Isolation, Purification, and Characterization of *Phakopsora pachyrhizi* Isolates

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Introduction:

Soybean rust, caused by *Phakopsora pachyrhizi*, was first reported in the continental United States in November 2004. Over the last 30 years, an international isolate collection has been maintained and used for research at the USDA-ARS Fort Detrick containment facilities. Since 2004, isolates have been collected by various researchers in the U.S. In our case, *P. pachyrhizi* isolates have been obtained from 2006 and 2007 across the U.S. Maintaining, purifying, and characterizing isolates requires a commitment since keeping live cultures of the pathogen requires multiple resources. The goal of this research is to maintain an isolate collection to measure the pathogenic and molecular variability of *P. pachyrhizi* across years and locations.

Objectives:

- Isolate and purify *P. pachyrhizi* isolates as single spore and composite isolates from across the U.S.
- Develop a differential set of soybean accessions for characterization of *P. pachyrhizi* isolates
- Evaluate differences in phenotypic reactions of *P. pachyrhizi* isolates on the differential set

Isolation and purification of *P. pachyrhizi* isolates:

- Field leaf samples are examined for sporulating uredinia away from other fungal contaminants
- Spores are picked with a needle from sporulating uredinia and placed on detached leaves to create a composite isolate (Fig. 4)
- Single spore isolates are obtained by transferring single spores, from sporulating uredinia, with a needle off of water agar plates to detached leaves (Fig. 2)
- Successful single spore and composite isolates from across the U.S. are available in detached leaf culture (Fig. 1)

Fig. 1. Locations of *P. pachyrhizi* isolates

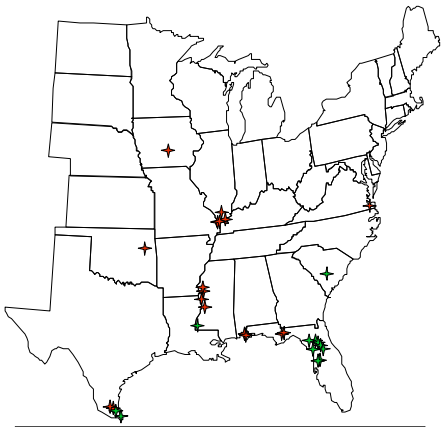


Fig. 2. Single spore isolation

Picking a single spore from water agar plate

Single pustule from single spore transfer

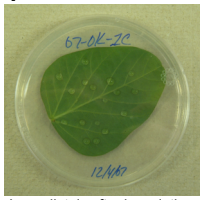
Inoculated detached leaves are incubated in a tissue chamber

Fig. 3. Reaction types for soybean rust

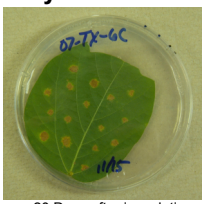
Developing a differential set of soybean accessions to characterize *P. pachyrhizi* isolates:

- Inoculation of soybean accessions with isolates is done in a detached leaf assay
- Lesion types are evaluated as a hypersensitive reaction (HR), a reddish-brown resistant reaction (RB), or a susceptible tan reaction (Fig. 3)
- Uredinia counts within lesions and sporulation are recorded

Fig. 4. Isolate maintenance and spore production on detached soybean leaves



Immediately after inoculation



20 Days after inoculation

Uses for the *P. pachyrhizi* isolate collection:

- Screening germplasm for resistance
- Measuring pathogenic and molecular variability among isolates
- Characterizing culture differences among isolates

Support:

United Soybean Board

Illinois Soybean Association

Soybean Disease Biotechnology Center

Thank you to those people who sent leaf samples from their states for inclusion in the isolate and specimen collections.