Plant Pathology

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Research in the Plant Pathology programme is focused on economically important diseases and pests of potato, barley and berry fruit. Our research extends from studies on pathogen genes and disease processes at the level of molecules and cells to the epidemiology and evolution of pathogen populations infecting field crops. The knowledge gained is used to underpin sustainable disease control methods by aiding the development of resistant plants and integrated management strategies.

Changes in environmental conditions, for example, changing temperatures, rainfall and CO_2 levels, may influence the incidence and severity of existing pests and diseases or bring new threats to Scottish crops. We have re-focused some of our efforts to investigate this. To this end a major new grant has been secured from RERAD by Dr Ian Toth and colleagues to study the potential threat to Scottish seed potatoes from the bacterium *Dickeya dianthicola* which infects potatoes in the warmer conditions of Southern Europe.

This year, research on the barley pathogen *Rhynchosporium secalis* has been strengthened by the appointment in Plant Pathology of Dr Anna Avrova to study pathogenicity effectors and Dr Mark Looseley in the Genetics programme to study barley resistance genes. Their research will be integrated with the ongoing epidemiology work with the major aim of developing durable resistance to one of the most destructive pathogens of barley. Bioinformatics research has also been strengthened with the appointment of Peter Cock.

Globodera pallida genomics (John Jones & Vivian Blok)
The sequencing project for *Globodera pallida* is now in progress. This is a joint project between SCRI, Rothamsted Research, The Wellcome Trust Sanger Institute and Leeds University. Genomic libraries have been produced and sequencing from these libraries is underway. Over the next few months a draft sequence will be completed along with extensive RNA sequencing using the Solexa Illumina platform. This transcriptome analysis will assist gene finding and annotation as well as providing quantitative information on the genes expressed in each life stage. We are also using a range of techniques to analyse the function of important

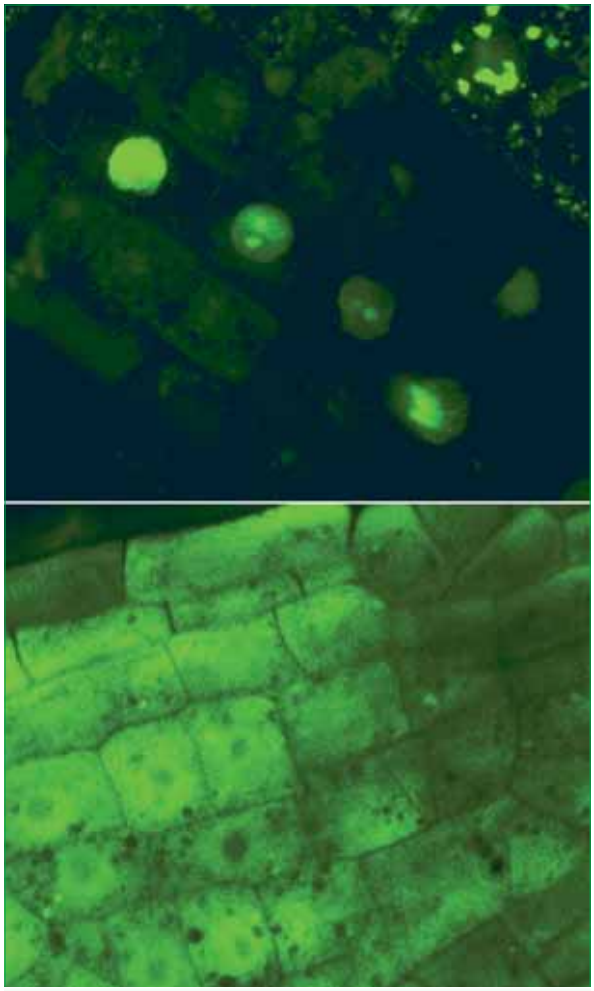

Figure 1 Different members of a family of nematode secreted proteins (SPRYSECs) localise to the nucleus/nucleolus (upper panel) or the cytoplasm (lower panel).

nematode secreted proteins that may play important roles in the plant–nematode interaction. In collaboration with colleagues at the French National Institute for Agricultural Research (INRA) Rennes we have identified a very large family of novel proteins (SPRYSECs) from *G. pallida*. We have examined the subcellular localisation of several members of this gene family and have found that while some remain in the cytoplasm others are targeted to the nucleus (Fig. 1).

Programmed cell death and its role in plant defence

(Michael Taliansky) Animals and plants exploit programmed cell death (PCD) as a means to eliminate redundant and damaged cells during their development and in response to various stresses. Although representatives of both kingdoms share several morphological features of PCD, plants lack homologues of caspases – a family of highly specific cysteine-dependent proteases that are critically involved in animal PCD. In collaboration with the Moscow State University team (Professor Andrey Vartapetian) we have identified and characterised a subtilisin-like plant protease (SLPP) that, being structurally distinct from animal caspases, appears to be a functional caspase analogue. In contrast to classical caspases which are Cys-dependent proteases, subtilisin-like proteases are serine-dependent (Ser-dependent). Using mutational analysis we have confirmed that SLPP is a real subtilisin-like protease. We next addressed a role of SLPP in plant cell death and development. For this purpose we constructed transgenic *Nicotiniana tabacum* Samsun NN plants either over-expressing tobacco SLPP or possessing a markedly decreased level of SLPP activity due to RNA interference (RNAi). Using these transgenic plants we have shown that the newly identified enzyme is essential for PCD activation in response to biotic (*Tobacco mosaic virus*) or abiotic (NaCl and methyl viologen) stresses.

To learn whether homologous enzymes that are responsible for this activity exist in other plant species/crops, we have now purified SLPP activities from potato and barley. Variable amounts of activity have been detected in different *Solanum tuberosum* isolates but only low levels of activity were detected in two *Solanum tuberosum* group *phureja* isolates. Variable amounts of SLPP activity were also detected in different barley cultivars with a transient elevation in activity being observed in plants treated with defence elicitors and infected with *Rhynchosporium secalis*. SLPP activity thus seems to play a role in the defence mechanism of plants against pathogen infection. This also applies to infection of plants with *Agrobacterium tumefaciens* where the agrobacteria VirD2 protein is a substrate for SLPP activity. Mutation of the VirD2 protein to remove SLPP cleavage sites improves the efficiency of gene transfer into a wide range of plant species by agrobacteria, a finding that has commercial significance.

Potato mop-top virus long distance movement

(Lesley Torrance & Eugene Savenkov) Previously it was shown that *Potato mop-top virus* (PMTV) does not infect all parts of the potato plant and that plants grown

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from infected tubers can 'escape' infection. This work was based mainly on the detection of the virus capsid protein. The PMTV genome encodes replicase, movement and capsid proteins on three different RNA molecules (RNA-R, RNA-M and RNA-CP respectively). Studies on the movement and systemic spread of the different PMTV RNAs have found that RNA-R and RNA-M can move to upper leaves independently of RNA-CP. More detailed studies of mutant RNA-CP infectious clones showed that systemic movement of RNA-CP is regulated by the capsid proteins but the movement of the other two RNAs is unaffected. It is most probable that RNA-CP free infections are 'dead ends' since RNA-CP is required for virion formation and vector transmission to new hosts. However, our results help to explain the earlier observations of uneven virus distribution and it is possible that the potato plants thought to have 'escaped' infection were actually carrying RNA-R and RNA-M.

iLOV, a new tool to study cellular processes

(Sean Chapman & Alison Roberts) Green fluorescent protein (GFP) and similar proteins have been used widely to study protein localisations and dynamics in living cells, and as reporters of virus infection and spread. However, currently used GFP-like proteins are large which limits their use. For instance, viruses expressing FP markers often move poorly between cells and fail to move systemically through plants. In addition, fusion of large FPs to proteins of interest can disrupt their function. In collaboration with Dr John Christie (Glasgow University) and Dr Karl Oparka (Edinburgh University) we have engineered a smaller (~10 kDa) alternative to GFP (~27 kDa) based on a flavin-binding motif derived from the light, oxygen, or voltage-sensing (LOV) domain of the plant blue-light receptor, phototropin. The procedure of DNA shuffling (molecular evolution) was combined with high-throughput, *Tobacco mosaic virus*-based screening to identify LOV derivatives with improved protein stability, optimised *in planta* fluorescence, decreased susceptibility to photo-bleaching and reduced bleaching recovery times. The resulting small molecule, termed iLOV, functions well as a genetically-encoded fluorescent reporter to track

fusion protein trafficking, and can be effectively targeted to label cellular structures such as the nucleus and Golgi apparatus in plant cells. The small size of the iLOV gene improves retention of the reporter gene by viruses and disrupts viral infection processes less. iLOV was expressed either as a free protein or as a fusion to a viral protein from both *Tobacco mosaic virus* and *Potato mop-top virus* and functioned as a superior reporter to GFP, allowing more natural cell–cell movement rates and systemic infections for both viruses. Further expression studies confirmed that iLOV also functions well as a fluorescent marker in animal and bacterial cells.

Phytophthora effectors (Steve Whisson & Paul Birch) Pathogen effector proteins play a key role in establishing infection and as such are often 'sensed' by the plant as a signal of invasion by a pathogen, triggering defence responses. There is currently an international focus on the effector proteins of *P. infestans* and other oomycete plant pathogens, to understand how effectors are translocated inside host cells, what host plant proteins or processes are being targeted, and which effectors act as avirulence proteins to trigger resistance in diverse host germplasm. We have shown that the RXLR-EER translocation motif is functionally interchangeable with the RXLXE/D/Q translocation motif from the virulence proteins from malaria parasites. Similarly, the RXLR or RXLR-EER motifs from avirulence proteins from the *Arabidopsis* downy mildew pathogen are functionally interchangeable with the translocation motifs from *P. infestans* Avr3a. This suggests that there may either be an ancient conserved mechanism for translocation of effectors inside host cells, or an evolutionary convergent solution from different groups of pathogens to this common problem.

The effector protein Avr3a was used to screen a yeast two-hybrid library of potato genes to identify interaction proteins that may represent the virulence target(s) of Avr3a. This revealed that Avr3a interacts with the potato CMPG1 protein, a ubiquitin E3 ligase. In this system, Avr3a functions as an E2 ubiquitin conjugating enzyme. Ubiquitination is a signalling process found in all cells, and reprogramming or inhibiting it can drastically alter cell fate.

Dr Alison Roberts, Cell Biology and Imaging.

Using a positional cloning strategy, the *Avr2* avirulence gene, matching the *R2* resistance gene in potato, was isolated from *P. infestans*. It also contains the dual RXLR-EER motifs. Interestingly, it is able to trigger a resistance response mediated by *R2*-like resistance genes in a broad range of *Solanum* species.

Monitoring the late blight pathogen (David Cooke & Alison Lees) Marked changes in the populations of the late blight pathogen *P. infestans* have been recorded over the past two seasons in a Potato Council funded survey of GB potato crops. Over the 2006, 2007 and 2008 seasons we have tested the mating type of over 3500 *P. infestans* isolates from almost 700 late blight outbreaks. On average, the A2 mating type was found in 74% of outbreaks with both mating types found together in almost 20% of outbreaks. The risks of sexual oospore formation are thus high. Such oospores may act as an additional source of primary inoculum and generate a more diverse and adaptable pathogen population. The threats of this dramatic shift have been investigated further by genetic fingerprinting using Simple Sequence Repeat (SSR) markers. We have shown that the population is made up of relatively few clonal lineages and is dominated by a single lineage of the A2 mating type known as genotype 13_A2. This single clone comprised over 70% of the pathogen populations sampled in 2007. Aggressiveness tests using a range of *P. infestans* genotypes against five commonly grown potato cultivars demonstrated that genotype 13_A2 is particularly aggressive, especially at lower temperatures. These results and their implications have been widely reported to the industry.

To further understand the impact of this new pathogen population on disease management and inform future breeding plans we have also re-evaluated the resistance ratings of a range of commercial cultivars and breeding material. As lead researchers in the Eucablight project (www.eucablight.org) we have also been responsible for coordinating the inclusion of information into a database of European isolate data. In 2007 the database was extended to allow the collection of *P. infestans* data from Central and South American countries.

Assays for soil borne pathogens (Alison Lees & Vivian Blok) Soil borne potato tuber blemish diseases including black dot (*Colletotrichum coccodes), Rhizoctonia solani* and powdery scab (*Spongospora subterranea*) affect the quality of seed and ware crops in the UK and worldwide. We have improved DNA extraction methods and sampling strategies so that a molecular test (based on real time PCR) can be used for the rapid and accurate quantification of potato pathogen DNA in plants, tubers and soils. Diagnostic tests are used to understand the relative importance of different sources of inoculum and the environmental factors influencing infection and disease development, with the aim of developing effective control strategies. In collaboration with SAC Aberdeen, commercial diagnostic tests along with the information needed to interpret the results and implement integrated control strategies have been delivered to growers.

The new EU PCN (potato cyst nematode) directive which comes into force in 2010 will require many more soil samples to be processed than before. Statutory testing is conducted by SASA – Science & Advice for Scottish Agriculture and is based on microscopic examination of samples which is labour intensive and not suited to processing very large numbers. Therefore, we jointly developed a quantitative molecular diagnostic to assay soil samples for PCN as an alternative. Excellent agreement in terms of sensitivity and accuracy was obtained in comparative tests and the assay is currently being adapted to a robotic platform for the high throughput of samples.

Enterobacteria in the environment (Nicola Holden & Ian Toth) The Enterobacteria are a large family of bacteria that can persist in many environments and are found in association with hosts from every biological kingdom. The outcomes of their interactions vary from beneficial through to commensal or pathogenic and include many devastating human, animal and plant diseases. Our research centres on bacteria–host interactions that occur naturally in the environment. We have shown that human pathogenic enterobacteria are able to colonise leafy vegetables and arable crops without causing obvious disease symptoms. We have also found plant pathogens that normally cause disease on potatoes are able to colonise other plants in an asymptomatic manner. This shows that differences in the host species can result in different outcomes from the bacterial infection. The recent increase in genomic information has led to a better understanding of these bacteria and has opened the way for research on hitherto unknown aspects of their lifestyle. Research is ongoing into the mechanisms of adherence during the early stages of plant colonisation by both human pathogenic and plant pathogenic enterobacteria. The objective is to identify adherence factors that are required for specific bacteria–host cell interactions. Further work on both groups of bacteria aims to understand the regulatory processes during different stages of infection. This area has progressed considerably over recent time for plant pathogenic enterobacteria and has revealed a complex network that is sensitive to the particular stage of infection as well as a number of environmental cues. The area is at an early stage for human pathogenic enterobacteria. An overarching goal that draws all aspects of the work together is to find the parallels between both groups of bacteria that facilitate colonisation of plants.