Southeastern Peanut Research Initiative 2017
FINAL REPORT

UF Project Number: P0047361

Project Title: Identifying molecular tools for assessing Sclerotium rolfsii populations and taxonomic classification of the white mold pathogen on peanut.

Reporting Period: 1/01/17-6/30/18

Prepared by:
Nicholas S. Dufault, Extension Plant Pathologist, University of Florida
352-273-4623
nsdufault@ufl.edu

1. Abstract
Further understanding of a pathogen’s population diversity is important for disease management through improved breeding programs and a better understanding of population responses to varying environments (e.g. temperature and fungicides). The goals of this project were to clarify the genetic diversity of *S. rolfsii* in the Southeastern U.S., and assess this diversity with isolates from around the world. A total of 26 *S. rolfsii* isolates were previously collected from Florida, Georgia, Alabama, and South Carolina with 18 new isolates being collected from Florida, Georgia, Alabama, Mississippi and Arkansas in this study. Type sequence data had also been previously collected from Spain, Italy, Portugal, Brazil and Chile. DNA was extracted from these isolates and amplified by PCR using ITS, RPB1, RPB2, and MS204 loci. The resulting sequences were aligned and analyzed using Geneious and BioEdit. Phylogenies were constructed with MEGA7 and a TCS (Templeton, Crandall and Sing, 1992) population network created in R. Using the 4 loci *S. rolfsii* isolates where separated into 2 groups with no differentiation related to host or geography. The network analysis using RPB2 indicated that isolates from different Florida counties had different haplotypes, and that the other isolates from Georgia and South Carolina, Europe and South America grouped in different haplotypes as well. This data shows that variation does exists in the *S. rolfsii* populations, which means pathogen populations should be considered when assessing disease management strategies (e.g. cultivar resistance).

A majority of the new isolates were collected during the 2018 growing season for this grant due to low levels of white mold in 2017. Novel genes (EF1α and β-tublin) were also examined in this study, however, data collected from these genes was not clean. Currently, tests are being done to re-run these genes and add the 18 new isolates to the data set.

2. Introduction
To effectively manage white mold caused by *Sclerotium rolfsii* in infested fields, it is critical that we understand the genetic diversity of the pathogen. This information is essential for breeding programs, assessing host specialization of *S. rolfsii*, and the efficacy of integrated management techniques. Preliminary research on 15 isolates of *S. rolfsii* collected throughout Florida’s major peanut producing regions indicates that a significant amount of diversity exists within this pathogen. Another study (Xie et al., Plant Disease) also observed that a considerable amount of variation is present for *S. rolfsii* in the Southeastern U.S., and that isolates from peanut may be specialized or adapted to optimal growth on peanuts. Further
characterization of this diversity is needed to better understand the pathogen's as it relates to
pathogenicity, virulence, and other epidemiological factors (e.g. climate). We hypothesized
that a high level of diversity is present within the S. rolfsii populations of Florida and the
Southeastern U.S., and that this diversity will be present at the regional scale. Our objectives
are to: 1.) Continue collecting isolates of S. rolfsii from production fields in Florida, Alabama
and Georgia; and 2.) Examine the diversity of S. rolfsii isolates collected during growing
seasons from 2012 to 2018 using multilocus sequencing typing - MLST with previously used
loci and novel loci EF1-α and β-tubulin.

3. Methods
Isolate collection and DNA extraction
Isolates were collected from Florida, Georgia, Alabama, Arkansas, Mississippi and South
Carolina to represent S. rolfsii population in the Southeastern US. Pure cultures of S. rolfsii
were isolated from infected peanut by surface sterilization. Actively growing mycelia were
then harvested from cellophane-covered acidified potato dextrose agar plates. Total DNA
was extracted using the standard protocol of DNeasy Plant Mini Kit (Qiagen, Valencia, CA).
Sequences from Mediterranean S. rolfsii isolates were downloaded from GenBank
(Accession Nos. JF267840, JF267859, JF267856, JF267855, JF267860, JF267861,
JF267832, JF267848).

Amplification and Sequencing
To amplify each locus polymerase chain reactions (PCR) were conducted using a
Mastercycler thermocycler (Eppendorf, Hamburg, Germany). Reaction mixtures included
USB Taq PCR Master Mix 2X (Affymetrix, Santa Clara, CA) and appropriate primers at
5μM concentration (Table 1). For each primer pair, the PCR cycling parameters described in
corresponding citations from Table 1 were used. Amplification products were checked on a
1.2% agarose gel stained with ethidium bromide. PCR products were purified using ExoSAP-
IT™ PCR Product Cleanup Reagent (ThermoFisher, Waltham, MA) following the standard
protocol. Cleaned PCR products were sent to Eurofin Genomics for sequencing with 2X
coverage (forward and reverse).

<table>
<thead>
<tr>
<th>Locus / gene</th>
<th>Primers</th>
<th>Length (base pairs)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously used loci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITS</td>
<td>ITS1 – ITS4 (White et al 1990)</td>
<td>~680 bp</td>
<td>Internal transcribed spacer region</td>
</tr>
<tr>
<td>RPB1</td>
<td>Af – Cr (Matheny et al, 2002)</td>
<td>~1300 bp</td>
<td>RNA polymerase subunit 1</td>
</tr>
<tr>
<td>RPB2</td>
<td>bSr.1F – b11R1 (Matheny et al, 2002 and Remesal et al, 2013)</td>
<td>~1000 bp</td>
<td>RNA polymerase subunit 2</td>
</tr>
<tr>
<td>MS204</td>
<td>E1F – E4R1 (Walker et al, 2012)</td>
<td>~1000 bp</td>
<td>guanine nucleotide-binding protein subunit beta-like protein</td>
</tr>
<tr>
<td>Further Loci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF1-α</td>
<td>EF1-526F – EF1-567R (Rehner, 2001)</td>
<td>~1500 bp</td>
<td>Eukaryotic translation elongation factor 1 alpha</td>
</tr>
<tr>
<td>β-tubulin</td>
<td>F-βtub2r – A-βtub2r (Elhaz et al., 2002)</td>
<td>~400 bp</td>
<td>Beta tubulin</td>
</tr>
</tbody>
</table>
Population analysis
DNA sequences were aligned using MUSCLE (Edgar, 2004) and edited manually using Geneious (Kearse et al., 2012) and BioEdit (Hall et al, 1999). Phylogenies were constructed using maximum likelihood approach with 1000 bootstrap replicates using MEGA 7 software (Kumar, 2016). Haplotype networks were constructed using the TCS algorithm (Templeton et al, 1992) and visualized using PopART (http://popart.otago.ac.nz). A haplotype is a group of genes within an organism that inherited from the same parent, indicating genetic similarity. To detect whether there is significant population structure, an Analysis of Molecular Variance (AMOVA) was conducted using Arlequin 3.5 software (Excoffier et al, 2010).

4. Results
Analysis of molecular variance had a p-value of 0.125. The AMOVA indicated that ~14% of the variation occurs among the populations. It also indicated that most (~86%) of the variation occurred within the populations.

Figure 1: Multilocus sequence typing (MLST) based on a neighbor-joining phylogeny using concatenated nucleotide sequences of ITS, RPBI, RPB2, MS204. Different colors represent isolates from different counties in Florida and the states of Georgia (g), South Carolina (sc), and Alabama (h). The multilocus phylogeny shows that the isolates are separated into two major groups, however no trends are apparent for the geographic regions based on county or state.
Figure 2: Haplotype Network for *S. rolfsii* isolates based on RPB2 loci in 3 US states, South America and Europe. This network reveals haplotype groups but also considerable haplotype diversity, especially within the state of Florida. Each tick mark indicates the relative difference between the haplotypes.
Figure 3: Haplotype Network for *S. rolfsii* isolates based on RPB2 loci with overlay for the various counties in which isolates were collected in Florida. Data shows that isolates from different counties have different haplotypes, indicating the Florida population to be diverse.
5. **Summary**

Unfortunately, due to low quality from the DNA extractions, the new loci were not able to be added to the analysis. The previous analysis is shown here, and it believed the new loci will improve the confidence in the diversity seen in the isolates already. Further isolates were collected (18) from two new states Mississippi and Arkansas. However, a majority of these isolates (15) were collected in 2018. The goal is to finish the analysis of these new in 2018 and add the new loci in 2019. A new factor (host type) will also be added to this data set as isolates were collected from tomatoes and soybeans.