Pathology

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The broad directions of work within the former Departments of Nematology, Virology, and Fungal and Bacterial Plant Pathology, have been described in previous Annual Reports. Although most of the elements within the three departments have now been merged into the Unit of Mycology, Bacteriology and Nematology, and the Unit of Virology, the themes remain the same. These are (1) to understand at the molecular level the biology of the pathogens and their interaction with their hosts (and vectors), especially with regard to host resistance, (2) to delimit the extent of pathogenic variation, integrating the results into wider epidemiological studies, and (3) to exploit those results in the production of rapid, sensitive and specific diagnostics, and, wherever possible, control strategies. Many of the fundamental questions are similar for each group of pathogens, although the approaches employed to study them must be affected by their different biology. A principal goal of the new Units is to integrate the different experience and knowledge of staff gained about bacteria, fungi, nematodes, or viruses into teams that can tackle important questions about host specificity, host resistance and evasion of host defence mechanisms, which are common to plant pathogens.

Diagnostics and epidemiology The standardisation and harmonisation of pathogen tests in Europe is an important issue in terms of open borders and free trade. Work is progressing to assist this important goal. The diagnostics developed for the potato tuber blemish pathogens (Ann. Rep. 1998/99, 136-138) have been extended for use with real time quantitative PCR (Taqman™), thereby making possible the rapid processing of large numbers of samples and the development of PCR diagnostics (PCRDs) for fungal tuber rots in store. All of the above PCRDs are based on the internal transcribed spacer (ITS) regions of rDNA.

Other PCRDs have been developed for distinguishing *Meloidogyne chitwoodi* and *M. fallax* from other root knot nematodes. *M. chitwoodi* and *M. fallax* are important pathogens of potato that are present in the USA and the Netherlands, from where they have spread to several other EU countries but not yet the UK.

Recent surveys in Scotland, in the Nordic countries and in Poland have shown that only about 60% of isolates of *Erwinia carotovora* subsp. *atroseptica (Eca)*, the causal agent of potato blackleg disease, belong to

serogroup I, vs. >90%, as previously believed. This has led to extensive studies on the population structure of *Eca* using AFLP and phage sensitivities. Different strains can now be tracked in the farm environment and sources of contamination identified. Closely linked is the development of new PCRDs for *Erwinia*: one for detecting all *Erwinia* spp. in potato tissue cultures and one specifically for *Eca*. The latter can quantify the number of *Eca* present in samples of potato peel. The gene for green fluorescent protein (GFP) has also been introduced into *Eca* and has been used to trace the movement of the bacterium in and on potato plants in the laboratory (Fig. 1). Early results have emphasised the close similarity of *Eca* in its aetiology to other vascular wilt pathogens. A spin-off of the phage typing of *Eca* is the development of phage typing kits for strain determination in clinical bacteria, especially for the very important pathogen *Escherichia coli* O157.

The interactive, PC-based, integrated control model for the white potato cyst nematode (PCN; *Globodera pallida*), mentioned in last year's Annual Report, has been shown to be very sensitive to small variations in the effectiveness of nematicides and PCN decline rates during the growing of rotational crops. In addition, the model has demonstrated the need for major changes in the protocols for sampling of PCN, including sampling post-harvest as well as pre-planting.

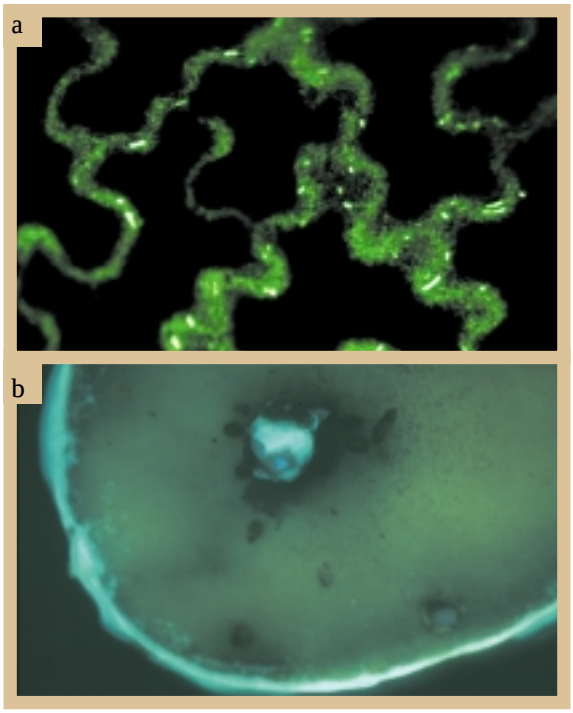

Figure 1 Fluorescence produced after infection of potato by *Eca* expressing the GFP. The bacteria (fluorescence) are seen in the intercellular spaces of an infected leaf (a) and concentrated under the epidermis and centre of pith in a potato tuber (b).

A detection method has been developed for *Tobacco rattle virus* (TRV) RNA by RT-PCR in extracts from potato tubers without interference of PCR inhibitors. TRV was shown to be distributed uniformly throughout the tubers of symptomlessly infected potato cv. Wilja. Tests on seedlings grown from botanical seed produced on Wilja plants, systemically infected with TRV, produced no evidence for seed transmission.

Resistance and control Since most of the work in Pathology is focused on potato as a host, wild *Solanum* species have been and will continue to be valuable sources of resistance to all potato pests and diseases.

In a study of *Solanum* mainly from Mexico, Bolivia, Argentina and Peru, 40 outbreeding and 28 inbreeding accessions were found to be uniformly resistant to the PCN *G. pallida* (races Pa 2/3) with a further 17 segregating for resistance. Eleven of these accessions which together represent 34 different species of wild potato, are new sources of resistance to *G. pallida.*

High levels of resistance to fungal and bacterial diseases (powdery scab, dry rot, blackleg and soft rot) have also been identified in clones of a long-day-adapted *Solanum tuberosum* group Phureja population. Exploitable levels of resistance to these diseases are often absent in *S. tuberosum* group Tuberosum. Selected clones of *S. tuberosum* group Phureja have been hybridised with each other, and with *S. tuberosum* group Tuberosum germplasm.

Recently, PLRV resistance has been assessed in various diploid species of potato including *S. raphanifolium* and *S. tuberosum* group Phureja. Some accessions of *S. tuberosum* group Phureja contained genotypes that had very strong resistance to PLRV accumulation, whereas other genotypes in the same accession were susceptible to PLRV. When resistance tests were made on *S. raphanifolium*, a proportion of genotypes in some accessions remained virus free after graft inoculation.

Molecular biology of pests, pathogens and host-pathogen interactions In collaboration with Dutch partners, one thousand ESTs from second stage juveniles of *G. rostochiensis* have been sequenced. *In situ* hybridisation and other techniques have been used to

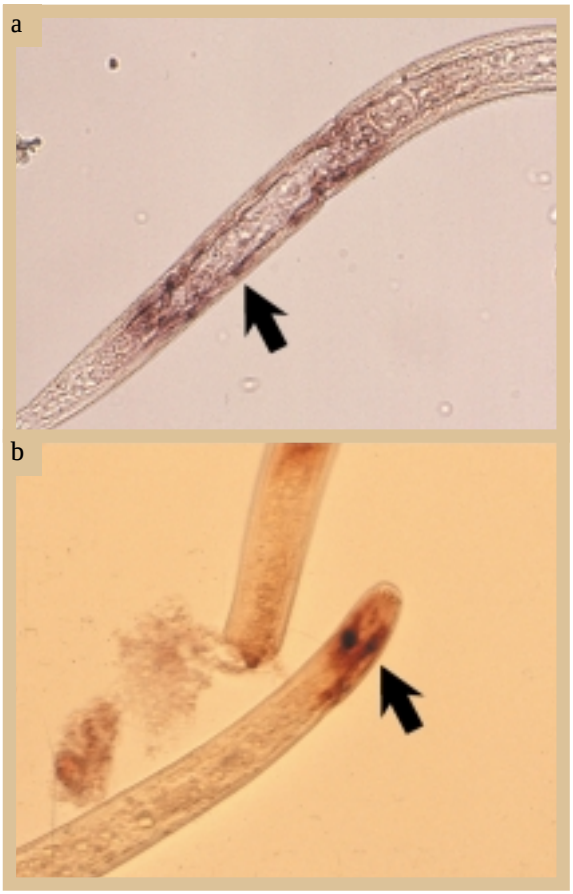

Figure 2 *In situ* hybridisation of digoxigenin labelled probe to fragments of *Globodera rostochiensis* second stage juveniles. Genes encoding secreted proteins expressed in the hypodermis (a) and the amphids (b) are shown. A positive reaction is indicated by a purple/brown deposit (arrows).

characterise some of their functions. (Fig. 2). Antioxidant proteins have been identified in secretions of *Globodera rostochiensis*. A secreted pectate lyase has also been identified in *G. rostochiensis* – the first gene of this type from any animal and the first demonstration of non-symbiotic degradation of plant cell walls by any animal.

The Ty3-*gypsy* group of retrotransposons has been found in *Globodera* and *Meloidogyne*; a first report for plant pathogenic nematodes. Sequence and phylogenetic analyses have shown that the reverse transcriptase sequences are different between the genera but highly homogeneous within each individual species.

Large insert DNA Bacterial Artificial Chromosome libraries have been generated from *E. carotovora* subsp. *carotovora*, and *P. infestans*. The *P. infestans* library is being used for map-based cloning of avirulence genes (*i.e.* genes that elicit the hypersensitive resistance response in potato). The *Erwinia* genomes are being reconstructed *in vitro*, for comparison with the closely related *E. coli*, in the search for novel sequences implicated in pathogenicity and host-range. An entire '*hrp*' cluster, of the type implicated in pathogenicity in other plant pathogenic bacteria, has been discovered in *Eca*.

The technique of suppression-subtraction hybridisation (SSH) has been used in the isolation of plant response genes activated by both *Eca* and *P. infestans* (Ann. Rep. 1998/99, 133-135). Signalling pathways distinct to the hypersensitive response, and to general elicitor-based activation, have emerged. The SSH system has also allowed *P. infestans* genes expressed specifically during early stages of late-blight infection to be isolated.

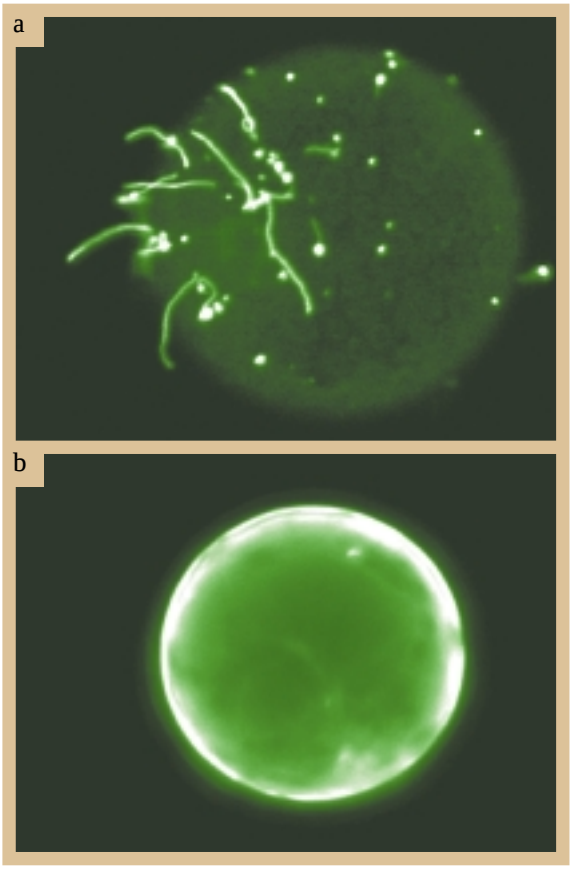

Figure 3 Confocal microscopy showing the presence of fluorescence due to the presence of CMV 3a-GFP. Wild-type 3a-GFP forms tubules on the surface of infected tobacco protoplasts (a). A 3a movement protein mutant fused to GFP does not form tubules, but rather shows fluorescence limited to the surface of the infected protoplast (b).

Tobacco plants transformed with a full-length copy of the genome of PLRV, contain very few infected cells (Ann. Rep. 1998/99, 142-145). When such plants were crossed with transgenic plants expressing the P1/HC-Pro genes from *Tobacco etch virus* (TEV), progeny plants contained about 10-fold more virus than those expressing the PLRV genome alone, and the number of infected cells increased substantially. HC-Pro produced by potyviruses such as TEV are known to suppress post-transcriptional gene silencing. This result suggests that the presence of the P1/HC-Pro genes from TEV may suppress some, but not all post-transcriptional gene silencing that occurs in transgenic plants containing the full length PLRV genome.

Bean yellow vein banding virus (BYVBV) is an umbravirus, isolated from faba beans in England, and reported to use the luteovirus *Pea enation mosaic virus* 1 (PEMV1) as its helper for aphid transmission. The nucleotide sequence of a PCR amplified region of BYVBV ORF3/4 was 97% identical to that of the equivalent region of the unbravirus PEMV2; the predicted sequences of the BYVBV ORF3 and ORF4 proteins were 93% and 97% identical, respectively, to those of the PEMV2 proteins. Thus, while BYVBV is a strain of PEMV2, natural infections of PEMV2 independent of PEMV1 have not previously been reported.

Various aspects of the interactions between *Cucumber mosaic virus* (CMV) and its hosts have been examined. CMV was shown to be able to form tubules protruding from the surface of infected protoplasts (Fig. 3a). These tubules were formed by the 30 kDa movement protein (MP) of CMV, and were observed if the MP was fused to the GFP. These types of tubules have been seen before with other viruses, and it was believed that they might provide a role in cell-to-cell movement. A MP mutant of CMV was generated that could not promote movement between epidermal cells, but could promote movement within infected plants, between other cell types. Tubules were not generated on the surface of infected mesophyll protoplasts from this mutant MP fused to GFP (Fig. 3b), indicating that tubule formation is not essential for movement of CMV between mesophyll cells.

Specific articles follow concerning aspects of the increasing importance of recombinant antibodies in diagnostics, nematode secretions and PCN pathogenesis, the population diversity of *P. infestans*, the development of PLRV-GFP, and the long-distance movement and transmission of plant viruses.