

# Standardization of *Tomato spotted wilt virus* Transmission to Aid in Screening of Peanut Genotypes

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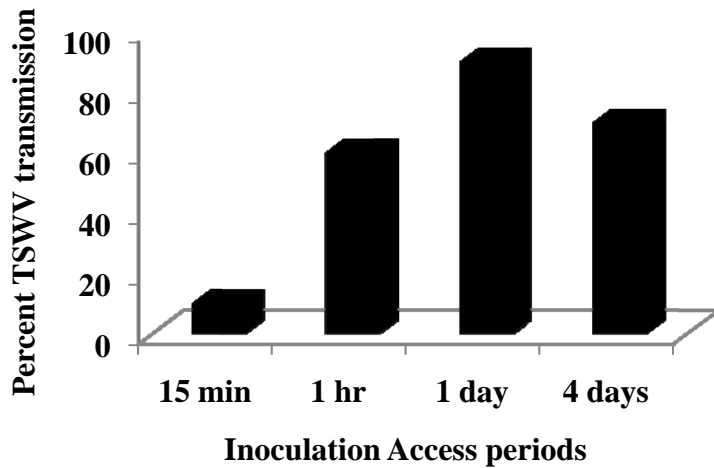
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*Tomato spotted wilt virus* (TSWV) continues to be a major concern to peanut production in Georgia and in Southeastern United States. Resistant genotypes are the most reliable tool for spotted wilt management. In order to consistently assess the status of genotype susceptibility to available TSWV isolates and to newer isolates a standardized transmission protocol could prove to be very useful. Most often mechanical inoculation is used to screen genotypes, though mechanical inoculation is a very valuable procedure, it should be noted that it discounts numerous intricate interactions between thrips and TSWV. This could be a concern especially due to the fact that thrips and the virus have coevolved with each other, and are involved in an intricate relationship. We evaluated mechanical and thrips-mediated transmission on a susceptible TSWV susceptible peanut genotype, Georgia Green. Our goal was to standardize several transmission parameters, such as number of thrips needed to perform a transmission assay, optimal inoculation stage of a genotype, and optimal latent period before detection by serological tools.

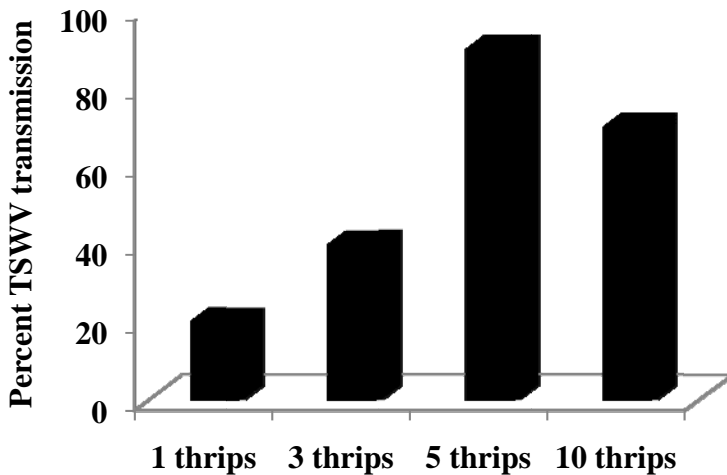
We used the most common peanut thrips species, tobacco thrips *Frankliniella fusca* for our experiments. Two separate populations of *F. fusca* (TSWV-infected and non-infected) were established and maintained on peanut foliage in growth chambers at 24-28<sup>0</sup>C. We used thrips larvae at various densities (1, 3, 5 and 10) for our experiments. We also tested various inoculation access periods for each one of the thrips numbers indicated (15 minutes, 1 hour, 1 day, and 4 days). Young peanut seedlings (7-10 days old) with the first fully expanded leaves were chosen for the experiments, and thrips were confined to a single leaflet with the help of 200 µl PCR tube and held in place with modeling clay. The insects were removed after the prescribed time intervals and the plants were enclosed in a Mylar<sup>®</sup> film (Grafix<sup>®</sup>, Cleveland, PA) cage with a copper mesh top (mesh pore size-170 microns) (TWP<sup>®</sup>, Berkeley, CA). Suitable controls were also included. The infection status of peanut seedlings was verified using double antibody enzyme linked immunosorbent assay (DAS-ELISA); (Agdia Inc., Elkhart, IN) following the manufacturer's protocol. Inoculated peanut plants were tested for TSWV-infection two-three weeks post inoculation using DAS-ELISA. A subset of the plants was also tested with reverse transcriptase polymerase chain reaction (RT-PCR) to confirm ELISA detections. Mechanical inoculations were conducted using TSWV-infected peanut foliage as an inoculum source and an abrasive (Carborundum<sup>®</sup>) in a phosphate buffer, additional chemicals such as β-mercaptoethanol and sodium sulfite were added to enhance transmission efficiency. The infection status of the mechanically inoculated plants was also estimated as per the above-mentioned detection procedures. We used twenty plants for mechanical inoculation and ten plants for each of the thrips-time interval assays. Experiments are also currently being conducted with another ten plants for all thrips treatments.

In general, we found that thrips inoculations were more efficient than mechanical inoculations in transmitting TSWV. We observed typical TSWV symptoms on both mechanically and thrips-inoculated plants. Plants with symptoms tested positive for TSWV through ELISA and RT-PCR. Symptoms appeared usually two weeks post inoculation; however, the symptoms on mechanically inoculated plants were delayed at least by another seven days. TSWV transmission efficiency was only 15 % in mechanically inoculated plants and on thrips inoculated plants it extended up to 90 % depending on the treatment. TSWV transmission efficiency increased with increase in inoculation access periods as well as with increase in thrips numbers used for inoculations (Figs. 1a and 1b).

**Fig 1a**



**Fig. 1b**



These results reiterate that thrips and TSWV are very closely associated, and several intricate interactions are involved in successful transmission of the virus. Mechanical inoculations may serve as a quick screening tool. Nevertheless, their reliability and usefulness should not be overestimated as it discounts thrips and virus interactions. Therefore, thrips transmission screening tests in the greenhouse and field situations with high thrips and inoculum pressure should be considered for genotype evaluations.

In this project we considered various parameters such as number of thrips, inoculation access periods, and detection tools with an intention of optimizing a simple protocol. The results so far have been very encouraging, we intend to replicate these experiments and also repeat them with various cultivars across the susceptibility-resistance spectrum.