%) and 6 (22%). Two isolates produced a previously unreported pattern of reaction on the differentials (compatible on all genotypes except *B. juncea* cv. Florida Broad Leaved Mustard) and were designated race 7.

Repetitive DNA polymerase chain reaction-based fingerprinting (rep-PCR) using REP (repetitive extragenic palindromic), ERIC (enterobacterial repetitive intergenic consensus) and BOX primers was used to assess the genetic diversity of the Nepalese isolates. A dendrogram derived from the combined rep-PCR profiles obtained with the three sets of primers, showed that isolates tended to cluster within races, independently of geographical origin. The results suggest that rep-PCR can be a useful tool for rapid initial selection of isolates for further analyses, including race typing. These results may also be useful for comparison with future studies on the distribution and diversity of *Xcc* in other countries, for instance in Northern Europe, where the predicted global warming may lead to increased incidence and impact of bacterial diseases.

Biocontrol and plant transformation as a new alternative integrated strategy to control *Botrytis cinerea* on lettuce

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Botrytis diseases are wide-spread on a number of hosts worldwide and account for significant yield losses to the horticultural industry. At the same time, there is now a focus on reducing the use of agrochemicals on plants due to the levels of residues and general concerns over human health and environmental safety. Therefore, we are developing an approach using biological control agents combined with the transformation of lettuce, with a chitinase gene to provide a viable alternative strategy to the use of fungicides. *Botrytis cinerea* (strain BCL) and lettuce (*Lactuca sativa*) cv. Evola have been used as a model system for this combined disease control approach and plant transformation study.

In total, 220 bacterial strains were isolated from indoor and outdoor lettuce leaves using leaf discs washed in maximum recovery diluent (MRD) solution, with and without 0.1% (v/v) Tween 20. Potential antagonists against *B. cinerea* were identified by *in vitro* bioassay. In the primary screen 29 isolates were categorised as excellent, 27 as moderate and 164 as weak, based on the zone inhibition given by the antagonists. Only the 29 most promising biological control agents (BCAs) isolated first time mostly spore forming bacteria, had the ability to suppress *B. cinerea* in secondary screening and showed high (up to 30-50%) *Botrytis* growth reduction using dual culture tests on three different media. The *in planta* assay for further testing of the efficacy of BCAs against *B. cinerea* was based on spraying the washed bacterial cells in MRD solution onto lettuce leaf discs prior to inoculation with the pathogen. From this experiment, strains 62p and 66p were chosen as the best putative BCAs for control of *B. cinerea,* which had given significant reduction of disease development on leaf discs when compared with the control (water) and with the other isolates tested. These two strains of bacteria gave almost equivalent levels of control when compared to use of the fungicide Rovral Flo (sprayed at the standard rate).

Lettuce cv. Evola transformation was successfully achieved using a protocol that involved co-culturing (for 2 days) 7 days-old excised cotyledons with *Agrobacterium tumefaciens* (strain LBA4404) on Uchimiya and Murashige medium. They were thereafter maintained on Murashige and Skoog -based shoot regeneration medium which contained 30 g L^{-1} sucrose, 0.04 mg L^{-1} naphthaleneacetic acid, 0.5 mg L^{-1} 6-benzylaminopurine, semi-solidified with 0.8% (w/v) agar at pH 5.8 and supplemented with 500 mg L^{-1} cefotaxime and 50 mg L^{-1} kanamycin the latter acting as selection agent. Strain LBA4404 contained the binary vector pBI101, which carried a chitinase gene (pChit1) linked to the 35SCaMV promoter and nos terminator sequences and the neomycin phosphotransferase II (*npt* II) gene as the reporter. Polymerase chain reaction (PCR) analysis confirmed the successful introduction of the chitinase gene into lettuce. In future work, transgenic lettuce plants will be evaluated for their resistance to *B. cinerea* and subsequently integrated into *in planta* assays with BCAs, to determine whether this combined approach leads to effective and durable control of *Botrytis*.

Identifying resistance to Fusarium Ear Blight in wheat

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Fusarium ear blight (FEB), caused principally by the fungal pathogens *Fusarium graminearum* and *Fusarium culmorum*, is a devastating disease of wheat (1). Infection occurs at anthesis when periods of high humidity prevail. The problems caused by ear infection are two fold: shrivelling of grain causes a reduction in yield and accumulation in the grain of *Fusarium* trichothecene mycotoxins, primarily deoxynivalenol (DON) and its acetylated derivates 3-ADON and 15-ADON and nivalenol (NIV), results in a reduction in quality and is a concern for food safety. Control of the disease is difficult, with fungicide application being impractical. The use of resistant cultivars is now considered to be the best control option (2).

In this project, hexaploid wheat genotypes from around the world have been screened in field trials over two years for resistance to FEB. Harvested grain from the trial was analysed using gas chromatography-mass spectrometry to assess the quantity of DON mycotoxin present. Genotypes which showed reduced disease symptoms and/or mycotoxin accumulation are now being analysed further.

Traditional breeding methods require regular screening for disease, which is costly and time-consuming. To screen a wheat genotype for resistance only once can take approximately four months from seed to maturity. We have developed a novel screening assay which reduces the time disease to three weeks. Using the attached leaf assay, lesions are seen 4 days post inoculation. We are currently determining whether it is possible to distinguish between resistant and susceptible genotypes and if a correlation exists between observed field disease resistance and leaf assay disease resistance. The mechanism of infection of leaf tissue will be investigated using transgenic isolates of *Fusarium graminearum* producing the reporter protein β-glucuronidase, as well as conventional trypan blue staining.

1. US Wheat and Barley Scab Initiative Website – http://www.scabusa.org
2. Parry, D.W., *et al*. (1995). *Plant Pathology*, **44**, pp. 207-238.

This PhD is funded by the EU within the FP6 integrated project BioExploit.

A whole genome transcriptomics approach to determine the quorum sensing regulon of *pectobacterium atrosepticum* during potato infection

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Pectobacterium atrosepticum (*Pba* – formerly *Erwinia carotovora* subsp. *Atroseptica*), is the causal agent of the economically-important potato disease blackleg. In *Pba*, plant cell wall degrading enzymes (PCWDEs) and other pathogenesis-related factors are controlled, at least in part, by quorum sensing (QS). QS is a population density-dependant regulatory mechanism controlled by the production of the hormone-like, *N*-acyl homoserine lactone (AHL) – the product of ExpI. Using whole-genome *Pba* microarrays (Agilent Technologies), we have generated global gene expression data based on a comparison between the fully sequenced *Pba* strain SCRI1043 (*Pba*1043) verses an *expI*::*Tn5* strain during a potato tuber infection time course. Our data showed a total of 605 differentially expressed genes ($P<0.05$) in response to *N*-AHL in potato tubers at 12 hour post inoculation (hpi), with 319 up-regulated and 286 down-regulated genes in the *expI* mutant compared to the wild type strain. We also confirmed that the production of many PCWDEs, and the virulence-associated genes, eg *nip* and *svx,* were QS regulated as they were down-regulated in *expI* mutant strain. Over 70 genes associated with regulation, and those associated with phytotoxins production, type II secretion and many other functions were also differentially regulated in the *expI* mutant at both 12 and 20 hpi. Our data demonstrates that the QS system has a far wider influence on the interaction between *Pba* and its host, potato, than has previously been shown, uncovering many genes previously not associated with the QS regulon. We are now further investigating the roles of some of these genes in both the QS regulon and in virulence.

DON mycotoxin biosynthesis by Fusarium species, a metabolomics perspective.

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Many Fusarium species are fungal plant pathogens causing disease on both cereal and non-cereal hosts. Infection of the wheat ear typically results in bleaching and a subsequent reduction in grain yield. In addition, an increasing proportion of the harvested grain may be spoiled by trichothecene mycotoxins, such as deoxynivalenol (DON). The biosynthesis of DON in the wheat ear is a critical event, because infection of approximately one ear per 600 is sufficient to prevent usage of grain consignments under EU legislation. Much progress has been made in the elucidation of genes required for trichothecene production most of which are clustered at a single locus in the Fusarium genome.

This project seeks to extend this field of research by describing the metabolic characteristics associated with DON biosynthesis. As part of a new BBSRC metabolomics initiative, this project will examine a wide range of well characterised wild-type Fusarium laboratory strains and single-gene deletion mutants under controlled DON-inducing conditions *in vitro*. A variety of analytical techniques are being employed to analyse the metabolome, including 1H-NMR, electrospray mass-spectroscopy (ESI-MS) and GC-TOF-MS. Initial results have shown that both NMR and ESI-MS techniques are sufficient to discriminate a range of wild-type isolates. Principle components analysis identified metabolic differences between the wild-type strains, and was able to resolve *F.graminearum*, *F.culmorum*, *F.pseudograminearum* and *F. venenatum* islates after growth in minimal medium. In addition, several single-gene deletion strains that are reduced in pathogenicity exhibit large shifts in primary metabolism relative to their parent strains. Future work will attempt to find correlations between observed metabolic trends and DON biosynthesis and then confirm these by targeted methods.

Acknowledgements:

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Science Research Council (BBSRC).

How and where do plants make salicylic acid?

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Salicylic acid (SA) is important for a number of responses in plants, including defence against infection, responses to heat, UV and salinity. Despite this importance, the synthesis of SA is not fully understood in plants. SA may be synthesised by hydroxylation of benzoic acid, which is derived from the phenylpropanoid pathway. Alternatively, SA may be synthesised directly from chorismate in a way analogous to bacteria, such as *Pseudomonas aeruginosa*. This alternative pathway requires two enzyme activities: an isochorismate synthase (ICS) and an isochorismate pyruvate lyase (IPL). In *Arabidopsis*, there are two ICS genes, which encode proteins that convert chorismate to isochorismate. *Ics1* was identified from the *sid2* mutant, which is severely compromised in SA biosynthesis, and subsequently demonstrated to be an ICS. *Ics2* has been proposed to be involved in the synthesis of phylloquinone, a photosynthetic electron acceptor.

This project is aimed at characterising the roles of these two ICS proteins in *Arabidopsis*. ICS1 has been demonstrated to be targeted to the chloroplast whereas ICS2 is predicted to be cytosolic. ICS fusions to green fluorescent protein (GFP) are being generated in order to determine whether the two enzymes are spatially separated within the cell, as this may be a way of controlling metabolic flux. Alternatively, the two proteins might have different kinetic characteristics. In order to test this hypothesis, recombinant ICS1 and ICS2 are being produced. Once sufficient amounts of protein can be produced, the crystal structures of the two proteins can be determined and compared in order to investigate any differences between the two enzymes. The regulation of *Ics1* and *Ics2* gene expression is being studied using reporter gene constructs and reverse transcriptase polymerase chain reaction (RT-PCR) under different biotic and abiotic stimuli. *Ics1* is known to be induced by different pathogens (including *Pseudomonas syringae* pv. *maculicola* and *Erysiphe orontii*), but little is known about *Ics2*, which is likely to be constitutively expressed. This work will therefore provide further evidence for the role of isochorismate synthesis in pathogen defence.

Unraveling gene regulatory networks governing the Arabidopsis response to *Botrytis cinerea* infection

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The interaction between the host plant, *Arabidopsis thaliana*, and the necrotrophic fungal pathogen, *Botrytis cinerea* is complex. The outcome of the interaction is governed by genetic variation in both Arabidopsis and *B. cinerea*. Arabidopsis accessions vary in their susceptibility to this pathogen, and *B. cinerea* isolates vary in their ability to cause disease. We are interested in how Arabidopsis cells recognise *B. cinerea* infection and mount a coordinated defence response. Drop inoculation of leaves generates a gradient of secondary metabolism and gene expression changes and these spatial patterns differ after infection with different *B. cinerea* isolates, indicating different isolates can be recognised to different degrees or produce varying amounts and/or type of a signal.

Global gene expression profiling identified several hundred genes with significant changes in expression in response to pathogen infection. Several of these expression profiles have been confirmed using quantitative PCR or GUS reporter gene fusions. We have focused on two groups of genes – those encoding potential regulatory components and those encoding potential secondary metabolism enzymes. RNAi and/or T-DNA insertion lines have been used to assess the effect of knocking out these genes on susceptibility to *B. cinerea* and hence identify genes with a key role in defence against this pathogen. Local networks are being built around these key players.

We have also carried out a high-resolution time series experiment using CATMA arrays to analyse gene expression profiles of infected and uninfected leaves every 2 hr over a period of 48 hrs. Bayesian State space modelling of this extensive data set will enable us to infer gene regulatory networks activated in response to *B. cinerea* infection. Information from RNAi /T-DNA knockout lines used to perturb the system will be incorporated into this network in an iterative manner to generate validated predictive models.

Barley non-host and host interactions with *Polymyxa* species elicit a similar basal resistance response

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Barley is susceptible to the obligate root-infecting plasmodiophorid cereal parasite, *Polymyxa graminis,* but exhibits non-host resistance to the closely related parasite of the *Chenopodiaceae, Polymyxa betae*. Although the two species are not truly pathogenic, *Polymyxa* zoospores vector a wide range of important soil-borne viruses of cereals and sugar beet. To develop an understanding of the barley-*Polymyxa* interaction we have used the Affymetrix Barley1 GeneChip microarray to investigate the transcriptional responses in barley roots during the early stages (up to seven hours post-inoculation) of the interactions with zoospores of *P. betae* and *P. graminis*. We detected a small subset (*c.* 100) of genes that were transcriptionally activated by zoospores of both species, and none that were down-regulated by either *Polymyxa* species. Functional classification showed that nearly 50% of the activated transcripts in both the host and non-host response were associated with known defence genes, although very few were related to signalling or metabolic processes. The majority of the identified genes encoded antimicrobial peptides, pathogenesis-related proteins, and proteases. There was no clear distinction between the genes activated in host and non-host interactions suggesting that the reaction of barley to both compatible and incompatible *Polymyxa* species elicits a similar basal resistance response during the earliest stages of this plant-pathogen interaction.

The Roles of Cathepsin B-like Proteases in Plant Disease Resistance

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The hypersensitive response (HR) in plants is a form of programmed cell death (PCD), which is under genetic control. Apoptosis in animals is also a form of PCD and it is thought that some mechanisms may be conserved between the HR and apoptosis; particularly with respect to the involvement of caspase-like enzymes and other proteases. Cathepsin B is a non-caspase cysteine protease which was identified in a screen in *Solanum tuberosum* for genes up-regulated in a compatible HR against the oomycete *Phytophthora infestans*. Previous work has demonstrated that silencing *Cathepsin B (CTB)* in *Nicotiana benthamiana* greatly reduced the HR and consequently increased susceptibility to non-host pathogens and compromised resistance (*R*)-gene mediated protection.

Further work carried out using GFP and mRFP protein fusions has localised *Nb*CTB to the apoplast where there is potential for it to interact with pathogen effectors. Also, overexpressed, active *Nb*CTB has been isolated from the apoplast giving us a system to assay the effects of various inhibitors and pathogen effectors on CTB activity.

Arabidopsis has three homologues of the potato and tobacco *CTB* genes. T-DNA insertion knockout lines have been isolated for each of these genes. As these genes have >80% similarity and functional redundancy is thought to occur, double and triple knockout lines as well as plants over-expressing *CTB* have been constructed and are currently being analysed. Preliminary results indicate that *Arabidopsis CTB* genes may be involved in cell death and defence signalling mediated by coiled-coil- nucleotide binding site-leucine rich repeat (*CC-NBS-LRR*) & Toll interleukin-1 receptor (*TIR*)-*NBS-LRR R*-genes. In contrast, these proteases do not seem to be required for basal disease resistance.

Agrobacterium suppresses P. *syringae*-elicited salicylate production in *Nicotiana tabacum*

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Agrobacterium tumefaciens strain GV3101 is widely used in transient gene expression assays in plants, particularly *Nicotiana* spp. Transient expression assays have previously been used to study the role of pathogen effectors and elicitors in modulating plant defences, and to investigate the signal transduction pathways involved in expression of defence responses. We have used *Agrobacterium*-mediated transient expression to monitor the fate of fluorescent proteins in healthy and *P. syringae*-infected tobacco leaves. However, while performing these experiments we observed that inoculation of *Agrobacterium* GV3101 into tobacco leaves followed by co-infiltration with the tobacco pathogen *Pseudomonas syringae* pv. tabaci 11528 resulted in delayed macroscopic symptoms and lower *P. syringae* population densities when compared with leaves infiltrated with a procedural control. We have previously used a bioluminescent SA biosensor to monitor SA levels in the apoplast of *P. syringae* infected leaves, and have shown that apoplastic SA levels increase during compatible and incompatible *P. syringae*-plant interactions. Infiltration of the SA biosensor into *Agrobacterium*-treated leaves shows that *P. syringae* induced SA induction is strongly reduced by pre-treatment with *Agrobacterium* in a time and dose-dependent manner. Heat-killed *Agrobacterium* cells fail to suppress SA. We are currently exploring the mechanistic basis of this interaction.

The effect of mycotoxigenic microorganisms on the quality of palm oil produced at the cottage industry level

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Oil seeds form a very important component of tropical agriculture, because they are readily available and provide highly nutritious human and animal food. Microbial activity on oil seeds often results in mycotoxin contamination of the products, the consumption of which could be fatal for both animals and humans. Mycotoxin contamination in some oil seeds and stored grain, for example aflatoxins in groundnuts and maize, has been extensively studied. However, most of the work done on oil palm has been related to the free fatty acid content and oil degradation, and no studies have been undertaken to determine the influence of microbes on mycotoxin contamination of the oil palm products. This work aimed to study microbial activity on palm fruits in Ghana, and to determine the quality and levels of mycotoxin contamination in palm oil at the cottage industry level.

To achieve these objectives, various culturing and PCR-based diagnostic methods have been undertaken to identify the predominant microbes on oil palm fruit from Ghana. Commercial aflatoxin detection kits (e.g. Aflacard) have also been used to monitor the levels of aflatoxin contamination in samples, and PCR primers based on the aflatoxin and ochratoxin biosynthetic gene sequences have been used on oil palm samples from various small-scale mills in Ghana to assess their potential for identifying the presences of mycotoxin-producing fungi.

A total of forty fungal and eleven bacteria species have been identified from sequencing and PCR fragment analyses. Lypolitic microbes identified on palm fruits and in oil have included *Aspergillus, Bacillus, Candida, Geotrichum, Pseudomonas* and *Rhodotorula species*. Fourteen samples derived from the small-scale mills amplified with the aflatoxin biosynthetic gene primers, and forty-five with the ochratoxin biosynthetic gene primers. The study also showed that the age of fruits plays a very significant role in determining palm oil quality.

Phenotypic and metabolomic analyses of the *Fusarium graminearum snf1* mutant

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The Ascomycete fungus *Fusarium graminearum* (Fg) is a ubiquitous plant pathogenic fungus in all major cereal growing areas of the world. Infections, which occur during plant anthesis, lower grain quality and contaminate grains with trichothecene mycotoxins. The genome was sequenced by the BROAD Institute and completely aligned to the genetic map. Fg has 4 chromosomes and minimal repetitive DNA (http://www.broad.mit.edu).

In an effort to identify the set of pathogenicity genes in Fg and other fungi, a database of known fungal and Oomycete pathogenicity genes was established at Rothamsted Research (http://www.phi-base.org). We have identified all the Homologues of verified pathogenicity and virulence (HvPV) genes in Fg, and created a chromosome visualisation software, called ChromoPaint (J. Antoniw, unpubl.) to inspect visually the chromosomes for clustering of HvPV genes. This analysis identified a region on chromosome 1, where 5 HvPV genes are clustered together within 24 kb. This includes the *S. cerevisiae SNF1* gene homologue, a regulator of catabolite derepression in yeast and a regulator of cell wall degrading enzymes in *Cochliobolus carbonum* and *F. oxysporum*. We deleted the *snf1* gene in Fg as the first member of the HvPV gene cluster in chromosome 1 to characterise the importance of catabolite derepression during plant pathogenicity and mycotoxin production. The availability of the *snf1* mutant, which exhibits highly reduced virulence on wheat ears, has allowed us to use 1H-NMR metabolomic analysis as a tool to identify fungal candidate metabolites with a role in plant pathogenicity.

Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council.

NpPDR1, a Pleiotropic Drug Resistance transporter from *Nicotiana plumbaginifolia* is implicated in plant-pathogen interactions

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ABC (ATP Binding Cassette) transporters are involved in the active transport of a large variety of metabolites and constitute a large gene family organized in subfamilies. Our laboratory has developed a functional study of ABC transporters of the Pleiotropic Drug Resistance (PDR) subfamily in *Nicotiana tabacum* and *N. plumbaginifolia*.

NpPDR1 was shown to transport the antifungal diterpene, sclareol. Expression analysis localized the NpPDR1 protein to the trichomes from which sclareol is secreted as a first defense barrier against pathogens. Furthermore, NpPDR1 expression was strongly induced in the whole leaf after microbial aggression, suggesting that this ABC transporter might be involved in plant defense. Finally, silencing of *NpPDR1* expression by RNA interference resulted in increased susceptibility of the plants to infection by *Botrytis cinerea*.

We reproduced this last observation under controlled conditions and extended this analysis to other agronomically important fungal pathogens such as *Fusarium oxysporum* or *Rhizoctonia solani*. Preliminary pathogenicity tests indicated that the *NpPDR1*-silenced plants were more susceptible than the wild-type to infection by these three pathogens under the tested conditions. These data thus support the hypothesis that *NpPDR1* is involved in the plant defense.

In parallel, we sought to identify the NpPDR1 substrates by a comparative GC-MS analysis of the leaf surface metabolites from wild-type and *NpPDR1*-silenced plants. Reduced concentration of sucrose esters as well as diterpenes was found in the silenced lines.

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New sources of resistance to UK isolates of *Hyaloperonospora parasitica* in *Brassica oleracea* L.

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Downy mildew of brassicas is an economically important disease caused by the oomycete *Hyaloperonospora parasitica* subsp. *brassica* (*HpB*) and can occur worldwide wherever Brassica crops are grown. Seedlings and young plants raised in glasshouses for transplanting are most susceptible, but the disease can affect plants at all stages of development causing seedling damage and death, lesions on leaves and curds, loss of quality of mature plants and affect seed quality. The disease is favoured by mild, humid weather. Fungicides are used to control downy mildew in *B. oleracea* crops, but repeated applications of fungicide can lead to fungicide insensitive variants in the pathogen populations; resistant cultivars could provide a practical, environmental-friendly, alternative mean of control.

Previously, three *B. oleracea* accessions (two borecoles and a summer cabbage) with strong resistance at the cotyledon stage were identified and doubled-haploid (DH) lines were produced from outcrosses with a rapid-cycling parent. The three derived DH lines were tested with 25 *HpB* isolates collected from major vegetable growing areas of the UK and confirmed to be resistant to all isolates. Crosses were made between the three sources of resistance and between each source and a susceptible DH line of broccoli and a susceptible rapid flowering cauliflower; F_2 and back-cross populations were produced. These lines were tested at the cotyledon stage with a representative *HpB* isolate from the UK. The results of the segregation indicate that resistance on all three lines is controlled by one single dominant gene that appears to be allelic, identical or tightly linked in all three lines.

A back-cross programme is under way to transfer the resistance to broccoli and cauliflower. Doubled-haploid lines derived from crosses between two sources of resistance and a susceptible broccoli were produced in order to fix the resistance in a 'broccoli-type' plant. Thirty-five DH lines were tested for resistance at the cotyledon stage to one *HpB* isolate. As expected, approximately half of the lines were very resistant, but an intermediate phenotype that shows restricted sporulation was observed in approximately one quarter of the plants. The inheritance and spectrum of this partial resistance will be further investigated.

We are now screening lines generated from wild C-genome species (e.g. *B. cretica*, *B. incana*, *B. macrocarpa*) to identify new sources of downy mildew resistance using representative isolates from the *HpB* collection. Preliminary results indicate that, as with *B. oleracea,* most of this wild germplasm is highly susceptible to downy mildew. However, several examples of partial resistance were observed in our first round of experiments.

The resistance genes identified in this work could be incorporated in commercial cultivars in order to control downy mildew at the seedling stage. Selected DH lines produced in this project will be used for epidemiology studies and will be a good starting point for more detailed studies on host-pathogen interactions.

Investigating the effects of fungicides and biocontrol on take-all of wheat

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Take-all of wheat is caused by the soil-borne fungus *Gaeumannomyces graminis* var. *tritici.* It is a widespread and destructive disease in temperate climates around the world and losses may vary from negligible up to 50%. Several practices can be used to limit the disease on susceptible crops, including tillage, rotation, choice of variety, N fertilizer, and chemical and biological seed treatments. However, there are public concerns over the use of chemicals in the environment in general, and fungicide resistance can occur in pathogen populations. Hence, it is necessary to better understand the effects of different treatments on the populations of pathogens, potential antagonists and the general microbial community in the rhizosphere and on roots.

The terminal restriction fragment length polymorphism (T-RFLP) was used to monitor changes in populations of pathogens, applied biocontrol agents and other components of the microbial community in the rhizosphere and on the roots of wheat. This technique has the advantage over conventional methods of recording the changes in population sizes for different microbes by allowing simultaneous evaluation, in response to different treatments. Moreover, an internal control was introduced into the technique to monitor any changes of microbial populations semi-quantitatively. The current experiments focused on investigating the effects of seed-applied fungicides and / or applied biocontrol agents, such as *Bacillus* and *Pseudomonas*, on the pathogen, the applied biocontrol agents and other root-associated microbial populations. Fungicide treatments included experimental formulations of micro-encapsulated active ingredients, which offer a water insoluble matrix as a complex delivery system for fat soluble chemicals. Four fungicides treatments were tested, being: Jockey (a commercially-available product based on fluquinconazole and prochloraz applied at 450mL per 100 Kg seed), encapsulated tebuconazole), conventional tebuconazole and prochloraz, 3:1) and encapsulated tebuconazole and prochloraz, 3:1). All treatments except Jockey were applied as 40g active ingredient per 100 Kg seed.

Current results show that micro-encapsulation is a promising fungicide treatment to control take-all in the glasshouse environment, and *Bacillus* and *Pseudomonas* were able to inhibit take-all fungus under *in-vitro* conditions. Furthermore, the fungicides used did not alter significantly the bacterial communities in the rhizosphere and on roots. *Pseudomonas* spp. were also found to be more competitive than *Bacillus* in colonization of the rhizosphere and wheat roots.

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Transcriptional regulation by a NAC transcription factor which regulates the penetration resistance in Barley and Arabidopsis

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Abiotic and biotic stresses of plants are perceived by a wide range of regulatory proteins. One class of regulatory proteins is the NAC transcription factors. NAC transcription factors are plant-specific gene products found in several agronomical important crops. Research on their biological functions originally focused on the involvement of NAC members in plant developmental processes. However, recent papers on both biological and biochemical functions of NAC members have shed light on their regulation of the plants sensing of environmental stresses.

We have used a functional genomics approach in barley to unravel the functions of NAC transcription factors found to be differentially displayed in powdery mildew (*Bgh*) infected epidermal cells compared to control cells. Using this approach we have identified a NAC member, termed HvNAC6 for its high sequence identity to the rice NAC protein OsNAC6. Transient RNAi of *HvNAC6* renders barley epidermal cells significantly more susceptible to attack by the compatible *Bgh* isolate A6 compared to control cells. In a reciprocal approach, we have performed transient overexpression studies, and found that *HvNAC6* overexpression leads to significantly more penetration-resistant cells compared to control transformed cells. Furthermore, T-DNA mutation in the Arabidopsis *HvNAC6* homologue, *ATAF1* gene significantly increases the penetration rate compared to wild-type Col-0 plants, when inoculated by the non-host pathogen *Bgh*. ATAF1 has been described recently as a repressor of stress-sensitive genes, making the plant more drought tolerant. To learn more about the down-stream targets of *HvNAC6*, we have decided to look into the large-scale transcriptional profiles of *ataf1* and Col-0 plants +/- *Bgh* attack using the Affymetrix microarray. Knowledge from this study will hopefully cast light on some of the complex regulatory networks involved with perception of abiotic and biotic stresses in plants.

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Towards *in vivo* activity-based profiling of *Hyaloperonospora*-infected Arabidopsis tissues

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Activities of enzymes can be displayed using activity-based protein profiling. This technology is based on the use of biotinylated inhibitors which react with the catalytic residues of enzymes in an activity-dependent manner. The labelling is covalent and irreversible which facilitates the display and identification of the labelled enzymes. ABPP has been established for papain-like cysteine proteases, but more probes for other enzymes have been developed and remain to be tested. A disadvantage of the biotinylated probes is that they are usually not membrane permeable, which restricts their use to *in vitro* experiments on extracts. We introduce a 2-step labelling procedure for membrane-permeable probes using click chemistry. This procedure will be applied to investigate enzyme activities during infection of Arabidopsis by *Hyaloperonospora parasitica*.