

## Understanding interactions among thrips, newly released peanut cultivars, and *Tomato spotted wilt virus* through transcriptomics

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### Summary

The goal of this study was to understand interactions between thrips, peanut genotypes, and TSWV in greater detail. Though a number of studies have biologically characterized these interactions, the underlying basis for such interactions at transcript/gene level has not yet been understood in detail. The premise behind undertaking this aspect of research is that, it would lay a foundation for identifying gene targets, which could be exploited for pest management down the road. Such an approach could lead to development of non-traditional pest management approaches that are more cost-effective and sustainable for peanut growers. We used transcriptomics to study tricomponent (thrips-TSWV-peanut) interactions. In simpler words, transcriptomics helps to identify genes and annotate their functions in a given organism. In 2013, much of our efforts concentrated on understanding thrips-TSWV interactions. Transcriptomes from three life stages viz. larvae, pupae, and adults were developed and analyzed. The sequences from our transcriptomes have been/will be shortly deposited in the NCBI (National Center for Biotechnology Information) database and will be available to peanut, thrips, and *Tospovirus* researchers Worldwide. The obtained transcripts were typically grouped into three categories viz. biological processes, cellular processes, and molecular processes and sub-categorized. Further, differential expression analyses helped us understand gene-level changes (upregulation/downregulation) following virus infection. Results indicate that transcripts associated with 28 functional groups were upregulated in the case of TSWV-infected adults when compared with a few in the case of non-infected adults. This information indicated that TSWV severely influences biological processes in adults of *F. fusca*. Our early experimental results corroborate these findings (Shrestha et al. 2012). In addition, transcripts associated with reproduction, embryo development, and cell differentiation were all upregulated implying that TSWV-infection could have a positive impact on the reproductive fitness of *F. fusca*. Our published results also substantiate this information (Shrestha et al. 2012). Fitness studies conducted earlier indicated that thrips ovipositional abilities were enhanced upon TSWV-infection (Shrestha et al. 2012). On the contrary, transcripts associated with cell death were upregulated. This suggested that TSWV-infection could have some deleterious effects on thrips themselves. Our earlier study revealed that TSWV-infected thrips were not feeding as well as the non-infected thrips suggesting that TSWV infection could have some negative impact (Shrestha et al. 2012). Nevertheless, in all these studies it is unclear if these effects are directly induced or whether they are indirectly induced

through the host plant. Moreover, obtained results indicated that TSWV-infection initiates a very strong immune response. Our approach forward is to identify gene targets that could play a pivotal role in thrips development and/or virus multiplication, which in turn could be extremely useful for management of thrips and/or TSWV. Additionally, we developed transcriptomes from one TSWV-susceptible genotype (Sunoleic<sup>®</sup>) and TSWV-resistant genotype (Tifguard<sup>®</sup>) with and without TSWV infection. We are in the process of performing differential expression analyses. All these information would help us understand the interactions between peanut and thrips, peanut and TSWV, as well as between thrips and TSWV in greater depth. This information has excellent potential for future peanut breeding and management of thrips and TSWV.

Shrestha, A., R. Srinivasan, D. Riley, and A. Culbreath. 2012. Direct and indirect effects of a thrips-transmitted *Tospovirus* on the preference and fitness of its vector, *Frankliniella fusca*. *Entomol. Exp. Appl.* 145: 260-271.

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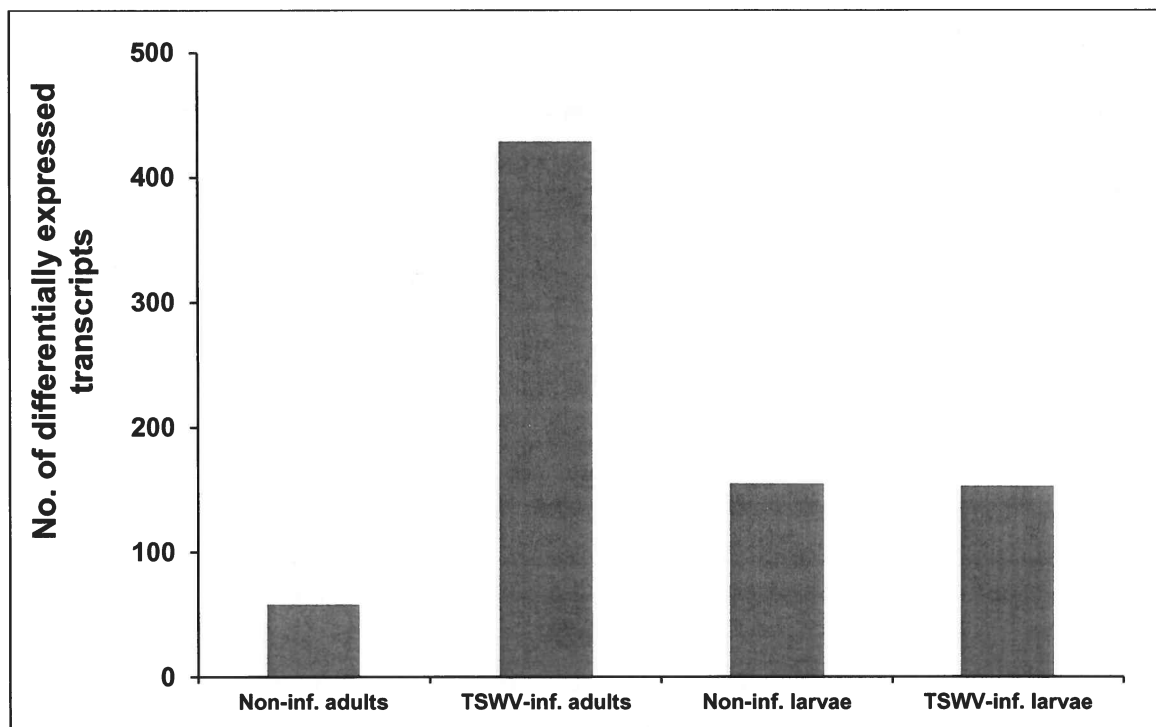
The objective of this proposal was to *identify genes and their putative functions by analyzing transcriptomes from thrips and newly released peanut cultivars with and without TSWV infection.*

*Tomato spotted wilt virus* (TSWV) is exclusively transmitted by thrips. Two thrips vectors viz. Tobacco thrips (*Frankliniella fusca*) and Western flower thrips (*Frankliniella occidentalis*) are found in the Southern United States. *F. fusca* is considered to be an efficient vector of TSWV in peanut, as it colonizes peanut plants more effectively than *F. occidentalis*. *F. fusca* also is known to occur early in the planting season when the peanut plants are more vulnerable to TSWV. The interactions among thrips, TSWV, and peanut plants are very complex and intricate. For instance, TSWV transmission by adults is possible only after acquisition of TSWV by early instar larvae. Further, evidence from our research indicates that recently developed peanut cultivars differentially interact with the virus as well as the vector (Shrestha et al. 2013, Sundaraj et al. 2014). Therefore, the goal of this study was to characterize tricomponent (peanut-thrips-TSWV) interactions in greater detail. We used transcriptomics to study these effects. Transcriptomics is used to identify transcripts in any given organism. In other words, it helps to identify genes and annotate their functions in a given organism.

My graduate student, Ms. Anita Shrestha has focused a substantial amount of her doctoral research towards this objective. She started with the thrips first. Three replicates of three life stages viz. larvae, pupae, and adults were selected. Thirty samples were selected for each sample. CDNA libraries were constructed and the samples were subjected to pair-end Illumina sequencing. The reads were further cleaned using Trimmomatic, and the transcripts were assembled using Trinity software. Differential gene expression was performed using RSEM. The sequences from our transcriptomes have been/will be shortly deposited in the NCBI

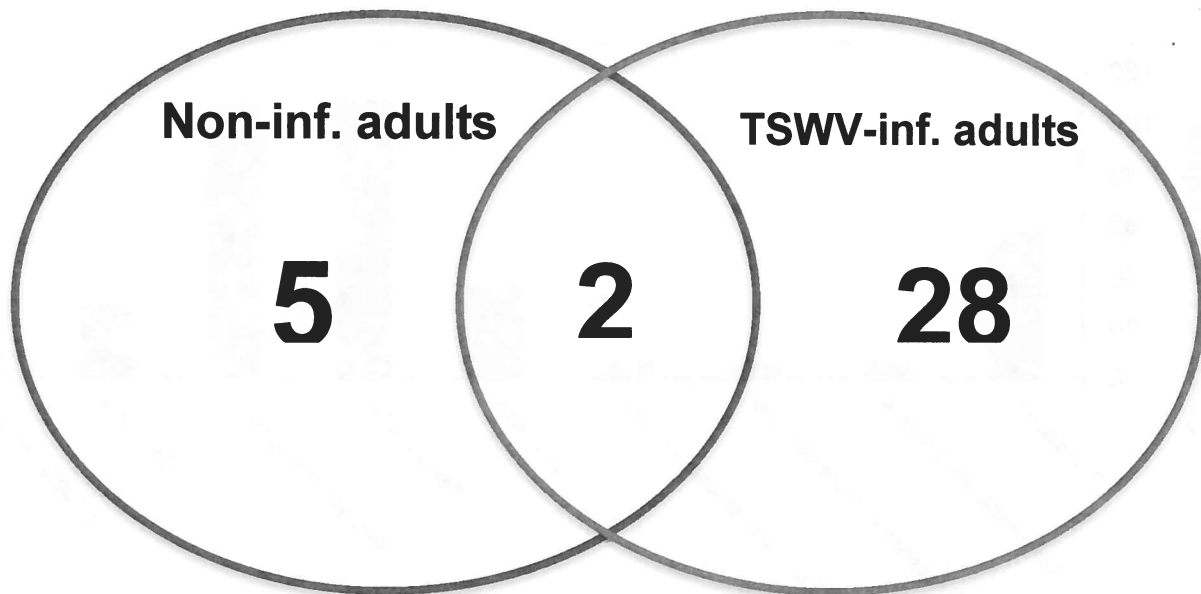
(National Center for Biotechnology Information) database and will be available worldwide. Differential expression analyses allowed us to obtain a plethora of genomic information. It should be noted that despite the availability of 7000 thrips species, prior to our study, transcriptome was available for only one species. Our efforts will be of tremendous benefit to the thrips and *Tospovirus* community worldwide.

Differentially expressed transcripts were assessed from the developed transcriptomes. Data revealed that there was an upregulation of transcripts particularly in the infected adults as opposed to non-infected adults. However, in larvae, no such upregulation was observed despite virus infection.



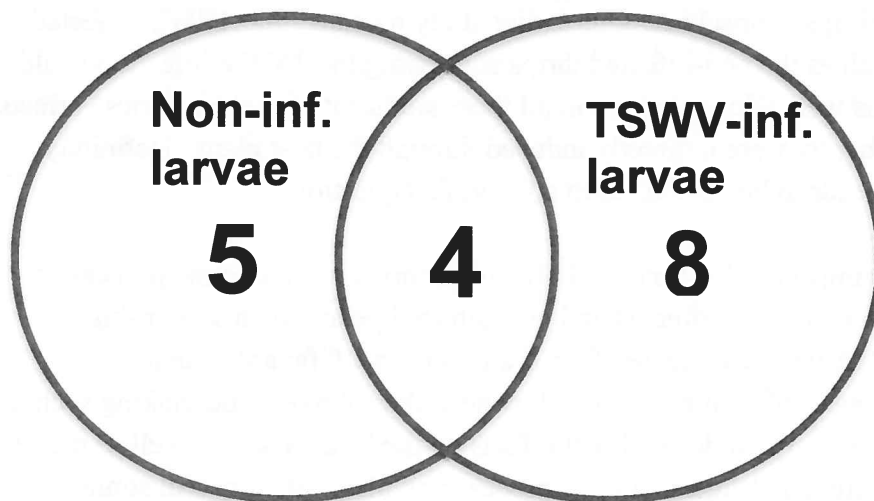
**Fig. 1. Number of differentially expressed transcripts in TSWV-infected and non-infected larvae and adult *F. fusca*. Three replicates of each sample were used for differential expression analyses.**

Upon obtaining the transcripts, the unique transcripts were annotated for putative functions using Blast2go, a non-redundant database from NCBI. This allowed my graduate student to categorize functions of transcripts. The transcripts were typically grouped into three categories viz. biological processes, cellular processes, and molecular processes. Further functional groups were grouped under each category. For instance, functional groups were characterized in the case of transcripts associated with biological processes. That information is presented in the figure presented below.



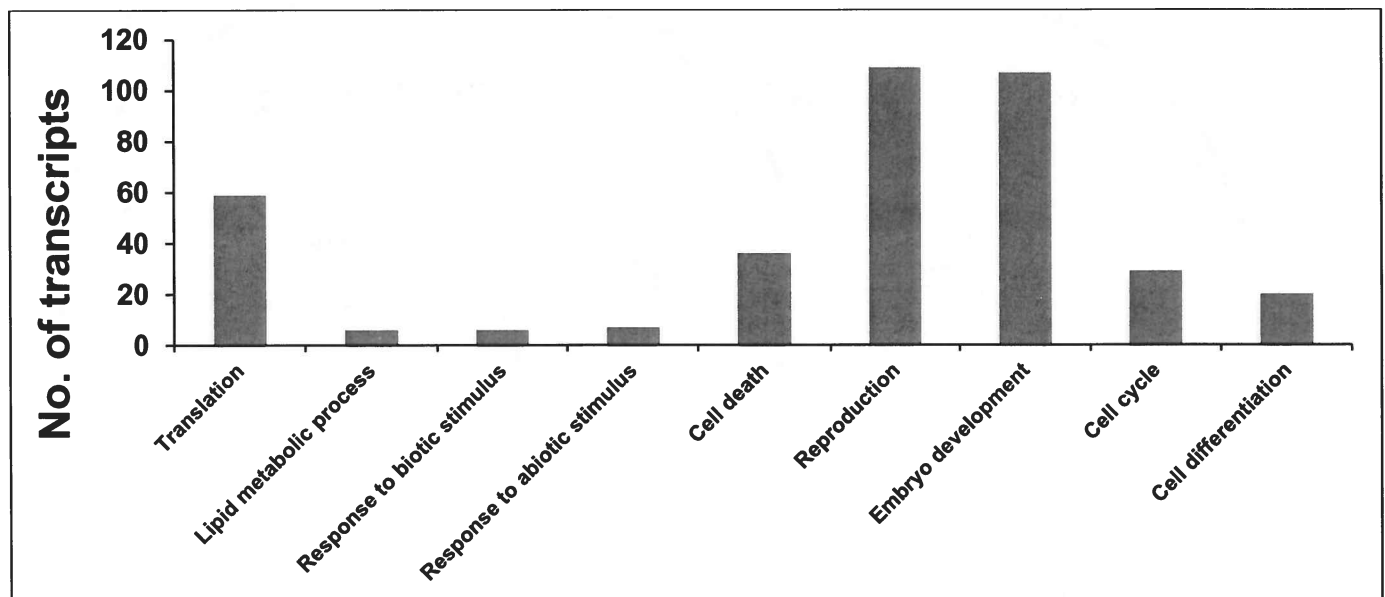
**Fig. 2. Venn diagram describing the functional groups of transcripts associated with biological processes in TSWV-infected and non-infected adults.**

Results indicate that transcripts associated with 28 functional groups were upregulated in the case of TSWV-infected adults when compared with a few in the case of non-infected adults. This information indicated that TSWV severely influences biological processes in adults of *F. fusca*. Our early experimental results corroborate these findings. The changes, however, were not so noticeable in the case of larvae.



**Fig. 3. Venn diagram describing the functional groups of transcripts associated with biological processes in TSWV-infected and non-infected larvae of *F. fusca*.**

The information presented below further characterizes the upregulation of unique transcripts in both TSWV-infected adults and larvae.

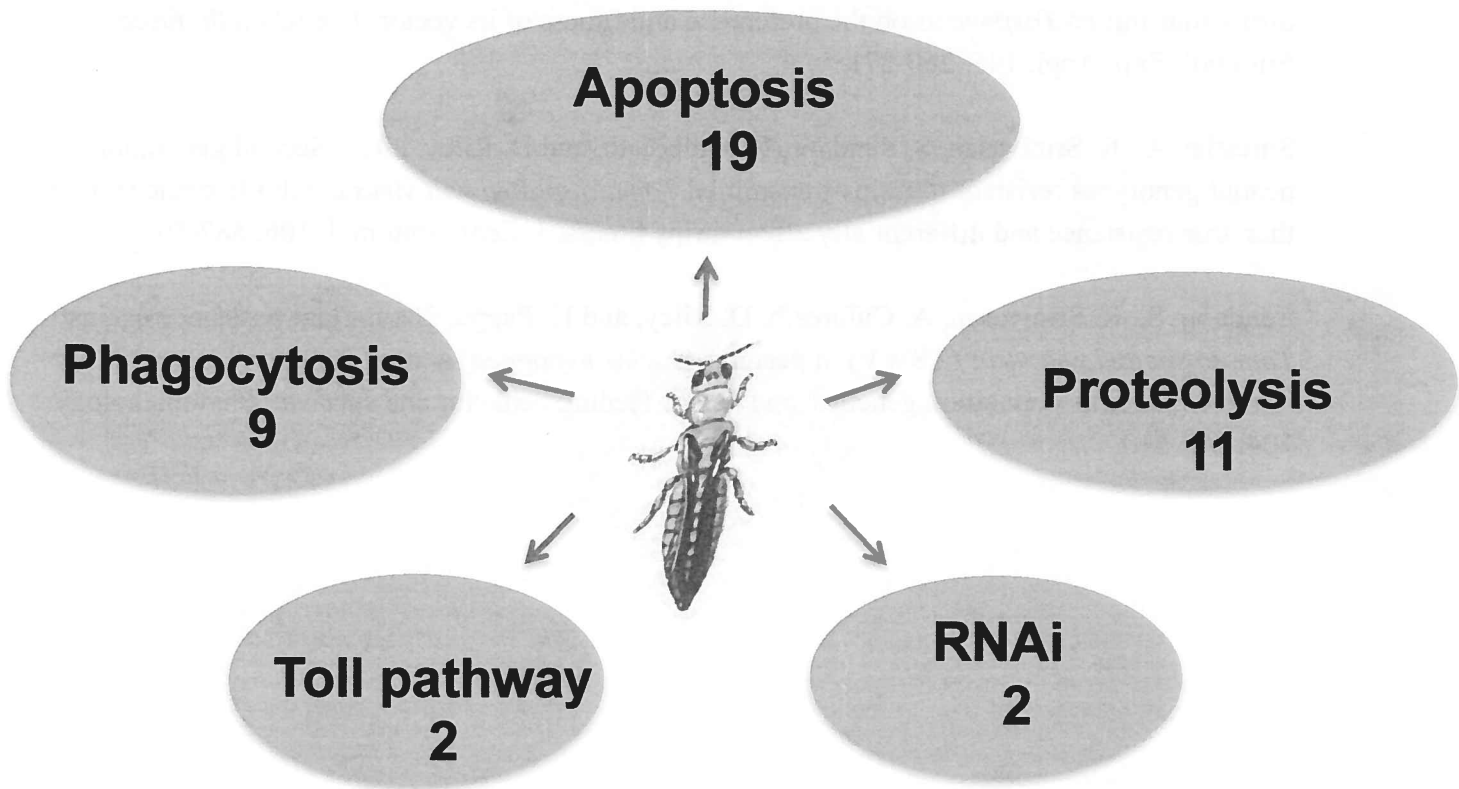


**Fig. 4. Up regulation of transcripts unique to TSWV-infected adult *F. fusca***

Fig. 4 indicates that transcripts associated with reproduction, embryo development, and cell differentiation were all upregulated implying that TSWV-infection could have a positive impact on the reproductive fitness of *F. fusca*. Our published results further substantiate this information (Shrestha et al. 2012). Fitness studies conducted earlier indicated that thrips ovipositional abilities were enhanced upon TSWV-infection. On the contrary, transcripts associated with cell death were upregulated. This suggested that TSWV-infection could have some deleterious effects on thrips themselves. Our earlier study revealed that TSWV-infected thrips were not feeding as well as the non-infected thrips suggesting the TSWV infection could have some negative impact as well. Nevertheless, in all these studies it is unclear if these effects are directly induced or whether they are indirectly induced through the host plant. Definitely, more downstream research needs to be conducted to address this question.

In order to assess the impact of host-induced effects on thrips, we are in the process of developing transcriptomes from TSWV-infected and non-infected peanut plants. For this purpose, three samples of RNA will be extracted from Sun-oleic and Tifguard. Then transcriptomes will be developed following the workflow described above. Undertaking such an effort will aid in parsing out the virus-induced direct effects on the insect itself as well as indirect effects mediated by host nutritional changes. Such a process will also further reveal some information on resistance in TSWV-susceptible (Sun-oleic) and TSWV-resistant (Tifguard) cultivars.

Besides developmental transcripts, other transcripts associated with defense responses were also characterized. Those details are described with the help of a cartoon below.



**Fig. 5. Upregulation of transcripts associated with innate-immune pathways in *F fusca*.**

The obtained results indicated that TSWV-infection initiates a very strong immune response. The fitness costs associated with such a response is yet to be fully understood. But preliminary results in our study system seem to indicate negative fitness effects on thrips.

These results clearly indicate that interactions between thrips, TSWV, and peanut plants could be very complex and very intricate. Transcriptomics is an appropriate approach to characterize such interactions. Analyses of differential expression of peanut plant transcriptomes will further aid in understanding these component interactions in our peanut-TSWV pathosystem more closely. Even though the obtained information is not immediately applicable as a management tactic, this would significantly improve our understanding on thrips-TSWV interactions as well as thrips-TSWV-peanut interactions. Peanut plant transcriptomes will further help us understand TSWV resistance mechanisms. In total, this information could provide clues for next-generation management options including benefitting the ongoing genomics-based approaches to develop pathogen resistant peanut plants.

## References

Shrestha, A., R. Srinivasan, D. Riley, and A. Culbreath. 2012. Direct and indirect effects of a thrips-transmitted *Tospovirus* on the preference and fitness of its vector, *Frankliniella fusca*. *Entomol. Exp. Appl.* 145: 260-271.

Shrestha, A., R. Srinivasan, S. Sundaraj, A. Culbreath, and D. Riley. 2013. Second generation peanut genotypes resistant to thrips-transmitted *Tomato spotted wilt virus* exhibit tolerance rather than true resistance and differentially affect thrips fitness. *J. Econ. Entomol.* 106: 587-596.

Sundaraj, S., R. Srinivasan, A. Culbreath, D. Riley, and H. Pappu. 2014. Plant resistance against *Tomato spotted wilt virus* (TSWV) in peanut (*Arachis hypogaea*) and its impact on susceptibility to the virus, virus population genetics, and vector feeding behavior and survival. *Phytopathology* 104: 202-210.