Executive Summary.
Project ID: 309; PI Dr. R. Srinivasan
Project Description: Variation of TSWV strains in peanut fields and its implications in sustainable peanut production in the SE US.
Institution: University of Georgia Research Foundation
Project fiscal year: 2010

Research in 2009 indicated that the characterization of TSWV isolates from weed species may not be feasible as the natural infection rates were less than in peanut. Instead, we attempted to collect and characterize various TSWV isolates from resistant and moderately-resistant peanut cultivars. Though the cultivars are known to exhibit field resistance, they can be infected with the virus and may not display typical TSWV symptoms.

Leaf samples from TSWV-resistant and moderately-resistant peanut cultivars were collected from peanut fields in ten major peanut producing counties in South Georgia. These samples were initially tested for the presence of TSWV using serological and molecular techniques. PCR positive samples were partially sequenced subsequently. Phylogentic analysis indicated that the nucleotide/amino acid composition in resistant and susceptible isolates were similar. In fact, these isolates also were compared with TSWV isolates from tomato and tobacco. These isolates were very similar to the isolates obtained from peanut. Sequences of isolates that were obtained in 2010 also were compared with the sequences of TSWV isolates in the 1990s. Results revealed that the virus had undergone some changes. But, these changes may not be enough to increase the virulence of newer isolates.

We evaluated several new cultivars such as GA-06G, GAGreener, Georganic, Tifguard, and GAGreen against TSWV and against tobacco thrips. Greenhouse inoculations induced typical TSWV symptoms on both resistant and moderately-resistant cultivars. Also, we examined TSWV titers in all the cultivars using real time PCR. Results revealed that the resistant cultivars were also infected at the same rate as that of the moderately-resistant cultivar GAGreen. TSWV titer levels in resistant cultivars were not significantly different from that of moderately-resistant cultivar GAGreen. Numerous choice and no-choice experiments were conducted using peanut cultivars and tobacco thrips. Plant injury/damage induced by thrips as well as ability of these cultivars to support thrips populations was examined. These cultivars were only bred to exhibit resistance against the pathogen but not against the vector itself. However, our results indicated differential responses of thrips to peanut cultivars. Cultivars such as Georganic and Tifgaurd had fewer thrips than other cultivars. On the contrary, a more in-depth biology study did not identify such differences. Based on the results, the resistant cultivars can be infected with TSWV and display typical TSWV symptoms under greenhouse conditions. But, these cultivars are already known to display field resistance. It is unclear as to how these resistance-inducing mechanisms operate in peanut. The variation among TSWV isolates does not indicate any evidence of development of resistance breaking isolates that may threaten the prolonged usage of these resistant cultivars.
Interpretation of results: Careful examination of TSWV isolates indicated that the resistant and moderately-resistant cultivars are in no direct threat from TSWV isolates in GA. There is no evidence of development of resistance-breaking isolates. However, consistent monitoring is required to identify such changes as RNA viruses such as TSWV are capable of evolving rapidly.

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NATIONAL PEANUT BOARD/SOUTHEAST
PEANUT RESEARCH INITIATIVE
QUARTERLY PROGRESS REPORT FOR WORK
DONE UNDER RESEARCH AGREEMENT

Final Report

INSTITUTION: University of Georgia

Project Title: **Variation of *Tomato spotted wilt virus* strains in peanut fields and its implications in sustainable peanut production in the Southeastern United States**

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GACCP Control NO:

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EXPIRATION DATE: June 30, 2011

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Peanut foliar samples exhibiting TSWV symptoms from ten counties (Tift, Colquitt, Berrien, Burke, Sumter, Decatur, Coffee, Toombs, Terrell, and Tattnall) were collected from resistant and susceptible peanut cultivars and were subjected to RT-PCR. The amplicons were sequenced and phylogenetic analysis was conducted to assess the differences among sequences. These sequences were compared with TSWV N-gene (whole gene) sequences obtained from Georgia crops ten years ago. Analysis indicated that there were minor variations associated with the isolates collected from peanut genotypes. However, these variations also were observed in the sequences collected ten years ago. In fact, there was only one amino acid change that was consistent in new isolates when compared with isolates collected a decade ago. These results emphasize that TSWV has not undergone substantial changes at least in the gene we tested. It is true that other genes could have undergone extensive variations and need to be examined. Nevertheless, our results indicate that the selection pressure induced by TSWV on resistant cultivars seems to be less intense.

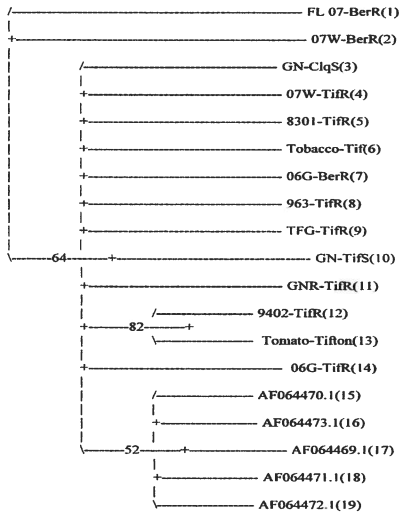

Fig. 1. TSWV N-gene amino acid sequences were used for analysis. The first two or three letters indicate the name of the cultivar/line; next three represent the county name, and R and S represent resistant and susceptible, respectively. The sequences beginning with A belong to sequences isolated from GA 10 years ago.

Experiments were further conducted to identify the various factors that could influence TSWV resistance in resistant cultivars. We attempted both mechanical and thrips-mediated inoculations on four resistant cultivars (GA-06G, Tifguard, GAGreener, Georganic) and GAGreen. Mechanical and thrips-mediated inoculation protocols have been standardized with assistance from previous NPG grants. Also, we attempted to quantify viral titers in the resistant and susceptible cultivars through Real Time Reverse Transcriptase PCR (RT-RT-PCR). Finally, we evaluated thrips feeding responses on the above-mentioned cultivars.

Fig. 2. TSWV-specific symptoms on TSWV resistant peanut cultivars. Peanut cultivars were inoculated mechanically.

Almost all the resistant cultivars inoculated upon mechanical inoculation were infected with TSWV and they displayed TSWV-specific symptoms. Figure 2 shows Tifguard and GA Greener with TSWV-specific symptoms. Also, RT-RT-PCR confirmed our mechanical and DAS-ELISA results. Results suggested that TSWV accumulated in similar levels in both resistant and moderately resistant cultivars. Several resistant cultivars accumulated more titer than GA Green, the number of viral copies varied from 700,000 to 1.8 million. This indicated that resistant cultivars also can accumulate high viral titers under the right conditions.

Further, thrips feeding response and thrips reproduction was evaluated on the same cultivars. This experiment was performed to delineate the effects of resistant cultivars on the virus as well as on the vector, respectively.

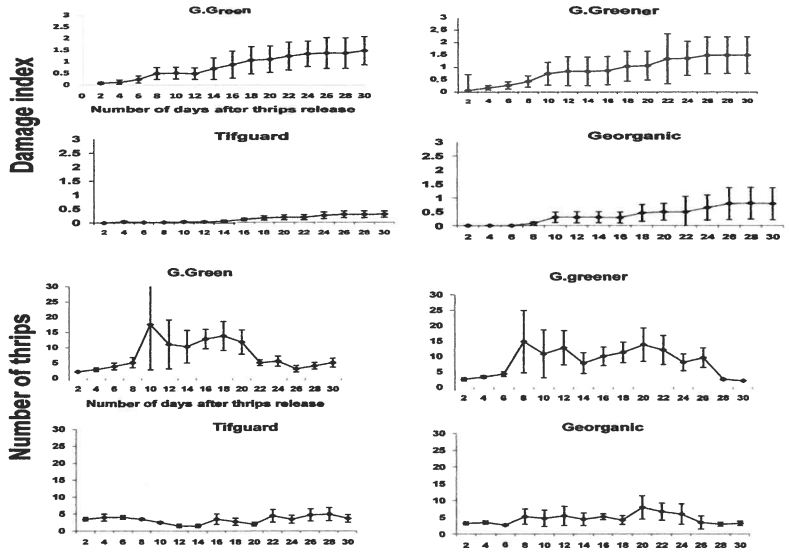

Fig. 3. Thrips feeding damage index in peanut cultivars

Fig. 4. Thrips reproduction in peanut cultivars

Results reiterated that thrips reproduction was differentially influenced by different cultivars. Reproductive performance of thrips was lower in some cultivars when compared with others such as Georgia Green and Georgia Greener. Similar results were obtained with no-choice tests. Therefore, field resistance observed in many of the newly-released second generation peanut cultivars seems to be influenced by a multitude of factors. More detailed studies on biology and thrips-mediated acquisition and transmission aspects are being conducted as a part of this year's National Peanut Board's proposal this year (2011). Results so far, have been very impressive and continue to shed more light on thrips-TSWV-peanut and their interactions.