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Southeastern Peanut Research Initiative 2016
FINAL REPORT

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Project Title: Increasing our understanding of the population diversity of Sclerotium rolfsii, the white mold pathogen, in Southeastern peanut production areas.

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1. Abstract

To effectively manage white mold of peanut caused by *Sclerotium rolfsii*, it is critical that we understand the pathogen's genetic diversity. 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 collected from Florida, Georgia, Alabama, and South Carolina. Type sequence data was also collected from Spain, Italy, Portugal, Brazil and Chile. DNA was extracted from these isolates and amplified by PCR using ITS, RPB_1, RPB_2, 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 RPB_2 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).

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 techinques. 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 2016 using multilocus sequencing typing – MLST.

3. Methods

Isolate collection and DNA extraction

Twenty-six isolates were collected from Florida, Georgia, Alabama, 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 thermomcycler (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 1.

Locus / gene	Primers	Length (base pairs)	Description
ITS	ITS1 – ITS4 (White et al 1990)	~680 bp	Internal transcribed spacer region
RPB1	Af – Cr (Matheny et al, 2002)	~1300 bp	RNA polymerase subunit 1
RPB2	bSr_1F – b11R1 (Matheny et al, 2002 and Remesal et al, 2013)	~1000 bp	RNA polymerase subunit 2
MS204	E1F1 – E4R1 (Walker et al, 2012)	~1000 bp	guanine nucleotide-binding protein subunit beta-like protein

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 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 Arelquin 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.

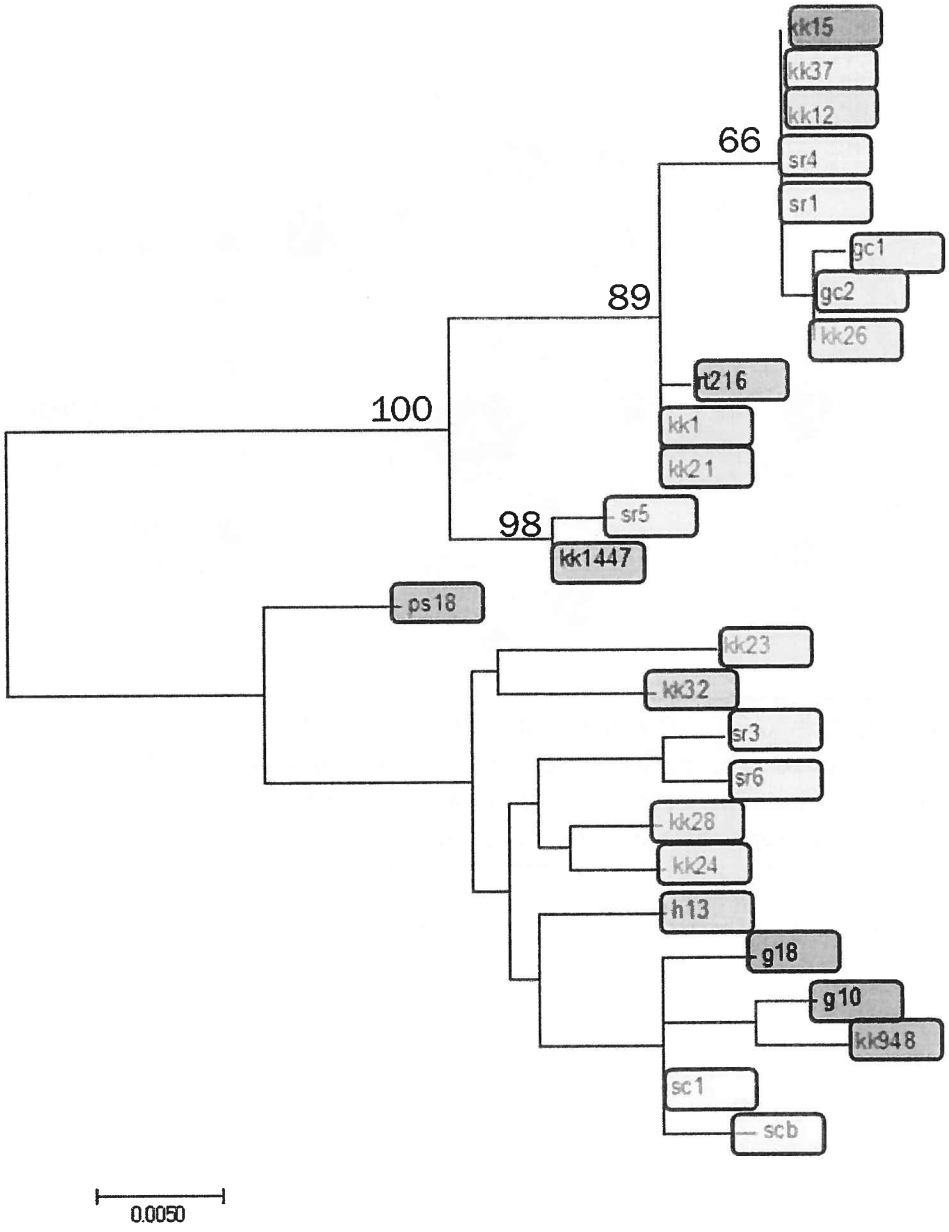

Figure 1: Multilocus sequence typing (MLST) based on a neighbor-joining phylogeny using concatenated nucleotide sequences of ITS, RPB1, RPB2, MS204. Different colors represent isolates from different counties in Florida and the states of Georgia (g), South Carolina (sc), and Alabama (h). The multilocus 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.

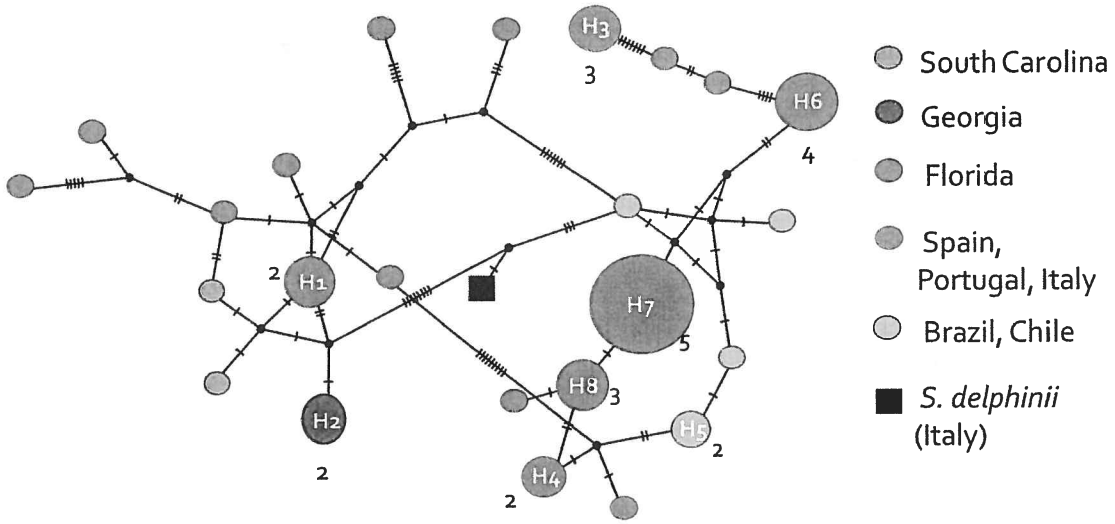

Figure 2: Haplotype Network for *S. rolfsii* isolates based on RPB_2 loci in 3 US states, South America and Europe. This networks reveals haplotype groups but also considerable haplotype diversity, especially within the state of Florida. Each tick mark indicates the relative difference between the haplotypes.

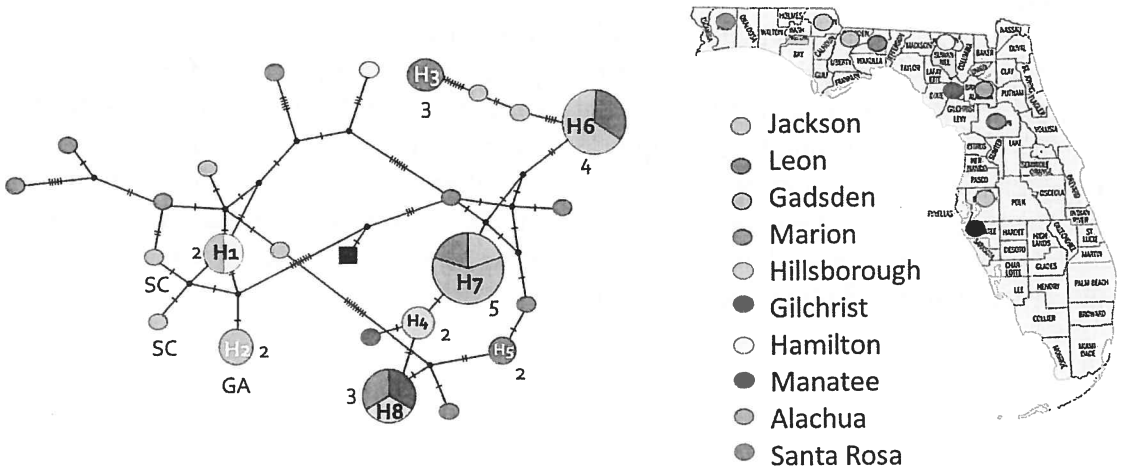

Figure 3: Haplotype Network for *S. rolfsii* isolates based on RPB_2 loci with overlay for the various counties in which isolates were collected in Florida. Data shows that isolates form different counties have different haplotypes, indicating the Florida population to be diverse.

5. Summary

Overall, these results indicate that a high level diversity is present in *S. rolfsii* populations from Florida and the southeast. In general, it appears that isolates from different regions (e.g. counties) could possibly have different haplotypes, however, MLSTing indicates that the grouping do not correspond to geography. In general, the isolates do form distinct genetic groups with at least 2 genotypes present in the Southeastern U.S. population. It is apparent from these results that diversity is present within the *S. rolfsii* populations and that these distinct population groups should be considered when assessing disease management (e.g. cultivar resistance and fungicide sensitivity).