

Plant Disease Molecular Diagnostic Initiative

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Executive Summary:

Plant pathogens cause billions of dollars of damage to crop production every year in Illinois. Because of this extensive damage it is essential that new technologies be applied to assist growers in managing plant diseases. Currently plant diseases are managed using chemical pesticides, resistant plant varieties, and crop rotation strategies. No matter what control strategy is employed, an assessment of control effectiveness is essential to determine what pathogen types are in the field and if these pathogen populations are increasing or decreasing over time. Plant pathologists spend enormous amounts of time and money identifying and counting organisms or rating disease severity. Pathogen detection needs to be performed in breeding programs, research labs, and as extension services. Recommendations to producers depend directly on the accuracy and speed of pathogen detection.

In this project we purchased equipment to aid in the rapid detection of plant pathogens. We used the funds to purchase a Zeiss microscope that speeds the counting soybean cyst nematodes, a microplate spectrophotometer that can perform high-throughput immunological and DNA quantification assays and two ABI sequence detection systems that can quickly quantitate pathogen DNA. This equipment has been extensively used for the detection of a wide range of plant pathogens and the diagnostic assays devised have made a significant impact on the speed and accuracy of plant pathogen detection in Illinois and the United States.

Primary Objectives and Goals

To devise and establish rapid, high throughput diagnostic tests for plant pathogens

- A. By computer assisted microscopy
- B. By High throughput spectrophotometer-based assays
- C. By Quantitative real-time polymerase chain reaction (PCR) assays

The opportunity that drove this project was the commercial development of new high-throughput instrumentation that would allow the detection very small numbers of plant pathogens in a rapid and cost effective manner. The project provided the funds to purchase the instrumentation and the collaborating investigators used this equipment to optimize plant pathogen assays. The assays and target pathogens that can now be detected are described below.

Outcomes

Soybean Cyst Nematode Detection:

Two methods were devised to detect soybean cyst nematode (SCN), a microscopy method and one based upon the use of the real-time PCR technique. This method is also called quantitative PCR (QPCR). While both methods were successful, the QPCR-based assay has been the most useful.

To conduct QPCR assays we purchased an ABI 7900HT and an ABI 7000 sequence detection system, which performs QPCR to detect any target DNA molecule. The ABI 7000

system processes 96 samples at once and is used for assay development; while the 7900HT processes 384 samples per run and is used for high-throughput sample processing using optimized assays.

We have devised and published an SCN QPCR assay that can detect a single SCN at any developmental stage (Bekal et al 2003 and Lambert et al 2005). This assay is so specific and sensitive that it is currently being evaluated as a “standard test” to confirm a nematode is SCN and not a closely related species. This new SCN molecular assay has also proved effective at monitoring SCN infection levels in infested soybean roots by detection of the pathogen in DNA preparations of field soybean roots; thus our SCN QPCR assay is a viable method to measure SCN infestation levels in the natural field populations.

The real-time PCR machine has also been a boon for our research on detecting virulent soybean cyst nematodes (SCN) (nematodes capable of infecting resistant soybean plants). In one project, we have used QPCR-based molecular markers to add markers to a genetic linkage map for SCN (Atibalentja et al 2005). The ability to genotype very small amounts of nematode DNA has allowed us to initiate our genetic studies of SCN. In fact, we have recently been awarded a large USDA-NRI grant to add markers to our SCN genetic map using QPCR based assays. This project is only possible because of the real-time PCR instrumentation purchased from this grant.

Impact:

The ability to map any SCN marker to the SCN genome is essential for map-based cloning of virulence genes. The ability to understand the genetics of SCN virulence, combined with the ability to quickly predict SCN virulence and monitor these populations over time, will allow us to advise growers on which SCN resistant soybean varieties to grow to control the nematode. The ability to rotate SCN resistant soybean will prevent the build up of highly virulent SCN populations and help preserve SCN resistant soybean germplasm.

Fungal and viral disease detection:

Dr. White’s laboratory studies *Fusarium* ear rot in corn, and routinely uses ELISA to detect mycotoxins in corn grain. These toxins are deadly to animals, including humans, in high concentrations. The White laboratory performs over 9000 mycotoxin assays every year in the process of breeding corn resistant to *Fusarium*. The ELISA plate reader and plate washer is the rate-limiting step in performing, thus the acquisition of a new high throughput 96-well plate spectrophotometer has allowed Dr. White’s breeding program to continue.

The ABI 7900HT and 7000 sequence detection systems have also been used to detect fungal pathogens. We are now able to detect brown stem rot (BSR) of soybean caused by *Philophora gregata*, Aphanomyces root rot (ARR) of alfalfa caused by *Aphanomyces eutieches*, as well as, Phytophthora rot on pumpkin, *Fusarium solani f.sp. glycines*, which causes sudden death syndrome in soybean and *Phakopsora pachyrhizi*, the causal agent of soybean rust.

In addition to fungal pathogens, our QPCR equipment has also been effective for developing assays to detect, Alfalfa mosaic virus, Bean pod mottle virus, Soybean dwarf virus, Soybean mosaic virus, Tobacco ringspot virus, Tobacco streak virus. QPCR was the only reliable method available for detection of Alfalfa mosaic and Soybean dwarf viruses. Alfalfa mosaic virus is highly variable and Soybean dwarf virus is present in plants at very low titers. These factors have made the production of diagnostic immunological reagents for the viruses very difficult. Using nucleotide sequence information available in GenBank, it was possible to design assays that detected all known isolates of the viruses. In non-epidemic years, the incidence of

the two viruses is very low. Because of the sensitivity of the QRT-PCR assays, it was possible to design sampling and detection procedures that allowed the estimation of virus incidence in grouped samples, which reduced labor and reagent costs.

Impact: Overall, the ability to detect plant pathogens allows researchers to investigate the biology, ecology, and infection processes of these poorly understood pathogens. Such knowledge will be essential to improve integrated management of these diseases. This initiative has had a wide impact, showing benefits to farmers, plant breeders, and researchers studying plant pathogens. The SCN diagnostic tests are helpful for breeding for resistance, for monitoring nematode levels in the field and for studying and monitoring the build up of destructive and virulent strains of SCN. The ability to detect low levels of fungal pathogens has shown to be important for monitoring the invasion and spread of emerging pathogens, i.e. the use of QPCR has been essential for tracking the movement of soybean rust and for the accurate diagnosis of this potentially destructive plant pathogen. For both SCN and for SDS disease the use of QPCR has been useful for measure pathogen biomass *in planta*, thus providing new tools to measure pathogen level, which is critical for breeding for plant disease resistance.

Publications:

Atibalentja, N., Bekal, S., Domier, L.L., Niblack, T.L., Noel, G.R., and K. N. Lambert. 2005. A genetic linkage map of the soybean cyst nematode, *Heterodera glycines*. *Molecular General Genomics*, 273:273-381.

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Kelly, K., A. G. Hager, K. N. Lambert, and D. E. Riechers. 2004. Quantitative expression analysis of GH3, a gene induced by plant growth regulator herbicides in soybean. *J. Agricultural and Food Chemistry* 52: 474-478.

Gao X., K. N. Lambert, T. A. Jackson, S. Li, G. L. Hartman and T. L. Niblack. 2004. Detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots using real-time quantitative polymerase chain reaction. *Plant Dis.* 88: 1372-1380.

Grants leveraged:

Lambert, K.N. Functional analysis of a nematode chorismate mutase and other parasitism genes. U. S. Department of Agriculture, National Research Initiative Competitive Grants Program. 2002-2004. \$165,000.

Niblack, T. L., K.N. Lambert, & G. R. Noel. Development of a marker for resistance breaking populations of the soybean cyst nematode. North Central Regional Integrated Pest Management Competitive Grants Program. 2003-2005. \$80,000.

Lambert, K.N., Niblack, T., Domier, L. Hudson, M. Bird, D. Whole genome analysis of the soybean cyst nematode. U. S. Department of Agriculture, National Research Initiative Competitive Grants Program. 2006-2008. \$611,842.