

TITLE: Physiological and Molecular Responses Associated with *Tomato spotted wilt virus* in Phorate-Treated Peanut; D. Rowland and M. Gallo

OBJECTIVES: 1) quantify the physiological responses to TSWV infection, especially related to gas exchange, in different peanut varieties through the growing season at different stages of TSWV infection, 2) examine the effect of pre-plant insecticide treatments on these physiological responses, and 3) correlate the expression of peanut defense response genes involved with increased resistance to TSWV with the above physiological responses.

PLANT MATERIAL AND FIELD TRIAL: Six peanut varieties were hand planted in three replications at the USDA-ARS, National Peanut Research Laboratory in Dawson, GA, USA. Peanut cultivars were selected based on their resistance level to represent the full range of *Tomato spotted wilt virus* (TSWV) resistance. Two highly resistant varieties, ANorden and AP3, three moderate resistant varieties C3424, GA-02C, and Georgia Green, and the highly susceptible cultivar SunOleic97R were chosen for this experiment. Seeds were planted in blocks consisting of a total of 12 rows; two rows per variety spaced 0.9m apart and with the distance between the seeds within a row being 10.2 cm. The early planting date and large interplant distance were chosen to maximize the incidence and severity of TSWV. Genotypes were treated with three different at-plant insecticide treatments: No treatment (Control), Temik, and Thimet. These insecticides are systemic and aimed at inhibiting cholinesterase: Temik (Aldicarb) is a carbamate; Thimet (Phorate) belongs to the group of organophosphates. Tissue of all six varieties in three treatments and three replications was collected on four dates throughout the season: 26 May (42 days after planting - DAP); 5 June (52 DAP); 11 July (88 DAP); 8 August (116 DAP). Except for the first collection date when plants were too small for measurement, gas exchange and other physiological measurements were taken just prior to leaf collection. After physiological characterization, leaf tissue was removed and placed immediately into liquid nitrogen, then stored at -80 C until genetic testing was initiated.

RNA EXTRACTION: Approximately 1.5 to 2.5 grams of peanut leaf tissue were used for RNA extraction. The extraction was done based on a small-scale protocol. The concentration of extracted RNA was measured by use of a UV spectrophotometer and the quality was determined. High quality RNA was insured by only using samples that had an optical density (OD) ratio of 1.8 to 2, and by determining visual quality on a gel.

RESULTS AND CONCLUSIONS: Northern hybridization was used to determine gene expression. Therefore, a formaldehyde agarose gel was used to denature the RNA. The separated RNA was transferred to a nylon membrane (Hybond-N) and UV cross-linked. The DNA probes were derived from cDNA clones that had been identified in a previous study. Two primary probes were examined: 1) PR-4a, a pathogenesis related protein likely to be induced by viral infection and involved in increased resistance to TSWV; and 2) glutathione reductase, an anti-oxidant that may help inactivate reactive oxygen species produced under viral infection. Up-regulation of these two proteins was measured under Thimet treatment for many of the genotypes tested; particularly for ANorden, AP3, C3424, and Georgia Green. Decreased protein expression was measured in the Temik treatment for these same genotypes. Up-regulation was hypothesized to be beneficial under viral infection, possibly conferring protection from infection or tolerance to infection. Some evidence of this phenomenon was seen in photosynthetic rates: up-regulation of photosynthesis was seen in ANorden, C3424, and Georgia Green after treatment with Thimet. TSWV infection rates were all reduced in the Thimet treatments for all six cultivars, while Temik applications showed increased TSWV infection in four of the cultivars. Therefore, Thimet appears to play a role in increased gene expression of beneficial proteins, increased physiological function, and decreased TSWV infection for most cultivars.

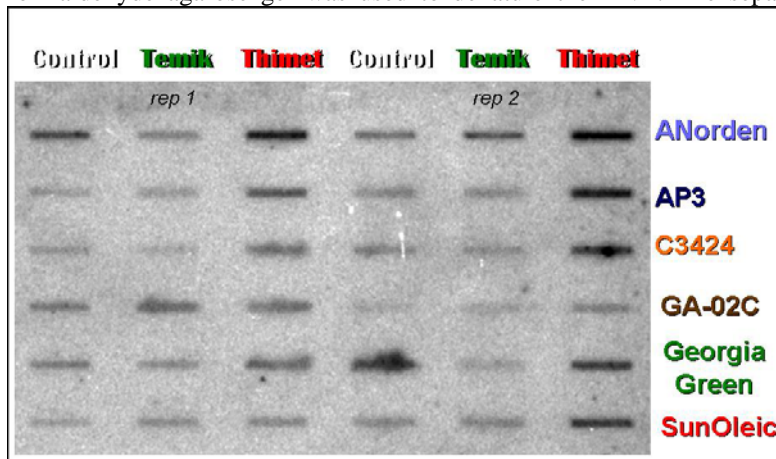
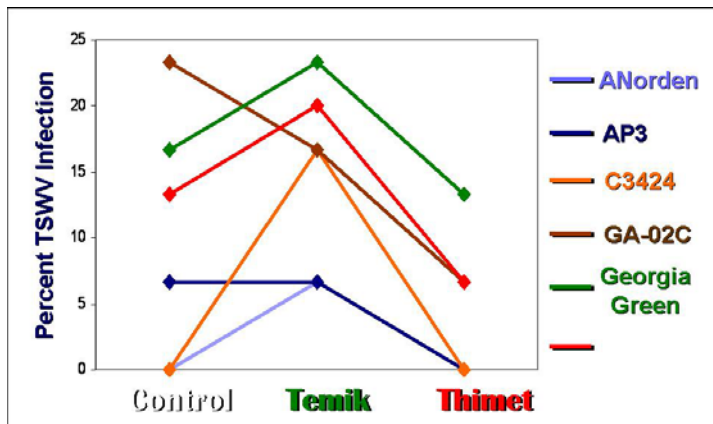


Figure 1: Northern hybridization gel for PR-4a protein showing expression levels for three chemical treatments and six peanut genotypes.



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Figure 2: TSWV infection rates for three chemical treatments and six peanut genotypes.