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Monitoring Airborne Soybean Rust Urediniospores Using Passive Wind-Vane Traps During the 2009 Season in the South and Central United States

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ABSTRACT:
Following the arrival of *Phakopsora pachyrhizi* in the continental U.S. in 2004, epidemiological studies have tracked the annual spread of soybean rust by field-scouting for disease and by reporting of urediniospores detected in rainwater trap sediments by Q-PCR, and in wind-vane adhesive traps by microscopic examination with confirmation by Q-PCR. It has been postulated that airborne translocation of this potential inoculum could be monitored to reveal the location of rust invasion before appreciable crop infection occurs. Wind-vane spore traps were established in June 2009 in the states of AL, AR (two), FL, IL (ten), IN, IA (two), KY, LA (two), MN, MS (four), MO (two), TN, and TX (two). At weekly intervals through October, local cooperators mailed the trapped material to Urbana where total DNA was extracted and any *P. pachyrhizi* DNA present was quantified by Q-PCR. Rust was eventually detected in every state except MN, but fewer than two spores was detected in five of the northwestern IL locations and the central IA location. On four instances a single spore was detected at the northern IA location, although no disease was reported in that state. Spores were detected in southeast AR and TN two and four weeks, respectively, before rust was reported by scouting. High numbers of spores (over 100) were found after mid-September in central AR, FL, LA, southeast MO, and TN. These results suggest that wind-vane spore trapping coupled with Q-PCR assays may be useful in the tracking, analysis, and forecasting of soybean rust outbreaks.

INTRODUCTION:

In the time since soybean rust arrived in the continental USA, initial disease diagnosis for each new state report based on microscopic examination of putatively infected tissues has required confirmation by molecular analysis. Similarly, wind-vane spore-traps have been initially evaluated microscopically, with followup confirmation of suspect positives by quantitative PCR (Q-PCR). For the 2009 season, we eliminated the microscopic evaluation of wind-vane spore-trap slides and utilized solely Q-PCR for quantification.

METHODS:

Wind-vane spore traps (**Fig. 1**) were deployed at 30 locations (**Fig. 2**), including ten in Illinois. These passive traps utilized white petrolatum-coated slides, which were exchanged weekly and mailed to Urbana for processing. DNA was extracted from any material adhering to the slide, as described previously (A). The DNA was assayed (in duplicate) by Q-PCR using the method of Frederick (B), with a multiplexed internal control reaction (C) to validate negative (zero spore) results. The data were then normalized to the assay result from ten reference spores (in quadruplicate), to determine the number of spores trapped. This method offers single-spore sensitivity, as previously demonstrated (D).

RESULTS:

The first *P. pachyrhizi* spore was detected in the week of June 5, in Quincy-FL (where rust had already been reported). In two cases, wind-vane spore traps detected rust spores well in advance of actual outbreaks of disease: Monticello-AR, where two spores were found (week of 8/11) and disease was reported on 8/28; and Jackson-TN, where two spores were found on 7/27 and disease was reported two months later (9/30). Single spores were detected repeatedly in Waterloo-IA (7/1, 7/15, 8/5, & 9/24), and twice in Germantown Hills-IL (7/6 & 7/28) and Vincennes-IN (7/15 & 9/16), while eight spores were detected once in Columbia-MO (9/2), and a pair of spores were detected in Shabbona-IL (9/18), although rust was never reported in any of these locations. Similarly, in several weeks, between one and 30 spores were detected in Urbana-IL (7/6, 9/8 & 9/15), and one spore was detected in Erie-IL (9/11), while a rust outbreak was never reported in those locations. In other areas where disease outbreaks were well-underway, spores were detected in greater numbers, e.g., over 500 in Baton Rouge-LA, Jackson-TN, and New Madrid-MO.

Figure 1. (Background photo) Wind-vane spore trap in a soybean field in Urbana, IL.

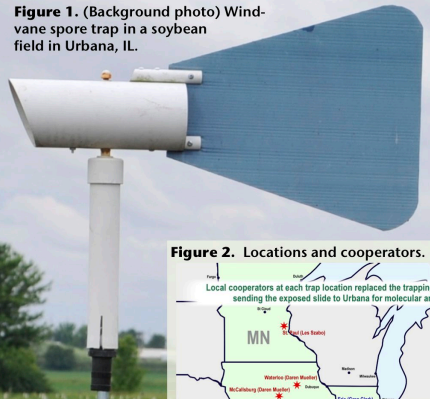

Figure 2. Locations and cooperators.

DISCUSSION:

Q-PCR can detect the DNA from a pathogen such as *P. pachyrhizi* with exquisite sensitivity, and these results show that in central Arkansas and in Jackson-TN, passive wind-vane spore traps coupled with Q-PCR were able to anticipate rust outbreaks. However, as DNA is a relatively stable molecule, this method is unable to differentiate between living and dead propagules. Because single spores were repeatedly detected in some of the northern locations, but no disease was reported in those states, it is reasonable to suggest that these urediniospores are unable to survive such long-distance airborne transport. As such, disease forecasting models that utilize Q-PCR data should account for the historical incidence of disease in a particular locale, before predicting an outbreak solely on the presence of *P. pachyrhizi* DNA. Current work is underway to develop a biosensor that can discriminate between living and dead spores, to better track the spread of viable rust propagules.

REFERENCES & DOWNLOADS:

(A) Haudenshield, et al. "Recovery of *Phakopsora pachyrhizi* urediniospores from passive spore trap slides and extraction of their DNA for quantitative PCR". *Poster presented at:* Annual Meeting of the North-Central Division of the American Phytopathological Society, June 21–23, 2009; Ames, IA, USA. *Abstract in:* Phytopathology 100(6)xxx. 2010.
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(C) Haudenshield and Hartman "Synthetic internal control sequences to increase negative call veracity in multiplexed, quantitative PCR assays for *Phakopsora pachyrhizi*". *Poster presented at:* Annual Meeting of the American Phytopathological Society, July 26–30, 2008; Minneapolis, MN, USA. *Abstract in:* Phytopathology 98(6):S66. 2008.
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(D) Haudenshield, et al. "Quantification and single spore detection of *Phakopsora pachyrhizi*". *Poster presented at:* 2007 National Soybean Rust Symposium; December 12–14, 2007; Louisville, KY, USA.
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Photo by Theresa Herman, 2009

Figure 3. Rust spores detected from wind-vane traps during the 2009 season.