Title: Factors Affecting Mycelium Pigmentation and Pathogenicity of Sclerotinia sclerotiorum on Peanut

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Abstract

This project was conducted to determine the relationship of mycelium pigmentation and oxalic production in Sclerotinia sclerotiorum to the pathogenicity of this pathogen on Valencia peanut. The following are the findings of the study: 1) there is no relationship between mycelium pigmentation and the ability of S. sclerotiorum to infect peanut plants; 2) oxalic production is associated with peanut infection by S. sclerotiorum; and 3) sugars such as glucose play a role in the production of oxalic acid and therefore in the pathogenicity of S. sclerotiorum.

Background

Sclerotinia sclerotiorum is a soilborne fungal pathogen that attacks a very wide range (>400 species) of host plants, including peanut, within and outside the United States. The normal phenotype of S. sclerotiorum that is found worldwide is white or non-pigmented and has fluffy mycelium when grown on laboratory growth media. However, Sanogo and Puppala, in 2007, described darkly pigmented isolates of S. sclerotiorum in New Mexico. In 2008, darkly-pigmented isolates of S. sclerotiorum were also found in western Texas.

The ecological significance of the occurrence of variants of S. sclerotiorum with pigmentation in mycelium is not known. A graduate student was hired to carry out research on the relationship between pathogenicity and the biological attributes such as mycelium pigmentation and production of oxalic acid.

Recently completed work has examined the effects of factors such as temperature, carbon source, and pH on mycelium pigmentation and pathogenicity. Melanin-deficient (non-pigmented) mutants were derived from the New Mexico darkly pigmented isolates of S. sclerotiorum using melanin inhibitors. Non-pigmented mutants were not pathogenic and did not infect peanut plants, while darkly pigmented isolates killed the plant within a week (Figure 1). To complement this finding, melanin precursors were used to restore pigmentation in the mutants, which, when tested on peanut, were not pathogenic. This finding ruled out pigmentation as being the main factor in pathogenicity of S. sclerotiorum. Research was then directed to assessing oxalic production.

Oxalic acid is well established as a major determinant of pathogenicity in S. sclerotiorum. We found that the darkly pigmented isolates of S. sclerotiorum did produce oxalic acid while the non-pigmented mutants did not produce observable amounts of oxalic acid within the medium.
This report describes in detail the work that has been done on quantifying oxalic acid in pigmented and non-pigmented mutant isolates of *S. sclerotiorum*.

**Objectives**

1. To quantify the amount of oxalic acid production in darkly pigmented (wild type) isolates in relation to non-pigmented mutant isolates of *Sclerotinia sclerotiorum*.

2. To re-establish pathogenicity in non-pigmented mutant isolates of *Sclerotinia sclerotiorum* through the initiation of oxalic acid production.

**Experimental Approach**

To quantify the amount of oxalic acid, a metabolomics study was initiated. Mycelial tissue was prepared by freeze drying, lyophilizing and homogenizing. The two polar and non-polar layers were separated and analyzed using gas chromatography and mass spectroscopy.

To evaluate carbon sources and re-establish pathogenicity in mutant isolates, potato dextrose agar (PDA) was amended with complete glucose or complete sucrose. Both mutant non-pigmented and darkly pigmented isolates were transferred to the amended plates for 72 hours. Then the isolates were used for both pathogenicity and oxalic acid testing. For pathogenicity testing, Valencia peanut plants were inoculated with darkly pigmented and non-pigmented isolates and maintained in a humidity chamber. Plants were monitored over two weeks for symptom development. Oxalic production by the isolates was tested using bromophenol blue medium for 72 hours in the dark. The presence of a yellow halo within the medium (signifying production of oxalic acid) was recorded.

**Results and Conclusion**

Gas chromatography and mass spectroscopy analysis revealed down regulation of oxalic acid in the mutant isolates when normalized and compared to the darkly pigmented isolates. Upon further analysis many sugars including glucose and fructose as well as many compounds within the citric acid cycle, were also down regulated in mutant isolates compared to wild type darkly pigmented isolates. This result provided us with a clue as to what the melanin inhibitors targeted within the pathogen and caused it to lose pathogenicity.

After incubation on medium containing different carbon sources, the darkly pigmented isolates remained pathogenic on Valencia peanut plants and produced a yellow halo within the bromophenol blue plate. The mutant non-pigmented isolates remained non-pathogenic on peanut
plants when incubated on PDA or complete sucrose plates. The same treatments also did not produce a yellow halo within the bromophenol blue plates. However, the mutant isolates on complete glucose plates re-established pathogenicity and killed peanut plants within a week like the darkly pigmented isolates. On the bromophenol blue plates, after 24 hours a yellow halo was present confirming oxalic acid production. These results indicate that glucose is needed to establish pathogenicity in non-pigmented mutant isolates of *S. sclerotiorum*.

The finding of this work may be useful in terms of disease control, particularly with a focus on identifying sugars that are associated with oxalic acid production. Suppression of oxalic acid production through manipulation of sugars may render *S. sclerotiorum* less pathogenic on peanut and other plant hosts.
Figure 1. Pathogenicity of a darkly pigmented (SD) isolate and a non-pigmented (SW) isolate of *Sclerotinia sclerotiorum* on whole Valencia peanut plants shown in A), side view and (B), top view. Note complete plant death with the SD isolate and no plant death with the SW isolate.