Australasian Plant Pathology (1999) 28: 263-268

The 'pathosphere', paradigms and enigmatic pathogens

J.W. Randles

Department of Applied and Molecular Ecology, The University of Adelaide, Waite Campus, Glen Osmond, South Australia 5064 Australia

Presidential address to the Australasian Plant Pathology Society, Canberra 1999

Introduction

Plant pathology is a complex science because social, economic and political issues impinge on core technical aspects such as microbiology, biochemistry and cell biology. My experience has been that plant pathology is fascinating and exciting because of these interactions. This presidential address is an opportunity for me to reflect on the profession of plant pathology, to consider what has been done well, what could be done better, and what still needs to be done.

The biosphere of our planet can perhaps be viewed as a scientific universe in which the boundaries of knowledge are still an unimaginable distance away, and plant pathology as a subset of this scientific universe. For the purposes of this address I have decided to adopt the term 'pathosphere' to refer to that part of the biosphere which has to do with the agents of disease. I realise that I have been fortunate in being able to explore an extremely small part of this pathosphere both in Australia and abroad. To tell you of some of my experiences I propose to structure my address around a review of what can be currently recognised as the pathosphere, a discussion of what we might regard as some concepts shared by plant pathologists (paradigms), and finish with a discussion of how consideration of some of these paradigms can lead to finding appropriate ways for approaching some of the more recalcitrant problems associated with identifying and working with plant diseases.

The pathosphere

The pathosphere can be described as the known range of disease-causing parasites, or pathogens, of cellular organisms. It covers the range from molecular to multicellular parasites, but excludes the parasites of molecular pathogens such as the satellites of viruses.

As shown in Figure 1 the components of the pathosphere vary in composition, size, structure, genomic complexity and their mode of information transfer.

Prions The discovery of pathogenesis related proteins (PrP's or prions) in association with slowly developing diseases of the brain has forced pathologists to review the role of genes in the transfer of information. Prions are currently considered to be structurally altered isoforms of a host-encoded glycoprotein. In the case of the scrapie prion, the pathogenic form (PrP^{Sc}) differs from the normal form (PrP^{C}) in structure, and is thought to produce disease following inoculation by inducing the PrP^{C} to convert to the scrapie associated PrP^{Sc}. The pathogenic mechanism may therefore involve a

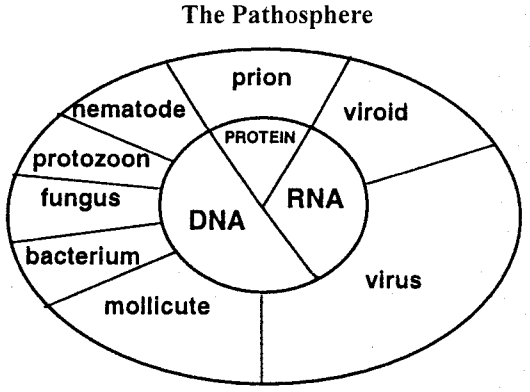

Figure 1 The 'pathosphere', illustrating the groups of known pathogens of cellular organisms in relation to their mode of information transfer.

(Volume 28 Number 3 was issued on 15 September 1999)

protein molecule passing on its conformation to other protein molecules, as no nucleic acid can be found to be associated with prions. Their high infectivity and high stability is exemplified by the recent epidemic of 'mad cow disease' in the United Kingdom and recent evidence that the agent has been transmitted to humans. Simple molecular diagnostic tests are not available and so it is not yet possible to test whether prions are associated with plant disease.

Viroids As plant pathogenic, uncoated, circular, small single-stranded infectious RNAs these also challenge the concept that the genes of a pathogen are needed for the production of disease because they do not have translatable genes or a messenger RNA function. It is therefore presumed that they are functional in the form of RNA and that they are replicated by a rolling circle system using host RNA polymerase. Their mode of pathogenesis is not known. About 30 viroids are known and they have been found only in plants.

Viruses The plant-infecting members of this large and complex group of macromolecular pathogens carry as a minimum a complement of genes required for replication, movement, structure and transmission. Viruses parasitise the host cell's translation machinery and this generally induces disease in the whole organism. They have been used as models of gene structure and function in molecular biology. A wide range of genome strategies have been described for viruses and they have been found in essentially all cellular organisms.

Mollicutes These are obligate cellular parasites without a cell wall. The mollicutes or mycoplasmas that infect plants are phytoplasmas. They are naturally transmitted only by vegetative propagation or by hemipteran vectors. Their widespread global distribution is now recognised due to the development of highly sensitive molecular diagnostics, and their taxonomy is currently a highly active area of research.

Bacteria These are prokaryotic extracellular or intracellular (fastidious) pathogens. *Agrobacterium* is probably the best known bacterial pathogen because of the utilisation of its tumour-inducing plasmid for plant genetic engineering. Extrachromosomal inheritance may be important in explaining the rapid changes in virulence and large number of biovars and ecotypes which occur with some bacterial pathogens such as *Ralstonia solanacearum.*

Fungi The fungi constitute the most diverse and economically important group of pathogens, with many modes of pathogenesis. The complexity of the fungi themselves means that fungus-host interactions are various and generally not well understood.

Nematodes and Protozoa These are animals which are included as pathogens because they produce disease by various mechanisms other than by direct feeding damage. They may be considered as the most complex members of the pathosphere because behavioural activity is superimposed on colonising activity.

The pathosphere concept reminds us that plant pathology is not only 'applied mycology'. It also reminds us that the study of diseases in conjunction with emerging technology has led in turn to the discoveries of viruses, viroids and prions. The technological developments include: the microscope; the use of bacterial filters which showed that filterable agents known as viruses existed; the use of sedimentation theory to find the sub-viral viroids, and the use of nuclear physics and molecular biology to demonstrate the insensitivity of prions to radiation, the presence of the gene for PrP in host animals and the absence of nucleic acid from infectious prion preparations.

The discovery of specific causal agents is central to understanding and eventually controlling diseases. In both animal and plant pathology there are still many diseases for which the cause is unknown. Are there still additions to be made to the pathosphere? It seems probable that the age of biotechnology will provide information that will contribute to the discovery of new pathogens.

Paradigms

The study of diseases of plants has to take into account several unique features of plants. Plant species outnumber animal species in number and variety of habitat, they are independent of external food supplies, they can be vegetatively propagated, they can reach great ages, and they do not recover from infection by a mechanism resembling an induced immune reaction. It seems probable therefore that a pool of unrecognised parasites exists in

the plant kingdom. Some of these may become new pathogens of crops. Some may remain latent but become detectable by the use of methods which detect non-host nucleotide sequences. Some may provide sequences which can recombine with other sequences to provide new genome components.

Whatever disease we work with, it could be helpful to categorise the concepts or assumptions within which we work. As I am unaware of any modern set of paradigms that apply to plant pathology, I thought that I should list a few that I find helpful to define the main features of plant pathology.

1. Disease epidemics are rarely recognised in diverse natural communities, whereas epidemics appear to be a product of host crop uniformity.
2. Epidemics can be considered as a balanced ecosystem in which control may be achieved by making one or more components suboptimal.
3. A disease should be studied on site in its natural ecosystem as well as in the laboratory, and laboratory technology needs to be made available on site.
4. Koch's postulates remain as the principle for identifying the cause of disease, but they have been modified for use with particular obligate plant pathogens by substituting the concept of 'constant association' and 'cure is associated with removal of the pathogen' for the rules devised by Koch for culturable pathogens.
5. Pathogens [except for prions] are genetically autonomous.
6. Pathogens mutate at rates greatly exceeding that of their hosts.
7. Pathogens may exist as quasispecies and this inherent variation in the pathogen population may lead to the rapid selection of new dominant variants.
8. Genetic recombination has been demonstrated for viroids and viruses ['viral genomes are .... best viewed as composed of exchangeable modules that have recombined' *Nature Structural Biology* **6**: 765, 1999].
9. Basic research is an investment and needs to be for the public good.
10. Research, knowledge and application are interdependent (Figure 2).

Diseases of unknown or uncertain cause and enigmatic pathogens

So how does a view of the pathosphere and a set of paradigms help to address problems in plant pathology. The accepted steps for studying and controlling pathogens are usually recognition of a disease, identification of its cause, development of diagnostics, epidemiology and control. But if these are not achievable, appropriate techniques need to be devised and used to achieve at least the primary goal of determining the cause (Randles 1993).

There are a number of diseases which for various reasons are regarded as having an unknown or uncertain cause. The problems that may lead to failure to find a cause include: (1) no vector is known or available (2) the disease is not transmissible experimentally (3) amounts of diseased tissue are limited (4) symptoms resemble abiotic or genetic disorders (5) symptoms are obscured or not apparent (6) incubation times are long (7) natural spread is slow or imperceptible (8) epidemiology provides no clues.

The genomic uniqueness of pathogens provides an opportunity to work with diseases of both unknown and uncertain cause. The type of nucleic acid making up the genome, its physical properties and features of the genes themselves may provide both generic and specific clues towards the presence and identity of a pathogen.

I wish to give a range of examples where targeting of the nucleic acid has given the essential information needed for identifying the cause of diseases, even to the level of isolate.

The pathogen genome as an indicator

Identification of unique species by physical methods The cause of the lethal coconut cadang-cadang disease in the Philippines was shown to be

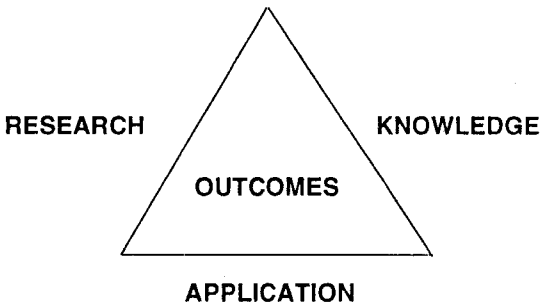

Figure 2 A representation of the inputs required to achieve outcomes in applied sciences such as plant pathology. The triangular format shows how optimisation of inputs from all sides of the triangle is necessary to maximise the outcomes (as depicted by the area of the triangle).

a viroid [CCCVd] as a result of concentrating and fractionating the nucleic acid components of leaf tissue from diseased coconut palms and showing that a low molecular weight RNA with unusual melting behaviour was uniquely associated with diseased palms (Randles 1975). Purified preparations of the RNA were infectious and reproduced the disease after mechanical inoculation to coconut palm (Randles *et al.* 1977) and cloned viroid is also infectious. These results also led to the discovery of coconut tinagaja viroid (CTiVd) in diseased palms in Guam (Boccardo *et al.* 1981). Two-dimensional gel electrophoresis is now available to identify viroids in nucleic acid extracts (Schumacher *et al.* 1983; Hodgson *et al.* 1998).

Coconut palms in Vanuatu affected with coconut foliar decay were shown to contain a unique, disease-associated DNA which by enzyme sensitivity, electron microscopy and 2-dimensional gel electrophoresis was shown to be single-stranded and circular (Randles *et al.* 1987). The DNA was shown to be associated with a new 20 nm spherical virus [CFDV] particle (Randles and Hanold 1989), and to have a unique sequence and apparently unique genome organisation (Rohde *et al.* 1990). CFDV is now a tentative member of the newly created genus *Nanovirus* which is the taxonomic home of banana bunchy top and subterranean clover stunt viruses. Koch's postulates have not been satisfied directly.

These two diseases are examples of novel pathogens being recognised in a poorly studied host species which grows in resource poor rural communities. It was necessary to transfer basic nucleic acid fractionating laboratory equipment to these areas for effective work to be done (Hanold and Randles 1998).

The next example comes from Australia in a crop which has had a long history of plant pathology research directed towards identification and control of disease.

Sugarcane striate mosaic disease has been recognised in the Burdekin River region of Queensland since 1961, and the evidence from soil treatment, symptoms and propagation via cane cuttings supported the view that it could be a virus disease. However, standard techniques to identify a virus were unsuccessful. Double stranded [ds] RNA was found in diseased samples, about 9000 nucleotides long, leading to the possibility that a rodshaped virus could be associated with the disease. Amounts of dsRNA were increased by a modified purification procedure (Choi and Randles 1997) and disease-specific clones representing part of the RNA were obtained and used diagnostically to monitor a virus purification procedure. Unusual rodshaped particles about 1 μm long were found (Choi *et al.* 1999) and work is continuing to fully describe this new sugarcane striate mosaic associated virus.

Analysis of sequences in diseased plants

Sequence information from pathogen nucleic acids can be used for analysis of pathogen genomes and to develop hybridisation and polymerase chain reaction (PCR) assays for identifying pathogen associated sequences. This information depends on having available databases which allow conserved or specific regions of genomes to be identified. Viroids have conserved regions and provide a good example of this means of finding new forms of pathogens.

Thus, even though cadang-cadang is a lethal disease of coconut palm, a new and much more severe form of disease known as 'brooming' was found in the Philippines and shown to be associated with the appearance of variant forms of the viroid in the affected palms. These variant forms differed in electrophoretic mobility, but it was not until a number of variants had been sequenced that it became clear that only two or three point mutations in the viroid were associated with this severe form of cadang-cadang (Rodriguez and Randles 1993). This provides a warning that if a pathogen or potential pathogen is present in a region, there is a possibility of it mutating to a severe form.

Sequence analysis identified sequences closely related to the coconut cadang-cadang viroid in oil palm in the Solomon Islands and this led to a survey of about 30 countries for sequences related to the cadang-cadang viroid. Many coconut and other palms contained sequences either closely or distantly related to CCCVd (Hanold and Randles 1991). Sequence analysis of some of these showed that they had major regions of nucleotide sequence similarity to CCCVd but the sequencing has not been completed (Hodgson and Randles 1999). The significance of this finding has yet to be determined. Oligonucleotide probes can be used to distinguish between the closely related CCCVd and CTiVd (Hodgson *et al.* 1998).

PCR amplification of specific regions of the ribosomal RNA gene provides a unique method of detecting and tentatively identifying phytoplasmas despite their low concentration and uneven distribution in plants. An example of the value of this method is with the recently described Mundulla

Yellows disease of *Eucalyptus* spp. in South Australia (Anonymous 1998). The detection of phytoplasma sequences in a small number of samples has provided a phytoplasma hypothesis for the disease which must now be exhaustively tested. In any case, this is the first evidence of phytoplasmas being detected in eucalypts in Australia (Y.G. Choi, J.W. Randles and K. Gibb, unpublished results).

Detection of sequences does not necessarily implicate a detected agent in the disease, but does provide a platform from which etiology can be examined. It suffers from the same inherent problem as other pathogen-specific diagnostic tests in that only pathogens which have known sequence information can be sought.

Identifying variation The genotype of pathogens may be analysed by a range of techniques. It is often useful to analyse specific genes rather than to look at overall sequence variation, because of the need for breeders to link genes of the pathogen to resistance genes in the host plant. In addition, ability to identify specific genetic markers can be useful to study the ecology of specific variants of a pathogen.

As an example, pea seedborne mosaic virus (PSbMV) has been segregated into three pathotypes by biological criteria, and two have been reported in South Australia. Allocation of isolates of PSbMV to pathotypes is laborious and dependent on the availability of differential genotypes (Ali and Randles 1997).

The genome of this virus has been well studied and regions of uniformity and variation have been identified. Molecular methods allow these conserved and variable regions to be exploited for comparing isolates at a more detailed level. The methods available include sequence comparisons, PCR and the ribonuclease protection assay (RPA). The RPA is particularly useful because a single labelled RNA transcript of a specific region can be hybridised with total nucleic acid preparations from infected plants and the degree of mismatching compared between isolates. A number of isolates can be placed into groups, and the members within the groups can be compared for genomic similarity or distance (Ali 1999).

Conclusions

This address has been directed towards both the finding of new pathogens, and better characterisation of known pathogens. At the physical or structural level, at the sequence level and the individual nucleotide level, nucleic acid methods provide novel approaches to seeking and identifying new pathogens. They provide an opportunity to employ more sensitive, more reliable, more specific and more economical methods to problem solving in plant pathology, either in conjunction with established methods, or alone.

To return to three questions that I raised in my introduction.

In Australasian plant pathology:

What has been done well?

Our populations are small, but our region has a well-educated team of practising plant pathologists who have an appreciation of the importance of their work to quarantine, industry and education.

What could be done better?

In Australasia there has been a recent loss of traditional skills and activities. Culture collections are in disarray, a review of Australian quarantine presented 109 recommendations to the government (Nairn *et al.* 1996), industry bodies are required to fund research related to the interests of their shareholders, the number of teachers of plant pathology has declined, and fewer skilled professionals are available to provide assistance overseas due to heavier commitments. Areas which are in particular need seem to be the smaller horticultural enterprises, and the environment. These, and in my opinion, even some better funded organisations such as AQIS, are failing to capture the benefits of new technology to improve plant health and production, and in particular the best techniques are not being picked up by the organisations that might benefit the most. As an example, viroid indexing of introduced germplasm has been applied to only a few species and we therefore have no knowledge of the viroid and probably also the virus status of many important commercial crop species grown in our countries. How can Pest Risk Analyses be done when the diagnostic methods that we use are probably not the best available and there is no national plan to fund research on ways to improve them?

What still needs to be done?

Most plant diseases of importance to our primary industries are shared with other countries, and information can therefore be obtained by contact with

colleagues worldwide. In contrast, we have a very poor knowledge of, and I consider a great responsibility to research parasites and pathogens unique to Australasia, and particularly those in our own native plant species and plant communities. No-one else can be expected to do this for us, and those of us with the will need to be given some relief from having to find friendly granting bodies to support the work.

Finally, it is clear that new tools are being fashioned by biotechnology, and their application to disease problems will be our challenge for the future.

References

Ali, A. (1999) – Pathology and molecular comparison of a range of pea seed-borne mosaic virus isolates. PhD Thesis. The University of Adelaide.

Ali, A. and Randles, J.W. (1997) – Early season survey of pea viruses in Pakistan and the detection of two new pathotypes of pea seedborne mosaic virus. *Plant Disease* **81**: 343-347.

Anon. (1998) – Mundulla Yellows disease of native plants; history, symptoms, distribution, impacts and likely causes. Proceedings of a workshop on the 16th September 1998 at Mundulla S.A. Department for Environment Heritage and Aboriginal Affairs, Government of South Australia.

Boccardo, G., Beaver, R.G., Randles, J.W. and Imperial, J.S. (1981) – Tinangaja and bristle top, coconut diseases of uncertain etiology in Guam, and their relationship to cadang-cadang disease of coconut in the Philippines. *Phytopathology* **71**: 1104-1107.

Choi, Y.G. and Randles, J.W. (1997) – Microgranular cellulose improves dsRNA recovery from plant nucleic acid extracts. *BioTechniques* **23**: 610-611.

Choi, Y.G., Croft, B.J. and Randles, J.W. (1999) – Identification of sugarcane striate mosaic associated virus by partial characterization of its dsRNA. *Phytopathology* **99**: 877-883.

Hanold, D. and Randles, J.W. (1991) – Detection of coconut cadang-cadang viroid-like sequences in oil and coconut palm and other monocotyledons in the South-West Pacific. *Annals of Applied Biology* **118**: 139-151.

Hanold, D. and Randles, J.W. (Eds) (1998) – Report on ACIAR-funded research on viroids and viruses of coconut palm and other tropical monocotyledons 1985–1993. ACIAR Working Paper No. 51. http://www.aciar.gov.au/viroids/viroids.htm ACIAR, Canberra.

Hodgson, R.A.J. and Randles, J.W. (1999) – Detection of coconut cadang-cadang viroid-like sequences. In *Current Advances in Coconut Biotechnology* (Eds C. Oropeza, J. Verdeil, G.R. Ashburner, R. Cardeña and J. Santamaria), *Current Plant Science and Biotechnology in Agriculture* **35**: 227-246. Kluwer Academic Publishers, Dordrecht, Boston, London.

Hodgson, R.A.J., Wall, G.C. and Randles, J.W. (1998) – Specific identification of coconut tinangaja viroid (CTiVd) for differential field diagnosis of viroids in coconut palm. *Phytopathology* **88**: 774-781.

Nairn, M.E., Allen, P.G., Inglis, A.R. and Tanner, C. (1996) – Australian Quarantine: a shared responsibility. Department of Primary Industries and Energy, Canberra.

Randles, J.W. (1975) – Association of two ribonucleic acid species with cadang-cadang disease of coconut palm. *Phytopathology* **65**: 163-167.

Randles, J.W. (1993) – Strategies for implicating virus-like pathogens as the cause of diseases of unknown etiology. In *Diagnosis of Plant Virus Diseases* (Ed R.E.F. Matthews), pp. 315-332. CRC Press, Boca Raton.

Randles, J.W., Boccardo, G., Retuerma, M.L. and Rillo, E.P. (1977) – Transmission of the RNA species associated with cadang-cadang of coconut palm and the insensitivity of the disease to antibiotics. *Phytopathology* **67**: 1211-1216.

Randles, J.W. and Hanold, D. (1989) – Coconut foliar decay virus particles are 20-nm icosahedra. *Intervirology* **30**: 177-180.

Randles, J.W., Hanold, D. and Julia, J.F. (1987) – Small circular single-stranded DNA associated with foliar decay disease of coconut palm in Vanuatu. *Journal of General Virology* **68**: 273-280.

Rodriguez, M.J.B. and Randles, J.W. (1993) – Coconut cadang-cadang viroid (CCCVd) mutants associated with severe disease vary in both the pathogenicity domain and the central conserved region. *Nucleic Acids Research* **21**: 2771.

Rohde, W., Randles, J.W., Langridge, P. and Hanold, D. (1990) – Nucleotide sequence of a circular single-stranded DNA associated with coconut foliar decay virus. *Virology* **176**: 648-651.

Schumacher, J., Randles, J.W. and Riesner, D. (1983) – A two-dimensional electrophoretic technique for the detection of circular viroids and virusoids. *Analytical Biochemistry* **135**: 288-295.