Promotion of Turf Health Through Early Pathogen Detection–*Development of a Turf PathoCHIP*

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Turfgrass and Environmental Research Online
Volume 12, Number 2 | March–April 2013

Objectives:

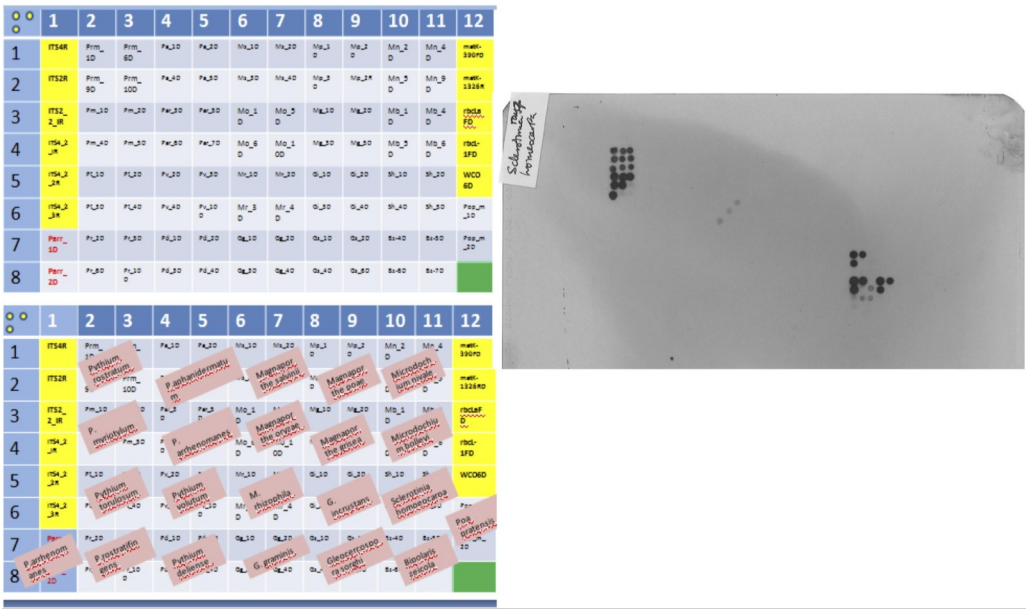
To develop and implement a highly sensitive DNA macroarray system––"Turf Patho–CHIP" for rapid detection of known and emerging turfgrass pathogens, based on the in–ternal transcribed spacer sequences of the rRNA genes that are used for DNA barcoding of fungi.

Fungi constitute the majority of pathogens that infect and damage turfgrasses. Over two hundreds fungal and fungus–like species have been recognized as turf pathogens, many of which are understudied. Early detection and rapid pathogen identification is essential for turf disease management. Identification of turfgrass pathogens poses a challenge because different pathogens may infect the same host concurrently and may produce similar symptoms. Traditionally, turfgrass diagnosticians use direct observations or culturing of pathogens from diseased plant samples to make a diagnosis. These methods are often time consuming and insufficient to identify pathogens to the species level.

DNA PathoCHIP is molecular technique that offers a fast, culture–independent alternative for the diagnosis of turfgrass pathogens from field samples. The advantage of the technique is its remarkably high throughput compared to other detection methods. Hundreds of different pathogens can be simultaneously detected with one array in one reaction within a few hours.

Most of the PathoCHIP (DNA array) platforms in use today have high specificity but reduced signal intensities. We initiated a study in 2009 to optimize the technique for use in detection of turfgrass pathogens. The goal was to develop a novel technical approach that could increase the sensitivity of a PathoCHIP to enhance its early pathogen detection power, while

Figure 1. Dimeric probes (D, DA) are more sensitive than monomeric probes (M). Dimeric probes can detect as low as 0.01 fg pathogen DNA, while monometic probes require 1000 times more DNA for a reliable detection for dollar spot, *Sclerotinia homoeocarpa*.



TERO Vol. 12(2):18–19 | March–April 2013
USGA ID#: 2011–07–417
TGIF Number: 219648

maintaining the detection specificity to ensure accurate pathogen identification. We found that dimeric oligonucleotide probes provided a low measurement variation and superior signal intensity (Fig. 1). The new technique was remarkable in detecting low quantities of pathogen, which was a thousand times more sensitive than the PCR detection technique. The method was also successfully validated with target species infected turfgrass or soil materials vis–à–vis disease free materials. Results of diagnostic array method improvement is published in J. Microbiological Method in 2011: Njambere, E. N., Clarke, B. B., and Zhang, N. 2011. Dimeric oligonucleotide probes enhance diagnostic macroarray performance. J. Microbiological Methods 86: 52–61. DOI:10.1016/j.mimet.2011.03.015

We also embarked on a sample collection exercise for important diseases of turfgrass and microbes co–inhabiting in turfgrass. Great progress has been made in this area. We have collected over 200 pathogen and pathogen strains, sequenced the ITS region of the rRNA gene (for probe design) and fully identified the pathogen to species and subspecies level. The purpose of this exercise is to use the ITS sequence to design sequence specific probes for all important pathogens infecting turfgrass. Starting 2011, different methods (bioinformatics software based and direct visualization) are being devised on how to detect the signature probes. This process requires all known related species sequence to be aligned and compared with sequences of target pathogen.

A total of 246 probes for 55 fungal, oomycete pathogens, 6 grass hosts, and 2 bacterial species have been designed and printed on the multi–pathogen diagnostic array (PathoCHIP). The array is currently being tested with various pathogens and field samples (Figs. 2–3). The process will also be validated with microscopy, culturing and real–time PCR. Once validated, the PathoCHIP will be ready for use in Plant Diagnostic Clinics.

Figure 2. Array design and detection results for dollar spot pathogen (from Njambere, Clarke and Zhang, 2011, *J. Microbiological Methods*).

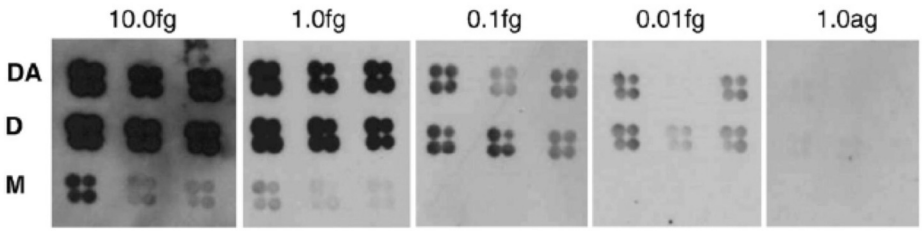
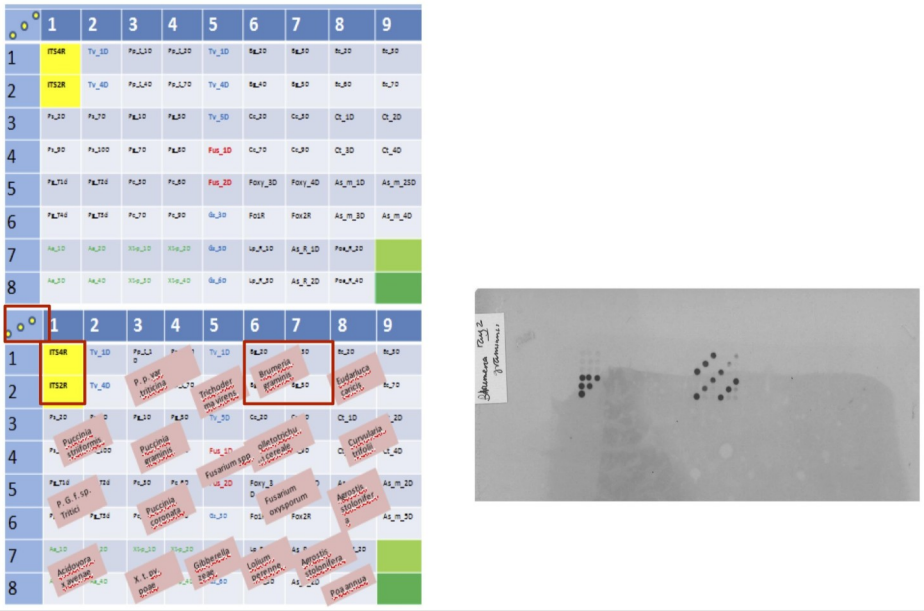


Figure 3. Array design and detection results for powdery mildew, *Blumeria graminis*.



Summary Points

- A database of 200+ turfgrass pathogenic fungal strains have been built. All pathogens have their ITS region sequenced (barcoded).
- The PathoCHIP (array) system has been optimized for sensitivity.
- Design of 246 probes specific to 50+ target pathogens of turfgrass is completed
- A full research paper has been published in *J. Microbiological Method* on diagnostic array method improvement.

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