

Examination of Sample Storage/Treatment Regimes on Detection and



Viability of *Phakopsora pachyrhizi*



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Abstract

Asian soybean rust (ASR) is caused by the filamentous fungus, *Phakopsora pachyrhizi*. This aggressive basidiomycete plant pathogen infects a wide range of cultivated and weedy legumes including kudzu (*Pueraria lobata*), which is present throughout the Southeastern United States. In Florida, ASR-infected kudzu serves as the over winter host which may provide an important source of inoculum for soybeans. Kudzu patches infected with ASR are routinely diagnosed based on visual symptoms (eruptive pustules on the underside of the leaf) using a dissecting microscope and are confirmed by PCR for newly infected areas or when symptoms are ambiguous. Rapid and accurate diagnosis of ASR-infected plant material is critical to minimize the economic impact of this devastating disease. Two widely-used diagnostic tools for ASR diagnosis, conventional PCR and ELISA, detect both viable and non-viable pathogens in ASR-infected samples. The relationship between pathogen viability, detection capability, and various environmental regimes was investigated in this study. Results will help identify optimal conditions for sample storage to preserve detection capability of each diagnostic test. Preliminary studies investigating spore viability have provided a foundation for future endeavors to investigate the impact of environmental conditions on pathogen survival in the field.

Materials and Methods

- ASR-infected kudzu leaves were collected from a site in Citra, Florida and were washed 3X with water, patted dry with a paper towel and incubated in a moist chamber for 24 hours.
- Three ASR-infected kudzu leaves with sporulating lesions were placed in labeled-Ziploc bags (3 bags per treatment) and were treated for 18 hours under various conditions.
- Treatment conditions included incubating leaves in a Ziploc bag at room temperature alone, at room temperature with 150 grams of desiccant, at 4°C in a refrigerator, and at -20°C in a freezer for 18 hours.
- Nine of 18 kudzu leaves that were kept at room temperature for 18 hours were then autoclaved for 30 minutes in three glass beakers covered with aluminum foil.
- Urediniospores were harvested from ASR-infected kudzu leaves by spraying the abaxial leaf surface with Tween-treated water and pouring the spore suspension onto water agar plates (1 plate/leaf for each treatment).
- Water agar plates were covered with aluminum foil, kept at room temperature, and allowed to incubate overnight.
- Spore germination was assessed by counting 100 spores (germinated and ungerminated) per plate after 12 hours.
- The SAS 9.1 program was used to analyze the spore germination data.
- Sample storage and germination experiments were conducted 3 times with 9 replications per treatment.
- ELISA and PCR were conducted three times as samples from each treatment were examined in triplicate.

Detection via Conventional PCR

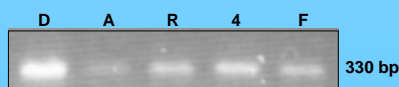


Figure 1. A 2.0% TBE agarose gel stained with ethidium bromide illuminated with UV light showing a 330bp amplicon.

- A 330bp amplicon was detected for all sample treatments examined using primer set Ppa1 and Ppa2 (Frederick et al., 2002).
- D (Desiccated), A (Autoclaved), R (Room Temperature), 4 (Refrigerated), F (Frozen=-20°C).

Detection using ELISA

Treatment	Mean Absorbance	Std Dev	+/-
ELISA + Control	1.43	0.020	+
Healthy Kudzu	0.170	0.013	-
Room Temperature	0.940	0.073	+
Desiccated	0.720	0.046	+
Autoclaved	0.240	0.047	-
4°C	0.830	0.074	+
-20°C	0.490	0.045	+

Table 1. Mean absorbance values for ELISA + Control, Healthy, Room Temperature, Desiccated, Autoclaved, 4°C and -20°C-treated samples.

- ELISA detected the pathogen following most treatments except autoclaving.
- A sample was considered positive when the A450nm was 2.5X greater than the absorbance of the healthy sample (>0.425).



Figure 2. Photo of ELISA plate before adding stop solution for quantification.

Key
P (Positive), H (Healthy), B (Blank), D (Desiccated), A (Autoclaved), F (Frozen=-20°C), 4 (4°C), and R (Room Temperature).

Spore Germination

Treatment	Mean %Germination	Standard Deviation
Room Temperature	40.4a	8.67
Desiccated	18.0c	9.09
Autoclaved	0d	0
4°C	29.4b	13.4
-20°C	10.3c	7.14

Table 2. Spore germination data from a representative experiment showing means and standard deviations for each treatment.

- All treatment regimes (Desiccated, Autoclaved, 4°C, and -20°C) reduced spore germination compared to the room temperature treatment.
- Means with the same letter are not significantly different according to Fisher's LSD.

Conclusions

- Conventional PCR detected *P. pachyrhizi*-infected plant material following all sample treatment regimes, whereas ELISA could not detect the pathogen following autoclave treatment for 30 minutes.
- All sample treatments (Desiccated, Autoclaved, 4°C, and -20°C for 18 hours) significantly reduced urediniospore germination compared to the room temperature control.

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