Executive Summary.

Project ID: 293; PI Dr. R. Srinivasan
Project Description: Role of weed flora as resistance-breaking TSWV strains and vector reservoirs in South Georgia's peanut pathosystem
Institution: University of Georgia Research Foundation
Project fiscal year: 2009

Since its introduction into the Southeastern U.S., *Tomato spotted wilt virus* (TSWV) has considerably affected peanut production, leading to losses extending up to tens of millions of dollars. At the time of its introduction, peanut cultivars grown in the region were extremely susceptible to TSWV. As a consequence, efforts towards TSWV eradication failed and focus shifted towards TSWV management. Breeding efforts resulted in the release of several peanut cultivars capable of exhibiting field resistance.

Today, resistant cultivars are the most important management option available to peanut growers. Thrips-transmitted TSWV is a RNA virus, and RNA viruses are known to change/evolve rapidly. The goal of this proposal was to identify if such changes can affect TSWV resistance in peanut cultivars. TSWV has a broad host range extending to almost 1000 host plants, including weed and cultivated hosts. Through this project we attempted to identify naturally-infected weed hosts, characterize TSWV isolates, and evaluate their impact on the available TSWV-resistant peanut cultivars. The idea was that the weeds of different families can exert varying levels of selection pressure and can differentially affect TSWV.

Hundreds of weed samples were collected from various peanut fields in Georgia. These weed samples were tested for the presence of TSWV using a normal serological procedure (DAS-ELISA). Numerous weed samples were infected. However, when the same samples were mechanically inoculated on an indicator host, there was no symptom development. Further examination through a RNA-based testing technique revealed that the weed samples were not infected with TSWV. This cast a big doubt on the ability of weeds to function as thrips reservoirs and inoculum sources. We focused on optimizing weed sample testing procedures and evaluating the role of weed hosts in TSWV epidemiology.

At least four suspected weed species were inoculated with TSWV using viruliferous thrips. Results indicated that the inoculated weed hosts were infected with TSWV at very high percentages (up to 50%). We also conducted experiments in the laboratory to evaluate the biology of thrips on selected weed species. Results indicated that weeds supported a healthy thrips population when compared with peanut. Also, back transmission experiments were conducted with weeds as inoculum sources and peanut as recipients. Results indicated that the inoculated peanut plants were infected with TSWV at very high rates (up to 50%). These results demonstrated that though the TSWV infection rates in weed species in the field were less than under laboratory conditions, there is tremendous potential for weeds to severely impact TSWV epidemiology. When conditions are optimal weeds can substantially induce TSWV epidemics.
Interpretation of results: Winter and spring weeds such as chickweed, cudweed, Carolina geranium, and sowthistle in peanut farmscape have the potential to significantly affect TSWV epidemics. At this point, it is not clear if weed management in the farm can result in reduced TSWV incidence. More research is being conducted.

Final

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

QUARTER ENDING

June 30, 2010

INSTITUTION: University of Georgia

Project Title: Role of weed flora as sources of resistance-breaking *Tomato spotted wilt virus strains* and vector reservoirs in South Georgia's peanut pathosystem

Res. Agr. No.:
GACCP Control NO: 4-930-653-5

PROJECT LEADER Dr. Rajagopalbabu Srinivasan

EXPIRATION DATE: December 31, 2009

NPB CONTACT Marie Fenn or M. Mehok
NPB Project NO.: 293

REPORT OF PROGRESS:

During the last five quarters weed survey was completed in all 10 counties. At least ten species of weeds were collected in each county. The infection status was tested using DAS-ELISA, and weed species that support vector thrips species (Tobacco thrips, *Frankliniella fusca*, Western flower thrips, *F. occidentalis*, and *F. bispinosa*) were also identified. Weeds infected with TSWV were identified in 7 out of 10 counties sampled, TSWV- infection ranged from 10% to 90 % in each location, and on an average across locations it ranged from 0-15%. Larvae from weeds collected in Berlese's funnels were identified under a compound microscope. Vector species larvae (*F. fusca*, *F. occidentalis*, and *F. bispinosa*) were identified only in four weed species *Viz.,* morning glory, purslane, horseweed, Florida pusley.

Mechanical inoculation on tobacco plants

Mechanical inoculations were performed using TSWV-infected weed tissue, a common abrasive (Carborundum®) and phosphate buffer. Inoculations were manually performed on tobacco seedlings (<10 cm tall) and were regularly monitored for symptom expression. However, no TSWV infection was recorded.

Approaches to resolve the issue: Approach I.

This led us to speculate that there may be some possible inhibitory materials in weeds. In order to test our hypothesis we spiked the RNA of weed samples with RNA isolated from TSWV positive peanut plants and conducted RT-PCR on the spiked samples and unspiked samples. The weed samples that previously tested negative through RT-PCR were now positive for TSWV. An approximately 700 bp band was detected in all the TSWV positive samples. These results suggest that the weed samples were not actually infected with TSWV and there are no RT-PCR inhibitory factors in weeds.

Approach II and progress for last quarter ending June 30 2010.

Through thrips-mediated inoculation, in the last few months we have been attempting to attest TSWV carrying abilities of several spring-winter weed flora. We used 20 viruliferous *F. fusca*/plant to inoculate at least four different weed host species, *Viz.* spiny sowthistle, cudweed, Carolina geranium, and chick weed. Peanut plants were inoculated with TSWV to test the transmission efficiency of our laboratory-reared *F.*

fusca and also to serve as positive controls. All the four weed hosts used in our transmission study *Viz.* Sowthistle, cudweed, Carolina geranium, and chick weed were all infected with TSWV and RT-PCR confirmed our results (infection percentages ranged from 10-60%, Fig 1.). Interestingly, this time, mechanical inoculations also produced typical TSWV symptoms on tobacco seedlings.

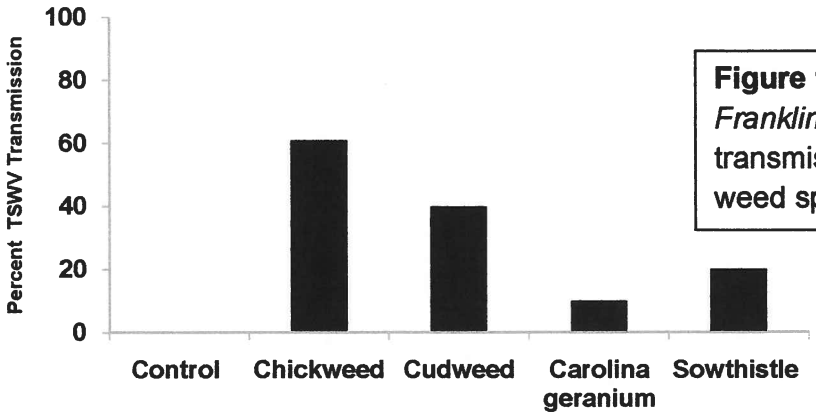

Figure 1. Tobacco thrips, *Frankliniella fusca* mediated transmission from peanut foliage to weed species

As a next logical step, we conducted transmission studies from weeds to peanuts to evaluate their role in TSWV epidemiology. The results were quite surprising, very high infection rates were observed on peanuts (up to 75%, Fig 2.) following back inoculation to peanuts.

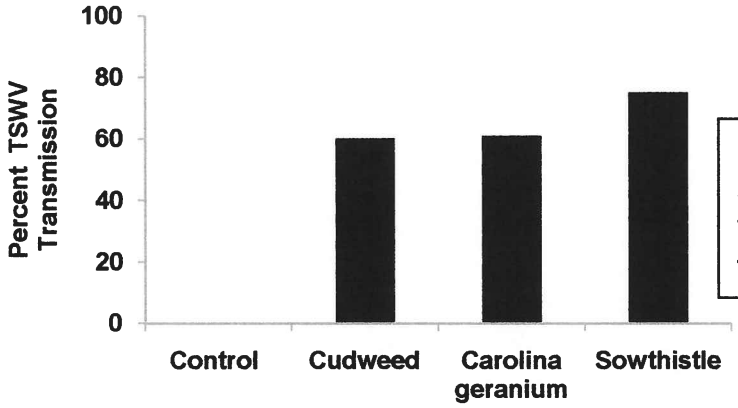

Figure 2. Tobacco thrips, *Frankliniella fusca* mediated transmission from weed species foliage to peanut seedlings

Interpretation and future plan

These results indicate that weeds have the potential to serve as primary inoculum sources early in the season, and can tremendously influence TSWV epidemics. Through this project we have clearly documented the transmission and back transmission of TSWV to weeds and peanut plants, respectively. The role of weeds has been clearly proven. No such published information is currently available and we hope to publish our results in the near future. Our studies also clearly demonstrated that thrips can actually survive and reproduce on these weed species at a very healthy rate re-emphasizing the pivotal role of weeds in initiating TSWV epidemics. In the future we intend to study the epidemiological contributions of these weed species in greater detail. Finally, thanks to the National Peanut Board for its continued support.