Southeastern Peanut Research Initiative 2012
FINAL REPORT

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Project Title: Determining the Impact of the Recently Discovered Virus, *Groundnut ringspot virus* on Peanut Production in the Southeastern U.S.

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1. Abstract

The presence of *Groundnut ringspot virus* (GRSV) in southern Florida poses a serious threat to peanut production systems throughout the Southeast. During the 2012 peanut production season a survey was conducted to determine the geographic range of this pathogen in peanuts. Peanut plants exhibiting viral symptoms were sampled from a total of 28 different field sites located in 13 separate counties of Florida and Georgia. These samples were processed using a GRSV specific ELISA reagent set, and positive samples were validated using PCR followed by sequencing. While a significant amount of the samples tested positive using the ELISA for GRSV, sequence analysis indicated that the virus in these samples was actually *Tomato spotted wilt virus* (TSWV). Our results indicate that GRSV is not currently present in peanut production fields of Florida and Georgia, and that ELISA is not currently an adequate test for confirming the presence of GRSV.

2. Introduction

Groundnut ringspot virus (GRSV) is a Tospovirus that was identified in the U.S. on tomato plants in south Florida during the 2009 growing season. The geographic range of this virus in the U.S. is still undetermined, but it has been confirmed on solanaceous hosts in 7 counties of southern Florida. GRSV causes severe symptoms in peanuts, such as considerable leaf chlorosis, shortening of the internodes and concentric ringspots on leaves. These symptoms can be confused with the devastating peanut disease *Tomato spotted wilt virus* (TSWV). GRSV is transmitted by various thrips, including the important TSWV vector *Frankliniella occidentalis* (the western flower thrips). GRSV poses a serious threat to southeastern peanut production systems and thus it is critical to assess the presence of this pathogen in peanuts.

The objective of this project was to sample peanut production fields throughout the state of Florida and into southern Georgia for the presence of GRSV. Assessing the presence of GRSV in peanut is the first step in evaluating the threat this new pathogen presents to peanut production in the Southeast and throughout the U.S. The results from this study also make it possible for researchers and industry personnel to effectively implement applicable management and control strategies for peanut viral diseases.

3. Methods

Peanut Field Survey

Samples of 10 viral symptomatic plants were collected from a total of 29 different field sites in 13 counties of Florida and Georgia (Figure 1 and Table 1). Sampling was completed between the dates of 7-25-12 to 8-17-12 approximately 60 to 90 days after planting at each peanut site (Table 1). The plant samples were placed in plastic bags (Ziploc™ Freeze Bag, 1 gallon size) and stored on ice until processed in the laboratory. Three separate tissue samples (leaves, crown and roots) were collected and were stored at -80°C until they could be analyzed using an Enzyme-linked immunosorbent assay (ELISA).

ELISA Analysis

The tissue samples collected from each site were screened using an ELISA reagent set from Agdia® designed specifically for GRSV and *Tomato chlorotic spot virus* (TCSV). The reagent set consisted of the virus specific antibodies, conjugate and a 96-well microtiter plates. A total of 84 samples were processed using the ELISA and a standard un-infected plant control was included in each test run (Table 2). The samples that tested positive for GRSV using the ELISA were then analyzed using PCR followed by sequencing to confirm the presence of the virus in peanuts.

PCR Analysis

ELISA positive samples were validated using PCR and sequencing molecular methods specific to GRSV, TCSV, TSWV and *Soybean chlorotic spot virus* (SCSV). Due to time and monetary constraints, a subsample of the ELISA positive samples was processed using these techniques. The subsample consisted of the ELISA positives that had the strongest assay reactions.

4. Results

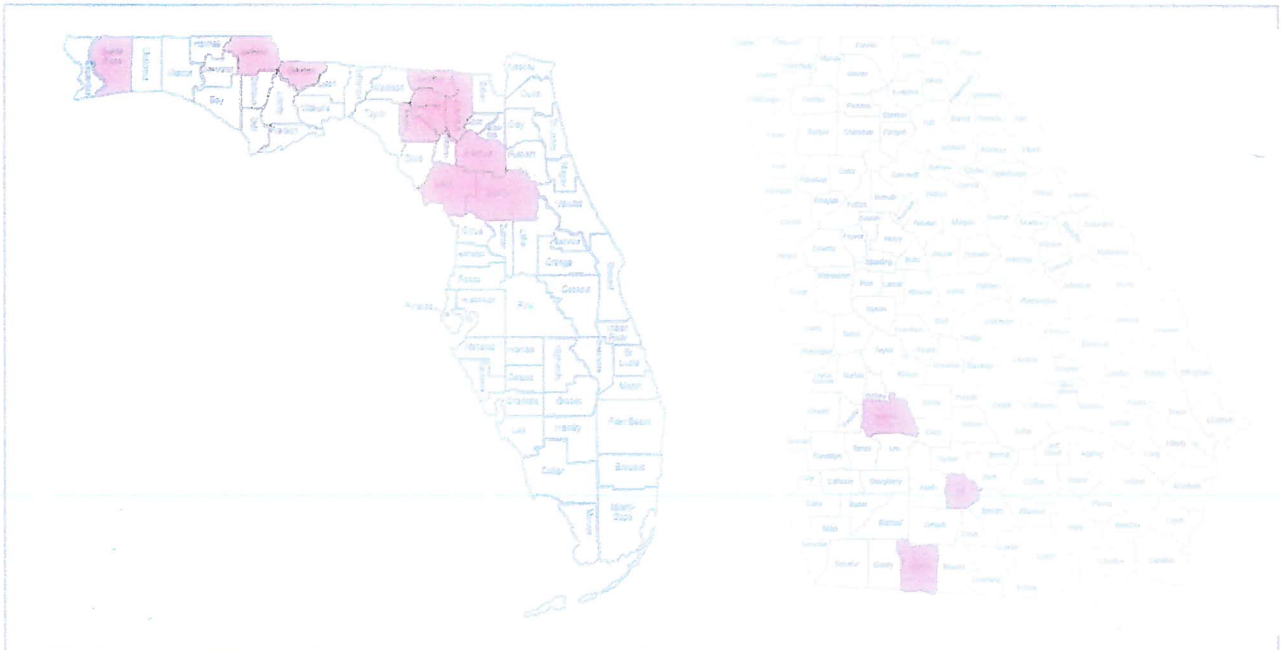

Figure 1. County maps of Florida and Georgia, U.S.A. Counties highlighted in red indicate where at least 1 field sample of 10 virus symptomatic plants was collected for the *Groundnut ringspot virus* survey in 2012.

Table 1. Site and collection information of the 10 symptomatic peanut plant samples collected from the 29 sites for the *Groundnut ringspot virus* survey during the summer of 2012.

State	County	Variety	Planting Date[a]	Collection Date
Florida	Marion	Georgia Valencia	4/4/2012	7/25/2012
Florida	Marion	UF12302 (3)	3/27/2012	7/25/2012
Florida	Marion	Georgia 06G	6/?/2012	7/25/2012
Florida	Levy	Tifguard	4/12/2012	7/25/2012
Florida	Levy	Florida 07	---	7/25/2012
Florida	Lafayette	Georgia 06G	4/25/2012	7/27/2012
Florida	Lafayette	Georgia 06G	4/25/2012	7/27/2012
Florida	Suwannee	Georgia 06G	4/17/2012	7/27/2012
Florida	Santa Rosa	Georgia 07W	5/8/2012	7/31/2012
Florida	Santa Rosa	Georgia 06G	---	7/31/2012
Florida	Santa Rosa	Georgia 06G	---	7/31/2012
Florida	Jackson	Florunner	---	7/31/2012
Florida	Jackson	Florida Fancy	---	7/31/2012
Florida	Jackson	Florida 07	---	7/31/2012
Florida	Jackson	TUFRunner 727	---	7/31/2012
Florida	Jackson	FloRun '107'	---	7/31/2012
Florida	Gadsden	Georgia Green	5/14/2012	8/1/2012
Florida	Gadsden	Georgia 06G	5/14/2012	8/1/2012
Florida	Alachua	Georgia 06G	---	8/8/2012
Florida	Hamilton	Georgia 06G	---	8/10/2012
Florida	Hamilton	Georgia 06G	4/26/2012	8/10/2012
Florida	Hamilton	Florida-07	---	8/10/2012
Florida	Columbia	Georgia 06G	---	8/17/2012
Florida	Columbia	Georgia 06G	---	8/17/2012
Georgia	Sumter	Georgia 06G	---	7/31/2012
Georgia	Thomas,	Tifguard	5/29/2012	8/1/2012
Georgia	Thomas	Georgia 10T	5/29/2012	8/1/2012
Georgia	Tift	Georgia 06G	4/25/2012	8/1/2012
Georgia	Tift	Georgia 06G	4/25/2012	8/1/2012

[a] Plant date was not available for cells with ---

Table 2. Results of the visual assessment of Tospovirus symptoms in field plants and the rate of detection of tospovirus using a GRSV/TCSV ELISA in samples collected from 29 fields in 10 counties in Florida and 3 counties in Georgia

State	County	No. Sites	% Symptomatic Plants/Field	% Samples Positive for Tospovirus by ELISA[1]
Florida	Alachua	1	<1	50
	Columbia	2	<1 - 2	100
	Gadsden	2	2	100
	Hamilton	3	<1	20-60
	Jackson	5	1 - 40	60-100
	Lafayette	2	1 - 3	60
	Levy	2	0 - 1	0-30
	Marion	3	< 1 - 20	20-40
	Suwannee	1	15	90
	Santa Rosa	3	<1 - 1	90-100
Georgia	Sumter	1	<1	100
	Thomas	2	<1	80-90
	Tift	2	<1 – 10	60-90

[1] ELISA results are based on results from crowns of 10 symptomatic plants per field

PCR and Sequence Analysis

All the samples submitted for PCR and sequence analysis were identified as TSWV, and not GRSV, TCSV or SCSV.

5. Summary

Overall, the data collected from this survey indicates that *Groundnut ringspot virus* (GRSV) is not currently present in the peanut production fields of Florida and Georgia. However, the presence of this pathogen in solanaceous crops of southern Florida is still a concern for peanut growers. It is important that the growers, industry personnel and extension educators continue to scout for peanut viral problems in the future. Monitoring for GRSV and other viral diseases is critical to determining proper management and control of these pathogens. Ultimately, resistant varieties are the best management strategy for viral pathogens, however, the early identification of GRSV in peanuts may allow growers to adjust their planting schedules appropriately to avoid major impacts from this disease.

The accurate identification of viral pathogens is a key step in determining the proper management approaches for these plant diseases. Enzyme-linked immunosorbent assays (ELISA) can provide researchers with a simplified method for identifying and monitoring the spread of viral pathogens. The non-specificity of the GRSV/TCSV reagent kit used in this study with *Tomato Spotted Wilt Virus* (TSWV) in peanuts is a concern for using ELISA as a monitoring tool. It is not uncommon for ELISA kits to be non-specific for viral pathogens from the same genus, such as GRSV and TSWV, but the inability to differentiate these pests means that more expensive and time consuming techniques are required for proper identification. In the future, if a significant viral outbreak is observed in peanuts it will be critical that researchers use PCR followed by sequencing to properly identify the pathogen. ELISA kits may still be useful to determine the viral genus (i.e. Tospovirus), but do not appear to be reliable enough to identify viral species.