MBPP 2008, Birnam, 17th – 19th September

Information for Delegates

Programme Outline

www.mbpp.co.uk

Information for delegates

The venue
All scientific sessions will be held in the main room at the Birnam Institute. Tea and coffee breaks will be taken upstairs in the BI.

Meals
Lunches will be served upstairs at the BI

Dinners **for all delegates** will be served in the Birnam House Hotel (across the road from the BI).
Dinner on Wednesday evening will be served at 7.30
Dinner on Thursday evening will be served at 8.00
Breakfast will be served in the hotel in which you are staying.

There will be live music in the Tap Inn bar (next to the Birnam House Hotel) on Wednesday evening. Delegates can avoid this by using the Birnam House Hotel bar as an alternative.

Accommodation
Delegates are housed in two hotels: The Birnam House Hotel (across the road from the conference centre) or The Atholl Arms situated in Dunkeld approximately 10 minutes walk from the BI). Details of how to access your rooms and how to get to your hotel are provided with individual delegate packs. If there are any problems with the accommodation you have been allocated please speak to John Jones as soon as possible.

Parking
You may park your car at your hotel or outside the BI.

Banks and other facilities
A Halifax/Bank of Scotland cashpoint that accepts all major cards (and does not charge for withdrawals) is located in the main square in Dunkeld. To reach this from the BI leave the BI to the left by the main entrance, turn left at the main road and then right towards Dunkeld at the road junction. Immediately after crossing the River Tay at Dunkeld bridge turn left towards the cathedral. The bank is immediately on the left hand side. Alternatively there are post offices in Birnam and in Dunkeld that will allow users of some banks to withdraw cash free of charge.

Presentations
Please ensure that your powerpoint file is loaded on to the conference computer well before the session you are speaking in starts. You can do this when you register or during any breaks. If you must use a Mac please speak to John Jones well in advance of your presentation so that we can check for compatibility. You can load your presentation from a CD or from a USB memory stick.

Things to do in and around Birnam

A variety of signposted walks are available around Birnam ranging from gentle strolls along the banks of the Tay to more challenging walks that include a climb of Birnam Hill. For those that prefer less strenuous activities Dunkeld Cathedral is a short walk (~15 mins) from the conference venue. The Cathedral is situated on the bank of the River Tay and is open to the public. The National Trust for Scotland has renovated 20 houses dating from the 17th and 18th Century in Dunkeld and has a shop and small visitor exhibition in Cathedral Street in Dunkeld

Congress Sponsors

The organizers of MBPP would like to express their gratitude to the following for their generous sponsorship of the meeting:

The Gatsby Charitable Foundation

The British Society for Plant Pathology

Programme Outline

Wednesday 17th September

12.00 – 14.00 Arrival and Registration
Lunches can be purchased from the Café at the Birnam Institute.

14.00 Welcome and Introduction (J. Jones SCRI)

14.00 – 17.10 – Session 1 – Chair: Miles Armstrong (SCRI)

14.00 – 14.40. Invited speaker: Dr Ari Sadanandom (University of Glasgow):
Ubiquitin mediated signaling and plant disease.

14.40 – 15.00. **Hazel McLellan**[1,2], Eleanor Gilroy[1], Gary Loake[2] & Paul Birch[1] ([1]SCRI, & [2] University of Edinburgh). The involvement of Cathepsin B-like genes in disease resistance in *Arabidopsis thaliana*.

15.00 – 15.15. **Anneke Prins,** Conrad Stevens, Andrew Plume & Murray Grant (University of Exeter). The role of protease inhibitors in the hypersensitive response

15.15 – 15.50. Break. (Tea & Coffee available upstairs at Birnam Institute.)

Session 2 – Chair: Maeve Price (University of Oxford).

15.50 – 16.10. **Nichola Spencer-Jones** (University of Leeds). The effect of nematode infection on the abiotic stress response in *Arabidopsis.*

16.10 – 16.30. **Siân Deller** John Keon, John Antoniw, Kim Hammond-Kosack and Jason Rudd (Rothamsted Research). Functional analysis of reactive oxygen species – related genes in the wheat pathogen *Mycosphaerella graminicola*

16.30 – 16.50. **Solange Mateo Montalcini,** Pari Skamnioti and Sarah Gurr (Oxford University). *Magnaporthe grisea* serine threonine protein kinase STA1 regulates stress resistance and aging and is a virulence determinant.

19.30 – Dinner (Birnam House Hotel).

Thursday 18th September

9.00 –10.30 – Session 3 – Chair: Solange Mateo Montalcini (University of Oxford).

9.00 – 9.40. Invited speaker: Dr Petra Boevink (SCRI): Plant Pathogen cell biology - illuminating the interaction.

9.40 – 9.50. Amarnath Thirugnanasambandam[1], Adrian Newton[1], Steve Whisson[1], Kath Wright[1], Neil Havis[2] and Simon Atkins[3]. ([1]SCRI, [2] Scottish Agricultural College, [3] Rothamsted Research). Role of seed-borne infection in *Rhynchosporium* and *Ramularia* epidemics in barley.

9.50 – 10.10. Miles Armstrong, Eleanor Gilroy, Jorunn Bos, Sophien Kamoun, Juan Morales, Ingo Hein, Leighton Pritchard, Steve Whisson and Paul Birch. (SCRI and JIC). Virulence and avirulence in *Phytophthora infestans.*

10.10 – 10.30. Ros Taylor, Miles Armstrong*, Petra Boevink*, Eleanor Gilroy*, Jorunn Bos+, Ratih, Sophien Kamoun+, Ari Sadanandom, Paul Birch*. (University of Glasgow, *SCRI and +JIC). Why does Avr3a Interact with StCMPG1?

10.30 – 11.15. Break. (Tea & Coffee available upstairs at Birnam Institute.)

11.15 – 12.15. Session 4 – Chair: Leighton Pritchard (SCRI)

11.15 – 11.35. Edgar Huitema, Osman Bozkurt, Mireille vanDamme, Liliana Cano and Sophien Kamoun (The Sainsbury Laboratory). A large and complex gene family from *Phytophthora infestans* encodes a novel and diverse class of effector molecules involved in virulence.

11.35 – 11.55. Volkan Cevik, Rebecca Allen, Peijun Zhang, Sharon Hall, Mary Coates, Rachel Baumber, Laura Baxter, Laura Rose and Jim Beynon (Warwick HRI& University of Munich). Genetic analysis of the *RPP13/ATR13* interaction complex between downy mildew and *Arabidopsis*

11.55 – 12.15. Angelique H. Riepsamen (University of Wollongong). The unusual mitochondrial genetics of the cyst-forming nematodes.

12.30 LUNCH (Available upstairs in the Birnam Institute).

13.30 – 17.00. Free time. Paul Birch will lead a walk in the Birnam area for those interested.

17.00 – 17.45. Adrian Newton. Uisge beatha: Everything you always wanted to know about whisky but were too inebriated to ask. Or: If it moves, distill it! Content of the talk will include: What whisky really is. How it is made. Why bourbon should be classed as a petro-chemical. Why burnt toast is dangerous. How to become a whisky expert (bore) in six easy steps.

17.45 – 19.30(ish) Whisky (and local beer) tasting. (Upstairs at Birnam Institute).

20.00 – Dinner (Birnam House Hotel).

Friday 19th September

9.15 – 9.30. Discussion on future format of MBPP. Anyone wishing to contribute is welcome to attend.

9.30 – 12.15. Session 5. Chair Siân Deller (Rothamsted Research).

9.30 – 10.10. Invited speaker: Dr J. Milner (University of Glasgow). An effector protein encoded by *Cauliflower Mosaic Virus* inhibits SA-dependent defence responses in *Arabidopsis* and suppresses innate immunity.

10.10 – 10.20. Kozlakidis, Z.[1], Brown, N. A.[1], Jamal, A.[1], Phoon, X.[1], Quicke, D.L.J.[2,3], Coutts, R.H.A.[1] ([1] Imperial College London, [2] Centre for Population Biology, Imperial College London, [3] Natural History Museum). Phylogeography and genetic diversity of *Phytophthora* species endornaviruses.

10.20 – 10.40. James Lord, Peter Urwin, Howard Atkinson (University of Leeds). Biofumigation for control of potato cyst nematodes and *Rhizoctonia solani*.

10.40 – 11.10. Break. (Tea & Coffee available upstairs at Birnam Institute.)

11.10 – 11.30. Maeve Price and Sarah Gurr (University of Oxford). SSW1 encodes a glycolipid-anchored surface protein and is a virulence determinant in *Magnaporthe grisea.*

11.30 – 11.50. **Jasper Johnson**, Mary Illes, Pari Skamnioti and Sarah Gurr (University of Oxford). NO: a nitric oxide synthase generated effector of pathogenesis in *Magnaporthe grisea*

11.50 – 12.00. **Nichola Hawkins [1],** Hans Cools [1], Michael Shaw [2], Helge Sierotski [3] and Bart Fraaije [1]. ([1]Rothamsted Research, [2]University Of Reading, [3]Syngenta Crop Protection). Fungicide resistance in *Rhynchosporium secalis*

12.00 LUNCH (Available upstairs in the Birnam Institute).

DEPART. (Bus time to Edinburgh to be arranged).

MBPP 2008, Birnam, 17th – 19th September

Conference organiser:

John Jones (SCRI)

Abstracts

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Hazel McLellan[1,2], Eleanor Gilroy[1], Gary Loake[2] & Paul Birch[1]
[1] Plant Pathology, SCRI, Invergowrie Dundee DD2 5DA
[2] IMPS, University of Edinburgh, Kings Buildings, Edinburgh , EH9 3JR

The involvement of Cathepsin B-like genes in disease resistance in Arabidopsis thaliana

Proteases have long been associated with apoptosis, a form of Programmed Cell Death (PCD) in mammals and there is considerable interest in investigating conserved roles for proteases in the Hypersensitive Response (HR), a plant defence response involving PCD, which shares some morphological characteristics with apoptosis. A cysteine protease, with homology to mammalian Cathepsin B proteases, was isolated in a screen for genes up-regulated in the HR. Previous work, investigating possible roles of a *Nicotiana benthamiana Cathepsin B* homologue in plant defence, found that either *NbCathB* silencing, or peptide inhibition of Cathepsin B activity, caused a reduction in the HR and corresponding increase in susceptibility in certain plant-pathogen interactions. The focus of this current research is to examine the roles of Cathepsin B genes in the model plant *Arabidopsis*: a more genetically tractable system. There are three Cathepsin B homologues in *Arabidopsis* for which knock-out lines were isolated and genetically crossed using a combination of T-DNA insert lines and RNAi to generate double and triple mutants. These genes were found to act redundantly with triple mutants showing increased susceptibility to virulent but not avirulent strains of *Pseudomonas syringae* DC3000. Moreover, these genes are also involved in non-host resistance to fungal pathogen *Blumeria graminis* f.sp. *tritici,* where they positively regulate the HR but negatively regulate *Pathogenesis-Related 1* (*PR1*) expression downstream of *Enhanced Disease Susceptible 1*(*EDS1*). In addition, this work also implicates *Cathepsin B* genes in senescence, a developmental form of PCD, via regulation of the senescence marker gene *Senescence Associated Gene 12* (*SAG12*). Furthermore, it was shown that NbCathB is localised to the plant apoplast where it is activated upon secretion. Partially purified Cathepsin B protein was inhibited by a variety of peptide inhibitors but evidence of inhibition by several pathogen-derived inhibitors that are secreted during infection was inconclusive

Anneke Prins, Conrad Stevens, Andrew Plume & Murray Grant
School of Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter, Devon, EX4 4QD

The role of protease inhibitors in the hypersensitive response

The plant immune system relies on the innate immunity of each cell and on systemic signals originating from infection sites to protect the plant from pathogen attack. Successful pathogens that escape the plant's initial response to pathogen associated molecular patterns deploy effectors that contribute to pathogen virulence. Effector-

triggered immunity is classsically encoded by R proteins whose activation following direct or indirect recognition of effectors results in localised programmed cell death known as the hypersensitive response (HR).
RPM1 is a typical NBS-LRR R protein that recognises the bacterial effector AvrRpm1 and AvrB. We have identified two proteins, RIN12 and RIN13 (Al Daoude et al. 2005) that interact with RPM1 in yeast 2-hybrid and whose mis-expression modifies the HR. RIN12 overexpression leads to a delay in HR and enhanced bacterial growth, while a reduction in RIN12 leads to faster HR upon elicitation with bacteria expressing the AvrRpm1 effector. The RIN12 protein shows structural homology to protease inhibitors, although the substrate specificity domain in the combining loop unusually contains a proline. RIN12 successfully inhibits serine endopeptidases of the subtilisin-type. Mutation of the combining loop abolishes both subtilisin activity and RPM1 interaction in yeast. Therefore our data are consistent with a "guard hypothesis" model in which bacterial effector activity relieves RIN12 association with RPM1 enabling activation of the RPM1 signalling network. Paradoxically, RIN12 also exhibits bacterial defensin activity, which – counterintuitatively – appears specific to bacteria that employ a type III secretion system. RIN12 defensin function is independent from the protease inhibitory activity. *RIN12* transcripts are induced by PAMPs. Collectively, these data suggest RIN12 is part of a secondary host PTI response to overcome ETS. We propose a model in which the ETI role of RIN12 evolved as a secondary function to the basal function of bacterial growth inhibition.

Nicola Spencer-Jones. IICB, Faculty of Biological Sciences, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT.

The effect of nematode infection on the abiotic stress response in *Arabidopsis.*

Plants respond to biotic and abiotic stress by activating signalling pathways leading to stress tolerance and resistance. Currently the effect of multiple simultaneous stresses on these pathways is largely unknown. This study investigates the effect of drought and nematode infection on stress signalling pathways. *Arabidopsis* plants infected with the nematode *H. schactii* were subjected to drought stress by withholding water, and RNA was isolated at points over a 10-day period. The expression of transcription factors in the drought response pathway was analysed by quantitative PCR and compared to that of uninfected plants. The results showed that when drought occurred the transcription factors ADR1 and RD26 were differentially expressed in nematode infected material in comparison to uninfected plants. This indicates that biotic stress may attenuate the normal response of plants to drought, suggesting significant overlap between biotic and abiotic signalling pathways.

Siân Deller, John Keon, John Antoniw, Kim Hammond-Kosack and Jason Rudd. Centre for Sustainable Pest and Disease Management, Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Functional analysis of reactive oxygen species – related genes in the wheat pathogen *Mycosphaerella graminicola*

Mycosphaerella graminicola (anamorph *Septoria tritici*) is a fungal pathogen of wheat leaves causing chlorotic / necrotic lesions that reduce the photosynthetically active leaf area. In the early stages of infection the fungus grows in the leaf intercellular spaces without causing visible disease symptoms. In the later infection stages fungal biomass increases, hyphal nutrition becomes necrotrophic and localised host programmed cell death occurs (1).

Reactive oxygen species (ROS)-specific stains have shown that hydrogen peroxide and superoxide are present in infected leaves, that levels increase with the appearance of disease symptoms (1, 3) and are closely associated with the fungal asexual fruiting bodies. Expression profiling of *M. graminicola* genes during infection of susceptible wheat genotypes has shown a number of ROS-associated genes that have greatly increased expression as disease symptoms become visible (1, 2, 3).

Candidate genes have been selected for functional analysis based either upon literature or their transcriptional up-regulation *in planta* during disease symptom formation. The sequenced genome of *M. graminicola* has enabled complete deletion of each gene separately through targeted *Agrobacterium*-mediated transformation. Results will be presented describing the functional characterisation of a superoxide dismutase, a peptide methionine sulfoxide reductase and a homologue of the *Yap1* transcription factor.

1. Keon *et al.* (2007) MPMI, **20,** 178-193
2. Keon *et al.* (2005) Fungal Genetics and Biology, **42,** 376-389
3. Keon *et al.* (2005) Molecular Plant Pathology, **6,** 527-540

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.

Amarnath Thirugnanasambandam[1], Adrian Newton[1], Steve Whisson[1], Kath Wright[1], Neil Havis[2] and Simon Atkins[3].
[1] *Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA.* [2] *Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG, UK.* [3] Rothamsted Research, Harpenden, Herts, AL5 2JQ

Role Of Seed-Borne Infection In Rhynchosporium And Ramularia Epidemics In Barley

Rhynchosporium secalis and *Ramularia collo-cygni* are important fungal pathogens of barley in the UK. *R. secalis* causes the disease 'Rhynchosporium' or 'scald' and *R. collo-cygni* causes Ramularia Leaf Spot (RLS) in barley. Rhynchosporium is the most damaging disease of barley necessitating 95% of crops being fungicide treated and causing ~£ 6.7M yield loss (2002). Ramularia also causes major yield loss (0.6 t/ha). *R.*

secalis can complete its life cycle within a host without producing any visible symptoms. Likewise *R. collo-cygni* usually produces symptoms during the heading stage but survives asymptomatically until then within the host. A greater understanding of the epidemiology of these pathogens, how they spread in symptomless phases of their life cycles, the role of seed-borne infection and subsequent proliferation and spread, will target resistance breeding, improve guidance to farmers for control measures and help optimizing fungicide application.
Visualization of these pathogens within the host during the asymptomatic phase while keeping them alive necessitates tagging them with fluorescent protein. An effective transformation protocol was developed using *Agrobacterium tumefaciens*. *Agrobacterium*-mediated transformation of *R. secalis* was performed and isolates were tagged with GFP and DsRed. These transformants were found to infect leaves and symptoms have been observed. Further isolates will be transformed using the same method. These transformed isolates will be visualized *in planta* using fluorescence microscopy including confocal microscopy which will yield a greater understanding of the spread of pathogen, significance of the asymptomatic phase, mechanisms that trigger the symptomatic phase (environmental or plant physiological) that are important in the development of visual symptoms.

Miles Armstrong, Eleanor Gilroy, Jorunn Bos, Sophien Kamoun, Juan Morales, Ingo Hein, Leighton Pritchard, Steve Whisson and Paul Birch.
SCRI and JIC

Virulence and avirulence in *Phytophthora infestans*

A combination of map based cloning and RXLR-EER translocation domain predictions, recently led to the identification of *Avr*2. When transiently expressed in R2 potato lines, one allele of *Avr*2 is recognised and causes a strong HR while the other does not. Interestingly, there is no correlation between the absence of the recognised allele in an isolate and virulence on R2. In addition, virulent and avirulent isolates appear to express *Avr*2 at similar levels. This has lead to the suggestion that an additional factor suppresses *Avr*2 HR induction in virulent isolates. This story is very different from *Avr*3a, where the occurrence of the AVR3a KI allele perfectly correlates with avirulence on R3a. In a survey of 51 isolates from the Tolluca valley in Mexico (a known centre of diversity for *P. infestans*), the AVR3a KI allele was found at lower frequencies compared to dominant AVR3a EM allele, even though the KI allele has been shown to have a virulence function in cell death suppression which is separate from its function in R3a activation and dependant on a tyrosine at position 147. A recent screening of wild potato species has identified lines that recognise both the KI and EM alleles of AVR3a and some that recognise the EM allele alone. This suggests that the EM allele must have a virulence function, that outweighs possible recognition, in order to account for its high frequency in populations. Screening of Y2H libraries with the KI and EM alleles of AVR3a has identified a number of potential interactors, some of which interact exclusively with the KI allele in a Tyr147 dependant manner. These are then candidate host targets for

AVR3aKI's involvement in cell death suppression, with the remainder more likely to be involved in R3a activation.

Ros Taylor, Miles Armstrong*, Petra Boevink*, Eleanor Gilroy*, Jorunn Bos+, Ratih, Sophien Kamoun+, Ari Sadanandom, Paul Birch*
Sadanandom Group, IBLS Division of Biochemistry, Bower Building, University of Glasgow, University Avenue, Glasgow, G12 8QQ, Scotland, UK
Birch Group, SCRI, Invergowrie, Dundee, DD2 5DA, Scotland, UK*
Karmoun Group, JIC, Norwich Research Park, Norwich NR4 7UH, UK+

Why does Avr3a Interact with StCMPG1?

CMPG1 is a U-box E3 ligase involved in plant defence. *Phytopthora infestans* effector AVR3a, possesses an RXLR motif shown to be required for delivery into the cytoplasm of host cells. There are two alleles of Avr3a: Avr3aEM and Avr3aKI. Avr3aKI is recognised by the potato R gene, R3a in the cytoplasm and suppresses cell death induced by the INF1 elicitin. A yeast two hybrid screen showed an interaction between CMPG1 and both Avr3a alleles. This talk will focus on work done to better understand this interaction. Firstly, transient expression assays in *Nicotiana benthamiana* of Avr3a and CMPG1 were carried out to look into the stability of these proteins. Secondly, confocal microscope analysis was used to investigate this interaction within the cell. Virus-induced gene silencing (VIGS) of CMPG1 in *N. benthamiana* has then been utilised to observe the role of CMPG1 during interactions with non-host bacterial pathogen, *Erwinia amylovora* and with virulent isolates of *P. infestans.* Lastly, ubiquitination assays carried out show an interesting role for Avr3a as part of plant ubiquitination. Future work will involve the conformation of these data as well as attempting to better understand the role of CMPG1 and it's interaction with Avr3a.

Edgar Huitema, Osman Bozkurt, Mireille vanDamme, Liliana Cano and Sophien Kamoun
The Sainsbury Laboratory, Colney Lane, NR4 7UH Norwich, UK

A large and complex gene family from *Phytophthora infestans* encodes a novel and diverse class of effector molecules involved in virulence

The plant pathogenic oomycete *Phytophthora infestans* forms intimate associations with its host, a feat that requires extensive reprogramming and suppression of defense responses. Perturbation of defense signaling is achieved by delivery of a suite of effector molecules that modify, mimic or eliminate host-signaling events during infection. The *crinkler* (*crn*) gene family encodes a large class of secreted proteins. Computational analyses unveiled a conserved N-terminal LxLFLAK motif, followed by diverse C-terminal domains with markedly different levels of conservation in related oomycete

species. Transient expression of these domains in plants result in cell death in some but not all cases, reflective of diverse functions and roles in virulence. To implicate the CRN protein family as intracellular effectors, we developed a novel and efficient transformation protocol for *P. capsici* and implemented reporter systems towards detection of effector translocation into host cells. Our preliminary work implicates the CRN protein family as a novel class of translocated and thus intracellular effectors. Corroboration of these results as well as further defining CRN translocation requirements forms part of our current objectives. These and further studies implicate the CRN protein family as an important and novel class of effectors in *Phytophthora* and provide novel insight into virulence strategies employed by Oomycetes.

Volkan Cevik[1], Rebecca Allen[1], Peijun Zhang[1], Sharon Hall[1], Mary Coates[1], Rachel Baumber[1], Laura Baxter[1], Laura Rose[2] and Jim Beynon[1]
[1]Warwick-HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK
[2]Section of Evolutionary Biology, University of Munich (LMU), Grosshadernerstr. 2, 82152 Martinsried, Germany

Genetic analysis of the *RPP13/ATR13* interaction complex between downy mildew and Arabidopsis

Elucidation of complex mechanisms driving host-pathogen co-evolution has been of great interest and presents a challenge. The studies involving the *Arabidopsis-Hyaloperonospora parasitica* pathosystem have contributed extensively to our understanding of the genetics of host-pathogen co-evolution. We have been studying a co-evolutionary model based on the *Arabidopsis RPP13* resistance gene with the highest reported level of sequence diversity among known R-genes, and the highly variable cognate effector gene *ATR13* from *H. parasitica*. Previously, sequence analysis of the *RPP13* gene resulted in numerous distinct clades, and the recognition of *ATR13* by *RPP13-Nd* has been attributed to the alleles in a single clade. In addition, ATR13 independent recognition of various *H. parasitica* isolates by an *RPP13* (Rld-2) allele in a different clade suggested the presence of novel ATR molecules. Furthermore, recognition of various alleles of *ATR13* by resistance genes other than the *RPP13* has been shown. Overall, this has revealed the presence of a complex co-evolution between *Arabidopsis* and *H. parasitica*. In order to provide an in-depth view of such a complex system, we are currently using map-based cloning approaches both in *Arabidopsis* and *H. parasitica* to identify and characterize the genetic components of the RPP13/ATR13 complex. Recent progress towards the cloning of these genes will be presented.

Angelique H. Riepsamen.
Biological Sciences, University of Wollongong, Wollongong NSW 2522, AUSTRALIA

The Unusual Mitochondrial Genetics of the Cyst-forming Nematodes

The cyst-forming nematodes, including the *Globodera* and *Heterodera* genera, contribute to millions of pounds in agricultural losses per annum through the parasitism and long-term infestation of host crops. Analysis of the mitochondrial DNA (mt-DNA) of this family of nematodes has revealed several unique characteristics uncommon to other animal mt-DNA. For example, in contrast to typical animal mt-DNA which consists as a large, single, circular DNA molecule, *Globodera* mt-DNA consists of at least 6 mini-circle DNA molecules, with many of the genes having discrete length variations at homopolymer tracts, such that non-functional protein products would be translated. Further, contrasting with typical animal mt-DNA, nucleotide sequence analysis indicates that these minicircles were the products of recombining mt-DNA molecules, a process considered rare if not absent in animal mt-DNA. Preliminary results suggest that while this unique genome organisation may be restricted to the *Globodera* genus, the presence of homopolymer length variation has been observed throughout the cyst nematode family. Further analysis is required to assess whether there is any correlation between unique mt-DNA features and evolution of host-parasite interactions in the cyst-forming nematodes.

Joel J. Milner[1], Andrew J. Love[1], Chiara Geri[1,2], Janet Laird[1], Byung Wook Yun[3], Gary Loake[3], Ari Sadanandom[1] &, [1]Plant Science Group, IBLS, University of Glasgow, Glasgow G12 8QQ, UK; [2]CNR, IBBA, Pisa, Italy and [3]Institute of Molecular Plant Science, University of Edinburgh, Edinburgh EH9 3JH, UK.

An Effector Protein Encoded by Cauliflower Mosaic Virus Inhibits SA-Dependent Defence Responses in Arabidopsis and suppresses innate immunity

CaMV infection stimulates SA-dependent responses in pre-invasion tissues, but these are down-regulated in tissues undergoing invasion. We show that protein P6, the main virus pathogenicity determinant, suppresses SA-mediated defence. Transgene-mediated expression of P6 in Arabidopsis and transient expression in *N. benthamiana* profoundly inhibits the expression of SA-responsive- but enhances expression of JA-responsive markers. by interfering with signaling downstream of SA. P6-transgenic plants show a greatly increased susceptibility to virulent, avirulent but not *hrpA* mutants of *Pseudomonas syringae*, and development of HR is inhibited. Conversely, the transgenics show greatly reduced susceptibility to the necrotrophic pathogen *Botrytis cinerea*. Expression of P6 enhances the accumulation of *NPR1* transcript- and NPR1 protein levels. Crossing *35S::P6* transgene into a line expressing an NPR1:GFP fusion protein, results in GFP expression confined to the nucleus even in the absence of SA, suggesting that P6 acts by inducing the mis-localization of NPR1.

Kozlakidis, Z.[1], Brown, N. A.[1], Jamal, A.[1], Phoon, X.[1], Quicke, D.L.J.[2,3], Coutts, R.H.A.[1]
[1] Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, Sir Alexander Fleming Building, Imperial College Road, London SW7 2AZ, UK
[2] Centre for Population Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK
[3] Natural History Museum, London SW7 5BD, UK

Phylogeography and genetic diversity of *Phytophthora* species endornaviruses.

The incidence of dsRNA endornaviruses in *Phytophthora* taxon douglasfir isolates originating from different plants on the east and west coasts of USA and *Phytophthora ramorum* isolates originating from various plants in the UK, USA and Europe was determined. DsRNA isolation from mycelia and RT-PCR amplification of genomic sequences characteristic for phytophthora endornavirus 1 (PEV1), which included conserved motifs for an RNA helicase, a putative UDP glycosyltransferase and an RNA-dependent RNA polymerase gene, revealed that all of the *Phytophthora* taxon douglasfir isolates and 20% of the thirty five *Phytophthora ramorum* isolates contained an endornavirus. Sequence analysis of the amplicons generated by RT-PCR and phylogenetic analyses showed that while the *Phytophthora* taxon douglasfir endornavirus isolates from the east and west coasts of USA could be separated cladistically from most, but not all of the *Phytophthora ramorum* isolates, intra-species genetic diversity was low over the three genetic regions. Since all of the *P. ramorum* isolates that contained an endornavirus were isolated and the same site this is suggestive of a clonal lineage and a high probability of horizontal transmission. This is also the first report of the incidence of endornaviruses in *Phytophthora ramorum*.

James Lord, Peter Urwin, Howard Atkinson
Plant Nematology Lab, Centre for Plant Sciences, University of Leeds

Biofumigation for control of potato cyst nematodes and *Rhizoctonia solani*

Biofumigation is the control of soil-borne pests by incorporating green manures of plants that produce volatile toxic isothiocyanates. The potential of this practice to at least partially replace environmentally destructive pesticides is both unknown and yet to be realised. This project aims to resolve the factors influencing the efficacy of biofumigation for control of two important pathogens of potato in the UK, the potato cyst nematode *Globodera pallida* and the fungus *Rhizoctonia solani*, causal agent of stem canker and black scurf.
In vitro assays were used to identify isothiocyanates and plants with toxicity to *G. pallida* and *R. solani*. Macerated tissues of some of the most promising biofumigant plants were then used to amend *R. solani*-infected soil and the degree to which the plant tissues reduced the level of fungal inoculum was determined by quantifying *R. solani* DNA using TaqMan PCR.

The most toxic compounds to *G. pallida* were benzyl, phenethyl, 2-propenyl, and 3-(methylthio)propyl isothiocyanate, which had similar ED_{50} values, ranging from 11 to 20 μM. The plants causing the greatest suppression were *Raphanus sativus*, *Nasturtium officinale* and *Brassica juncea*, the principal isothiocyanates of which are 4-methyl-3-butenyl, phenethyl and 2-propenyl isothiocyanate, respectively. These plants caused over 90% suppression of *G. pallida* second stage juveniles at concentrations that could feasibly be attained in the field. Treatment of *R. solani* infected soil with brassica green manures at 5% (w/w) had little effect on the density of fungal inoculum as quantified by TaqMan but disease severity on subsequently planted potatoes was reduced. Disease suppression bore no relation, however, to the isothiocyanate production of the brassica tissues. Possible mechanisms of action and future work will be discussed.

Maeve Price and Sarah Gurr
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SSW1 encodes a glycolipid-anchored surface protein and is a virulence determinant in *Magnaporthe grisea*

Knockout of a putative glycolipid-anchored surface gene, SSW1 in the rice blast fungus *Magnaporthe grisea*, via a split marker approach, gave rise to a mutant strain compromised in its ability to produce appressoria and penetration pegs. SSW1 transcript levels are highly elevated in the conidia as compared with other stages of early germling differentiation and penetration. Various assays, including atomic force microscopy, show that the *Δssw1* spore is "stiffer", more hydrophobic and less susceptible to degradation by cell wall degrading enzymes. The current hypothesis is that SSW1, a homologue of GAS1 gene, required for pathogenesis in *Candida albicans* and *Fusarium oxysporum* (Saporito-Irwin et al. 1994; Caracuel et al. 2005), and for maintenance of cell shape in *Saccaromyces cerevisiae* (Popolo and Vai 1999), plays a role in cell wall biosynthesis and/or morphogenesis and perhaps perception of host-derived signals required to drive germling differentiation.

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NO: a nitric oxide synthase generated effector of pathogenesis in *Magnaporthe grisea*

M. grisea carries 4 putative nitric oxide synthase (NOS) genes in its genome. One, *NOS3*, shows highly elevated transcript levels at the mature appressorium stage of fungal morphogenesis. The *Δnos3* mutant shows greatly attenuated disease levels in rice and barley, as compared with the wild-type and complemented strains.

Exogenously applied NO scavengers and inhibitors reduce the WT to *Δnos3* mutant phenotype. Conversely, NO donors restore the mutant to wild-type phenotype (and pathogenicity). The results will be discussed in the context of our current quest to image and quantify the NO burst, to place it in known signalling cascade(s) and to elucidate the role of NO in this model Ascomycete.

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Fungicide resistance in *Rhynchosporium secalis*

Barley leaf blotch or scald, caused by the fungus *Rhynchosporium secalis*, is the most economically damaging foliar disease of barley in the UK, and fungicides are a major component of control programmes for this disease. However, fungicide use can exert a strong selective pressure for the development of fungicide resistance. Resistance has already rendered the MBC fungicides ineffective against *R. secalis*, and a reduction in sensitivity to some triazole fungicides has been found in the field. Strobilurin resistance has been reported in many pathogens, but not yet in *R. secalis*.

Understanding the genetic basis of fungicide resistance enables improved detection through molecular diagnostics, facilitating a better understanding of the spread and management of resistance. This project aims to identify genetic changes responsible for reduced fungicide sensitivity in *R. secalis*, and to study their occurrence and spread in populations.

A fungicide sensitivity bioassay has been developed for *R. secalis*. This has revealed a hundred-fold reduction in sensitivity to some triazoles over the last 10-15 years, and the molecular basis of this reduction is now being investigated, initially looking for target-site mutations.