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Southeastern Peanut Research Initiative 2015
FINAL REPORT - Summary pg 5

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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 population diversity of this pathogen. This information is essential for breeding programs that are examining resistant peanut lines, and is also important for assessing the evolutionary potential of this pathogen, which can impact crop rotation management strategies as well as fungicide programs. Our research indicates that a significant amount of diversity exists within this pathogen throughout Florida's major peanut producing regions as well as Southeastern U.S. (Xie et. al., Plant Disease).

2. Objectives

The objectives were to 1.) Characterize peanut isolates of *S. rolfsii* using morphological and genetic tests; 2.) Assess long term storage techniques of *S. rolfsii*; 3.) Test the pathogenicity of *S. rolfsii* to 3 different peanut varieties in a field setting.

3. Methods

Objective 1.) Isolates of *S. rolfsii* will be collected in different peanut growing regions of Florida, and an USDA permit was requested to sample isolates from Georgia and Alabama in 2016. Isolates were stored and DNA was extracted for molecular analysis using the ITS gene. DNA was extracted using the Extract N Amp protocol (Gardes M & Bruns TD. 1993. Mol Ecol 2:113–118), amplified by PCR using the IST5 and IST4 primers, and Sanger sequenced. Sequences were assembled with BioEdit and aligned with Muscle using default parameters. A maximum-likelihood phylogeny was constructed with 1000 bootstrap replicates using MEGA7.

Objective 2.) A subset of representative isolates obtained in objective 1 will be exposed to multiple fungal storage methods. These methods will include, dry sclerotia storage, refrigerated agar plug storage in water, glycerol cold storage at -80°C and mycelial filter paper storage at -80°C.

Objective 3.) A field trial was conducted at the University of Florida's Plant Science Research and Education Unit in Citra, FL on 5 May. The experiment was a split plot design with four replicates, with fungicide spray program serving as the main plot and peanut cultivar as the subplot. Peanut varieties 'Georgia-06G', 'TUFRunner 511', and 'Georgia 12Y' were planted in Gainesville loamy sand soil at a density of six seeds per foot of row. Rows were 25 ft in length on 36-in. centers. Treatment plots consisted of two treatment rows buffered by two untreated rows on the ends. A 10-ft buffer of no plants separated each replicate, and each replicate consisted of nine plots. Plots were inoculated on 28 May with *Sclerotium rolfsii* colonized corn and oat grains buried in a narrow, 2-in. furrow next to each

treatment row. Three fungicide programs were assessed in each main plot: untreated check, chlorothalonil only (Echo 720 @ 1.5 pt/A), and a rotational program consisting of chlorothalonil (Echo 720 @ 1.0 pt/A) and tebucanazole (TebuStar 3.6 @ 7.2 fl oz/A). All fungicide applications were made using a CO^2 backpack sprayer calibrated to deliver 20 gal/A at a pressure of 22 psi; the boom consisted of flat-fan 8004 spray tips spaced 36 in. apart. Fungicides were applied at two week intervals on (A) 10 Jun, (B) 24 Jun, (C) 8 Jul, (D) 29 Jul, (E) 13 Aug, (F) 28 Aug, and (G) 12 Sep. Disease incidence for stem rot and disease ratings for leaf spot were first recorded 40 days after inoculation on 7 Jul and every two weeks afterwards until 17 Sep. Stem rot was evaluated by counting the number of 1-ft sections that had visible signs within the 50-ft plots. Disease ratings for leaf spot were done using the Florida 1 - 10 scale. Peanuts were dug on 2 Oct and harvested on 5 Oct. Results were analyzed using JMP Pro v12 software with Fisher's protected least significant difference (LSD) test ($P \leq 0.05$) being conducted for mean comparison.

4. Results

Isolate collection and Population Diversity of S. Roflsii.

A total of 18 isolates were collected in 2015 from 5 counties in Florida. The application process for out state permits was initiated in May of 2015 and received in November of 2015, so isolates from Alabama and Georgia, as well as other states, will be collected in 2016.

Initial genetic analysis of 7 *S. rolfsii* isolates from Florida indicated that there is diversity present with in this pathogen, and that the current taxonomic structure of one species may not be correct. Further analysis with other genes are needed to better understand this pathogen's genetic diversity and species complex.

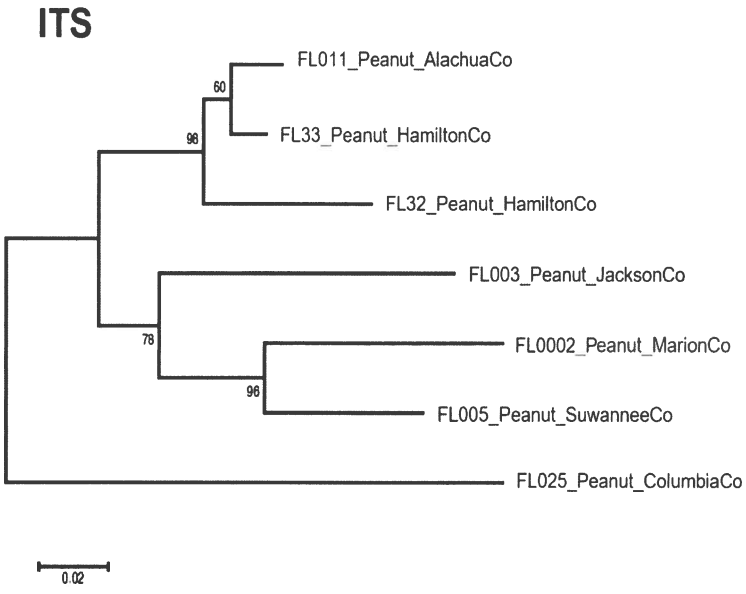

Figure 1: *S. rofsii* phylogenetic tree showing variation associated with 7 isolates from 6 Florida counties based on the ITS loci. In general, separate branches were observed for the isolates, and no correlation to region (Panhandle, Big Bend, and Central Florida) was noticed.

Isolate storage

In general, the isolates stored under the various methods germinated at each time point. However, since this was only one year that is a common result for isolate storage. This study will continue for 3 more years.

Field Trial Results:

During the 2015 peanut production season, total rainfall for May - Oct was 32 in, with an average temperature of 77ºF. Soil pH was 6.5, with an average soil temperature of 83.9 ºF. No significant interaction effects were observed between variety and fungicide program in this trial (Table 1). Disease intensity for both stem rot and leaf spot was observed to be significantly less in the two fungicide programs compared to the untreated check. Georgia-12Y had significantly lower disease pressure for leaf spot and stem rot compared to the other 2 varieties, except on 1 Sep when stem rot incidence was similar for Georgia-12Y and TUFRunner 511. Average yields were significantly higher for the 2 fungicide programs compared to the untreated check, and for Georgia-12Y compared to Georgia-06G and TUFRunner 511 despite significant stem rot incidence.

Table 1. Results from the 2015 field trial looking at the effects of cultivar and fungicide program on disease control and yield.

	Stem Rot		Leaf Spot	Yield (lbs/A)[w]	Stem Rot AUDPC[z]	Leaf Spot AUDPC[z]
	Sept-1[y]	Sept-17[y]	Sept-17[x]	Oct-5		
Split plot analysis (*F* value)[v]						
Fungicide treatment	36.48***[w]	15.37**	24.52***	38.26***	63.07***	87.48***
Variety	10.36***	10.30***	16.06***	121.6675***	11.04***	0.46
Fungicide treatment X Variety	1.96	0.939	0.18	0.83	1.69	0.16
MSE[t]	17.18	10.32	0.16	1.15	97.43	338.93
Peanut variety means[u]						
Georgia-06G	19.75a	14.58a	6.08a	1798.06a	619.83a	321.00a
TUFRunner 511	13.00b	13.33a	6.00a	1724.25a	509.50b	320.95a
Georgia-12Y	13.17b	8.91b	5.25b	3447.29b	443.37b	314.70a
LSD[s]	6.28	5.34	1.01	869.14	102.59	16.43
Fungicide treatment means[u]						
Untreated	23.50a	18.08a	7.25a	1062.38a	779.04a	375.92a
Echo 720, 1.5 pt/A (A-G)	13.00b	10.08b	5.00b	2674.10b	431.25b	296.00b
Echo 720, 1.5 pt/A (A,B,E,G) TebuStar 7.2 fl oz/A + Echo 720, 1.0 pt/A (C,D,F)	9.42b	8.67b	5.08b	3233.12b	362.38b	284.75c
LSD[s]	4.48	3.59	0.56	770.83	177.68	37.89

[z] AUDPC is the calculated area under the disease progress curve for the disease stem rot and leaf spot.
[y] Stem rot hits/foot; a hit is defined as any noticeable fungal signs (i.e. mycelia and/or sclerotia) within 1 foot of row.
[x] Disease ratings for foliar diseases were completed using the Florida 1 - 10 scale (Chiteka et al, 1988)
[w] Dry weight
[v] Significance of *F*-value at the 0.05, 0.01, and 0.001 levels is indicated by *, **, or ***, respectively.
[u] Means in each column followed by the same letter are not significantly different according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).
[t] Mean Square Error
[s] Least Significance Difference ($P \leq 0.05$)

5. Summary

Initial genetic analysis confirms the phenotypic results previously observed for *S. rolfsii* in that this pathogen has a diverse population structure. This diversity can have significant impacts on disease management and resistance breeding programs. The results from 7 isolates tested using the ITS loci not only indicates that there does not appear to be a regional grouping to the pathogens, but that it may be possible to separate this pathogen into different species. The multinucleate nature of this pathogen makes it difficult to get clean sequence data from DNA isolation, and thus further loci need to be explored. It may be possible that using a single copy loci could provide high quality information about this multinucleate pathogen. This would also provide researchers with a simple means for identifying this pathogen in the future.

Field studies were conducted in 2015 to assess the response of various varieties to *S. rolfsii* provided interesting results. In general, there was no interaction between fungicide and variety treatments in this trial, indicating that under the conditions of this study the varieties responded similar to the fungicide programs. Significant white mold pressure was observed in this study which would explain why the fungicide program with tebuconazole provided the highest numerical yields. Leaf spots did have some effects, but did not reach 100% defoliation in the untreated controls. The most unique effect noticed in this study was that the white mold resistant variety Georgia-12Y could be infected by the disease, however, produced twice the yields of TUFRunner 511 and Georgia-06G. This indicates that despite infection, Georgia-12Y can retain its yields under high disease situations. More research is needed to better understand this effect.

Overall, the results indicate the importance of understanding pathogen's population as well as its response on various varieties. Many factors can affect disease intensity, and continued research to understand these affects is critical to obtaining optimal peanut yields. As new resistant and tolerant varieties are developed, researchers will need to determine disease inputs carefully and over multiple seasons. This study provides more information about *S. rolfsii* genotypic and phenotypic traits and indicates that further assessment of the pathogen's diversity is critical to understanding white mold disease management.