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

Report: Final report 2014

INSTITUTION: University of Georgia

Project Title: Utilization of new peanut cultivars for the management of thrips-transmitted *Tomato spotted wilt virus* (TSWV) and understanding interactions among thrips, newly released peanut cultivars, and TSWV through transcriptomics

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The two objectives outlined in the NPB 2014 proposals were:

a) Evaluate the compatibility of new cultivars such as GA 10T and GA 12Y with chemical and cultural management tactics for thrips and *Tomato spotted wilt virus*.

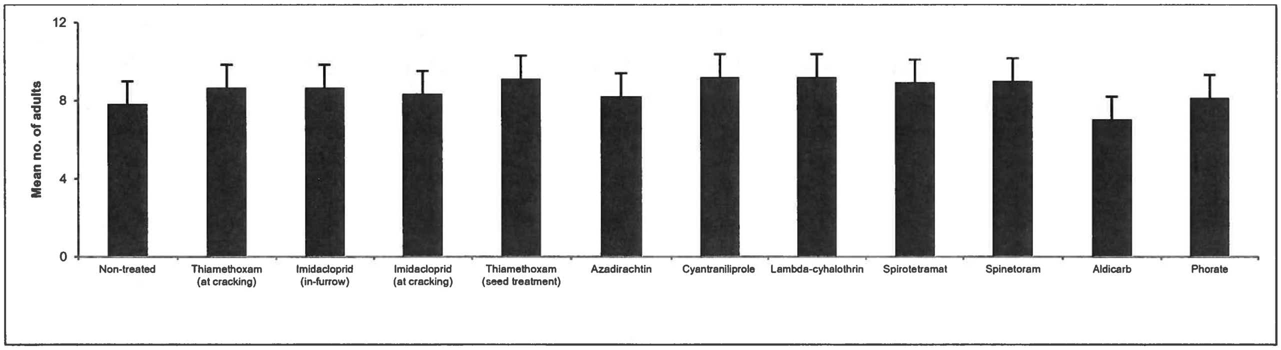
b) Identify genes and their putative functions in newly released peanut cultivars with and without TSWV infection, and also in thrips with and without TSWV infection.

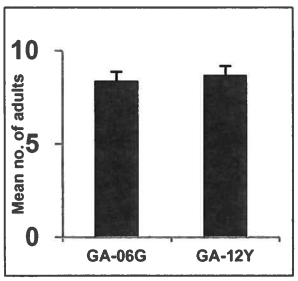
Below, I will briefly describe how these objectives were completed and highlight some of the results that were obtained.

- Peanut cultivars, Georgia-06G and Georgia-12Y, were planted and eight potential alternative insecticides were selected and evaluated in a trial at the Belflower Farm, Coastal Plain Experiment Station, Tifton, GA in 2013.
- A split plot design was adopted with peanut cultivars representing main-plot effects and insecticide treatments representing sub-plot effects. Each plot representing a replicate included six 30 feet rows spaced 3 feet apart.
- Thrips samples were collected from peanut terminals and peanut blooms three weeks after planting date for six consecutive weeks. Thrips feeding damage was recorded at five weeks after planting using an arbitrary scale from 0 to 10 (0 represent no feeding injury and 10 represent a dead plant). Spotted wilt incidences were rated two weeks prior to harvest by measuring the percentage of TSWV-infected plants per row ft. All peanuts were harvested at ~135 days after planting.
- All raw data from the trial were subjected to generalized linear mixed models using PROC GLIMMIX in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Tukey-Kramer

Grouping, as a revision for multiple comparisons at P=0.05, was used to identify the statistical significance of differences among treatments and between cultivars.

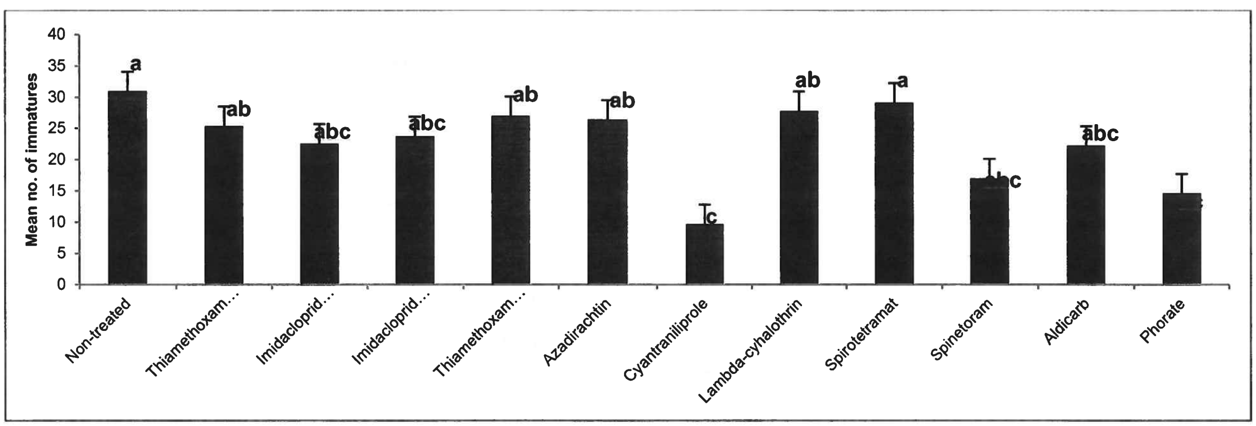
Evaluation of alternative insecticides to Temik and Thimet with GA-06G and GA-12Y.





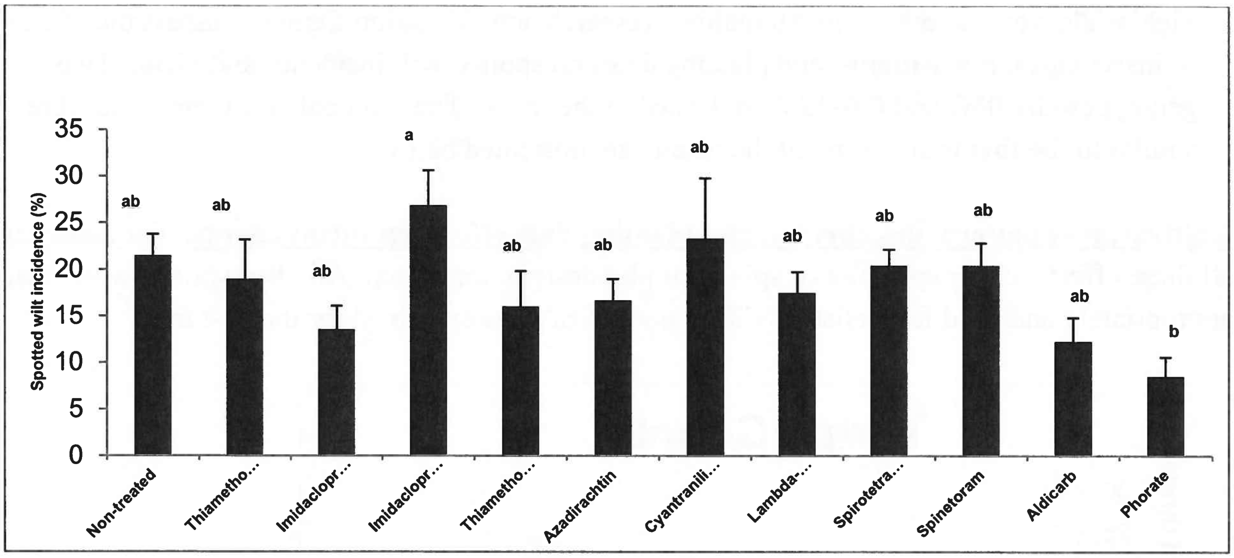
(top) Bars represent cumulative means(±SE) of adult thrips on two cultivars across insecticide treatments (bottom) Bars represent cumulative means(±SE) of adult thrips from plots treated with various insecticides across cultivars.

In general, insecticides were not so effective in suppressing adult thrips. However, they seem to be effective in suppressing thrips immatures.

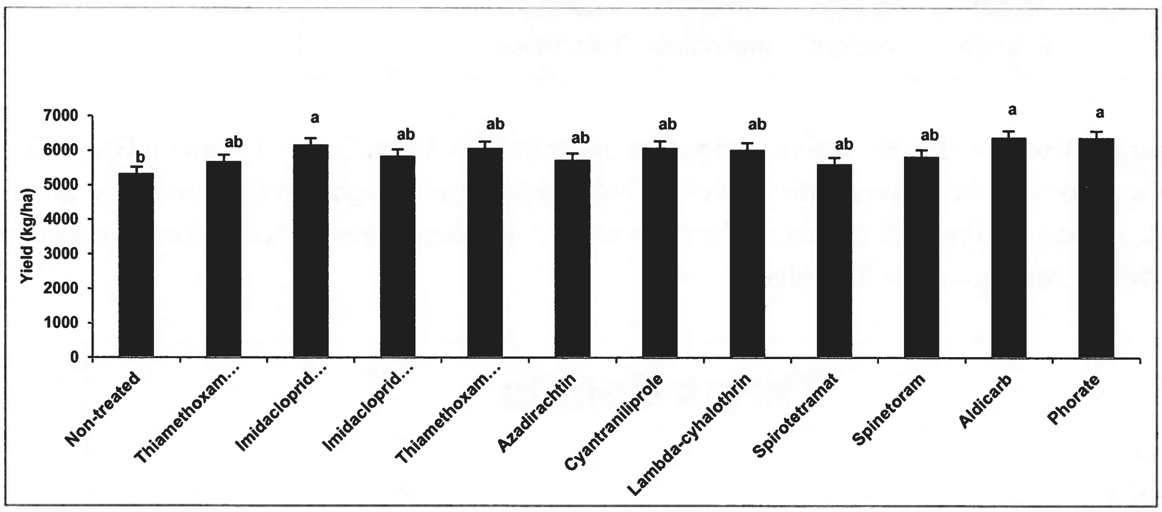


Bars represent cumulative means (±SE) of thrips immatures on two cultivars across insecticide treatments. No differences between the two cultivars were noticeable.

- None of the insecticides tested had any effect on adult thrips.
- However, alternative candidates such as imidacloprid, spinetoram, and cyantraniliprole suppressed immatures as effectively as aldicarb and phorate.
- Cultivar differences did not affect adult and immature thrips counts. Nevertheless, thrips feeding damage was slightly more on GA-12Y than on GA-06G.



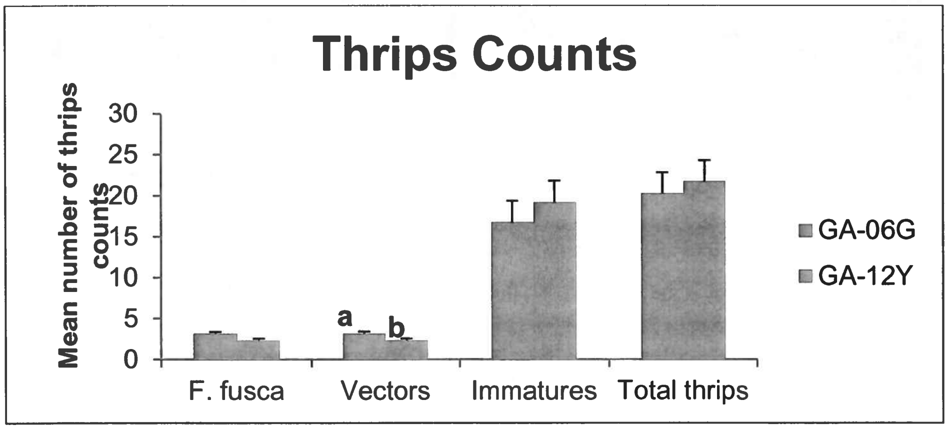
- Spotted wilt incidence on GA-06G was greater than GA-12Y.
- None of the insecticides including aldicarb and phorate suppressed spotted wilt incidence.



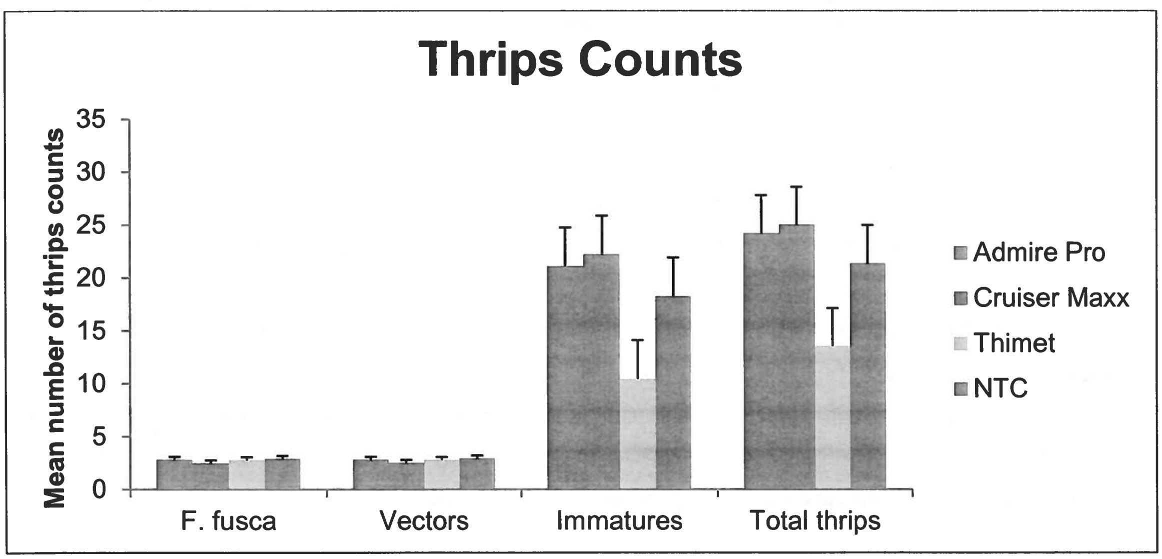
- Yields from non-treated plots were less than yields from insecticide treated plots.
- Yields from alternative insecticides and aldicarb and phorate treated plots did not differ from each other.
- Yields from the cultivar with more field resistance (GA-12Y) were greater than yields from the cultivar with less field resistance (GA-06G).
- These results suggest that, in the presence of cultivars with improved field resistance such as GA-12Y, it would be possible to replace older broad-spectrum toxicity insecticides with less toxic insecticides without compromising yields.

Field trials were conducted in Attapulgus Research and Education Center to assess the effects of insecticides, row patterns, and planting dates on spotted wilt incidence and yield. Two genotypes GA-06G and GA-12Y were used in the trials. The protocols for these trials were similar to the first trial. Some of the results are presented below.

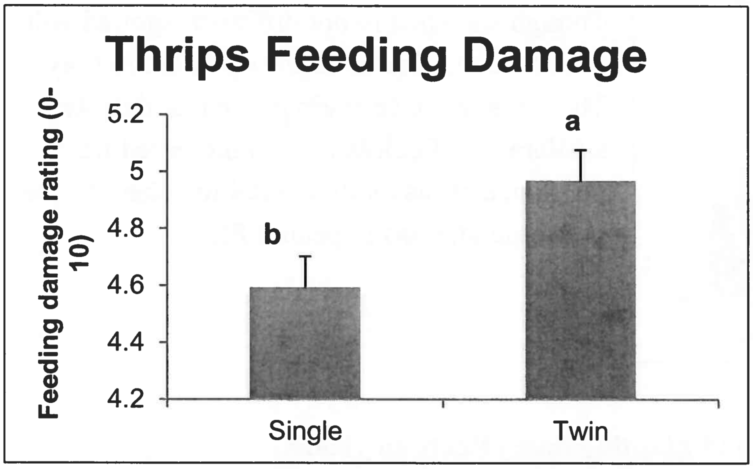
Cultivar, row pattern, insecticides, and planting date effects on thrips counts. For assessing all these effects, either split plot or split-split plot designs were used. All observations were then appropriately analyzed for statistical differences in SAS as explained for the first trial.



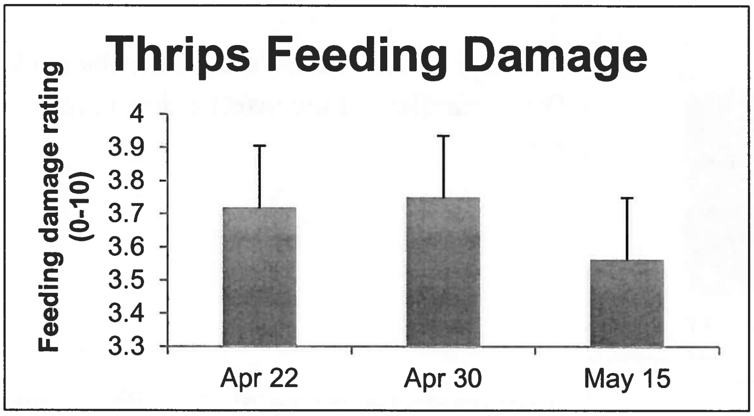
In general, there were fewer vectors (thrips that transmit TSWV) on GA-12Y than on GA-06G. The precise reason for this scenario is unclear, but it appears as though that there could be some cultivar induced differential effects on thrips as well. This phenomenon needs to be examined in depth before confirming such results.



Application of insecticides, as in the previous case, does not appear to have any impact on adult thrips. However, there seems to be some effect on immatures (larvae), especially with Thimet.

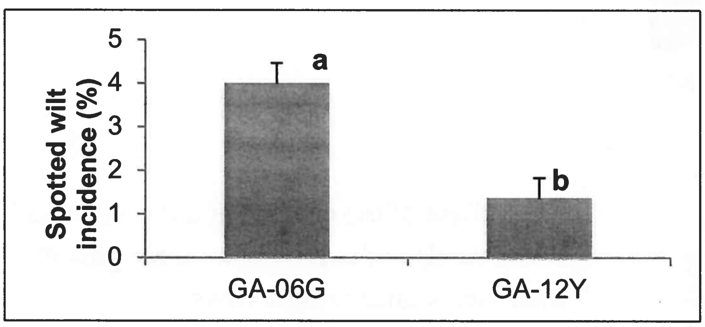


On the contrary to the common belief that thrips counts on twin row plots would be less than thrips on single row plots, we found more thrips on twin row plots than on single row plots.

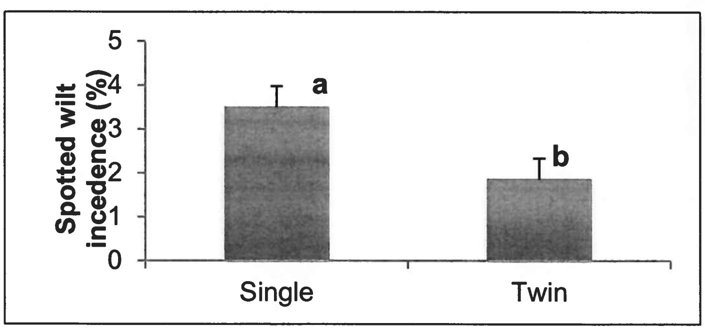


Though, statistically not very different, planting during the time recommended in the Peanut Rx seems to have some effect on thrips reduction

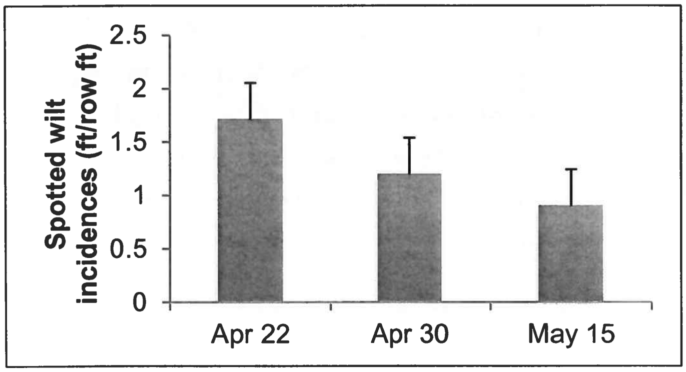
<u>Cultivar, row pattern, insecticides, and planting date effects on spotted wilt incidence.</u>



Despite the low incidence of spotted wilt in 2014 at Attapulgus, cultivar differences were noticeable. Spotted wilt incidence was lower in GA-12Y than in GA-06G.

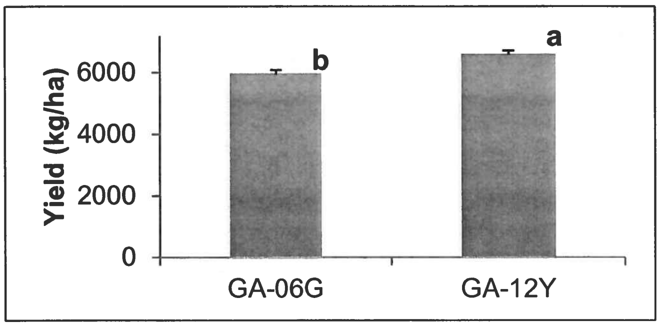


Despite more thrips on twin row plots, TSWV incidence was higher in single row plots than in twin row plots.

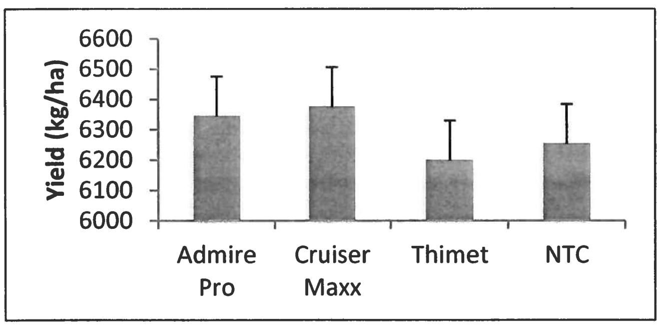


Though statistically not different, spotted wilt incidence was lower in plots planted in May. These results once again prove that despite availability of cultivars with increased field resistance, it may still be vital to adhere to the recommendations in peanut Rx.

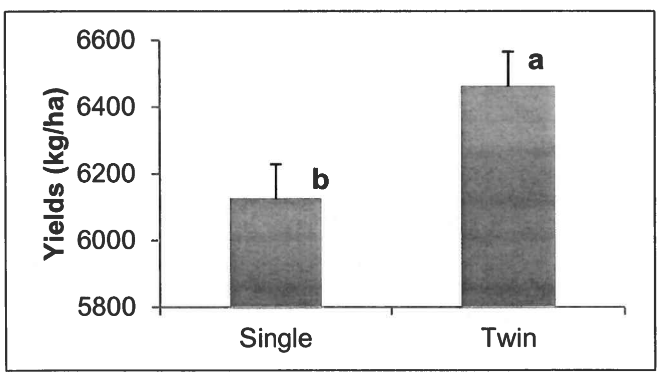
Cultivar, row pattern, insecticides, and planting date effects on yields.



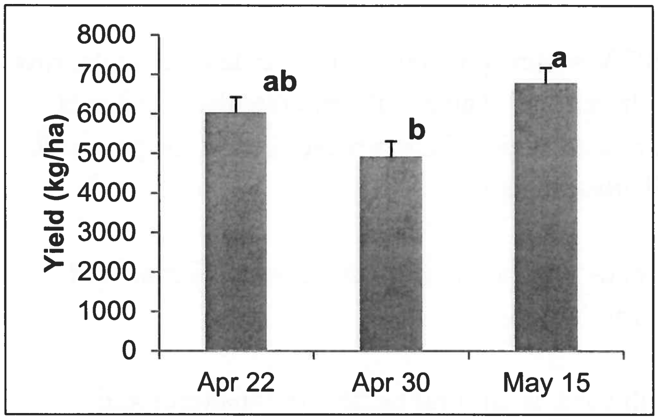
Yields from GA-12Y were greater than GA-06G regardless of the insecticide and row type.



Insecticide applications did not affect yields on either cultivar.

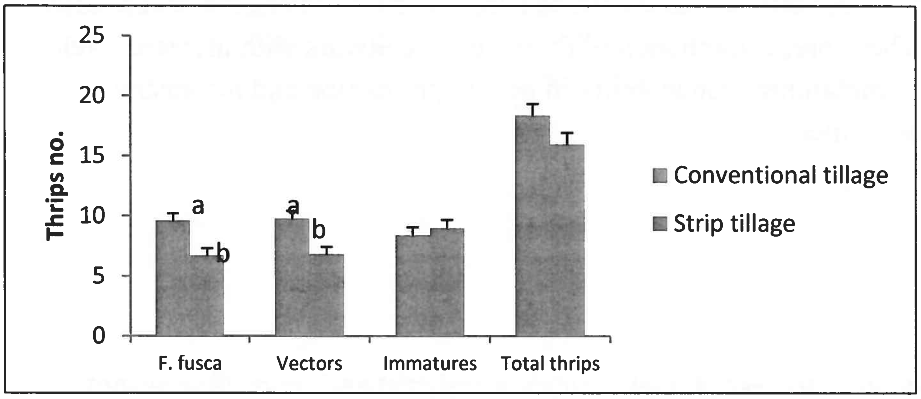


Regardless of the cultivar and the insecticide used, yields under twin rows were greater than yields under single rows.

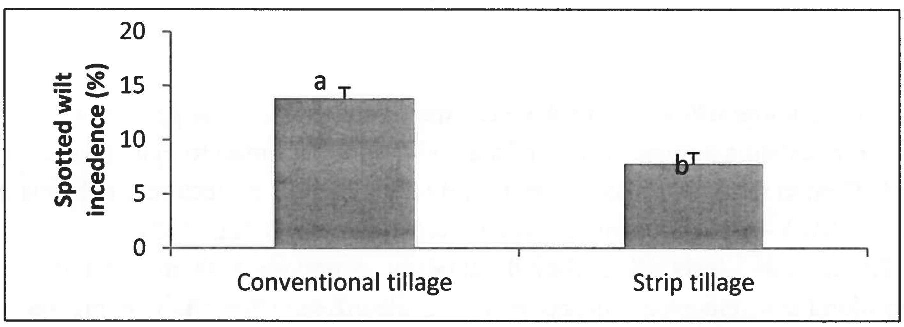


Regardless of the cultivar and insecticide treatments, yields were greater with May 15 planting than with earlier planting dates.

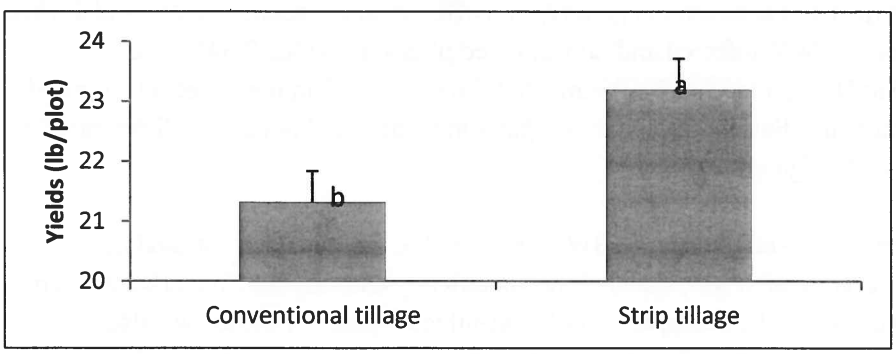
Tillage effects split plot trial in Tifton, GA.



Adult vector thrips were in general less on strip tillage plots than under conventional tillage plots



Spotted wilt incidence was reduced in plots under strip tillage than in plots under conventional tillage.



Yields, again, were better under strip till conditions than under conventional tillage.

Results indicate that thrips and TSWV incidence can be suppressed under strip tillage regardless of the cultivar used. The yields obtained were also greater under strip tillable than under conventional tillage.

Interpretations: /Summary

Cultivars with increased field resistance to TSWV still responded to insecticide treatments, row patterns, tillage conditions, and planting date alterations. The results reiterate that the newer cultivars are compatible with all the available risk reduction factors prescribed in the peanut Rx. Integrating these newer cultivars could be still advantageous:

They would allow the transition from using broad-spectrum insecticides such as Temik and Thimet to less toxic insecticides without compromising yields.

Newer cultivars still respond to integration with various cultural tactics (row patterns and tillage), as well as planting date alterations. Despite a substantially low spotted wilt year, such differences were realized. These differences would be even more visible under a severe spotted wilt year more readily. Therefore, combination of these newer cultivars with increased field resistance is very vital to maintaining sustainability in peanut production and increasing profitability to GA Peanut Farmers.

Objective 2.

***Tomato spotted wilt virus* induced transcriptional changes in resistant and susceptible peanut genotypes**

Thrips transmitted *Tomato spotted wilt virus* (TSWV) is a major constraint to the peanut production in the US. The use of resistant genotypes is one of the major strategies used to manage spotted wilt disease caused by TSWV. Though field resistance is extensively documented, molecular mechanisms underlying TSWV resistance in TSWV-resistant peanut genotypes are unknown. In this study, we sequenced TSWV-resistant (Tifguard) and susceptible (SunOleic) peanut genotypes with and without TSWV-infection using HiSeq Illumina sequencing. Subsequently, we identified differentially expressed (DE) contigs within the genotypes and between the genotypes. Differential expression analysis identified a total of 7,950 contigs between TSWV-infected and non-infected plants, of which 2,345 were upregulated in non-infected SunOleic, 4385 in TSWV-infected SunOleic, 432 in non-infected Tifguard, and 788 in TSWV-infected Tifguard. Further, pairwise comparison identified 448 contigs differentially expressed between SunOleic and Tifguard.

Functional annotations of non-infected and TSWV-infected DE contigs demonstrated that in SunOleic and Tifguard, contigs associated with metabolism specifically carbohydrate metabolism were upregulated in TSWV-infected plants when compared with non-infected plants. Further, we also documented downregulation of contigs associated with photosynthesis, photosystem II assembly, and thylakoid membrane organization in TSWV-infected SunOleic and Tifguard. Consistent with our results, several studies have also characterized virus-infected plants with increased carbohydrate metabolism and decreased photosynthetic rate. Among DE contigs in TSWV-infected plants, several homologs of plant defense related proteins such endoribonuclease dicer and pathogenesis-related proteins were present in both genotypes. Also, homologous contigs of plant disease resistance gene including receptor-like protein kinase and leucine rich repeat receptor-like serine threonine-protein kinase were upregulated in TSWV-infected plants when compared with non-infected plants of both genotypes.

Further, annotations of DE contigs between SunOleic and Tifguard provided insights into genes that are specific to resistant and susceptible genotypes. Both genotypes consisted of homologs of R- gene associated disease resistance genes including nucleotide binding site plus leucine-rich repeat (NBS-LRR), receptor-like kinase, and Toll and Interleukin-1 Receptor (TIR-NBS-LRR). A homolog of *Tobacco mosaic virus* (TMV) N resistance gene that prevents systemic movement of the virus was upregulated in non-infected SunOleic when compared with non-infected Tifguard. However, following TSWV-infection, three homologous contigs of TMV N gene were upregulated in Tifguard only. Also, a homolog of resistance protein pltr, which is a (NBS-LRR) type disease resistance protein found in *Arachis hypogaea* (L.) was only upregulated in TSWV-infected Tifguard. Further, several homologs of a lectin protein that is known to inhibit systemic movement of *Tobacco etch virus* was only upregulated in non-infected and TSWV-infected plants of Tifguard genotype. In case of SunOleic, more homologous contigs of recpetor-like-kinase were present than in Tifguard. Furthermore, TSWV-infection upregulated homologs of gene-silencing protein, argonaute 4a in SunOleic when compared with Tifguard.

This study provided in depth knowledge on changes that occur in SunOleic and Tifguard following TSWV-infection at transcript levels. It also provides insights into transcripts specific to resistant and susceptible genotype. These sequences are now available in a public database and could be used by peanut researchers widely. More downstream research is planned to examine how this information could be used to elucidate resistance mechanisms and also assess if the resistance in these cultivars is sustainable.

Transcriptional changes associated with TSWV-infection in *Frankliniella fusca* at various life stages

Thrips transmitted *Tomato spotted wilt virus* (TSWV) is a type member of the genus *Tospovirus* in the family *Bunyaviridae*. TSWV is transmitted in a persistent propagative manner. Also, TSWV acquisition during early larval stages is a requisite for TSWV transmission. Fitness studies conducted on tobacco thrips, *Frankliniella fusca* [Hinds] and TSWV demonstrated positive effects of TSWV-infection on *F. fusca* oviposition but TSWV-infection negatively affected adult survival rate. To elucidate TSWV-induced changes in *F. fusca* at molecular levels, in this study, we adapted a transcriptomic approach. We examined transcriptional changes associated with TSWV-infection in *F. fusca* at various life stages. With differential expression analysis, we identified 395, 204, and 562 differentially expressed (DE) contigs following virus infection in larvae, pupae, and adults, respectively.

Functional annotations of DE contigs revealed upregulation of contigs associated with virus movement such as clathrin-mediated endocytosis in viruliferous adults. Also, as expected, homologs of proteins associated with virus replication were upregulated by several folds in viruliferous adults. We further documented upregulation of contigs associated with immune pathways including apoptosis, phagocytosis, and RNAi, mostly in viruliferous adults when compared with viruliferous larvae and pupae. In viruliferous larvae, contigs associated with development including neuron development and cell division were downregulated. Despite evidence for potential negative effects, upregulation of contigs associated with egg production and embryo development were also documented in viruliferous adults.

This study provided insights into stage specific transcriptional changes associated with TSWV–infection in *F. fusca*. This information is also available through the public database and could be accessed by thrips and peanut researchers widely. This platform developed has innumerable uses, and our downstream work will focus on how we can use this information to identify targets for pest management. A few preliminary targets have already been identified and are currently being evaluated in our laboratory. This approach though nascent for now, has tremendous implications in the future. Besides, a number of tangential benefits were also obtained due to the development of transcriptomes. For instance, we can actually use the available information and develop protocols that are reliable for evaluating resistance to neonicotinoids in thrips. This is of growing concern, primarily because of the widespread use of neonicotinoids such as imidacloprid (Admire Pro) and thiamethoxam (Criser Maxx), and also due to the recent reports on increased resistance to neonicotinoids in cotton production systems. We are also currently monitoring resistance development in thrips against commonly used insecticides in peanut production.