METHODS TO DETECT SEED-TRANSMITTED PLANT PATHOGENS IN CORN: A PERSPECTIVE FOR THE FUTURE

Dr. Gary P. Munkvold, Professor
Department of Plant Pathology & Microbiology
Seed Science Center
Iowa State University, Ames, IA 50011 USA
munkvold@iastate.edu

Seed-transmitted pathogens are a concern in many crops, due to their effects on crop production as well as their potential to be spread to new geographical areas through the movement of seed. Maize seed is frequently moved across international borders, both during product development and in commercial trade, therefore the relevance of seed health testing is widely recognized. The needs for seed health testing are dynamic because of shifting populations of plant pathogens; the recent outbreak of *Maize lethal necrosis* in Africa is a striking example. Cases such as this underscore the importance of accurate, accessible seed health testing methods. Unfortunately, the implementation of standardized, validated phytosanitary seed health testing is slow; it is not responsive to rapidly changing needs and it lags behind the most current technologies available. For example, pathogen detection methods based on polymerase chain reaction (PCR) have been developed for hundreds of pathogens, and are in use by industry and regulatory agencies, but very few of these methods have been standardized, validated and approved for phytosanitary seed testing by internationally recognized bodies.

The potential advantages of PCR-based tests are not in dispute, considering the potential for accuracy, rapidity, quantification, objectivity, and simultaneous detection of multiple pathogens (e.g., Mumford et al., 2006; van Doorn et al., 2009). Many of these methods are very useful for quality control purposes, but their implementation in phytosanitary certification has been slow and inconsistent. For an assay to be useful in phytosanitary seed health testing, the scope of organisms that will react positively must be carefully investigated and defined, the assay must be demonstrated to be effective with naturally infected seeds, results must be compared against existing (non-PCR) methods, and the entire procedure must be shown to provide repeatable results with seedlots of diverse origin. A key obstacle to the effective implementation of PCR-based tests is a poor understanding of the relationship between test results and the actual risk of disease or pathogen introduction.

This relationship between test results and pathogen risk is affected by numerous factors. Although nucleic-acid based methods such as PCR are potentially highly sensitive, their sensitivity can be impaired by the difficulty in extracting high-quality DNA or RNA from seeds. Many PCR-based assays that are very effective for leaf tissue perform poorly on seed extracts, due to the occurrence of inhibitory compounds in seed extracts, which interfere with PCR reactions. This problem has been addressed by altering extraction methods, or using enrichment methods such as magnetic-capture hybridization (Ha et al., 2009). Extraction steps must be developed specifically for infected seeds, and must be diligently followed. In addition, sample

size has a significant influence on the likelihood of detecting low incidences of contamination, yet sample sizes are not always designed around risk assessment for specific pathogens. On the other hand, nucleic-acid based tests can be perceived as “too sensitive”. One reason for this is the possibility of amplifying nucleic acids from non-viable pathogens. There are effective strategies for addressing this, such as BIO-PCR (e.g., Schaad et al., 2007) or the use of propidium monoazide, but these approaches have limitations and they add extra steps; an ideal solution is not yet evident. Another issue, particularly for viral diseases, is that virions outside the embryo can be detected but may not be capable of being transmitted to seedlings. This question is currently extremely relevant in relation to MCMV.

PCR-based tests also can be subject to specificity issues. An assay can be validated against only a finite number of non-pathogenic strains that may be closely related to the target pathogen. There is always a risk that an untested strain of a closely related taxon will falsely react positively with the assay. This problem has possibly been most evident in relation to *Pantoea stewartii* in maize (Block et al., 2011). False positive results for this pathogen can (and have) led to costly delays and rejections of seed shipments, and the needless destruction of valuable seedlots. Conversely, an assay can be validated against only a finite number of strains of the target pathogen. If these strains do not adequately represent the diversity of seedborne pathogenic strains, the assay may be too specific, detecting only a subset of pathogenic strains; false negative tests will be the result. These issues underline the need for extensive validation studies preceding the inclusion of PCR-based assays in phytosanitary testing protocols.

Risk assessment is a universally accepted component of Pest Risk Analysis that is used to develop phytosanitary regulations, and components of risk assessment often have been the basis for the development of thresholds for seedborne pathogens. Our understanding of the connection between seed inoculum levels and seed transmission frequency is dependent on accurate measurement of seedborne inoculum levels using seed health tests. In fact, research that establishes the disease risk associated with specific seedborne inoculum levels is a mainstay of seed pathology. However, this area of research has not been actively pursued with regard to inoculum levels as measured by PCR-based assays. Given that the sensitivity & specificity of PCR-based methods may differ from those of traditional methods, research is needed to specifically establish the relationships between results of these methods and disease risk. Unfortunately, information is still lacking in this area. A strong research effort (and funding to support this effort) will be needed in order to establish this relationship, not only for maize, but also for many other crops. Even as we take stock of the uncertainties of PCR-based assays, newer technologies for seed health testing methods are coming on the scene with many similar unanswered questions.

References

Block, C.C., Shepherd, L., and Munkvold, G.P. 2011. Comparison of nine PCR primer sets designed to detect *Pantoea stewartii* subsp. *stewartii* in maize. Phytopathology 101:S16

Ha, Y., Fessehaie, A., Ling, K. S., Wechter, W. P., Keinath, A. P., and Walcott, R. R. 2009. Simultaneous detection of *Acidovorax avenae* subsp. *citrulli* and *Didymella bryoniae* in cucurbit seedlots using magnetic capture hybridization and real-time polymerase chain reaction. Phytopathology 99:666-678.

Mumford, R., Boonham, N., Tomlinson, J., and Barker, I. 2006. Advances in molecular phytodiagnostics – new solutions for old problems. Eur. J. Plant Pathol. 116:1–19

Schaad, N. W., Berthier-Schaad, Y., and Knorr, D. 2007. A high throughput membrane BIO-PCR technique for ultra-sensitive detection of *Pseudomonas syringae* pv. *phaseolicola.* Plant Pathol. 56:1–8

van Doorn, R., Szemes, M., Bonants, P., Kowalchuk, G.A., Salles, J.F., Ortenberg, E., and Schoen, C.D. 2007. Quantitative multiplex detection of plant pathogens using a novel ligation probe-based system coupled with universal, high-throughput real-time PCR on OpenArrays. BMC Genomics 8:276.