
Preface

Pathogens pose a threat to plants in natural communities (i.e., forests, grasslands), horticultural commodities, or cultivated crops. Risks of pathogen spread have increased with increased human mobility and the globalization of trade. In addition, factors such as environmental changes (local or global climate fluctuations) and changes to pesticide legislation impact on whether pathogens and their vectors establish in different habitats and the selective pressures that will give rise to new pathotypes and pesticide- or antibiotic-resistant variants. Damages caused worldwide by either emerging, re-emerging or endemic pathogens are significantly important. The International Plant Protection Convention, Regional and National Plant Protection Organizations, have developed phytosanitary measures to prevent the spread of regulated pathogens (particularly quarantine pathogens) between countries in order to protect agricultural and natural plant systems.

Safeguarding plant biosecurity relies heavily on the early detection and diagnosis of the pathogen. Other than diagnoses based on morphological characteristics, diagnostic methods can be separated into three main categories: bioassay, serological and molecular methods, and sometimes a combination of these methods will be used. Since the late 1970s, the serological method of ELISA, using polyclonal and especially monoclonal antibodies, has been the method of choice for most diagnostic laboratories, due to its cost effectiveness and capacity to provide reliable detection and diagnosis for a large number of samples. However, over the past decade an increasing number of DNA/RNA-based assays, particularly PCR-based assays, are routinely used in diagnostic laboratories because of their increased sensitivity and specificity, the relative ease with which tests can be developed, their adaption to detect multiple targets, their requirement for minimal quantities of target, and their capacity to be automated for high-throughput testing. Moreover sequencing has contributed considerably to the increased knowledge of plant and microbial genomes and is now widely used either as stand-alone methods or in addition to other methods for diagnosis. Techniques such as end-point (conventional) PCR, real-time PCR, and diagnostic microarrays are versatile and can be used as either a generic or species-specific detection/diagnostic method. One of their drawbacks, however, is their reliance on prior knowledge of the genome of the target pathogen or pathogens. The rapid evolution of bioinformatics and computing technology to analyze very high numbers of complex datasets will make next-generation, high-throughput parallel sequencing platforms (also known as deep sequencing) accessible as a detection and diagnostic method. The application of these metagenomic approaches to diseased material offers the possibility to identify pathogens that have yet to be fully characterized or described. Importantly, recent advances in plant pathogen diagnoses have delivered field deployable portable diagnostic systems that do not require thermal cycling equipment. This allows rapid on-site identification of pathogenic agents, thereby passing the need for laboratory-based analysis. The development of any diagnostic assay requires thorough validation to ensure for example sensitivity, specificity, repeatability, and reproducibility and that the assay is fit for purpose.

This second edition of *Plant Pathology Techniques and Protocols* covers diagnostic methods that are currently used in laboratories for a broad range of plant species and matrixes. These include serological and molecular methods that have one or more of the

following characteristics: suitability for high-throughput testing, detection of a group of pathogens or of sometimes uncharacterized pathogens, detection and identification of specific pathogens, and high sensitivity. Qualitative and quantitative tests are described, as well as recently developed cutting-edge diagnostic methods. These chapters target an audience of plant pathologists and molecular biologists who will find information on how to perform the tests in their laboratories. Also provided is background information on many pathogens, which are endemic, nonendemic, or emerging and with different lifecycles that cause diseases of significant importance in a wide variety of hosts. Finally I would like to thank all authors that have contributed to this second edition of *Plant Pathology Techniques and Protocols*.

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