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Movement of *Phakopsora pachyrhizi* (soybean rust) spores by non-conventional means

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Abstract Soybean, caused by the rust fungus *Phakopsora pachyrhizi*, is the most important foliar pathogen infecting soybean. Historically, the disease was important only in the Eastern Hemisphere, but since 1994 the disease has been reported in many countries in Africa and the Americas. In the U.S.A., soybean rust has been perceived as a threat to soybean production and monitoring of the disease occurs throughout the country where soybean is grown. The objectives of this study were to show conclusive evidence that soybean rust spores can be transported by non-conventional means such as clothing. The implication may affect how researchers approach monitoring this disease in research and sentinel plots.

Keywords Epidemiology · Quantitative PCR · Spore detection · Spore movement

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There has been much effort to document the occurrence of *Phakopsora pachyrhizi* in sentinel and mobile locations throughout the continental U.S. (USDA 2008). Before the introduction of soybean rust to the continental U.S. in 2004 (Schneider et al. 2005), there were concerns about its entry in ways unrelated to air movement (USDA-APHIS 2004), including human-assisted introduction. Although there has been no apparent documentation of such transport, conveyance by humans remains a potential for spore movement. Currently, the fungus appears to overwinter in the warmer areas of the U.S. including kudzu (*Pueraria lobata*) patches in Florida, Louisiana, Mississippi, Texas, and possibly other states along the Gulf Coast, and other countries including Mexico (USDA 2008). The general trend of rust occurrence has been from the southern U.S.A. states to the northern U.S.A. states during the summer, often not reaching the northern soybean growing areas until late in the season, and having little or no consequence on crop production.

The first author returned from a research trip to the southern part of the U.S.A. during which several days (15–16 October 2007) were spent in soybean fields and/or plots where rust occurred. Before the trip the trousers and shoes were clean and had not been in contact with any plants with rust. The trousers and shoes from those 2 days were isolated from other

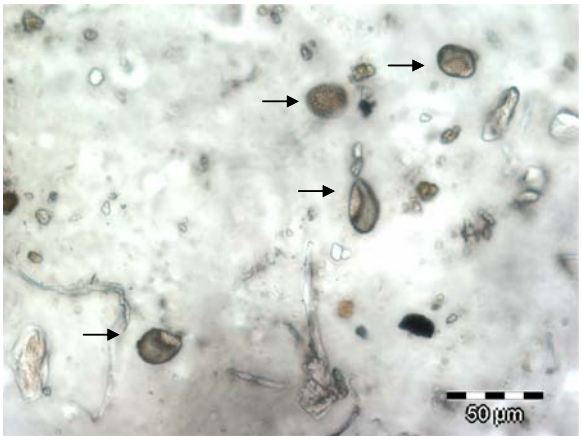

Fig. 1 *P. pachyrhizi* urediniospores obtained from double-sided cellophane tape applied to infested trousers

clothing in double-layered polyethylene bags. Five days later, double-sided cellophane tape (1.9 cm wide) was stuck to microscope slides and then pressed to various parts of the trousers and shoes. Seven samples were taken in all—four from shoes and three from trousers. For the shoes, samples were taken from each shoe at the base of the tongue, and the very front of the shoe where the rubber sole and toe meet. From the trousers, each leg was sampled at the hemline (lowest part of the inner legs) as well as the inner liner of the right pocket. Urediniospores were observed under a compound microscope (×20) in all samples (Fig. 1), and tabulated as spores cm^{-2}. There were four and 48 spores from the shoe tongue and seven and nine spores cm^{-2} from the front toe of the left and right shoes, respectively. From the trousers, there were 99 and 22 spores cm^{-2} from the left and right leg, respectively, and three spores cm^{-2} from the pocket (Fig. 2).

These results were further confirmed using quantitative polymerase chain reaction (q-PCR). Four sections (0.2×1.9 cm) of tape were excised from the left-leg trouser sample and individually extracted using the FastDNA SPIN kit and FastPrep instrument, essentially as recommended by the manufacturer (MP Biomedicals, Solon, OH, USA), with final DNA cleanup and concentration using QIAquick spin columns (Qiagen Inc., Valencia, CA, USA). Real-time q-PCR (Frederick et al. 2002) was performed on these eluates as well as on dilutions of reference DNA previously extracted from a known quantity of spores. DNA detection crossed the quantitation threshold between 23 and 36 amplification cycles (the C_t value; Fig. 3). Estimates of the number of *P. pachyrhizi* spores were made by comparing the C_t values determined with the C_t values of the reference DNA plotted as a standard curve (Fig. 4). In the first experiment, an average of 250 spores per tape section was determined, and when repeated, an average of 190 spores was determined.

Although there was no demonstration of the viability of hitchhiking *P. pachyrhizi* spores, and there is no documented evidence to indicate that viable *P. pachyrhizi* spores have been spread by human garments, it seems plausible that this could occur considering the large number of spores that may be found on clothing. The transport of *Puccinia striiformis* f. sp. *tritici* urediniospores on clothing was implicated in the movement of that fungus to Eastern Australia (Wellings et al. 1987). In addition, in New Zealand it was estimated that 70,000 viable rust urediniospores of diverse species were brought in on clothing and luggage over a 4-week period (Sheridan 1989). It is justified to raise awareness that transport of *P. pachyrhizi* spores to other locations via non-conventional means may play a role along with aerial transport in its spread and distribution.

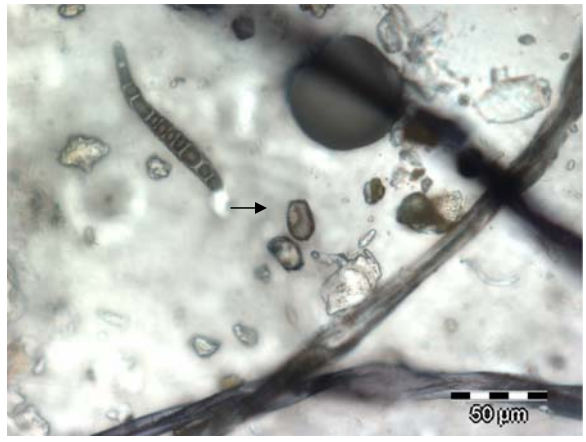

Fig. 2 *P. pachyrhizi* urediniospores obtained from double-sided cellophane tape applied to inside trouser pocket

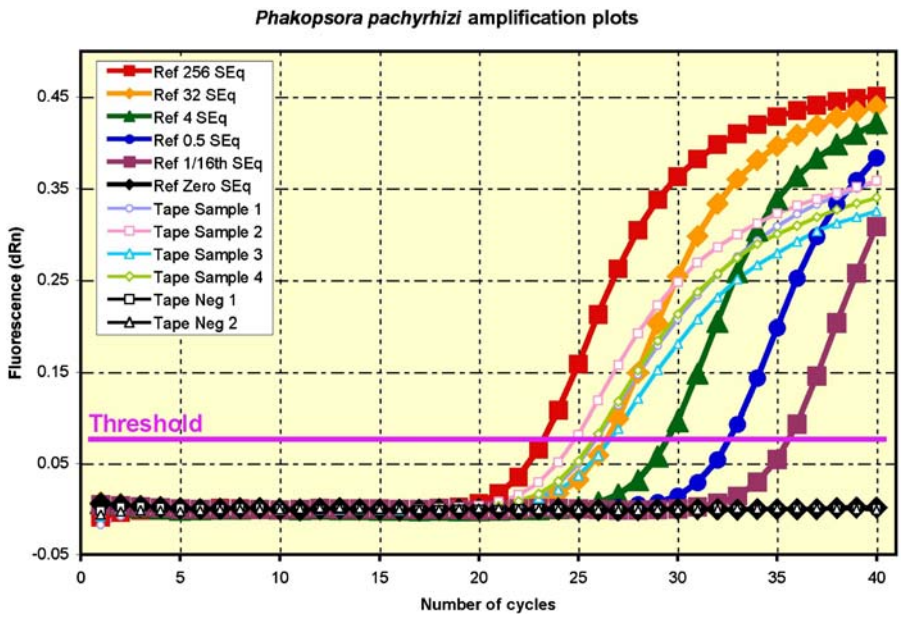

Fig. 3 *P. pachyrhizi* DNA amplification plots

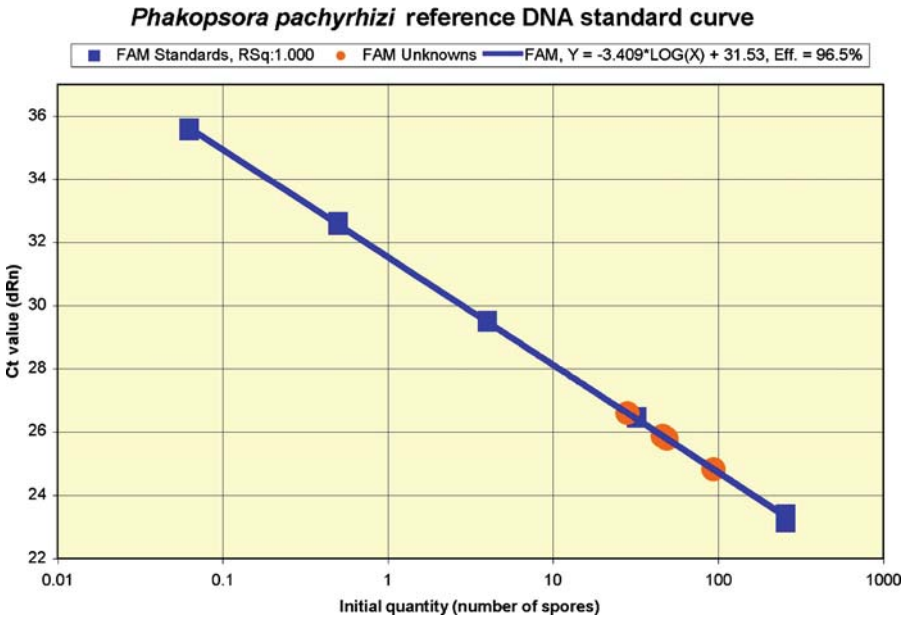

Fig. 4 Standard curve for estimation of urediniospores counts of *P. pachyrhizi*

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