

Microbial threat– a growing challenge for plant biosecurity



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Australia is relatively free from many of the plant pathogens that seriously impact on agricultural production and natural environment in other countries. This provides a valuable competitive advantage for Australia's plant industries in terms of securing market access and maintaining lower production costs. The increasing growth in global trade, travel and tourism is exposing Australia's plant industries and environment to ever-increasing risk of exotic microbial pathogens. At risk are approximately \$14 billion per annum in crop exports¹, the environment and its associated tourism, the sustainability of regional communities with plant industries contributing approximately \$25 billion annually², and indirectly animal and human health and safety. In addition, biosecurity threats are recognised as a serious risk to global food security³.

What is plant biosecurity?

"Plant biosecurity is a set of measures which protect the economy, environment and community from the negative impacts of plant pests"⁴.

A simpler definition is "safeguarding of resources from biological threats"⁵ and biosecurity under this definition should be implemented at a national, regional and farm level. These biological threats can be defined as any organism that has the

potential to harm people's health and life, food and agriculture, the environment and the economy.

Biosecurity is often thought of as simply being quarantine – preventing the introduction of exotic organisms into a country. However, just as important to an economy such as Australia is the impact of exotic organisms on exports and access to vital markets. The mere presence of an organism in a country can halt exports and there are examples of when the presumption of presence has resulted in temporary market closure until freedom from the organism could be substantiated.

Microorganisms are well represented in these biological threats, with a large list of plant pathogens considered serious threats to Australia. Microorganisms such as fungi, bacteria, viruses, viroids, phytoplasmas and nematodes all provide new challenges for plant biosecurity which require significant investment in research and infrastructure to ensure Australia has a functional and effective plant biosecurity system.

Plant biosecurity is not a single discipline but rather the melding of many disciplines. Plant pathology is one of the core disciplines. Plant biosecurity is achieved by bringing together pathologists, economists, engineers, modellers, entomologists, mathematicians, climatologists, chemists and others to address research and operational needs as demonstrated in the National Plant Biosecurity Strategy⁴.

Biosecurity continuum

The biosecurity continuum refers to the need to operate at the pre-border, border and post-border levels if a biosecurity system is to operate effectively. By operating at these levels the system

can gain knowledge of the organisms (preparedness), have the capacity to detect and identify the organism (diagnostics and surveillance) and be able to respond (incursion management) to provide the best possible outcome for industries and the environment.

Plant pathogens can enter Australia through numerous pathways. These pathways are regulated but given the size of microorganisms it is not feasible to consider that 100% watertight border protection can be maintained. Pathways for introduction include but are not limited to tourism, flower, seed, timber and plant imports, machinery movement and natural phenomena.

Microbial threats

Microorganisms provide challenges to the Australian biosecurity system that are distinct from those caused by insects. Two of the most significant challenges are the differentiation between disease and organism and the access to reliable and accurate diagnostics.

Disease versus organism

The International Plant Protection Convention (IPPC) is established with 177 country signatories to prevent the introduction and spread of plant pests (including plant pathogens). Through the IPPC numerous *International Standards for Phytosanitary Measures (ISPMs)* have been developed that define the standards by which countries can regulate plant biosecurity (www.ippc.int). In the context of this article, when stating pests we are referring to both insect pests and plant pathogens. The ISPMs have all been developed with reference to pests (organisms) and not the disease(s) they cause.

Currently trade in a plant product can be halted due to the presence of a plant pathogen even if no disease is evident. *Why is this a problem?* If we were confident that we could diagnose every microorganism to the species (or pathovar or subspecies) level accurately and know that it is the causal agent for a known disease then it would not be an issue. In addition, our knowledge of pathogenic and saprophytic microorganisms is increasing at an unprecedented rate and this increases the complexity of pathogen diagnostics. Despite the fact that the majority of diseases for plants are caused by fungi, new and emerging diseases of plants are dominated by viruses and bacteria. Plant viruses and bacteria have been identified as the cause of more than two-thirds of the emerging infectious diseases of plants (1996–2002)⁶.

The definition of a microbial species is also changing with the continuing development of the many 'omics' and other molecular methods which have resulted in a clearer understanding of the vast genetic diversity of microflora, including fungi, bacteria, phytoplasmas, viruses and viroids. Examples of the complexity faced by the plant world are the high rate of horizontal gene transfer that occurs within and between bacterial species⁷; the generation of new and more virulent viral species through the recombination of viral genomes⁸; and the Ug99 example of fungal evolution⁹. Should a future goal of the IPPC be to only quarantine

microbial species that are known pathogens and have been shown to cause a disease? The dilemma posed is whether the biosecurity decision be based on the presence of the disease and not on the organism as occurs in the animal world (OIE, <http://www.oie.int/en/about-us/>). Without this change the implications are significant as we risk quarantining imports and losing market access for valuable exports due to the presence of organisms that are not pathogens.

Diagnostics

The problems encountered in plant bacterial disease diagnosis and microbial species identification clearly illustrate the dilemma. Industry biosecurity planning conducted by Plant Health Australia identified 56 plant pathogenic bacteria as threats to Australian agricultural and horticultural industries; 11 are considered high risk. Australia currently lacks the capability to diagnose these pathogens; many are difficult to differentiate from closely related pathovars of endemic bacteria. In 1997, an inaccurate diagnosis of the fire blight pathogen, *Erwinia amylovora*, in the Melbourne and Adelaide Botanic Gardens resulted in the false declaration that the disease was present. Losses due to trade export restrictions during this incident were in excess of \$15 million¹⁰.

False positive and false negative detections that result from inaccurate diagnostics often result in large and unnecessary costs and risks to government and to plant industries. In the past decade the inability to reliably differentiate among bacterial pathovars and between closely related species has compromised the ability to intercept and prevent the establishment of exotic bacterial pathogens into Australia. Bacterial pathogens generally offer few, if any, morphological characteristics to aid in identification. Consequently, the implementation of molecular detection and diagnosis has become the only way to safeguard against unwanted incursions of bacterial plant pathogens.

Robust diagnostics are underpinned by an intimate knowledge of the ecology, phylogeny, diversity and evolution of the target organisms at the species and/or pathovar level. Species and subspecies categorisation is fundamental to the accurate identification of the causal agent of a plant disease epidemic and critical to effective management of an exotic disease incursion¹¹. Many bacterial pathovars are difficult to identify and in some instances the taxonomy is not yet resolved¹². By definition, pathovars are distinguished by their host specificity¹³ and bioassays on plants are currently the only definitive means to differentiate between closely related pathovars. However, bioassays are fraught with difficulties, including the length of time it may take to conduct them, the facilities, materials and equipment that may be required, and the expense. Consequently, pathovar differentiation can delay diagnosis, thus precluding rapid response.

Current international research in bacterial diagnostics indicates that a bacterial genomics approach can identify genes that encode certain proteins (effectors, precursors of secondary metabolites, and so on) that are unique to the target bacterial

species or pathovar¹⁴. Examination of sequence data of hundreds of bacterial genomes has identified the enormous amount of genetic diversity between bacterial species and among genomes of strains from the same bacterial species. A multilocus molecular diagnostic approach is emerging as the preferred strategy to differentiate between pathovars and strains of bacterial species¹⁵.

Rapid, robust, low-cost and reliable molecular-based diagnostic tools for detecting exotic plant pests are the foundation for secure border protection, underpinning large-scale active surveillance programs and enabling a more rapid response to incursions. Yet these tools are not currently available for many of the microbial pathogens that threaten Australia's agricultural and horticultural industries and the environment.

Conclusion

The issue of plant biosecurity is not going to end for Australian governments, plant industries or the environment. The volume of trade is only going to increase as will the number of travellers entering Australia. Taxonomic revisions will continue to exacerbate the dilemma as our plant biosecurity system is based on the identification of the organism and not the diagnosis of the disease. Given current predictions of climate change, the severity of weather events will intensify and these changes may increase the role played by natural phenomena in moving microbial pathogens.

Through plant biosecurity initiatives, the melding of disciplines will be essential to ensure the Australian plant biosecurity system is as robust as possible and can respond to any threat at the pre-border, border or post-border level.

References

1. Australian Bureau of Agricultural and Resource Economics [ABARE] (2010) Australian commodities June quarter 2010, p. 141, Commonwealth Government, Canberra, ACT, Australia.
2. Australian Bureau of Statistics [ABS] (2008) Value of Agricultural Commodities Produced, Australia, 2007–08. Catalogue no. 7503.0, Canberra, ACT.
3. Strange, R.N. and Lodovica Gullino, M. (2010) The role of plant pathology in food safety and food security. Springer, ISBN 978-1-4020-8931-2.
4. Plant Health Australia (2010) National Plant Biosecurity Strategy. Plant Health Australia, Canberra, ACT, Australia.
5. Sharma, S.B. (2008) Global issues in plant biosecurity: opportunities and challenges for Nematologists. Proceedings of the 5th International Congress of Nematology, Brisbane, Queensland, Australia; page no 71.(abstract).
6. Anderson P.K. *et al.* (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol. Evol.* 19, 535–544.
7. Koonin, E.V. and Wolf, Y.I. (2008) Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. *Nucleic Acids Res.* 1–32.
8. Singh, R.P. *et al.* (2008) Discussion paper: the naming of Potato virus Y strains infecting potato. *Arch. Virol.* 153, 1–13.
9. Singh, R. *et al.* (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Ann. Rev. Phytopathol.* 49, 465–481.
10. Rodoni, B., Merriman, P., McKirdy, S. and Wittwer, G. (2006). Costs associated with fire blight incursion management and predicted costs of future incursions. 10th International workshop on fire blight. Bologna, Italy, July 5-9, 2004. *Acta Horticulturae* 704:55-61.
11. Buckley, M. and Roberts, R.J. (2006) Reconciling microbial systematics and genomics. Report of a colloquium sponsored by the American Academy of Microbiology. American Society Microbiology, Washington, DC.
12. Alvarez, A.M. (2004) Integrated approaches for detection of plant pathogenic bacteria and diagnosis of bacterial diseases. *Ann. Rev. Phytopath.* 42, 339–366.
13. Agrios, G.N. (2005) Plant Pathology. Fifth Edition. Academic Press, New York, USA.
14. Vincelli, P. and Tisserat, N. (2008) Nucleic acid-based pathogen detection in applied plant pathology. *Plant Disease* 92, 660–669.
15. Lang, J.M. *et al.* (2010) Genomics-based diagnostic marker development for *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. *Plant Disease* 94, 311–319.

Biographies

Dr Simon McKirdy (PhD) is the CEO for the Cooperative Research Centre for National Plant Biosecurity (CRCNPB). Dr McKirdy is a plant pathologist and has published widely in the area of plant virology, addressing a range of crops across horticulture, grains, legumes and viticulture. Dr McKirdy's experience in plant biosecurity includes: development and review of import risk analyses, development and implementation of diagnostic capacity, development and operation of surveillance strategies, active participation in responses to incursions of exotic plant pests at both state and national level and development of industry biosecurity plans.

Ms Jane Moran is Deputy Research Director Biosciences (Victorian Department of Primary Industries) and has over 35 years experience as a plant virologist. She has responsibility across plant and animal health biosecurity research and development projects and diagnostic services. Ms Moran is also a program leader with the CRCNPB. She is currently chair of the subcommittee on plant health diagnostic standards (SPHDS), a subcommittee of Plant Health Committee. SPHDS is charged with the development of national diagnostic protocols, guidelines for laboratory accreditation, diagnostic training and the development of a national diagnostic strategy. During her career Jane Moran has been involved in the development of the Biosecurity Strategy for Victoria and the VicDPI R&D investment strategy.

Dr Brendan Rodoni (PhD) is a senior research scientist with Victorian Department of Primary Industries Agribio and has over 25 years experience as a plant microbiologist with a focus on the detection and epidemiology plant viruses and phytopathogenic bacteria of temperate and tropical crops. Dr Rodoni hold a joint appointment with La Trobe University as a Senior Research Fellow and is a Honorary Staff Member with the Melbourne School of Land and Environment (MSLE) at The University of Melbourne

Dr Shashi Sharma is Director, Plant Biosecurity, Department of Agriculture and Food, Government of Western Australia. He is Chairman of three industry committees: Grains Industry Biosecurity Committee, Horticulture Industry Biosecurity Committee, and the Bee Industry Consultative Committee. Dr Sharma is Adjunct Professor at the School of Biological Sciences and Biotechnology, Faculty of Natural Sciences, Murdoch University, WA, Australia. He has been internationally active in the area of pest management for the last 35 years.