New technologies for the detection and identification of pathogens, pests and environmental pollutants

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The need for new technologies Plants have many enemies and these can cause a variety of diseases. However, plants can respond to attack in only a limited number of ways, which can make disease diagnosis – identifying the pathogen responsible – difficult and confusing. For example, yellowing of leaves, wilting or die-back are symptoms that can be induced by a variety of pathogens or pests and even by stresses such as herbicide damage or a lack of nutrients or water. Added to this is the propensity of pathogens to respond to plant defences by producing novel variants that can then induce novel diseases. On the other hand, plants can display a variety of symptoms when affected by closely related pathogens; sometimes a pathogen can cause no obvious disease symptoms in a tolerant cultivar but can have devastating effects in a susceptible one.

Figure 1 Unprotected potato plants infected by *Phytophthora infestans*, photographs were taken 10 days apart.

These problems are serious because plant pathogens and pests can cause large economic losses in yield and quality of food. For example, damage caused by potato cyst nematode alone costs the UK an estimated £50million annually[1].

Increased world trade in plants and plant propagation materials, and the rapid movement of plants between countries, have increased the risk of spread of harmful organisms. New diseases are emerging that are caused by the appearance of resistance-breaking strains, or by the introduction of pathogens to new hosts in other countries or continents. For example, new strains of potato blight (*Phytophthora infestans*), *Potato virus Y* and *Raspberry bushy dwarf virus* and the spread of the Columbia root knot nematode (*Meloidogyne chitwoodi*) are all posing serious threats to growers world-wide. Therefore, it is vital to ensure that only disease-free plants and materials are traded.

To facilitate trade whilst preventing the spread of disease, it is essential to have rapid, simple and accurate methods by which to detect and identify plant pathogens. In the European Union, plant passports are issued to guarantee plant health, but must be validated to be worthwhile. Tests and test protocols vary between countries, and in order to harmonise testing and build confidence, there is a need to devise standardised tests and methodologies that can be used to detect and identify economically important pathogens in different countries. These tests must be specific, robust and simple.

Rapidity of diagnosis is also an important aim as farmers and growers need to identify the cause of disease symptoms quickly and accurately so that the appropriate remedial action, such as spraying with anti-fungal agrochemicals, can be taken (Fig. 1).

Early detection by means of rapid and sensitive tests would assist control measures. They would also help to minimize chemical inputs and thereby be an important feature of attempts to develop sustainable crop production.

Basic and strategic research is done in the Pathology Division to understand the biodiversity and ecology of pathogens and pests. The results have both produced the background underpinning knowledge, and contributed to the development of the new technologies (antibodies, nucleotide sequences, novel methodology) needed to identify harmful plant pathogens. This article presents a brief overview of the technologies and applications.

Recombinant antibodies Antibodies can be highly specific and highly sensitive probes for the structure of virus proteins. They have been used in virus research to study the roles of surface features of virus coat proteins in interactions with host plants and vector organisms (e.g. aphid, nematode, protozoan). This combination of the specificity and sensitivity of antibodies has made them valuable reagents for the diagnosis of virus diseases. It is possible to express genes encoding the binding portions of antibody molecules by phage display. We have used this technology for antibody production to obtain valuable reagents (recombinant antibodies) (see Ann. Rep. 1995, 125-127). There are many advantages in using antibodies derived by phage display techniques. For example, the DNA encoding the recombinant antibody gene can be stored indefinitely, and it can also be manipulated so as to encode fusion proteins of antibody + tag (AFP) for use in a variety of different test formats. AFP can be produced cheaply in bacteria or in other systems (Ann. Rep. for 1997/98, 111-113 and 1998/99, 139-141), and this method therefore guarantees continuity of supply. These qualities make AFP ideally suited for standardised test methods.

AFP preparations that bind to particles of *Potato leafroll virus* (PLRV) readily detect PLRV in potato (Fig. 2). Currently, we are collaborating with four European partner laboratories, to devise and evaluate standard test kits that incorporate AFP that bind to particles of PLRV, *Tomato spotted wilt virus* or *Beet necrotic yellow vein virus.* The ultimate aim of this project is to produce prototype test kits for commercial exploitation.

An unexpected benefit from these developments has been the extension of this detection technology into the field of environmental pollution. We have successfully isolated recombinant antibody fragments that bind to microcystin-LR, a toxin produced by cyanobacteria, such as those in the genus *Microcystis.* These algae are common in reservoirs and lakes and are usually harmless. But in certain environmental

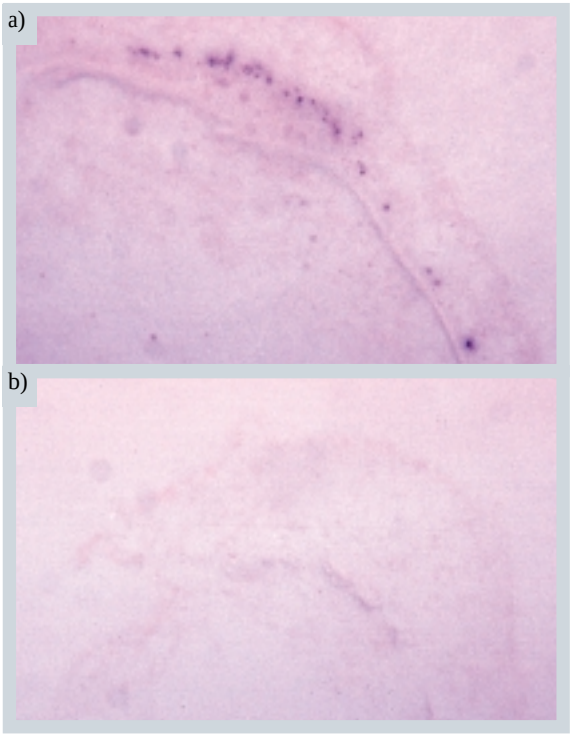

Figure 2 Detection of PLRV in potato stems by AFP stem print test a) the black spots indicate presence of PLRV in the vascular tissue b) non-infected stem.

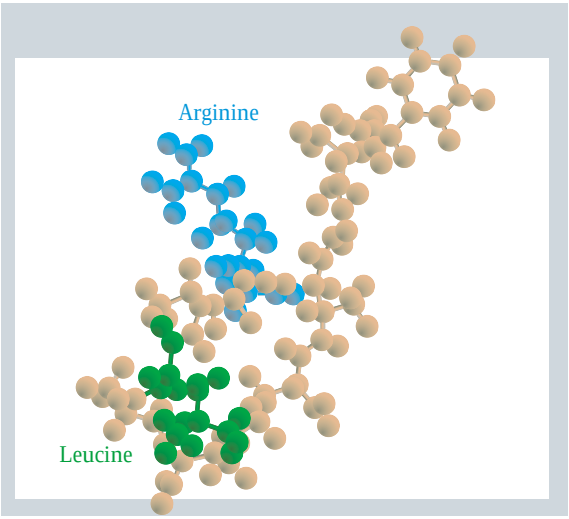

Figure 3 Model of the structure of microcystin-LR derived by NMR spectroscopy (Protein Database ID number 1LCM[2]).

conditions, they grow rapidly and the populations expand to produce floating masses of cells on the surface of water. These blooms can be public health hazards because they can contain toxins. For example, blooms of *Microcystis aeruginosa* release the toxin microcystin-LR into the water when the cells die and disintegrate. Microcystins are cyclic heptapeptide molecules (Fig. 3) that can persist in water for long periods. They are potent inhibitors of eukaryotic protein phosphatases, and can cause a range of health problems particularly in the liver, nerves and skin of humans and animals.

The next experimental steps are to optimise the AFP test and evaluate it against other methods used currently to detect microcystins. If AFP tests are shown to be effective, it should be possible to produce rapid simple tests for environmental pollutants based on our novel antibody technology. Such tests would provide a rapid way to identify the toxins in water.

Mimotopes Mimotopes are structures that bind strongly to antibodies in the same way as, and with similar affinities to, those of the homologous antigens. By using libraries of random composition peptides displayed on phage particles, it has been possible to select mimotopes that bind specifically to monoclonal antibodies that recognise particles of *African cassava mosaic virus*[3], Tomato yellow leaf curl viruses and PLRV. (Fig. 4). These mimotopes are robust and cheap to produce and provide a convenient and precisely defined positive control. The inclusion of mimotopes in test kits will help to validate the tests wherever the kits are used. This 'field' application should help to avoid the risk of spread of pathogens.

The use of peptide libraries as sources of controls for immunoassays could be extended to other pathogens, especially those difficult to obtain (such as some fungal and bacterial pathogens). The technique is being explored further in the EC project 'Standardisation of the immunodiagnosis and quantification of plant viruses by development of synthetic antigens.'

High affinity peptides, obtained by selection from phage-displayed libraries, could also be used in new ways of interfering with plant diseases and pests. For example, the appropriate peptide can be expected to inhibit the activity of enzymes coded for, or induced by, pathogens or pests, or inhibit the interactions between plants and either pathogens or pest-encoded proteins. For example, work is in progress to analyse peptides that bind to nematode surface proteins.

Nucleic acid technologies Techniques to study the genetic structure and function of pests and pathogens have made great strides in recent years. Over the past 15 years a major new technique, the polymerase chain reaction (PCR), has revolutionised the way we study the biology of pathogens. PCR, which allows specific amplification of a target DNA sequence, has made it possible to distinguish certain pathogens from their closest relatives, to quantify accurately the amounts of these pathogens present in diseased tissues and to detect them specifically even when they are present at vanishingly low concentrations.

At SCRI, PCR-based diagnostics have been designed for the assay of a number of fungal, bacterial and nematode pathogens. For example, quantitative PCR tests have been developed for *Erwinia carotovora* (blackleg) and *Spongospora subterranea* (powdery scab). These, and other, diagnostics have been made available to growers either directly, where growers are performing the tests in-house, or via a testing service provided by SCRI and others, including the British Potato Council. These tests are providing valuable information to growers to help them manage their stocks effectively. We will continue to improve these diagnostics as new technologies become available.

Tracking plant pathogens in the environment is an important area of research as this information allows

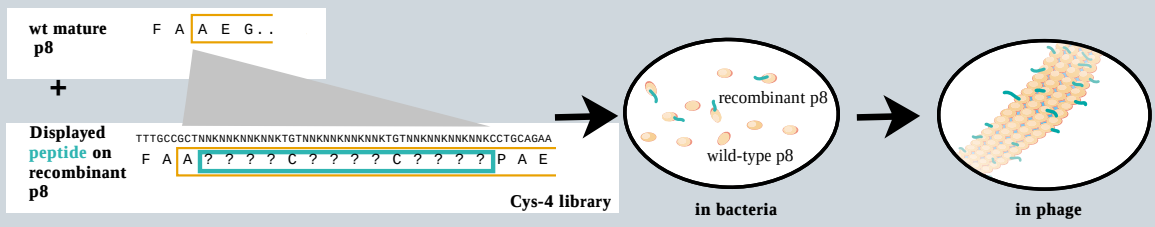

Figure 4 Phage display peptide library. Filamentous phage in the libraries have 2 copies of gene 8 (that encodes the major capsid protein). One is modified to have an N-terminal extension with randomised amino acid composition except for a pair of cysteine residues that constrains a peptide loop.

scientists to understand better how a pathogen adapts to new environments, how it spreads from one plant, field or even country to the next, and how to track and eliminate the source of a disease. Perhaps the main advance to this area in recent years has been molecular fingerprinting. Molecular fingerprinting of a pathogen provides a unique profile for a particular genus, species or even strain of a pathogen. The sensitivity of the fingerprint is determined by the particular technique chosen. For example, studying the intergenic transcribed region (ITS) region of the rDNA has allowed great strides to be made in the identification of fungal pathogens, including *P. infestans*, the causal agent of late blight, and other *Phytophthora* species. This technique has ensured that SCRI is a world-leader in the identification of these pathogens. Another technique called amplified fragment length polymorphism (AFLP) has allowed scientists to track individual strains within the environment, thus providing detailed information on disease spread and development.

Similarly, the intergenic spacer (IGS) region of the rDNA has been used at SCRI to develop a diagnostic for distinguishing individual root-knot nematodes (*Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*). These nematodes infest potato and were recently designated as quarantine organisms by the EU (Ann. Rep. 1996/7, 191-3). Other PCR-based techniques such as random amplified polymorphic DNA (RAPD) and AFLP have allowed scientists to distinguish different introductions of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* into Europe (Ann. Rep. 1995, 151-4). Also, microsatellites have been used to track individuals within the environment, and to follow population changes following selection pressures, thus providing detailed information on disease spread and development.

Phage typing Even high resolution methods like those described above are sometimes insufficient to discriminate between a harmless organism and a harmful one. A good example is the discrimination of *E. coli* O157 from other strains of *E. coli*. For this, we have turned to phage typing. This method was developed in the 1920s to fingerprint bacterial pathogens based on their susceptibilities to infection by bacteriophages. It has been recently adopted by scientists at SCRI for the study of plant pathogens and is now being applied to the detection of *E. coli* O157. The annual costs of infections by *E. coli* O157 in the USA alone from lost productivity and health care are currently estimated at $913 million. Effective investigation of the serious outbreaks caused by *E. coli* O157, and the saving of lives and money, depends on rapid, accurate fingerprinting of many isolates from different sources. The method, developed in association with Grampian Health Trust, has been patented and attempts are being made to commercialise it in kit format.

Conclusions and future perspectives The expertise and diagnostic tools produced at SCRI have been transferred to farmers and processors to assist the commercial production of high quality food crops for Scottish consumers and beyond. Furthermore, some spin-out research has been of value in the fields of medical and environmental diagnostics.

It seems clear that the need for such state-of-the-art diagnostics is unlikely to diminish. Pathogens evolve all the time and agricultural practices seldom remain static for long. A skilled research base has been, and looks certain to continue to be, the essential resource upon which pathologists can rely so as to rise to the diagnostic challenges posed by ever-resourceful plant pathogens. With the continuing support of SERAD, the European Commission, the British Potato Council and other sponsors, research at SCRI will continue to improve and devise new methodologies to meet these needs.

References

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