European Journal of Plant Pathology **101**: 351–363, 1995.

Mini review

Use of monoclonal antibodies in plant pathology

Lesley Torrance
Virology Department, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK

Accepted 20 January 1995

Introduction

Unlike the diverse population of antibodies found in the sera of immunised animals, a preparation of monoclonal antibodies (MAbs) consists of homogeneous antibody molecules. They all have the same specificity and affinity for an antigenic determinant or epitope. MAbs are produced *in vitro* from a clonal population of hybridoma cells [Goding, 1983; Campbell, 1984; Harlow and Lane, 1988]. MAbs have been widely applied in many areas of the medical, veterinary and agricultural sciences because their use confers the advantages of defined specificity and the ready availability of unlimited quantities of a standardised reagent.

Polyclonal antisera have been used to identify plant viruses and provide information on serological relationships since the early days of plant virus research [Matthews, 1991], and since the early 1980s MAbs have been produced to more than 60 viruses in 20 different virus groups [Van Regenmortel and Dubs, 1993]. Most plant viruses have simple genomes (c. 6 kb of ssRNA) protected by a protein coat consisting of multiple copies of a single polypeptide. The coat protein, although encoded by only a small part of the genome, can have other important functions besides a protective role. It can interact with virus nucleic acid molecules to initiate particle assembly [Bloomer *et al.*, 1978], regulate replication [Houwing and Jaspers, 1987], elicit plant resistance genes [Goulden *et al.*, 1993], mediate transmission by vectors [Harrison and Robinson, 1988] or be required for systemic movement of virus within the host plant [Saito *et al.*, 1990; Quillet *et al.*, 1989]. Subtle changes in regions of the coat proteins involved in these biological functions that result in alteration or destruction of an epitope can be detected by MAbs. MAbs are therefore useful tools to study the antigenic structure and molecular ecology of viruses. In addition they have been widely used to detect and identify viruses at the strain-, species- and genus level.

Until the advent of MAbs, serological methods were not widely used to study plant pathogenic fungi, bacteria, mycoplasma-like organisms (MLOs) or nematodes mostly because of the relatively wide range of cross-reactivity of polyclonal sera raised to these more complex organisms [Davies, 1994; Clark, 1992; Dewey, 1992]. However, MAbs have now been developed that are specific for genus, species and isolate (or life-stage) of these organisms. MAbs are used in detection and diagnosis, as well as in ultrastructural studies of host-pathogen interactions. The following sections highlight some recent research involving the use of MAbs to help answer questions in both fundamental and applied areas of plant pathology with particular emphasis on plant virology.

Viruses

Antigenic structure

Virus epitopes are usually classified according to whether they are composed of amino acid residues that are contiguous in the linear sequence of a polypeptide called 'continuous epitopes', or whether they are formed by amino acids that come together on folding or association of polypeptide chains called 'discontinuous epitopes' [Van Regenmortel, 1990]. In addition viral epitopes are classified depending on whether they are present on different states of the particle proteins e.g. cryptotopes are only reactive when the particles are disassembled, neotopes when the particles are intact, and metatopes are reactive in both intact

and disassembled particles [Van Regenmortel, 1990]. Some information about the location of epitopes can be obtained by testing MAb reactivity with synthetic peptides, fusion proteins or mutant strains, and by visualizing binding in the electron microscope. In this way detailed information about the antigenic structure has been obtained for tobacco mosaic tobamovirus [TMV; Van Regenmortel and Dubs, 1993], beet necrotic yellow vein virus [BNYVV; Commandeur *et al.*, 1992, 1994], potato mop-top furovirus [PMTV; Pereira *et al.*, 1994], tobacco rattle tobravirus [Legorburu, 1993], potato leafroll luteovirus [PLRV; Torrance, 1992a] and some potyviruses [Andreeva *et al.*, 1994; Jordan, 1992].

In studies with TMV, a group of MAbs were identified that reacted with a metatope present on one extremity of the virus particles, and by using immunoelectron microscopy and gold-labelled MAbs it was established that they reacted with the end that contained the 5′ end of the RNA [Dore *et al.*, 1990]. These MAbs were used to show that the stacked disk structures formed by TMV subunits are bipolar. Further studies showed that some (but not all) of the MAbs specific for the end containing the 5′ extremity inhibited translation of TMV particles in an *in vitro* translation system, and therefore may have inhibited cotranslational disassembly of the virus particles [Saunal *et al.*, 1993].

Using the Pepscan technique [Geysen *et al.*, 1987], continuous epitopes have been identified by the reactions of MAbs with short (6–8 amino acids) peptides in the linear sequence of the coat protein [Trifilieff *et al.*, 1991; Torrance, 1992b; Pereira *et al.*, 1994; Commandeur *et al.*, 1994]. For example, in the PMTV coat protein, MAb SCR 69 reacted with the octapeptide AEIGERKA (amino acids 2 to 9, numbered from the N terminus). SCR 69 was also shown to react on the surface along the sides of intact particles by immunogold labelling [Pereira *et al.*, 1994], providing evidence that the N terminus is surface-located. These results are in contrast to findings with BNYVV (a possible member of the furovirus group that has a different soil-borne plasmodiophorid fungal vector), where the C terminus of the coat protein is located on the surface along the sides of particles, and amino acids at the N terminus form an epitope exposed at one extremity of the particles [Lesemann *et al.*, 1990; Koenig *et al.*, 1990; Commandeur *et al.*, 1992].

Differences in binding affinity of TMV MAb 57P to a set of related 13-mer peptides spanning amino acids 134–146 in the TMV coat protein sequence were measured using the BIAcore instrument produced by Pharmacia Biosensor AB, Sweden [Altschuh *et al.*, 1992]. The tests showed that three residues (140-N, 143-S and 144-F) were key contributors to MAb binding, and changing any one of them abolished binding. The authors postulate that these residues are brought together in a helical conformation (that mimics the structure in the protein) to form the epitope. They also showed that amino acid changes outwith the key residues affected MAb association and dissociation rate constants to a small extent.

A different approach using site-specific mutagenesis was taken to identify a key amino acid that influences binding of MAb MCA-13. MCA-13 reacts with isolates of citrus tristeza closterovirus (CTV) causing severe symptoms on citrus hosts, but not with isolates causing mild symptoms. Examination of the coat protein sequences of 8 mild and 10 severe isolates revealed only a few differences at the amino acid level, of these the amino acid at position 124 was the most conserved, being either phenylalanine in the severe isolates or tyrosine in the mild isolates. MAb MCA-13 reactivity was abolished in the coat protein of a severe isolate of CTV when phenylalanine-124 was changed to tyrosine by mutagenesis. Conversely, MCA-13 reacted with the coat protein of a previously non-reactive mild isolate when tyrosine-124 was changed to phenylalanine [Pappu *et al.*, 1993]. These results show that MAb binding can be abolished by a difference of a single amino acid residue.

Knowledge of the antigenic structure of viruses also helps to characterise the MAbs. MAbs that bind to specific epitopes, or regions on the surface of the particles that may influence function (e.g. a feature important for recognition in transmission by the vector) can be used per se, or used to produce anti-idiotypic antibodies [Hu and Rochow, 1988; van den Heuvel *et al.*, 1994], to investigate that function.

Molecular ecology

For several viruses it has been shown that the

particle proteins are adapted for transmission by vectors [Harrison and Robinson, 1988]. The following examples show that MAbs can be used to detect variation among particle protein epitopes that correlate with transmission. Potyviruses are a very large group of viruses (over 150) which have filamentous particles. They are transmitted by aphids in a non-persistent (non-circulative) manner, virus particles are acquired and inoculated in a few minutes. Transmission is not highly aphid-specific and most viruses can be transmitted by several aphid species. The N- and C-termini of the coat protein sub-units are located at the outer surface of the particles [Shukla and Ward, 1989]. The N-terminal part of potyvirus coat protein plays a role in aphid transmission and the amino acid triplet DAG, found near the N terminus of many aphid transmissible potyviruses, is associated with transmissibility [Harrison and Robinson, 1988; Atreya *et al.*, 1990]. The triplet DAS is associated with aphid non-transmissibility in tobacco etch potyvirus [Allison *et al.*, 1985]. Recent work has shown that there is a correlation between aphid transmission of potato virus A (PVA) and reactivity with MAb A5B6 [Andreeva *et al.*, 1994]. This MAb readily detected aphid non-transmissible isolates of PVA by ELISA and immunoblotting assays, but reacted weakly or not at all with transmissible isolates. Pepscan analysis showed that MAb A5B6 reacted with the octapeptide AETLDASE (amino acids 1to 8) in the non-transmissible isolate B11 [Andreeva *et al.*, 1994]. Recent work indicates that aphid transmissible isolates differ from non-transmissible isolates in this N-terminal sequence [L Andreeva, personal communication].

A different form of aphid transmission is found in the luteoviruses. This group of phloem-limited viruses are transmitted persistently. Vector specificity of luteoviruses is well developed, and each virus is transmitted efficiently by only one or a few aphid species. Vector specificity is correlated with the antigenic properties of the capsid protein [Rochow, 1970; Harrison and Robinson, 1988]. The virus particles pass selectively from the hindgut into the haemocoele and circulate in the haemolymph through the aphid body [Gildow, 1993]. Evidence from ultrastructural studies with barley yellow dwarf luteoviruses (BYDV) indicates that vector specificity is mainly determined by the ability of luteovirus particles to cross from the haemocoele into the cells of the accessory salivary glands, in particular the ability to cross the salivary basal lamina and basal plasmalemma [Gildow 1990; Gildow and Gray, 1993]. Virus particles were identified in thin sections by labelling the excised salivary glands with virus-specific antibodies and then ferritin-labelled second antibodies before fixation and embedding the tissue [Gildow and Rochow, 1980]. Particles of PLRV have also been identified in thin sections of aphids by direct labelling of particles (pre- and post-embedding) with MAb conjugated to gold [Garret *et al.*, 1991]. The passage of the MAV strain of BYDV through the basal lamima was impeded (as judged by visual examination of thin sections in the electron microscope and transmission studies) when virions were mixed with MAV-specific MAbs but not when mixed with RPV-specific MAbs before micro-injection into the aphid haemocoele [Gildow and Gray, 1993]. The three MAV-specific MAbs tested (either as whole antibody or Fab fragments) impeded transmission of virus particles: 0–18% of aphids transmitted virus compared with 68% of aphids injected with an unrelated anti-RPV MAb. No virus particles were observed in thin sections of the basal lamina or intracellularly in the accessory salivary gland cells in treatments with the specific MAbs, whereas many particles were observed in these tissues in the control treatments. These results indicate that MAb binding interfered with virus capsid recognition and penetration of the basal lamina. The attached MAbs may have prevented recognition of particles by steric hindrance or it is possible that these MAbs reacted with epitopes important for recognition of virus capsid by membrane receptors. The authors concluded that Fab fragments of MAbs were not useful in studying interactions between virus capsid and salivary gland membranes. However, further work on these lines may be more productive if smaller fragments of antibodies such as single chain Fv or Fd fragments (produced by recombinant antibody techniques, see below) that are specific for determinants found on transmissible isolates are used.

PLRV isolates that differ in transmission efficiency were serologically indistinguishable when tested with polyclonal antisera but were distin-

guished in tests with a panel of MAbs. Two MAbs, SCR 8 and 10 reacted weakly with poorly transmitted isolates of PLRV [Tamada *et al.*, 1984; Massalski and Harrison, 1987]. These poorly transmitted isolates were shown to be acquired and retained in the aphid, indicating that the particles were unable to pass from haemolymph to salivary gland cells [Tamada *et al.*, 1984]. Therefore the MAbs possibly recognize an epitope on the transmissible virus particles that is important to mediate passage across the basal lamina and/or basal plasmalemma.

Using sensitive forms of ELISA incorporating MAbs, it is possible to measure virus concentration in individual aphids, and to measure virus acquisition and retention by single aphids [Torrance, 1987; Barker and Torrance, 1990]. Tests with MAb MAC 92 specific for an English BYDV- RPV-like isolate showed that both *R. padi* (an efficient vector) and *S. avenae* (an inefficient vector) acquired virus and the virus was detected in individual *S. avenae* after the aphids had fed for a further two days on healthy oats [Barker and Torrance, 1990]. These tests provide a different line of evidence that the virus can pass from the gut to the haemocoele in both efficient and inefficient vectors. The tests also showed that virus concentration in aphids does not increase after leaving source plants providing evidence that the virus does not multiply in the vector, and similar results have been obtained with the same kinds of tests on PLRV in aphids [Tamada and Harrison, 1981].

Panels of MAbs have been used to build epitope profiles to distinguish serologically related viruses that cause similar diseases. Cassava mosaic disease was described at the beginning of this century but it was only shown to be caused by a virus, called African cassava mosaic geminivirus (ACMV), in the early 1980s [Bock and Woods, 1983]. Subsequent work with MAbs has helped to show that cassava mosaic disease is, in fact, caused by three different geminiviruses. A panel of 17 MAbs produced to a west Kenyan isolate of ACMV gave different reaction patterns with different isolates of viruses causing mosaic in cassava. For example, MAb SCR 20 reacted with 11 different virus isolates, whereas other MAbs (SCR 11 and 33) reacted with only two or three [Swanson, 1992; Swanson and Harrison, 1994]. The MAbs were used to compare reactivities of cassava-infecting geminiviruses and the resulting epitope profiles distinguished three different groups of isolates. These groups also differ in geographic location, each region separated by major natural barriers to spread. Viruses in group A were found in countries in Africa west of the Rift valley and South Africa, those in group B, in countries east of the Rift valley in coastal Kenya, coastal Tanzania, Malawi and Malagasy, and those in group C, in India and Sri Lanka. These serogroups could also be distinguished in other ways including when comparisons were made of nucleotide sequences, and the differences were considered great enough to justify separation into three different viruses called ACMV, East African cassava mosaic virus and Indian cassava mosaic virus [Hong *et al.*, 1993]. Cassava was brought to Africa and India from South America during the 16 to 18th centuries. Since geminiviruses have not been found infecting cassava in South America, Harrison and Robinson [1988] proposed that the cassava became infected by isolates of geminiviruses that were already present in the particular region in other plant species, so the distribution of the three viruses may reflect the different routes of introduction of cassava.

Virus detection

The use of MAbs in routine virus detection has been reviewed recently [Van Regenmortel and Dubs, 1993; Torrance, 1992b; Jordan, 1992] and the key points can be summarised as follows: (i) MAbs possess a very high degree of specificity for an epitope and therefore can distinguish epitopes unique to individual virus strains or common to a virus group. (ii) Use of MAbs in routine testing ensures a uniformity and standardisation between tests that is highly desirable. (iii) However, the epitope recognised by the MAb must be stable under the conditions of the test or false negatives will result. (iv) Some MAbs are sensitive to environmental conditions (pH, salt, freezing), or do not work in certain assay formats (e.g. lose activity when used to coat microtitre plates). Production of a MAb (or MAbs) with all of the desired properties, correct binding specificity, as well as the necessary robustness to be used in different test formats, is time-consuming and expensive. In purely commercial terms the expense may

be justified for MAbs used in large-scale routine testing for viruses of crops where the health status is guaranteed by certification schemes (e.g. potatoes, tree fruit, bulbous ornamentals), or other plant health monitoring (e.g. screening micropropagated plants, breeding lines for virus resistance, or virus infection of seed lots) where very large numbers of tests are done annually. Nevertheless, there are other kinds of tests that may need to be done on a smaller scale, such as for the provision of plant passports for movement of plants between different countries within the EU, and for which it is highly desirable to have uniform standard tests with readily available defined reagents. In these instances the development costs may need to be funded by the EU or individual state governments.

There are several groups of viruses where individual members have epitopes in common with others in the group, and these viruses can potentially pose a problem when using MAbs for diagnosis because reaction with a single MAb does not necessarily confirm virus identity. For example, in the luteovirus group, tests have shown that there is a complex network of serological interactions among luteoviruses [D'Arcy *et al.*, 1989; van den Heuvel *et al.*, 1990]. Sometimes this cross-reactivity can be used to advantage, for example, MAb PAV IL-1 raised to the PAV strain of BYDV can distinguish beet infecting beet mild yellowing virus from non-beet infecting beet western yellows. This MAb has been used to detect and identify the viruses in individual aphids as part of a disease forecasting scheme to prevent unnecessary use of pesticides [Smith *et al.*, 1991]. However, for accurate diagnosis of luteovirus diseases, if virus-specific MAbs are not available, it is best to use a panel of MAbs with defined epitope specificity.

Examination of multiple sequence alignments of 10 luteovirus coat proteins showed that they contained similar or identical amino acids in many areas [Mayo and Ziegler-Graaf, 1995], suggesting a reason why there are so many cross-reacting luteovirus MAbs. The sequences were examined using a secondary structure prediction programme, and they were compared with a general model for the shell domain of the sub-units of other small icosahedral viruses comprising an eight stranded β barrel. Such alignments can be used to predict peptide sequences that are exposed at the surface of the particles, and virus-specific or group-specific MAbs might be raised using peptides containing unique or common sequences.

The potyviruses are another group where there is a great deal of homology in the coat protein sequences [Shukla and Ward, 1989]. Virus-specific epitopes are found especially in the N-terminal part of the coat protein where there is a large amount of variation in amino acid sequence between viruses, whereas regions of conserved sequence are located in the central part or so-called trypsin resistant core, and potyvirus group-specific MAbs have been produced that react with epitopes in this region [Shukla and Ward, 1989; Jordan, 1992]. A subgroup-specific MAb (PTY-1) was obtained by immunisation with a mixture of intact and denatured virions of seven potyviruses [Jordan and Hammond, 1991]. PTY-1 detects a cryptotope present in the majority (135/143) of aphid-transmitted potyviruses tested [Jordan and Hammond, 1991; Jordan, 1992]. Analysis of bacterially-expressed coat protein deletion mutants of BYMV isolate GDD indicated that the PTY-1 epitope was in the region of amino acids 135–160 [Jordan, 1992], in the so-called trypsin resistant core [Shukla and Ward, 1989]. Examination of the known amino acid sequences of the coat proteins of the different potyviruses shows that in the sequence 136-QGEWTM-MDGEEQVTYPLKP of BYMV-GDD there are nine amino acids (letters in bold type) in common. Moreover, in some of the viruses 140-T is changed to a somewhat similar amino acid V, and 145-E is changed to the similar amino acid D, therefore the putative epitope may be located in the heptapeptide 139-WTMMDGE. A MAb, SCR 39 produced to potato V potyvirus reacted with 20/30 aphid-transmitted potyviruses in a sandwich ELISA where the virions were trapped by homologous antisera [Farmer, 1989]. It was deduced that SCR 39 detected a cryptotope just on the border of the trypsin-resistant core comprising amino acids 242-QEENTER. This sequence is also common to many potyviruses and one would predict that MAb SCR 39 would react with a greater number of potyviruses in a test where the virus antigen is denatured, such as the format used with PTY-1. MAbs specific for the amino acid sequences in the N-terminal region of potyvirus coat proteins have

been found to be virus-specific [Jordan, 1992] and it might be expected that virus-specific MAbs would be obtained by using unique peptide sequences as immunogens. Werkmeister *et al.* [1991] could not produce PVY-specific MAbs by immunising mice with peptides and performing the standard PEG-mediated fusion on the splenocytes, but they did produce several specific MAbs by a process of peptide-mediated electrofusion. The technique involves performing an electrofusion of the following mixture: splenocytes from mice immunised with PVY coat protein, biotin-labelled peptide (comprising the 30 N-terminal amino acids of PVY coat protein), streptavidin and biotin-labelled myeloma cells.

Non-structural virus-coded proteins

There are many reports of the use of antisera to locate, and investigate serological relationships among non-structural virus-coded proteins [Purcifull and Hiebert, 1992; Niesbach-Klösgen *et al.*, 1990; Berna *et al.*, 1986; Wellink *et al.*, 1988]. However, work with MAbs has mostly been limited to potyvirus inclusion proteins; for example, Suzuki *et al.* [1989] investigated the distribution of cylindrical (CIP) and amorphous (AIP) inclusion proteins in pumpkin leaves infected with watermelon mosaic virus 2 (WMV2) with MAbs specific for these proteins. Also, two MAbs to WMV2 AIP reacted with the AIP-like protein found in leaves infected with soybean mosaic virus but no cross-reactions were found with the AIPs of four other potyviruses [Suzuki *et al.*, 1991]. A MAb specific for the 49 kDa nuclear inclusion proteins of tobacco etch potyvirus was shown to inhibit the self-processing reaction in which the 49 kDa proteinase is cleaved from its 75 kDa precursor [Slade *et al.*, 1989]. A MAb raised to a 112 kDa protein produced in *Cucumis metuliferus* plants infected with papaya ringspot potyvirus also reacted with the 51 kDa AIP, a second MAb reacted with both the 112 kDa protein and proteins of 70, 64 and 47 kDa which are also present in the infected plants, but not with the 51 kDa. These results provide evidence for the hypothesis that the proteins are derived from the 112 kDa protein *in vivo* [Yeh *et al.*, 1992].

The non-structural protein 3A from cucumber mosaic virus was detected in *in vitro* translation mixtures by immunoprecipitation with each of five 3A-specific MAbs. In addition, it was located in the nucleoli of CMV-infected tobacco cells by immunogold labelling of ultrathin sections with 3A specific MAb 3H12 [MacKenzie and Tremaine, 1988].

Tomato spotted wilt tospovirus (TSWV) is transmitted to a range of economically important plant hosts (including vegetables and ornamentals) in a persistent manner by species of thrips, of which the western flower thrips (*Frankliniella occidentalis*) is thought to be the most important [Ullman *et al.*, 1993]. A polyclonal antiserum raised against the NSs non-structural protein was used to detect this protein in insect and plant cells [Ullman *et al.*, 1993]. Presence of NSs was taken as proof of replication as the NSs protein is found in TSWV-infected cells but not in virions. MAbs raised to TSWV NSs have been used to devise an ELISA to identify thrips that may be potential vectors of TSWV [Bandla *et al.*, 1994]. The zwitterionic detergent Empigen BB was incorporated in the antibody diluent in the tests on thrips because it substantially decreased non-specific background reactions [Bandla *et al.*, 1994].

Fungi and bacteria

Detection, diagnosis, quantification

Recent reviews by Dewey [1992], Dewey and Thornton [1994], Stead [1992] and Schots *et al.* [1994] show that it is possible to raise genus-, species- and pathovar or isolate-specific MAbs to detect and quantify fungal and bacterial plant pathogens. Because of the more complex structures and life cycles of these organisms, it is obviously important to select an appropriate immunogen for MAb production, for example, a component unique to isolate, species, or life-stage. However, it is not always possible to identify these types of compounds and some degree of serendipity is often involved. Species-specific MAbs have been raised against several fungal pathogens by immunising mice with fluid obtained by simply rinsing the surface of a mycelial colony grown on a slant culture with 1 ml phosphate buffered saline (PBS), spores, zoospores and hyphal fragments have also been used [Dewey, 1992].

Eyespot, *Pseudocercosporella herpotrichoides*,

is one of several stem base pathogens that attack cereals. These stem base pathogens cause similar symptoms but eyespot is the only one that causes significant yield losses in winter cereals and responds to fungicide treatment. Identification is usually confirmed by laboratory culture but eyespot is slow growing in culture and its presence is often masked by the other faster-growing stem base pathogens. The results of the laboratory tests are usually obtained too late to give effective prior warning for spray application [Priestley and Dewey, 1993]. Eyespot-specific MAbs were raised by immunizing mice with an ammonium sulphate precipitated extract from freeze-dried mycelia [Priestley and Dewey, 1993]. The MAbs were used to detect eyespot in plants at 8 and 15 days after infection (visible symptoms only appeared after 21 days). Development of these kinds of assays are important for early identification and diagnosis of disease in order to prevent unnecessary applications of fungicides.

Rhizoctonia solani is a soil-borne pathogen of a wide range of crop plants and ornamentals. MAbs specific for *R. solani* were produced by immunising mice with the acetone-precipitated fraction of the solution from a mycelial surface rinse or with a suspension of lyophilised mycelia [Thornton *et al.*, 1993]. Immunofluorescence microscopy showed that the MAbs reacted with antigens present on the surface of the fungal hyphae [Thornton *et al.*, 1993]. Immunofluorescence and ELISA based assays were devised to detect live propagules of *R. solani* in soil by a nutrient enrichment technique where the fungus is cultured for 48 h before testing. These assays were used to quantify *R. solani* inoculum in soils.

The blackleg disease of potato caused by *Erwinia carotovora* subsp. *atroseptica* (Eca) is tuber-borne. Eca is often present together with *E. carotovora* subsp. *carotovora* (Ecc; associated with soft rot of stored tubers) at low levels on potato tubers. Contaminated tubers are the main source of inoculum producing infection in the daughter plants and it is important to quantify the numbers of viable Eca and Ecc cells on seed tubers [Pérombelon *et al.*, 1993]. MAbs have been produced that distinguish between these closely related bacteria and they have been used successfully in ELISA [Vernon-Shirley and Burns, 1992; Hyman *et al.*, 1995].

Quantification is important to successful integrated pest and disease management, in risk assessment work, and in screening for efficacy or to optimize the application of fungicides or pesticides. Development of disease prediction models requires accurate monitoring of pathogen populations over time, identification of strains or determining the incidence of recombination events. ELISA formats are well suited to quantitative studies, they can be used successfully with a variety of different matrices (they are usually not affected by components of soil/plant tissue) and have a wide dynamic range (i.e. can detect high or low levels of pathogen). ELISAs provide cheap, sensitive tests, that are well suited to large-scale testing (thousands of samples), as well as requiring minimal technical/laboratory skills. Furthermore, incorporation of MAbs in ELISA provides improved standardisation and specificity.

Dot-binding or dipstick assays have proved useful in detection of fungal pathogens. In these tests, the sample is applied to a support membrane (e.g. nitrocellulose, polyvinylidene difluoride or nylon) and then the immunolabelling, washing and staining steps are done directly on the membrane. A positive reaction is denoted by an insoluble coloured precipitate [Miller *et al.*, 1992; Thornton *et al.*, 1993; De Ruiter *et al.*, 1994]. This method has been further developed by Cahill and Hardham [1994] to detect *Phytophthora cinnamomi* zoospores by chemotaxis. They impregnated membranes with different compounds so that the motile zoospores of the fungal pathogen were attracted to the membrane where they encysted and were detected by immunoassay with species-specific MAbs.

The surface located molecules on zoospores and cysts are likely to play an important role in the early stages of infection. MAbs raised to *P. cinnamomi* antigens have been used in immunofluorescence and immunogold labelling techniques for ultrastructural studies on the cell coat and internal organelles of zoospores, as well as studies of changes in the surface properties of zoospores during encystment [Hardham *et al.*, 1991; Gubler and Hardham, 1991].

Host-pathogen interactions

MAbs raised to bacterial and fungal antigens have also been used to study host-pathogen interactions.

Compatible and incompatible interactions between *Xanthomonas campestris pv. vesicatoria* and its pepper host were studied by immunogold labelling in the electron microscope using MAbs raised against xanthan (the main component of extracellular polysaccharide; EPS). The contribution of EPS to colony development and differentiation in the intercellular space was examined, and the product of avirulence gene AvrBs3 was located within the cytoplasm of the bacterial cells by labelling with specific polyclonal serum [Brown *et al.*, 1993].

MAbs raised to plant cell surface components expressed during colonization of pea by *Rhizobium* were used to investigate common features of pea roots colonized by the arbuscular mycorrhizal fungus *Glomus versiforme*. Antigens detected by the MAbs accumulated to different extents during invasion of pea root by rhizobial or mycorrhizal infection. The antigen recognised by MAb MAC 265 is thought to play a role in plant defence [Perotto *et al.*, 1994].

MAbs specific for cellulase enzymes, cellobiohydrolase I (CBHI) and endoglucanase I (EGI), of the soft rot fungus *Trichoderma reesei* have been used for affinity purification of EGI, and in ultrastructural studies conjugated to gold sol to locate enzyme binding sites and help to define their action on aspen cellulose microfibrils [Nieves *et al.*, 1991].

Mycoplasma-like organisms

MLOs are phloem-limited obligate pathogens and hence it is difficult to prepare immunogen substantially free of host components. Nevertheless MLO enriched extracts of vascular tissue from infected hosts such as *Vicia faba* or *Catharanthus roseus* [Clark, 1992; Caudwell *et al.*, 1988] have been used to produce MAbs specific for several MLOs including Molières [Kenyon and Clark, 1993], flavescence dorée [FD; Schwartz *et al.*, 1989] and primula yellows diseases [Clark *et al.*, 1989]. These MAbs have been used in a range of tests such as ELISA, immunofluorescence microscopy and electron microscope examination of immunogold-labelled, glutaraldehyde-fixed tissue sections [Clark, 1992] to detect and identify the MLOs, as well as for affinity purification of FD-MLO [Seddas *et al.*, 1993].

Nematodes

Although serological techniques have had more limited use in plant nematology, two recent reviews by Davies [1994] and Forrest [1995] highlight the utility of MAbs to identify and quantify nematodes, and to investigate host-pathogen interactions. As with fungal and bacterial pathogens, some consideration must be given to the immunogen used to obtain the desired MAbs. There is a wide choice of potential target nematode antigens that can be used, and different antigens may be present to a greater or lesser extent during different stages of the animals' development. MAbs obtained by immunising animals with thermostable proteins from the eggs, or homogenates of eggs and second stage juveniles of potato cyst nematode species *Globodera rostochiensis* or *G. pallida* have been produced that differentiate between these species [Schots *et al.*, 1989 and 1990; Robinson *et al.*, 1993].The MAbs were used to devise assays to detect and quantitate the two species [Schots *et al.*, 1992; Robinson *et al.*, 1993].

Nematode secretions such as saliva, amphidial exudates and secreted proteins on the surface of the cuticle play an important role in the interaction of the nematode with plant root cells, and in pathogenesis. MAbs specific for components of nematode secretions have been used to investigate such host-pathogen interactions [Davies, 1994; Forrest, 1994; Davis *et al.*, 1992]. Binding of the MAbs to the different structures can be seen using immunofluorescent microscopy but improved resolution of binding to internal components was obtained by examination of cryostat sections of 3rd stage nematode larvae by laser scanning confocal microscopy [McGillivery *et al.*, 1992].

Recombinant antibodies

The production of MAbs can be a time-consuming and a relatively inefficient process. Sometimes many fusion experiments must be done before

stable hybridomas secreting MAbs of the desired specificity are obtained. New ways to produce antibodies have been developed using DNA-technology to clone and express fragments of antibody genes in bacterial systems [Orlandi *et al.*, 1989]. Also, antibody fragments specific for a wide range of antigens can be obtained from antibody expression libraries [Marks *et al.*, 1991; Hoogenboom and Winter, 1992; Nissim *et al.*, 1994]. The binding affinities and specificities of these fragments can be improved by mutagenesis and chain shuffling [Hawkins *et al.*, 1992; Marks *et al.*, 1992a,b]. Production of recombinant antibodies by selection from large expression libraries has the potential eventually to replace conventional methods of antibody production involving animal immunisation, and culture of hybridoma cells. Cloned antibodies may also be useful in genetic engineering of crop plants to develop novel forms of resistance to plant disease [Schots *et al.*, 1992]. Cloned antibody genes have been expressed and shown to produce functional antibodies in tobacco plants [Hiatt *et al.*, 1989]. Also, transgenic *Nicotiana benthamiana* plants expressing single chain Fv fragments of antibody specific for artichoke mottled crinkle tombusvirus (AMCV) showed a delay in symptom expression when challenged by mechanical inoculation with AMCV compared with control plants [Tavladoraki *et al.*, 1993].

Concluding remarks

MAbs are sensitive and specific tools that can be used to help answer questions in both fundamental and applied plant pathology. This review has presented some selected examples of the use of MAbs: (i) to identify species-specific molecules; (ii) to compare molecular changes in pathogen populations at the level of individual gene products; (iii) to identify structural features important in vector-pathogen or plant-pathogen interactions; (iv) to locate pathogen-specific molecules in plants; and (v) to detect and quantify crop disease.

In the future, selection of scFv fragments from antibody gene libraries should make the production of useful antibodies more efficient, and the cloned genes may prove useful in developing resistance strategies. Application of MAbs to ultrastructural studies of plant-pathogen interactions using the confocal microscope should provide increasing clarity of resolution. Application of MAbs (both idiotypic and anti-idiotypic) might also facilitate the investigation of vector-pathogen interactions, such as the location of virus-specific receptors in vector organisms; or plant-pathogen interactions such as the ultrastructural location of avirulence gene products, or identification of the corresponding host gene products.

In the more applied areas, production of pathogen-free propagation and planting material, worldwide movement of germplasm for plant breeding, and the introduction of integrated pest and disease management systems all require rapid and sensitive tests to detect, identify and quantify plant pests and diseases. Use of appropriate MAbs in an enzyme immunoassay format can meet these requirements for many pathogens.

Acknowledgements

I thank those who provided pre-prints and unpublished information, and the Scottish Office Agriculture and Fisheries Department for financial support.

References

Allison RF, Sorenson JC, Kelly ME, Armstrong FB and Dougherty WG (1985) Sequence determination of the capsid protein gene and flanking regions of tobacco etch virus: evidence for synthesis and processing of a polyprotein in potyvirus genome expression. Proceedings of the National Academy of Sciences, USA 82: 3969–3972

Altschuh D, Dubs M-C, Wiess E, Zeder-Lutz G and Van Regenmortel MHV (1992) Determination of kinetic constants for the interaction between a monoclonal antibody and peptides using surface plasmon resonance. Biochemistry 31: 6298–6304

Andreeva L, Järvekülg L, Rabenstein F, Torrance L, Harrison BD and Saarma M (1994) Antigenic analysis of potato virus A particles and coat protein. Annals of Applied Biology 125: 337–348

Atreya CD, Raccah B and Pirone TP (1990) A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. Virology 178: 161–165

Bandla MD, Westcot DM, Chenault KD, Ullman DE, German

TL and Sherwood JL (1994) Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of the tomato spotted wilt tospovirus to identify viruliferous thrips. Phytopathology 84: 1427–1431

Barker I and Torrance L (1990) The relationship between barley yellow dwarf virus content in aphids and their ability to transmit. In: Burnett PA (ed) World Perspectives on Barley Yellow Dwarf (pp. 166–188) CIMMYT, Mexico DF, Mexico

Berna A, Briand J-P, Stussi-Garaud C and Godefroy-Colburn T (1986) Kinetics of accumulation of the three non-structural proteins of alfalfa mosaic virus in tobacco plants. Journal of General Virology 67: 1135–1147

Bloomer AC, Champness JN, Bricogne G, Staden R and Klug A (1978) Protein disk of tobacco mosaic virus at 2.8Å resolution showing the interactions within and between subunits. Nature 276: 362–368

Bock KR and Woods RD (1983) Etiology of African cassava mosaic disease. Plant Disease 67: 994–995

Brown I, Mansfield J, Irlam I Conrads-Strauch J and Bonas U (1993) Ultrastructure of interactions between *Xanthomonas campestris* pv. *vesicatoria* and pepper, including immunocytochemical localization of extracellular polysaccharides and the AvrBs3 protein. Molecular Plant-Microbe Interactions 6: 376–386

Cahill DM and Hardham AR (1994) Exploitation of zoospore taxis in the development of a novel dipstick immunoassay for the specific detection of *Phytophthora cinnamomi*. Phytopathology 84: 193–200

Campbell AM (1984) Monoclonal Antibody Technology. Elsevier, Amsterdam

Caudwell A, Kuszala C and Fleury A (1988) Antigen preparation from plant tissues of pathogenic mycoplasmas (MLO) causing flavescence dorée. Journal of Phytopathology 123: 124–132

Clark MF (1992) Immunodiagnostic techniques for plant mycoplasma-like organisms. In: Duncan JM and Torrance L (eds) Techniques for the Rapid Detection of Plant Pathogens (pp. 34–45) Blackwell Scientific Publications, Oxford

Clark MF, Morton A and Buss SL (1989) Preparation of mycoplasma immunogens from plants and a comparison of polyclonal and monoclonal antibodies made against primula yellows MLO-associated antigens. Annals of Applied Biology 114: 111–124

Commandeur U, Koenig R, Lesemann D-E, Torrance L, Burgermeister W, Liu Y, Schots A, Alric M and Grassi G (1992) Epitope mapping on fragments of beet necrotic yellow vein virus coat protein. Journal of General Virology 73: 695–700

Commandeur U, Koenig R, Manteuffel R, Torrance L, Lüddecke P and Frank R (1994) Location, size, and complexity of epitopes on the coat protein of beet necrotic yellow vein virus studied by means of synthetic overlapping peptides. Virology 198: 282–287

D'Arcy CJ, Torrance L and Martin RR (1989) Discrimination among luteoviruses and their strains by monoclonal antibodies and identification of common epitopes. Phytopathology 79: 869–873

Davies KG (1994) A nematode case study focusing on the application of serology. In: Hawksworth DL (ed) Identification and Characterisation of Pest Organisms (pp. 395–413) CAB International, Wallingford, Oxford

Davis EL, Aron LM, Pratt LH and Hussey RS (1992) Novel immunization procedures used to develop monoclonal antibodies that bind to specific structures in *Meloidogyne* spp. Phytopathology 82: 1244–1250

De Ruiter GA, Bos W, van Bruggen-van der Lugt AW, Hofstra H and Rombouts FM (1994) Development of an ELISA and dot-blot assay to detect *Mucor racemosus* and related species of the order Mucorales. In: Schots A, Dewey FM and Oliver R (eds) Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification (pp. 157–164) CAB International, Wallingford, Oxford

Dewey FM (1992) Detection of plant-invading fungi by monoclonal antibodies. In: Duncan JM and Torrance L (eds) Techniques for the Rapid Detection of Plant Pathogens (pp. 47–62) Blackwell Scientific Publications, Oxford

Dewey FM and Thornton CR (1994) Fungal immunodiagnostics in plant agriculture. In: Skerrit J and Appels R (eds) New Diagnostics in Crop Sciences (in press) CAB International, Wallingford, Oxford

Dore I, Ruhlmann C, Oudet P, Cahoon M, Caspar DLD and Van Regenmortel MHV (1990) Polarity of binding of monoclonal antibodies to tobacco mosaic virus rods and stacked disks. Virology 176: 25–29

Farmer M J (1989) Studies on Monoclonal Antibodies to the Particle Proteins of a Potyvirus and a Nepovirus. Dundee University, PhD Thesis. 264 pp

Forrest JMS (1995) Monoclonal antibodies: nematodes. In: Singh RP and Singh VS (eds) Advanced Methods in Plant Pathology (pp. 429–441) CRC Press, Boca Raton

Garret A, Kerlan C and Thomas D (1991) Visualization of a luteovirus in the vector aphid's body by two gold immunolabelling techniques: a comparative study. Journal of Virological Methods 35: 93–107

Geysen HM, Rodda SJ, Mason TJ, Tribbick G and Schoofs PG (1987) Strategies for epitope analysis using peptide synthesis. Journal of Immunological Methods 102: 259–274

Gildow FE (1990) Barley yellow dwarf virus transport through aphids. Peters DC, Webster JA and Chlouber CS (eds) Proceedings Aphid-Plant Interactions: Populations to Molecules (pp. 165–177) Oklahoma State University, Stillwater, Oklahoma, USA

Gildow FE (1993) Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. Phytopathology 83: 270–277

Gildow FE and Gray SM (1993) The aphid salivary gland basal lamina as a selective barrier associated with vector-specific transmission of barley yellow dwarf luteoviruses. Phytopathology 83: 1293–1302

Gildow FE and Rochow WF (1980) Role of accessory salivary glands in aphid transmission of barley yellow dwarf virus. Virology 104: 97–108

Goding JW (1983) Monoclonal Antibodies: Principles and Practice. Academic Press, London

Goulden MG, Köhm BA, Santa Cruz S, Kavanagh TA, and

Baulcombe DC (1993) A feature of the coat protein of potato virus X affects both induced virus resistance in potato and viral fitness. Virology 197: 293–302

Gubler F and Hardham AR (1991) The fate of peripheral vesicles in zoospores of *Phytophthora cinnamomi* during infection of plants. In: Mendgen K and Lesemann D-E (eds) Electron microscopy of plant pathogens (pp.197–210) Springer-Verlag, Berlin

Hardham AR, Gubler F and Duniec J (1991) Ultrastructural and immunological studies of zoospores of *Phytophthora*. In: Lucas JA, Shattock RC, Shaw DS and Cooke LR (eds) Phytophthora (pp. 50–69) Cambridge University Press, Cambridge

Harlow E and Lane D (1988) Antibodies A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Harrison BD and Robinson DJ (1988) Molecular variation in vector-borne plant viruses: epidemiological significance. Philosophical Transactions of the Royal Society of London B 321: 447–462

Hawkins RE, Russell SJ and Winter G (1992) Selection of phage antibodies by binding affinity mimicking affinity maturation. Journal of Molecular Biology 226: 889–896

Hiatt A, Cafferkey R and Bowdish K (1989) Production of antibodies in transgenic plants. Nature 342: 76–78

Hong YG, Robinson DJ and Harrison BD (1993) Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. Journal of General Virology 74: 2437–2443

Hoogenboom HR and Winter G (1992) By-passing immunization. Human antibodies from synthetic repertoires of germline V_H gene segments rearranged *in vitro*. Journal of Molecular Biology 227: 381–388

Houwing CJ and Jaspars EMJ (1987) *In vitro* evidence that the coat protein is the programming factor in alfalfa mosaic virus-induced RNA synthesis. FEBS Letters 221: 337–342

Hu JS and Rochow WF (1988) Anti-idiotypic antibodies against an anti-barley yellow dwarf virus monoclonal antibody. Phytopathology 78: 1302–1307

Hyman LJ, Wallace A, Lopez MM, Cambra M, Gorris MT and Pérombelon MCM (1995) Characterization of monoclonal antibodies against *Erwinia carotovora* subsp *atroseptica* serogroup I: specificity and epitope analysis. Journal of Applied Bacteriology (in press)

Jordan R (1992) Potyviruses, monoclonal antibodies, and antigenic sites. Archives of Virology Supplement 5: 81–95

Jordan R and Hammond J (1991) Comparison and differentiation of potyvirus isolates and identification of strain-, virus-, subgroup-specific and potyvirus group-common epitopes using monoclonal antibodies. Journal of General Virology 72: 25–36

Kenyon J and Clark MF (1993) Detection of Molières disease MLO with monoclonal antibodies. Phytopath. Medit. 32: 78

Koenig R, Commandeur U, Lesemann D-E, Burgermeister W, Torrance L, Grassi G, Alric M, Kallerhoff J and Schots A (1990) Antigenic analysis of the coat protein of beet necrotic yellow vein virus by means of monoclonal antibodies. Journal of General Virology 71: 2229–2232

Legorburu FJ (1993) Immunogenic structure of tobacco rattle virus particles and its possible relation to vector transmission. University of Dundee, PhD Thesis. 285 pp

Lesemann D-E, Koenig R, Torrance L, Buxton G, Boonekamp PM, Peters D and Schots A (1990) Electron microscopical demonstration of different binding sites for monoclonal antibodies on particles of beet necrotic yellow vein virus. Journal of General Virology 71: 731–733

MacKenzie DJ and Tremaine JH (1988) Ultrastructural location of non-structural protein 3A of cucumber mosaic virus in infected tissue using monoclonal antibodies to a cloned chimeric fusion protein. Journal of General Virology 69: 2387–2395

Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD and Winter G (1991) By-passing immunization. Human antibodies from V-gene libraries displayed on phage. Journal of Molecular Biology 222: 581–597

Marks JD, Griffiths AD, Malmqvist M, Clackson TP, Bye JM and Winter G (1992a) By-passing immunization: building high affinity human antibodies by chain shuffling. Bio/Technology 10: 779–783

Marks JD, Hoogenboom HR, Griffiths AD and Winter G (1992b) Molecular evolution of proteins on filamentous phage. Journal of Biological Chemistry 267: 16007–16010

Massalski PR and Harrison BD (1987) Properties of monoclonal antibodies to potato leafroll luteovirus and their use to distinguish virus isolates differing in aphid transmissibility. Journal General Virology 68: 1813–1821

Matthews REF (1991) Plant Virology, Third Edition. Academic Press, London

Mayo MA and Ziegler-Graaf, V (1995) Recent developments in luteovirus molecular biology. Advances in Virus Research (in press)

McGillivery DJ, Morton RE, Yong WK, Riffkin GG, Johnson C and Adler B (1992) Confocal microscopy of the antigen-antibody reaction site in nematode larvae. Australian Veterinary Journal 69: 41–42

Miller SA, Rittenburg JH, Petersen FP and Grothaus GD (1992) From the research bench to the market place: development of commercial diagnostic kits. In: Duncan JM and Torrance L (eds) Techniques for the Rapid Detection of Plant Pathogens (pp. 208–221) Blackwell Scientific Publications, Oxford

Niesbach-Klösgen U, Guilley H, Jonard G and Richards K (1990) Immunodetection *in vivo* of beet necrotic yellow vein virus-encoded proteins. Virology 178: 52–61

Nieves RA, Ellis RP, Todd RJ, Johnson TJA, Grohmann K and Himmel ME (1991) Visualization of *Trichoderma reesei* cellobiohydrolase I and endoglucanase I on aspen cellulose by using monoclonal antibody-colloidal gold conjugates. Applied and Environmental Microbiology 57: 3163–3170

Nissim A, Hoogenboom HR, Tomlinson IM, Flynn G, Midgley C, Lane D and Winter G (1994) Antibody fragments from a 'single pot' phage display library as immunochemical reagents. The EMBO Journal 13: 692–698

Orlandi R, Güssow DH, Jones PT and Winter G (1989) Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. Proceedings of the National Academy of Sciences, USA 86: 3833–3837

Pappu HR, Pappu SS, Manjunath KL, Lee RF and Niblett CL (1993) Molecular characterization of a structural epitope that is largely conserved among severe isolates of a plant virus. Proceedings of the National Academy of Sciences, USA 90: 3641–3644

Pereira LG, Torrance L, Roberts IM and Harrison BD (1994) Antigenic structure of the coat protein of potato mop-top furovirus. Virology 203: 277–285

Perotto S, Brewin NJ and Bonfante P (1994) Colonization of pea roots by the Mycorrhizal fungus *Glomus versiforme* and by *Rhizobium* bacteria: immunological comparison using monoclonal antibodies as probes for plant cell surface components. Molecular Plant-Microbe Internations 7: 91–98

Pérombelon MCM, Jones D and Hyman LJ (1993) Assessment of blackleg inoculum on potato seed. Report of Scottish Crop Research Institute 1992 (pp. 78–82)

Priestley RA and Dewey FM (1993) Development of a monoclonal antibody immunoassay for the eyespot pathogen *Pseudocercosporella herpotrichoides*. Plant Pathology 42: 403–412

Purcifull DE and Hiebert E (1992) Serological relationships involving potyviral nonstructural proteins. Archives of Virology Supplement 5: 97–122

Quillet L, Guilley H, Jonard G and Richards K (1989) *In vitro* synthesis of biologically active beet necrotic yellow vein virus RNA. Virology 172: 293–301

Robinson MP, Butcher G, Curtis RH, Davies KG and Evans K (1993) Characterisation of a 34 kD protein from potato cyst nematodes, using monoclonal antibodies with potential for species diagnosis. Annals of Applied Biology 123: 337–347

Rochow WF (1970) Barley yellow dwarf virus: phenotypic mixing and vector specificity. Science 167: 875–878

Saito T, Yamanaka K and Okada Y (1990) Long-distance movement and viral assembly of tobacco mosaic virus mutants. Virology 176: 329–336

Saunal H, Witz J and Van Regenmortel MHV (1993) Inhibition of *in vitro* cotranslational disassembly of tobacco mosaic virus by monoclonal antibodies to the viral coat protein. Journal of General Virology 74: 897–900

Schots A, De Boer J, Schouten A, Roosien J, Zilverentant JF, Pomp H, Bouwman-Smits L, Overmars H, Gommers FJ, Visser B, Stiekema WJ and Bakker J (1992) 'Plantibodies': a flexible approach to design resistance against pathogens. Netherlands Journal of Plant Pathology 98 Suppl 2: 183–191

Schots A, Dewey FM and Oliver R (1994) Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification. CAB International, Oxford

Schots A, Gommers FJ, Bakker J and Egberts E (1990) Serological differentiation of plant-parasitic nematode species with polyclonal and monoclonal antibodies. Journal of Nematology 22: 16–23

Schots A, Gommers FJ and Egberts E (1992) Quantitative ELISA for the detection of potato cyst nematodes in soil samples. Fundamental and Applied Nematology 15: 55–61

Schots A, Hermsen T, Schouten S, Gommers FJ and Egberts E (1989) Serological differentiation of the potato-cyst nematodes *Globodera pallida* and *G. rostochiensis*: II. Preparation and characterization of species specific monoclonal antibodies. Hybridoma 8: 401–413

Schwartz Y, Boudon-Padieu E, Grange J, Meignoz R and Caudwell A (1989) Monoclonal antibodies to the mycoplasma-like organism (MLO) responsible for grapevine flavescence dorée. Research in Microbiology 140: 311–324

Seddas A, Meignoz R, Daire X, Boudon-Padieu E and Caudwell A (1993) Purification of grapevine flavescence dorée MLO (mycoplasma-like organism) by immunoaffinity. Current Microbiology 27: 229–236

Shukla DD and Ward CW (1989) Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. Advances in Virus Research 36: 273–314

Slade DE, Johnston RE and Dougherty WG (1989) Generation and characterization of monoclonal antibodies reactive with the 49-kDa proteinase of tobacco etch virus. Virology 173: 499–508

Smith HG, Stevens M and Hallsworth PB (1991) The use of monoclonal antibodies to detect beet mild yellowing virus and beet western yellows virus in aphids. Annals of Applied Biology 119: 295–302

Stead DE (1992) Techniques for detecting and identifying plant pathogenic bacteria. In: Duncan JM and Torrance L (eds) Techniques for the Rapid Detection of Plant Pathogens (pp. 76–111) Blackwell Scientific Publications, Oxford

Suzuki N, Kudo T, Shirako Y, Ehara Y and Tachibana T (1989) Distribution of cylindrical inclusion, amorphous inclusion and capsid proteins of watermelon mosaic virus 2 in systemically infected pumpkin leaves. Journal of General Virology 70: 1085–1091

Suzuki N, Shirako Y and Ehara Y (1991) Reactivities of monoclonal antibodies to cylindrical inclusion, amorphous inclusion, and capsid proteins of watermelon mosaic virus 2 with corresponding proteins of other potyviruses. Annals of the Phytopathology Society Japan 57: 706–710

Swanson MM (1992) Antigenic variation and serological relationships of whitefly-transmitted geminiviruses. University of Dundee, PhD Thesis. 259 pp

Swanson MM and Harrison BD (1994) Properties, relationships and distribution of cassava mosaic geminiviruses. Tropical Science 34: 15–25

Tamada T and Harrison BD (1981) Quantitative studies on the uptake and retention of potato leafroll virus by aphids in laboratory and field conditions. Annals of Applied Biology 98: 261–276

Tamada T, Harrison BD and Roberts IM (1984) Variation among British isolates of potato leafroll virus. Annals of Applied Biology 104: 107–116

Tavladoraki P, Benvenuto E, Trinca S, De Martinis D, Cattaneo A and Galeffi P (1993) Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack. Nature 366: 469–472

Thornton CR, Dewey FM and Gilligan CA (1993) Development of monoclonal antibody-based immunological assays for the detection of live propagules of *Rhizoctonia solani* in soil. Plant Pathology 42: 763–773

Torrance L (1987) Use of enzyme amplification in an ELISA to increase sensitivity of detection of barley yellow dwarf virus in oats and in individual vector aphids. Journal of Virological Methods 15: 131–138

Torrance L (1992a) Analysis of epitopes on potato leafroll virus capsid protein. Virology 191: 485–489

Torrance L (1992b) Serological methods to detect plant viruses: production and use of monoclonal antibodies. In: Duncan JM and Torrance L (eds) Techniques for the Rapid Detection of Plant Pathogens (pp. 7–33) Blackwell Scientific Publications, Oxford

Trifilieff E, Dubs MC and Van Regenmortel MHV (1991) Antigenic cross-reactivity potential of synthetic peptides immobilized on polyethylene rods. Molecular Immunology 28: 889–896

Ullman DE, German TL, Sherwood JL, Westcot DM and Cantone FA (1993) *Tospovirus* replication in insect vector cells: immunocytochemical evidence that the nonstructural protein encoded by the S RNA of tomato spotted wilt tospovirus is present in thrips vector cells. Phytopathology 83: 456–463

van den Heuvel JFJM, De Blank CM, Goldbach RW and Peters D (1990) A characterization of epitopes on potato leaf roll virus coat protein. Archives of Virology 115: 185–197

van den Heuvel JFJM, Verbeek M and van der Wilk F (1994) Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. Journal of General Virology 75: 2559–2565

Van Regenmortel MHV (1990) Plant viruses. In: Van Regenmortel MHV and Neurath, AR (eds) Immunochemistry of Viruses, II. The Basis for Serodiagnosis and Vaccines (pp. 505–515) Elsevier, Amsterdam

Van Regenmortel MHV and Dubs M-C (1993) Serological procedures. In: Matthews REF (ed) Diagnosis of Plant Virus Diseases (pp. 159–214) CRC Press, London

Vernon-Shirley M and Burns R (1992) The development and use of monoclonal antibodies for detection of *Erwinia*. Journal of Applied Bacteriology 72: 97–102

Wellink J, Van Lent J and Goldbach R (1988) Detection of viral proteins in cytopathic structures in cowpea protoplasts infected with cowpea mosaic virus. Journal of General Virology 69: 751–755

Werkmeister JA, Tebb TA, Kirkpatrick A and Shukla DD (1991) The use of peptide-mediated electrofusion to select monoclonal antibodies directed against specific and homologous regions of the potyvirus coat protein. Journal of Immunological Methods 143: 151–157

Yeh SD, Wang CH and Chen MJ (1992) Detection and immunological analysis of a 112K protein of papaya ringspot virus produced *in vivo*. Botanical Bulletin Academia Sinica 33: 1–8