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

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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.

There are numerous fungal pathogens that infect and damage turfgrasses. Based on a very conservative estimate, about 200 fungal species were recognized as turfgrass pathogens causing 40 different turf diseases. 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 a 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 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.

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

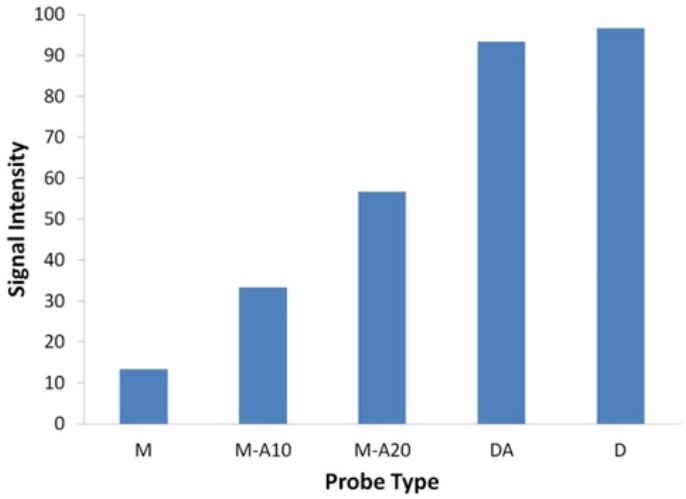

Figure 1. Dimeric probes (D, DA) are more sensitive than monomeric probes (M). Di–meric probes can detect as low as 0.01 fg pathogen DNA, while monometic probes require 1000 times more DNA for a reliable

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86: 52–61. DOI:10.1016/j.mimet.2011.03.015, and in Mycologia: Zhao, S., Clarke, B. B., Shen, Q., Zhang, L., Zhang, N. 2012. Development and application of a TaqMan real–time PCR assay for rapid detection of Magnaporthe poae. Mycologia 104:1250–1259. DOI:10.3852/11–365

A patent has been filed for the technique developed:

Zhang, N. 2013. "DIMERIC DIAGNOSTIC ARRAYS" RU Docket #2011–133, U.S. Publication No. 2013–0023445.

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, Table 1). The array has been tested with various pathogens and field samples (Fig. 2). The PathoCHIP is ready for the next phase, which is to develop a portable tool for onsite detection of turf pathogens that can be easily used by Plant Diagnostic Clinics and golf course superintendents.

Summary Points

- Completed design for a TurfPathoCHIP that can detect 55 turfgrass pathogens.
- The technique has been published, presented, and filed for patent.
- A database of over 200 turfgrass pathogenic fungal strains has been built.
- This project is completed and we are ready for the next phase—To develop a portable tool for onsite detection of turf pathogens. .

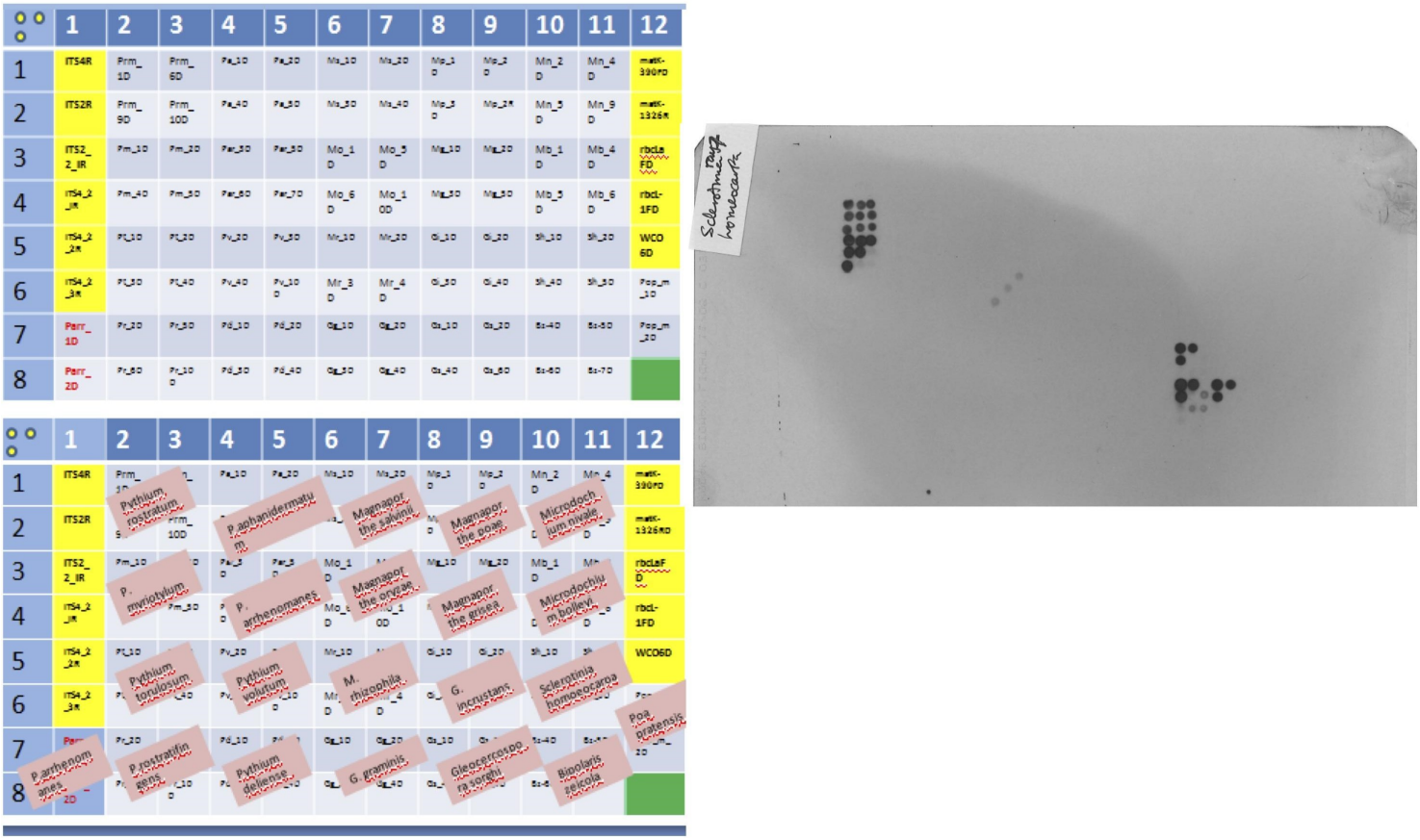

Figure 2. Array design and detection results for dollar spot pathogen.

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