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**NPB 2011. Summary; PI: Rajagopalbabu Srinivasan**

**Title:** Variation of *Tomato spotted wilt virus* isolates in resistant and susceptible peanut cultivars and its influence on durability of resistance and thrips sampling and *Tomato spotted wilt virus* detection in thrips and their usefulness as a component of Peanut Risk Index

Our research with funding (2010-2011) from NPB evaluated the diversity of TSWV isolates in resistant and susceptible peanut varieties. Our goal was to assess if there were any isoates that might pose a threat to the resistant cultivars. TSWV isolates were partially sequenced. The sequences of these isolates were compared with each other and also with sequences of TSWV isolates submitted from the same location ten years ago. However, the comparison only indicated minor differences. In general, these differences were not consistent and varied within resistant and susceptible cultivars. In 2011-2012, to understand the mechanism/s of resistance, we tested the newly released second generation TSWV resistant peanut cultivars by mechanical inoculation. We estimated infection rates, virus loads, and thrips feeding patterns. Results indicated that the resistant genotypes can get infected with TSWV, accumulate TSWV, and yet can be tolerant of TSWV infection. Though our results with mechanical inoculations indicated that TSWV-resistant cultivars can be infected with the virus and can accumulate TSWV, TSWV is almost exclusively transmitted by thrips in the nature. Thrips-mediated inoculations were also conducted. Results, however, indicated that they were not markedly different from mechanical inoculations. Experiments conducted in 2011 revealed that thrips can acquire TSWV from both resistant and susceptible genotypes. These results reiterated that under a high thrips and TSWV pressure the resistant genotypes may serve as inoculum sources and influence TSWV spread. Therefore, the effectiveness of these cultivars could be increased by continuing to grow these genotypes in conjunction with other cultural and chemical tactics. More research is currently being conducted to evaluate the compatibility of these new resistant genotypes with other cultural and chemical tactics.

These new varieties were not believed to possess any thrips resistance. But, they have never been evaluated for thrips resistance in depth. Our results indicated differential effects on thrip fitness and feeding patterns, though not substantial.

TSWV resistant peanut cultivars are the number one management option the growers have, loss of usefulness of these cultivars may be a major blow to peanut production. Our results suggested that there was no immediate selection pressure on the TSWV resistant cultivars and their usefulness may not be compromised in the near future. However, it is very important to realize that these cultivars alone might not be sufficient to suppress thrips and TSWV incidence as they can still get infected with the virus, accumulate TSWV, and support thrips populations. A management package is still necessary. With that said, the new cultivars are providing us with more flexibility. With the impending loss of one or more key insecticides we believe that our efforts will lead to updating the thrips-TSWV management package and maintain sustainability in peanut production in the southeast.

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INSTITUTION: University of Georgia

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Res. Agr. No.:  
GACCP Control NO:

PROJECT LEADER Dr. Rajagopalbabu Srinivasan

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NPB CONTACT Marie Fenn or M. Mehok

NPB Project NO:

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The goals of this proposal were to evaluate the variation among TSWV isolates obtained from TSWV-resistant and susceptible peanut genotypes, to assess the response of TSWV-resistant genotypes to thrips and TSWV, and to evaluate detection techniques in both thrips and peanut plants. TSWV isolates recovered from 80 different TSWV-resistant and susceptible peanut genotypes indicated that the virus isolates did not reveal significant differences between TSWV-resistant and susceptible genotypes. Furthermore, nucleotide sequences were also compared to TSWV N-gene sequences from Georgia isolated ten years ago and deposited in the NCBI database. Again, no significant differences were noticed (only one amino acid was consistently different). Most of this research was performed with grants from NPB in 2010. However, these results prompted us to consider other relevant questions. What are the mechanisms of resistance that confer resistance in peanut genotypes? How the genotypes that display field resistance to TSWV would respond to TSWV inoculations and thrips? It is important to note that none of these cultivars have ever been evaluated in the greenhouse or in the laboratory against either thrips or TSWV.

In 2011, in first two quarters, we began by evaluating interactions between thrips and new cultivars as well as with TSWV through thrips mediated transmission experiments. We inoculated TSWV-resistant cultivars (Georganic, Tifguard, GA06G, NC94022) and a moderately resistant peanut cultivar (Georgia Green) through potentially viruliferous thrips. As in the case of mechanical inoculations, thrips mediated inoculations also induced typical TSWV symptoms in all the cultivars tested (Figure 1). However, Georgia Green was infected at a higher rate than other cultivars (Figure 2). Estimations of TSWV viral loads in second-generation TSWV resistant cultivars indicated differences among TSWV N-gene copies. In general, Georgia Green accumulated more TSWV copies than others. Though NC-94022 was infected at a lower rate, TSWV N-gene copies in NC-94022 were comparable to Georgia Green (Figure 3). These results

reiterated that all the second-generation cultivars were indeed susceptible to TSWV albeit at varying levels. The second-generation cultivars seem to exhibit tolerance to TSWV. Prior research from our laboratory has already shown that TSWV variation in resistant and susceptible genotypes is not consistent, and does not pose a grave threat to development of resistance breaking strains. However, these results should be cautiously interpreted as only a portion of TSWV genome was sequenced.

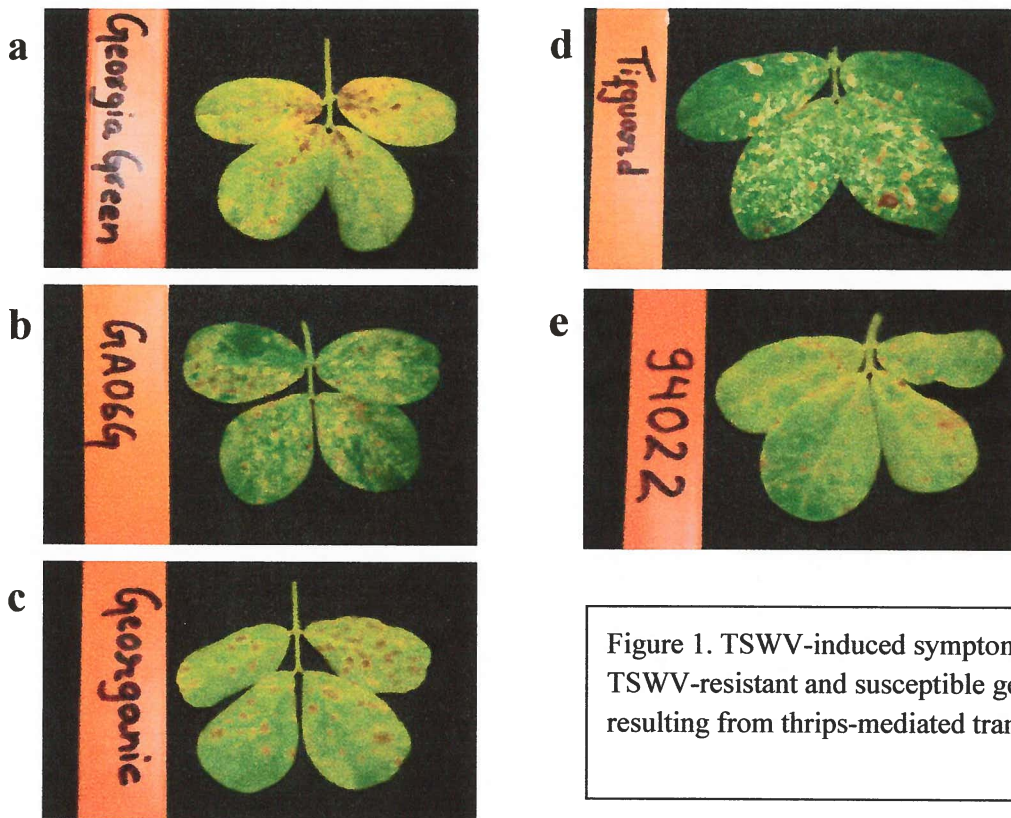


Figure 1. TSWV-induced symptoms in TSWV-resistant and susceptible genotypes resulting from thrips-mediated transmission

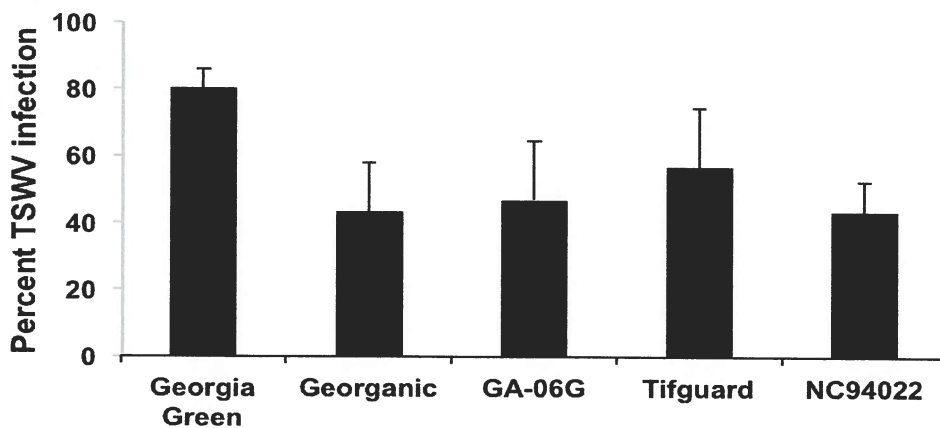


Figure 2. TSWV infection rates in TSWV-resistant and susceptible genotypes resulting from thrips-mediated transmission.

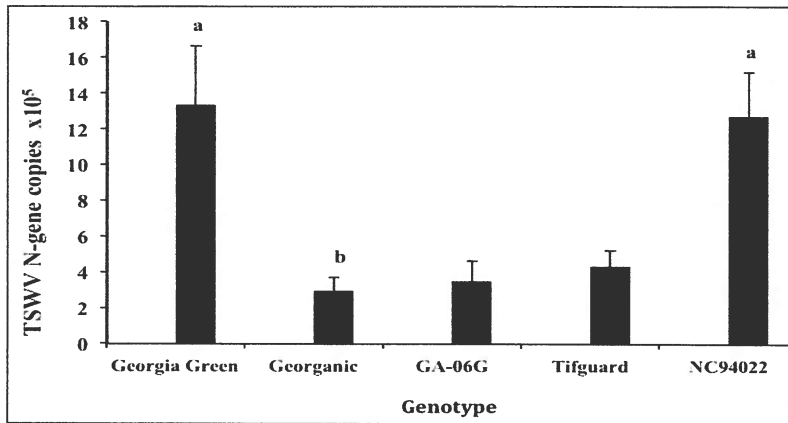


Figure 3. TSWV N-gene copies in TSWV-resistant and susceptible genotypes resulting from thrips-mediated transmission.

In the third and fourth quarters, we evaluated the effect of second-generation TSWV resistant genotypes on thrips biology and thrips TSWV acquisition ability from resistant genotypes.

Biology studies were conducted using Munger cells and leaflets of different cultivars. The experiment was replicated two times with ten Munger cells for each cultivar in each repeat of the experiment. Ten female tobacco thrips, *Frankliniella fusca* were introduced into each cage. Results indicated that *F. fusca*'s reproduction efficiency was greater in Georgia Green than in other resistant cultivars. However, this just could be a cultivar effect as thrips were maintained on Georgia Green for several generations. This hypothesis is in line with the notion that the second-generation cultivars were resistant only to the virus but not thrips. The time required to complete one thrips generation did not vary with peanut cultivars. However, it was also found that the development time in Tifguard was the lowest when compared to the other genotypes (median development time 11 days when compared with 13 and 15 days in other

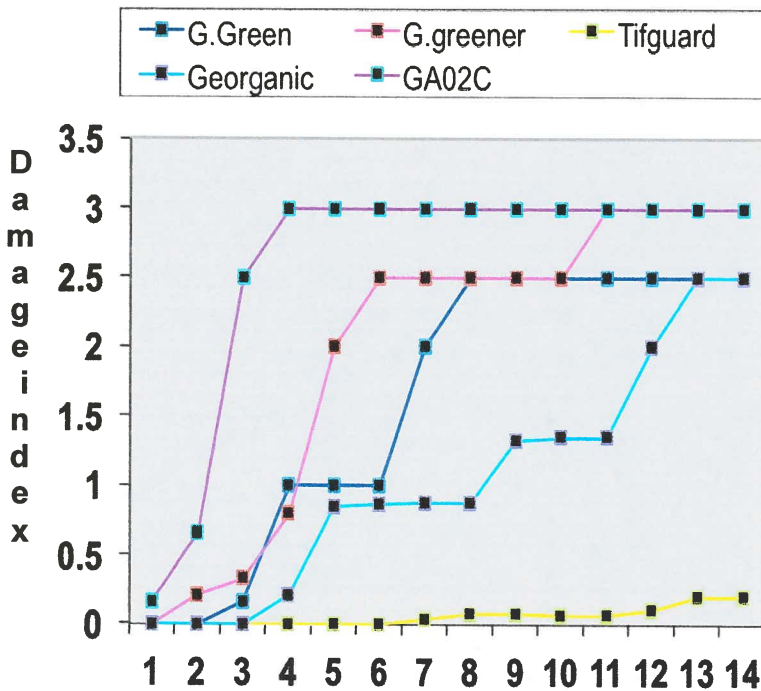


Figure 4. Thrips feeding damage index in TSWV-resistant and susceptible genotypes. Observations were taken at 2-day intervals

genotypes). Tifguard has a different parentage when compared to other genotypes. Tifguard has nematode resistance and it is likely that it could have some thrips resistance also. These results also correspond with our feeding damage index studies (Figure 4). Even in that study both Georganic and Tifguard had reduced feeding damage than the others, it is not clear as to what would cause these effects. More research is clearly needed to understand these interactions in detail. We believe that our current transcriptomics research will be a useful avenue to answer some of these questions.

In the fourth quarter, the ability of thrips to acquire and transmit TSWV from resistant cultivars was evaluated using western blotting and ACP-ELISA. The presence of TSWV non-structural protein was targeted using specific antibodies by ELISA as well as by western blotting. The presence of non-structural protein in thrips is indicative of virus replication and virus replication is a requirement for virus transmission. Experiments conducted with ACP-ELISA also indicated that thrips could acquire TSWV from both second generation resistant cultivars as well as from Georgia Green. However, the assays were met with consistent background effects. This indicated that more optimization of the protocol has to be undertaken before we can routinely use this assay for detection of NSs proteins in thrips. In order to circumvent this issue and answer our question as to whether thrips can acquire TSWV from second-generation TSWV-resistant cultivars, we used western blotting. Results with individual thrips indicated that thrips could acquire TSWV from second-generation TSWV-resistant cultivars. The results also suggested that thrips were able to acquire TSWV from second-generation TSWV-resistant peanut genotypes than from Georgia Green. Together with transmission studies, these results implied that the second-generation cultivars also could serve as thrips and TSWV reservoirs, but to a slightly lower degree than susceptible cultivars. Under high thrips and/or TSWV pressure these cultivars could likely succumb to spotted wilt disease. More studies were conducted to address this issue in 2012.

ELISA and/or western blots targeting the non-structural protein of TSWV could be useful to identify thrips as transmitters of TSWV. However, they are both very tedious, expensive, and time consuming procedures. Both procedures take two days to complete and the cost is prohibitive (1 ml of antiserum currently costs <\$700). For instance, last year the incidence of TSWV was very low in almost all the sampled sites, and the detection rate in thrips was extremely low despite testing hundreds of thrips. It might be worthwhile to devise a simpler strategy to detect TSWV in plants directly in the field (in peanut plants/weed hosts) early in the season (even prior to planting). This strategy could be more efficient and might not require extensive thrips sampling. A simple immunostrip assay in the field followed by PCR confirmation might just be sufficient. We intend to investigate this further in the following season. We believe that winter and spring season weeds (as well as volunteer hosts) and their TSWV-infection status could be assessed fairly easily. This assessment might be useful from a spotted wilt prediction standpoint and Peanut Risk Index estimation than sampling for thrips exclusively.

In the subsequent quarter, we also conducted biology experiments, feeding experiments, oviposition experiments. Results indicated that interactions between thrips and TSWV are complex and might also be influenced by the crop host. TSWV infection did not affect settling of thrips, but the infection status of host plants as well as thrips seem to significantly affect their feeding patterns as well as their oviposition and biology. TSWV infection in thrips seems to enhance their egg-laying capacity and the underlying physiological factors that could have

contributed to greater egg production were examined in greater detail. Results suggest that despite some minor benefits associated with thrips fitness, in general, their growth and development seems to be affected by TSWV infection in thrips as well as in peanut plants.

In the last quarter, most of the time was devoted to conducting statistical analysis and interpretation of results. Two manuscripts were prepared for two peer-reviewed journals. One of them has already been accepted and is currently in press (*Entomologia Experimentalis et Applicata*) and the other one will be submitted in the next week. All these results will also be discussed in the Peanut Risk Index meeting in November/December 2012.