Host pathogen interactions and crop protection

Peter Palukaitis, James M. Duncan & David L. Trudgill

Plant pathology continues to play an important role in the interactions between plants and their environment, and in developing strategies for disease management. The focus of plant pathology research at SCRI continues to encompass the four broad areas of diagnosis, epidemiology, control, and plant-pathogen interactions, at the population, organismal, cellular and molecular levels. Very exciting progress has been made over the last year in a number of areas. These will be described in this section. Some of those achievements will be described in detail in selected articles, while other results and conclusions will be summarised below.

Diagnosis and epidemiology The investigation of the main sources of contamination of potatoes by *Erwinia carotovora* subsp. *atroseptica* (*Eca*) in seed and ware production, using a variety of molecular fingerprinting techniques, has been funded jointly by SOAEFD and the BPC, as a new FF project. Preliminary results show that particular isolates out of mixtures of strains soon predominated on tubers. Whether this is due to their saprotrophic or pathogenic abilities, remains to be determined. The project relies strongly on both a quantitative PCR test for *Eca*, which can detect fewer than 100 bacteria, as well as a simple and rapid extraction procedure for DNA. Such recently developed procedures allow the ready detection of plant pathogens in soil. Thus, over the last year, PCR tests have been developed for the blemish diseases of potato: silver scurf (*Helminthosporium solani*), black dot (*Colletotrichum coccodes*) and common scab (*Streptomyces scabies*). These tests can

detect c. 3 spores of each pathogen per gram of soil. Other tests include a competitive PCR for use with soil and tubers for powdery scab of potatoes (*Spongospora subterranea*), as well as several formats for PCR detection of strawberry red core (as part of the EU project REDCORE). More details on the detection of potato pathogens are contained in the specific article by D. Cullen and K. Bell.

The EU-REDCORE programme has benefited from the completion of a molecular phylogeny for *Phytophthora* based on the sequence from the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA). This valuable database, which now includes data on c. 300 isolates representing 56 species, has been used to confirm new species such as *P. quercina*, and to spot natural hybrids with potentially new host ranges, such as the *Phytophthora* spp. causing alder decline throughout Europe. This has attracted considerable interest and commercial contracts have been awarded already for the routine identification of unknown isolates using the database, as well as for characterisation of important collections of tropical species, with the results to go on the World Wide Web for use by tropical plant pathologists.

Molecular and biological analyses indicate that *Globodera pallida* (the white potato cyst nematode – PCN) is more variable in the UK, and consequently, will be much more difficult to control with resistant cultivars than *G. rostochiensis* (yellow PCN), which it has replaced. Populations of *G. pallida* most commonly contain a wide spectrum of virulence genes, so that none of the resistance genes being used by plant breeders is completely effective. Virulence genes that occur at even a low frequency will be very numerous, since the populations of PCN are immense – 10 eggs of PCN per gram of soil is equivalent to 30,000 million PCN per ha.

Parthenogenesis is frequent in soil nematodes, complicating the delimitation of species in groups, such as *Xiphinema americanum*, species of which transmit several plant viruses. An RFLP analysis of DNA from populations sampled around the world revealed at least 11 groups, confirming the distinctness of some species. However, even within recognised species clusters, substantial morphological variation was evident. It was confirmed that *X. americanum, sensu stricto* is restricted to the eastern USA, but other species were more widely distributed, e.g., populations from Crimea, South Africa, Slovakia, and the Moscow region of Russia formed a cluster identified as *X. taylori*.

In the UK, trichodorid nematodes transmitting *Tobacco rattle virus* (TRV), causing spraing in tubers, are an increasing problem, but because the incidence of spraing cannot be predicted, nematicides are widely applied as a precautionary measure. To improve detection, the ITS of the rDNA was analysed and shown to provide the basis for the rapid identification of trichodorid species. To detect TRV in the nematodes, RT-PCR has been tested and shown to be a highly sensitive method, detecting TRV even when as little as 1% of the population is viruliferous.

Chlorogenic acid, at concentrations that might realistically be found in potato tuber extracts, was found to be a potent inhibitor of PCR. Chlorogenic acid is known to be distributed unevenly in tubers and the concentration may be increased on infection. Therefore, previous reports on the erratic distribution of TRV in infected tubers, based on RT-PCR results, may, in part, be due to the uneven distribution of inhibitor(s).

A screen of 13 potato cultivars showed the presence of TRV in leaves and/or roots of plants from 11 cultivars. Moreover, virus was detected in tubers of eight cultivars, although only two (Pentland Dell and Maris Bard) developed spraing symptoms. Plants generated from the infected symptomless tubers produced symptomlessly infected daughter tubers, for the three generations tested. These daughter tubers were shown to be sources of acquisition of TRV by nematodes. Thus, movement of symptomlessly infected seed tubers may result in the dissemination of TRV and its introduction into previously unaffected sites. Recent work at SCRI has demonstrated that the virus can have a significant effect on yield and quality attributes of some of these symptomlessly infected cultivars[1].

Epidemiological studies with the virus complex responsible for groundnut rosette disease demonstrated spatial and temporal separation in the transmission of *Groundnut rosette virus* (GRV) vs. *Groundnut rosette assistor virus* (GRAV). In addition, resistance to GRV and GRAV and their aphid vector was found to vary, depending upon both age and inoculum dose, with different potentially-resistant cultivars showing different responses.

scFV antibodies have been generated against *Potato leafroll virus* (PLRV) and also to a synthetic antigen related to *Tomato yellow leafcurl virus*, for use in diagnostics and standardisation.

Other articles in this chapter focus on the variation among aphid vectors of PLRV, (Woodford *et al.*), and

on a comparison of different expression systems for production of antibody fragments for use in diagnosis or control (Zeigler *et al.*).

Control Knowledge of the host selection on fungal pathogen variation has been applied in the development of cultivar mixtures of malting quality barley, leading to smaller inputs of fungicides for control of fungal diseases. The science base for this approach and its practical application are described in the article by Newton and Swanston.

A computer-based program to aid management of *G. pallida* is well advanced. Its use has already demonstrated the difficulties of controlling *G. pallida* vs. *G. rostochiensis*, and the crucial value to farmers of having a range of commercial, resistant cultivars effective against *G. pallida* (Fig. 1). To prevent populations of *G. pallida* from increasing, the computer programme emphasises that granular nematicides are most effective when used to treat populations while they are small (< 1 egg/g soil)(Table 1). Large populations (> 50 eggs/g soil) are difficult to decrease without resorting to a fumigant followed by granular nematicides, or by rotations, which may have to be extremely long because of the slow rate of decline of some populations (Fig. 1).

The root-knot nematodes (RKN, *Meloidogyne* spp.) are the most serious nematode pests world-wide, because of their wide host ranges and ability to have several generations on one crop. This makes them difficult to control by crop rotation. As an alternative to chemical control, a collaborative EU project, co-ordinated by SCRI, examined the potential of the bacterial biocontrol agent *Pasteuria penetrans*. Laboratory assays showed that isolates of the bacterium varied in their ability to bind to and infect populations and species of RKN. Field trials in Tanzania and Ecuador showed that introducing an exotic isolate resulted in a rapid increase in spore populations and suppression of RKN infection and damage. However, this effect was observed only at sites where *P. penetrans* was already present, and the bacterium did not increase in the control plots to which the exotic isolate was not added.

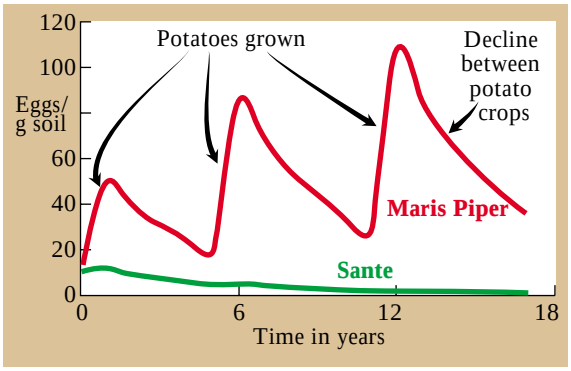

Figure 1 Changes in numbers of eggs of *Globodera pallida* per g soil in a 6 year rotation growing either susceptible cv. Maris Piper or partially resistant (80% resistant) cv. Sante, both treated with a granular nematicide which was 80% effective. Between potato crops the *G. pallida* population declines by 20% per annum.

At planting 1999	1999 Post harvest		2005 At planting	
	With nematicide	Without nematicide	With nematicide	Without nematicide
1	10.5	51.1	3.4	16.7
10	99	377	32.3	123.5
100	558	564	183	185

Table 1 Effect at planting susceptible cv. Maris Piper of differences in numbers of *Globodera pallida* (eggs/g soil) on numbers at harvest and 6 years later, for crops treated and untreated with a granular nematicide which was 80% effective. Between potato crops the *G. pallida* was assumed to decline by 20% per annum.

The TRV vector was used to express the snowdrop lectin (GNA2) in the roots of test plants. The expressed GNA2 lectin caused a reduction in the number of galls formed when plants were challenged with the RKN.

Transgenic plants expressing the coat protein (CP) gene of *Potato mop-top virus* (PMTV) showed resistance to PMTV in cvs Saturna and Pentland Marble, whether inoculated mechanically or via soil containing the infective powdery scab fungus vector of PMTV. The transgene prevented the infection of the tubers and development of spraing symptoms (PMTV and TRV both cause spraing).

In collaboration with colleagues at the National Institute of Biology, Ljubljana, Slovenia, the potato cv. Igor, particularly sensitive to the potato tuber ring necrosis disease caused by the NTN strain of *Potato virus Y* (PVY), was transformed with the CP gene of this strain. Some transgenic lines were identified which were immune to infection following graft inoculation.

A separate article in this chapter describes the integrated pest management of insect pests (Gordon *et al.*).

Plant-pathogen interactions The work on isolating potato genes involved in resistance to potato late

blight (caused by *Phytophthora infestans*) continues. Sequencing of c. 100 clones from a subtracted cDNA library, comprising 4,000 clones, has led to the identification of about 40 genes, 60% of which are involved in defence and stress reactions, and especially cell signalling. A library has also been constructed that is enriched for potato genes involved in rapid responses to infection by *Eca*. Again, many signalling genes and transcription associated factors have been identified from among c. 100 clones so far sequenced. Some of the same genes are also up-regulated upon infection with *P. infestans* in both compatible and incompatible interactions. The results obtained are described in detail in the accompanying article by Birch *et al*.

Many enquiries have been received regarding the application of this approach to other diseases, including cereal pathogens, and to the regulation of genes activated by the application of defence elicitors. Commercially-supported work on the development of elicitors as a practical control measure has also made good progress.

Complementing the work on gene expression in potato is a new core programme on the genomics of the major potato pathogens *P. infestans* and *Eca*. The development of a system for studying differential gene expression in *Eca*, allows studies on its gene regulation during the infection process. Thus, bacterial artificial chromosome (BAC) libraries are being developed for both potato pathogens. The work on *P. infestans* is part of an international *Phytophthora* Genome Initiative co-operation.

Wild tomato species have been shown to be potential sources of resistance to *G. pallida*. One such gene is the *Hero* gene, which confers about 80% resistance to most UK populations of *G. pallida*. In collaboration with M. Ganal (Gatersleben, Germany), work on the cloning of the *Hero* gene continues. However, due to changes in UK priorities for funding, no further work is continuing on searching other *Lycopersicon* spp. for new sources of resistance effective against a greater proportion of *G. pallida* populations.

The novel, multi-partite structure of the mitochondrial genome (mtDNA) of *G. pallida* has been confirmed. Instead of all the genes required for mtDNA function being contained within a single circular molecule, they are divided between several smaller molecules, each of which contains only part of the total genome. The results of sequence analysis of one of these mtDNAs and the implications of such mtDNA populations are described in an article by Armstrong *et al.*

Host resistance to nematodes may be increased by blocking the functions of nematode 'defence' genes. A screening programme of large numbers of small proteins (peptides) from a 'peptide display library' for their ability to bind to PCN secretions has detected two peptides, which bind to PCN thioredoxin peroxidase. Nematode cuticular proteins may also be involved in various recognition processes, including host resistance. Information from the international project completing the sequence of the entire genome of the free-living nematode *Caenorhabditis elegans* has been used to isolate three members of a *G. pallida* collagen multi-gene subfamily. Their expression in transformed *C. elegans* is being studied using constructs containing a 'reporter' gene using collagen promoters from both *G. pallida* and *C. elegans*.

Electron microscopy studies of roots infected by wildtype and RNA 2 mutants of the tobravirus *Pea early browning virus* (PEBV) revealed that PEBV was able to invade all tissue types in the roots, including the root tip and lateral root meristems. Whereas wildtype PEBV formed roughly spherical clumps of aggregated virus particles, mutants lacking the 2b gene (encoding a 29K nematode transmission factor) aggregated as extended columns of virus particles (Fig. 2). This

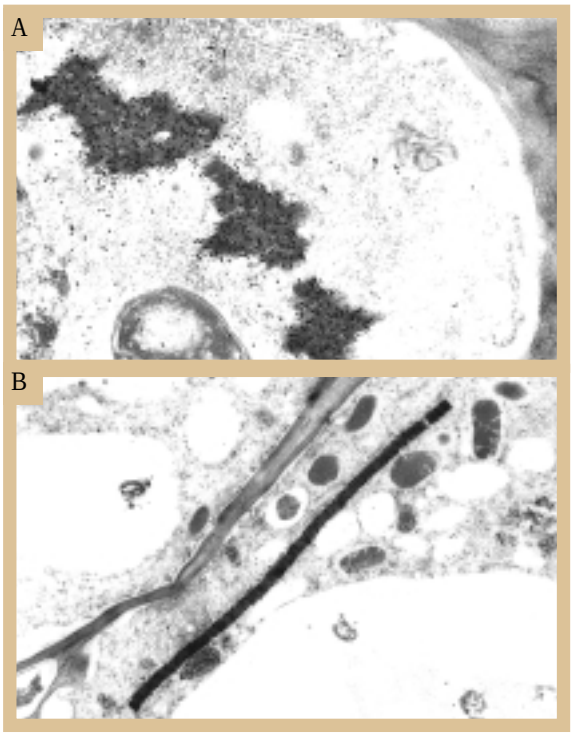

Figure 2 Electron micrographs of PEBV particles in root cells from wildtype PEBV (A) and PEBV lacking the 2b gene (B). Note the differences in aggregation profiles.

change in aggregation structure may explain why PEBV 2b mutants can no longer be transmitted by root feeding nematodes.

Expression vectors have been constructed for all three tobraviruses: TRV (described in the 97/98 Annual Report), PEBV (expressing foreign genes from a TRV CP subgenomic promoter), and *Pepper ringspot virus* (expressing from a PEBV CP subgenomic promoter). These different vectors allow the expression of non-viral proteins in a wide range of plant species.

The TRV isolate that breaks resistance in potato cv. Bintje also breaks resistance in cv. Arran Pilot, but not in cvs Record, Saturna, or Climax. (Record is one of the parents of both Saturna and Climax.) This suggests that there may be two distinct genes for resistance to TRV in cultivated potatoes.

Studies on the mechanism of CP-mediated, transgenic resistance to PMTV have revealed that the replication of RNA 3 (encoding the CP gene) was inhibited, while replication of RNAs 1 and 2 was not. Surprisingly, RNAs 1 and 2 of PMTV were able to systemically infect both the 'resistant' plants, which are resistant to the disease induced by PMTV, as well as non-transgenic plants, after sap inoculation from the resistant plants. This demonstrates both that CP is not needed for systemic movement and accumulation of PMTV RNAs 1 and 2, and that the resistance mechanism is targeted against the RNA (3) encoding the CP gene. Moreover, the accumulation of CP and its mRNA in the transgenic plants does not support a mechanism of resistance *via* virus-induced gene silencing, thus suggesting that another, potentially novel, mechanism of resistance is operating in these transgenic plants.

The mechanism of resistance to *Cucumber mosaic virus* (CMV) in transgenic tobacco expressing CMV RNA 1 was also analysed. A number of factors all argue against a virus-induced gene silencing model as the mechanism of resistance. These include: the level of expression of the transgene; the presence of a functional transgene translation product; the absence of induced resistance in a grafting experiment involving transgenic (susceptible) scion and transgenic (resistant) rootstock; the inability of the CMV-resistant, transgenic plants to inhibit the replication of *Potato virus X* (PVX) expressing CMV RNA 1 sequences; and the ability to break the resistance in transgenic scion grafted to susceptible, infected rootstock. This again suggests the operation of a novel form of resistance, but one similar to that observed in transgenic plants expressing a defective form of CMV RNA 2.

Tobacco and potato plants transformed with a full-length copy of the PLRV genome were found to accumulate virus particles in phloem cells, as do non-transgenic plants infected with PLRV by its aphid vector. Although PLRV accumulated to high levels in transgenic potato plants, comparatively little virus was found in the transgenic tobacco plants. Some of the characteristics of the transgenic tobacco plants resemble those of plants with post-transcriptional gene silencing (PTGS). The specificity of this fascinating system is described in the article by Barker *et al.*

GRV continues to produce surprising results. The ORF4 protein of GRV was able to localise to plasmodesmata when expressed from the PVX vector, while the ORF3 protein localised to the nucleolus. GRV ORF4 was able to promote CP-independent cell-to-cell movement of CMV, when expressed in place of the movement protein gene, while long-distance movement of the hybrid virus required CMV CP in the common host *Nicotiana benthamiana*, but did not occur in the CMV host *N. tabacum*. GRV ORF3 did not promote long-distance movement of CMV when it replaced the CMV CP gene, but did promote the long-distance movement of TMV, in place of the TMV CP gene, although only in the common host *N. benthamiana*. Thus, GRV ORF3 encodes a host-specific long-distance movement factor.

Thus, while we adhere to the tenets of Tennyson ("let knowledge grow from more to more", and "to follow knowledge like a sinking star, beyond the utmost bound of human thought"), the more we learn, the more surprised we become about the numerous levels and strategies of interactions between plants and their pathogens. Hence, we have to agree with Socrates, who said the more we know the more we realise how little we know.

Reference

[1] Xenophontos, S., Robinson, D.J., Dale, M.F.B. & Brown, D.J.F. (1998). *Potato Research* 41, 225-265.