Detection of Low Numbers of *Phakopsora pachyrhizi* Spores by Quantitative Real-Time PCR

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Introduction

Detection and quantification of airborne *Phakopsora pachyrhizi* urediniospores is a critical component of early warning systems and epidemiological models. Quantitative real-time PCR (qPCR) is extremely sensitive and specific, allowing for fast, reliable detection. We describe efforts to determine the detection limits of our qPCR system, as well as the application of this technology to field samples from Florida during the 2006 growing season.

Materials and Methods

Detection Limit Experiment

- *Phakopsora pachyrhizi* urediniospores collected at NFREC, applied to Vaseline-coated slides.
- Spores picked from slides were placed in FastDNA Lysing Matrix A tubes (Qbiogene, Inc.) in groups of 1, 4, 8, 16, and 100 spores, four replicates each.
- DNA extracted by FastDNA SPIN kit protocol, precipitated in ethanol.
- Quadruplicate 5μl DNA samples tested by qPCR per extraction, primers Ppm1/ Ppa2 and the FAM probe described by Frederick, et al. (2002).
- Experiment repeated once.

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- 22 locations in 18 counties.
- 408 Vaseline-coated slides (from 22 passive traps) washed with xylene and ethanol, extracted by FastDNA.
- 408 samples (from 22 rainwater traps) filtered through 8μm membranes, extracted by FastDNA.
- Extracts diluted 1/10 and tested by qPCR as above.

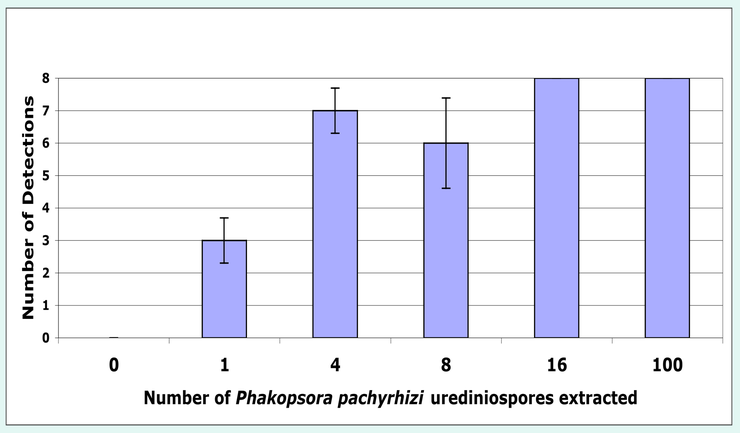

Fig. 1. Number of detections of *Phakopsora pachyrhizi* using qPCR, for eight replications of each of the six treatments. A positive reaction for any quadruplicate 5μl sample resulted in detection.

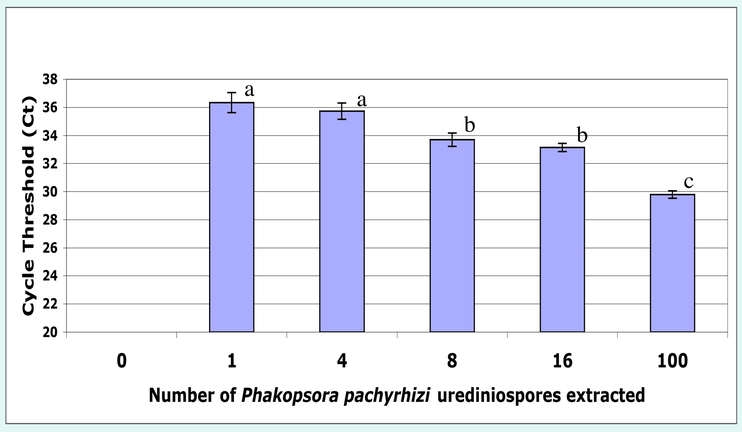

Fig. 2. Mean cycle threshold (Ct) in qPCR for eight replications of each treatment. Treatments consisted of the given number of *Phakopsora pachyrhizi* urediniospores.

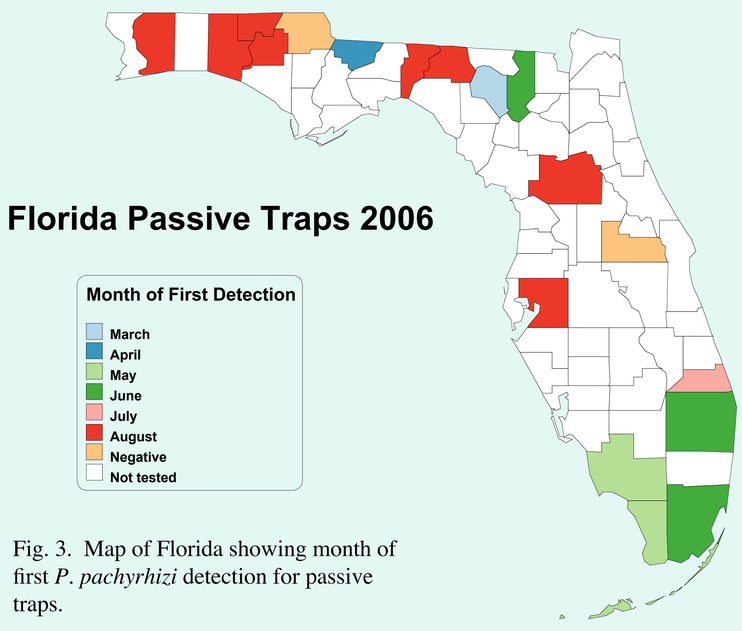

Fig. 3. Map of Florida showing month of first *P. pachyrhizi* detection for passive traps.

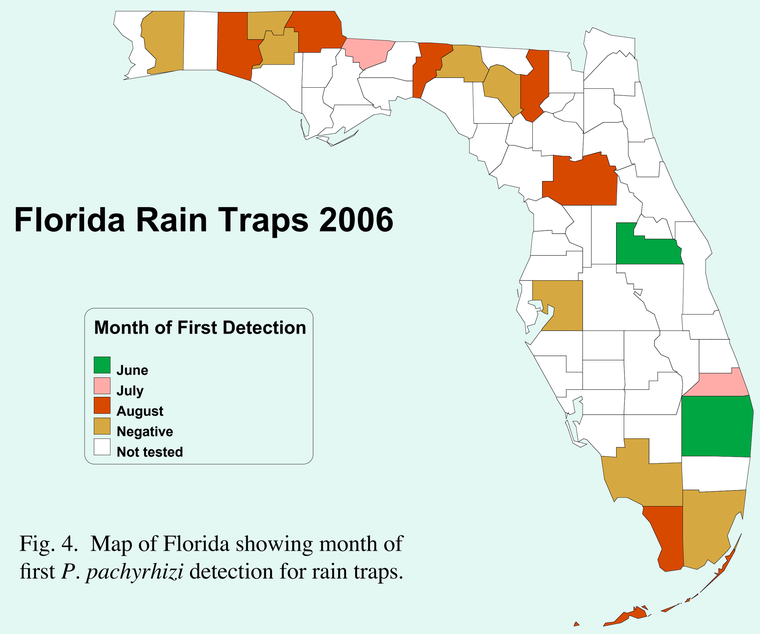

Fig. 4. Map of Florida showing month of first *P. pachyrhizi* detection for rain traps.

Results and Conclusions

Detection Limit Experiment

- Detection was positive 38% for single spore extractions, 88% for 4 spores, 75% for 8 spores, 100% for 16 spores and 100% for 100 spores (Fig. 1).
- Ct values did not differ between single and 4 spores, but were different between 4 and 8 spores, and again different between 16 and 100 spores ($p<0.05$) (Fig. 2).
- Detection was positive for low numbers of spores, but not consistent for single spores.

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- 44 passive trap samples were identified as positive.
- 17 rainwater trap samples were identified as positive.
- Positive samples from passive traps were identified as early as March 2006 in Northern Florida, but most positive detections occurred in August (Fig. 3).
- Positive samples from rain traps were identified in June, but again most positive detections occurred in August (Fig. 4).

Acknowledgements

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Reference

Frederick, R.D., Snyder, C.L., Peterson, G.L., and Bonde, M.R. 2002. Polymerase chain reaction assays for the detection and discrimination of the soybean rust pathogens Phakopsora pachyrhizi and P. meibomiae. Phytopathology 92:217-227.