Differential Response of Selected Peanut (*Arachis hypogaea*) Genotypes to Mechanical Inoculation by *Tomato spotted wilt virus*

B. Mandal, H. R. Pappu, and **A. K. Culbreath,** Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton 31793; **C. C. Holbrook,** USDA-ARS, Coastal Plain Experiment Station, Tifton, GA 31793; **D. W. Gorbet,** North Florida Research and Education Center, University of Florida, 3925 Highway 71, Marianna 32446; and **J. W. Todd,** Department of Entomology**,** University of Georgia, Coastal Plain Experiment Station, Tifton 31793

ABSTRACT

Mandal, B., Pappu, H. R., Culbreath, A. K., Holbrook, C. C., Gorbet, D. W., and Todd, J. W. 2002. Differential response of selected peanut (*Arachis hypogaea*) genotypes to mechanical inoculation by *Tomato spotted wilt virus*. Plant Dis. 86:939-944.

Screening of peanut germ plasm for resistance to *Tomato spotted wilt virus* (TSWV) has been largely inefficient due to the lack of a screening technique based on mechanical transmission of the virus under controlled environmental conditions. We have studied the reaction of three peanut cultivars (Georgia Green, Georgia Runner, C-99R) and one breeding line (C11-2-39) using a highly efficient mechanical inoculation procedure. The disease response was studied at two temperature regimes, 25 to 30°C (low temperature) and 30 to 37°C (high temperature). Based on percent transmission, symptomatology, distribution of TSWV, and relative levels of TSWV nucleocapsid (N) protein, Georgia Runner and Georgia Green were found to be susceptible, whereas C-99R and C11-2-39 were resistant. Of the four genotypes tested, C11-2-39 had the highest level of resistance to TSWV. The results correlated with the field performance of the genotypes except in the case of Georgia Green, which could not be distinguished from TSWV-susceptible Georgia Runner. Exposure of the inoculated plants to higher temperature (30 to 37°C) resulted in a better resistant response as reflected by reduced systemic infection, localized symptom expression, restricted viral movement, and reduced levels of TSWV antigen. To our knowledge, this is the first report of differential response of peanut genotypes to TSWV using mechanical inoculation. The four peanut genotypes should be useful as reference standards for the initial screening and identification of sources of TSWV resistance in peanut germ plasm.

Additional keywords: *Bunyaviridae,* groundnut, host plant resistance, *Tospovirus,* varietal resistance

Tomato spotted wilt virus (TSWV), the causal agent of spotted wilt disease, is one of the most economically important members of the genus *Tospovirus*, family *Bunyaviridae* (12,16). TSWV is transmitted by several species of thrips (18). In the United States, the virus has become a major constraint to peanut production in Alabama, Florida, Georgia, Mississippi, North Carolina, and Texas. Annual losses of several million dollars were attributed to TSWV in Georgia alone (19).

Progress in the management of spotted wilt disease in peanut was made possible by identifying and combining some critical management tactics, such as use of resistant cultivars, manipulating planting date, use of a higher density of plant populations, twin-row pattern, and application of phorate in the furrow (1–3). Among these tactics, the choice of cultivar is considered to be the single most important factor for spotted wilt suppression in peanut. Cultivars with moderate levels of field resistance to TSWV were developed following several years of screening and evaluation under natural conditions. However, incidence of TSWV varies from season to season, and the response of cultivars and breeding lines in a particular field may not be an accurate indication of the true level of genetic resistance–susceptibility. Successful mechanical inoculation eliminates some of the variability in the field such as variable thrips feeding and macro- and micro-environmental variation. Limited studies have been reported regarding the varietal response to TSWV by mechanical inoculation, and these studies found no significant differences among peanut cultivars in terms of their susceptibility to the disease (10,14). Based on these reports, a screening technique using mechanical inoculation appeared to be of limited value due to the inability to differentiate resistant and susceptible responses among genotypes. As a result, screening peanut lines for TSWV resistance usually has been conducted under field conditions relying on the natural occurrence of the disease.

Mechanical transmission of TSWV to peanut is relatively difficult (7,9,13,14). We previously have studied various factors affecting TSWV transmission to peanut and developed a highly efficient mechanical inoculation procedure (11). Culbreath et al. (5,6) studied the reactions of numerous peanut cultivars and breeding lines to TSWV under field conditions in Georgia and Florida. From these studies, three cultivars and one breeding line (henceforth referred to as genotypes) with varying levels of field resistance to TSWV were selected to determine their responses to TSWV by mechanical inoculation.

MATERIALS AND METHODS

Source of viral inoculum. TSWV-infected tobacco (*Nicotiana tabacum*) leaves were collected from the Black Shank Nursery at the Coastal Plain Experiment Station, University of Georgia, Tifton. Presence of TSWV in the leaf samples was confirmed by enzyme-linked immunosorbent assay (ELISA) using a TSWV nucleocapsid (N) protein specific kit from Agdia Inc. (Elkhart, IN). The virus was transmitted mechanically to the peanut cultivar Georgia Runner maintained in a growth chamber. Transmission of TSWV to Georgia Runner was confirmed by ELISA. A symptomatic plant including roots at 10 to 15 days postinoculation (DPI) was used as the source of inoculum to study the responses of peanut genotypes to TSWV by mechanical inoculation.

Genotypes. Three peanut cultivars, Georgia Runner**,** Georgia Green, and C-99R, and one breeding line, C11-2-39, were evaluated in this study. Georgia Runner and Georgia Green are medium-maturing cultivars with high yield potential, which were developed at the University of Georgia's Coastal Plain Experiment Station in Tifton. Georgia Runner is not widely grown because of its susceptibility to TSWV. Georgia Green has moderate field-resistance to TSWV, and as a result, it is the predominant cultivar grown in Alabama, Florida, and Georgia. C-99R is a

Corresponding author: H. R. Pappu
E-mail: hanu.r.pappu@aphis.usda.gov

Current address of H. R. Pappu: USDA-APHIS, Unit 133, 4700 River Road, Riverdale, MD 20737.

Accepted for publication 23 April 2002.

Publication no. D-2002-0703-01R
© 2002 The American Phytopathological Society

late-maturing runner-type cultivar (formerly known as F48/9B-4-2-1-1-2-b2-B), and C11-2-39 is a late-maturing breeding line. Both showed significantly lower incidence of TSWV in the field than Georgia Green (5,8).

Test plants were grown in an environmental growth chamber at 25 to 30°C, 60 to 95% relative humidity, and 12 h alternating light (intensity of 12 to 15 klx) and dark periods. Five to eight seeds of each genotype were sown in plastic pots (16 cm diameter and 14 cm height) containing all purpose professional growing mix consisting of Canadian sphagnum peat moss 75 to 85%, perlite 15 to 20%, and vermiculite 5 to 10% (Berger Peat Moss-Lee Berger Itee, Saint-Modeste, Quebec, Canada). Due to variability in germination, more pots were planted than required, and five to eight seedlings with uniform growth were then transplanted into new pots.

Mechanical inoculation. Inoculation procedure used was as described previously (11). Inoculum was prepared by grinding infected tissues at the rate of 1:6 (wt/vol) tissue to buffer ratio in freshly prepared ice cold 0.1 M potassium phosphate buffer, pH 7.0, containing 0.2% sodium sulfite and 0.01 M mercaptoethanol, with a chilled mortar and pestle. Debris was removed by squeezing the extract through a pad of nonabsorbent cotton. Celite 545 and Carborundum 320 grit (Fisher Scientific, Fair Lawn, NJ) were added to the inoculum at the final concentration of 1 and 2%, respectively. The inoculum was maintained on ice until the inoculation was completed.

Peanut plants at two- to three-leaf stages (7 to 8 days after planting [DAP]) were dusted with Carborundum, and inoculum was applied by rubbing both surfaces of the leaf with a Johnson's cotton swab (Johnson & Johnson, Skillman, NJ). After inoculation, the plants were sprayed with distilled water and kept in two growth chambers having the same environmental conditions except that one had a lower temperature regime of 25 to 30°C and the other had a higher temperature regime of 30 to 37°C. Light intensity in these two growth chambers was higher (15 to 20 klx) than in the growth chamber that was used for raising the test plants (see previous section).

Evaluation of inoculated plants and analysis of transmission data. When chlorotic rings or concentric rings developed on the inoculated leaves without any symptoms on the newly developed leaves, the plants were considered to have localized infection. When chlorotic spots followed by mosaic rings and necrotic spots developed in the newly emerging leaves, the plants were considered to be systemically infected. For each genotype, 10 to 12 inoculated plants were considered as a replication. There were three replications for each genotype conducted at different times. The experimental design was a split plot, where the temperatures were the main plots and the genotype was the subplot. Since only one growth chamber for higher temperature and one for lower temperature were used, the appropriate measures of error for testing temperature difference were the pooled replication effect from each growth chamber. Data were analyzed by ANOVA procedures of SAS, 2000 (SAS Institute Inc., Version 7, Cary, NC).

Detection, distribution, and relative level of TSWV by ELISA. Inoculated plants were monitored visually for symptoms, and TSWV infection was confirmed by ELISA. Recording of symptomatic plants and ELISA were done at 2 weeks postinoculation unless otherwise mentioned. ELISA values three times greater than the average value of negative controls (noninoculated plants) were considered positive (17).

To determine the extent of distribution of TSWV in C11-2-39 plants showing localized or systemic symptoms, individual plants at 10 to 15 DPI were divided into nine parts, which consisted of folded terminals, individual quadrifoliate (four inoculated and three noninoculated newly developed), and roots (Table 1). Three individual plants were tested for TSWV in each plant part. At 30 to 45 DPI, distribution of TSWV was analyzed by testing all the quadrifoliates, including terminal and roots, of three individual plants separately for each genotype.

To determine the relative level of TSWV in different genotypes, 100 mg of the

Table 1. Distribution of *Tomato spotted wilt virus* (TSWV) in C11-2-39 showing localized symptoms after mechanical inoculation at 30 to 37°C as determined by enzyme-linked immunosorbent assay (ELISA)

Plant part assayed	Symptoms	ELISA[x] readings	Scores[y]
Folded terminals	No	0.01	–
7th quadrifoliate	No	0.00	–
6th quadrifoliate	No	0.00	–
5th quadrifoliate	No	0.00	–
4th quadrifoliate	No	0.01	–
3rd quadrifoliate	Ring spot	2.28	+[z]
2nd quadrifoliate	Ring spot	2.36	+[z]
1st quadrifoliate	Ring spot	2.11	+[z]
Roots	No	0.01	–
Noninoculated control	No	0.03	–
Positive control	Mosaic	2.93	+

[x] Each value is the mean absorbance from three different plants tested separately.
[y] Presence or absence of TSWV.
[z] 1st, 2nd, and 3rd quadrifoliates of each plant were the site of inoculation.

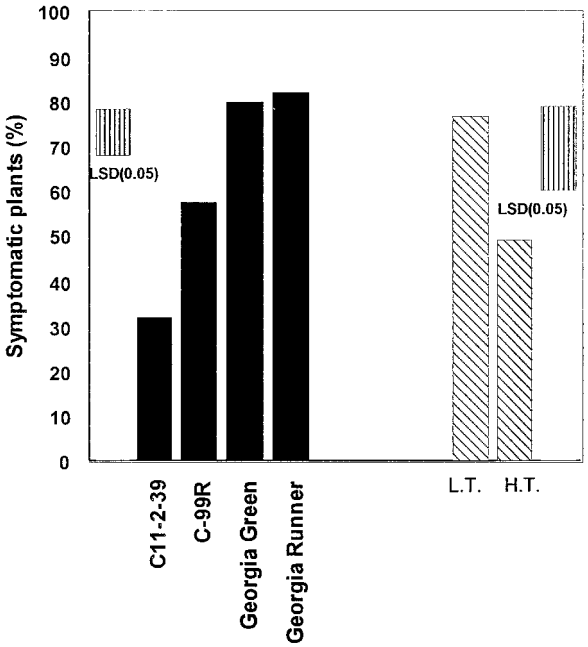

Fig. 1. Differential transmission of *Tomato spotted wilt virus* (TSWV) to the peanut cultivars Georgia Runner, Georgia Green, and C-99R and the breeding line C11-2-39 as determined by percent systemically infected plants at higher (30 to 37°C) and lower (25 to 30°C) temperature regimes. Bars are means from three inoculation trials (N = 10 to 12 at each trial). Temperature by genotype interaction was not significant; therefore, only main effects are shown. Slashed bars represent transmission of TSWV averaged over genotypes at low temperature (L.T.) and higher temperature (H.T.).

youngest symptomatic leaves at 10 to 15 DPI were assayed in 10-fold dilution series. For each genotype, three tests of separate dilution series of three individual plants at each test (N = 9) were conducted (C11-2-39 at high temperature had N = 6, as there were only six systemically infected plants in the three trials). Absorbance values were analyzed using Proc GLM (SAS, 2000, SAS Institute, Cary, NC). The experimental design was a split-split plot, where temperature was the main plot. Three independent runs were made, subplots were the three plants tested for each genotype, and sub-subplots were the five successive dilutions of the sap from each plant. The variance associated with temperature and genotypes were homogeneous, while the variance among the five dilutions was heterogeneous. Cubic effects for dilution were found using Proc GLM, where the model allowed comparison within temperatures and genotypes of the linear, quadratic, and cubic coefficients.

RESULTS

Percent transmission of TSWV. At lower temperature (25 to 30°C), 90% (mean of 100, 80, and 90%) Georgia Runner, 100% (mean of 100, 100, and 100%) Georgia Green, 70% (mean of 80, 50, and 80%) C-99R, and 46.7% (mean of 64, 36, and 40%) C11-2-39 plants had systemic TSWV infection. At higher temperature (30 to 37°C), 70% (mean of 80, 60, and 70%) Georgia Runner, 64.3% (mean of 70, 73, and 50%) Georgia Green, 45% (mean of 50, 45, and 40%) C-99R, and 17.6% (mean of 20, 25, and 8%) C11-2-39 plants had systemic TSWV infection. Statistical analyses of the transmission data are presented in Figure 1, which indicated that there was no temperature by genotype interaction. Irrespective of temperature regimes, C11-2-39 showed the lowest percentage of symptomatic plants compared with the three cultivars. Irrespective of genotypes, higher temperature resulted in significantly lower percentage of symptomatic plants for each of these four genotypes (Fig. 1).

Localized and systemic disease responses. C11-2-39 plants that were kept at higher temperature produced one or two yellow rings or concentric rings on most of the inoculated lamina at 5 to 10 DPI, and in most of these plants no symptoms were observed in the newly developed quadrifoliate, indicating only localized symptoms. Localized symptoms as produced by C11-2-39 at higher temperature were not observed in the three cultivars, Georgia Runner, Georgia Green, and C-99R. At 5 to 10 DPI, 76.1% (mean of 70, 75, and 83%) of the C11-2-39 plants produced localized symptoms, and 17.6% (mean of 20, 25, and 8%) of the plants produced chlorotic spots followed by mosaic and ring spots on the newly developed quadrifoliate indicating systemic symptoms (Fig. 2).

At the lower temperature regime (25 to 30°C), C11-2-39 did not produce localized ringspot symptoms, but a few chlorotic blotches were observed on the inoculated leaves. A significantly higher percentage (46.7%) of systemically infected C11-2-39 plants was observed at the lower temperature than at the higher temperature (17.6%) (Fig. 2).

In general, the systemically infected plants, irrespective of genotype, developed more ringspot symptoms at higher temperature (30 to 37°C) than the systemically infected plants kept at lower temperature (25 to 30°C), which produced more of the mosaic symptoms (Fig. 3).

Distribution of TSWV in plants showing localized and systemic symptoms. In C11-2-39 plants that showed localized ringspot symptoms at higher temperature, TSWV was detected only in the inoculated leaves showing ring spots but not in the younger quadrifoliates, terminals, or roots (Table 1). ELISA data indicated that TSWV replicated and produced symptoms at the site of inoculation but did not move to the developing plant parts at 30 to 37°C (Table 1). At the same time (10 to 15 DPI), in systemically infected C11-2-39 plants at higher temperature, TSWV was found to be distributed in the roots and newly developed symptomatic quadrifoliates (data not shown).

One to one and a half months after inoculation, symptoms on the newly developed quadrifoliates of systemically infected plants for all the genotypes in general were observed to be suppressed at higher temperature. All infected plants of C-99R and C11-2-39 recovered from symptoms and grew normally, having up to four to six symptomatic quadrifoliates at the bottom and two to three asymptomatic quadrifoliates on the top. In the top asymptomatic quadrifoliates of C-99R and C11-2-39, TSWV was not detected by ELISA, whereas TSWV was detected in the lower symptomatic quadrifoliates and roots. In Georgia Green and Georgia Runner, however, the top leaves showed one or two ring spots, and TSWV could be detected by ELISA in all the symptomatic quadrifoliates, including terminals and roots.

Relative levels of TSWV N antigen in peanut genotypes. There was variation in the levels of TSWV in individual plants of any genotypes. ELISA values irrespective of genotype increased at 10^{-2} dilution of

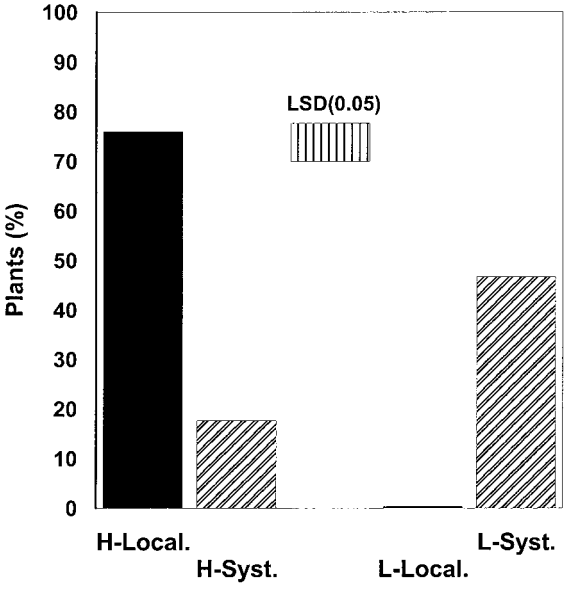

Fig. 2. Systemic and localized disease response by the breeding line C11-2-39 after mechanical inoculation of *Tomato spotted wilt virus* at higher (H = 30 to 37°C) and lower (L = 25 to 30°C) temperature regimes. Bars are mean from three inoculation trials (N = 10 to 12 at each trial).

Fig. 3. Temperature-dependent symptomatology of peanut plants (cultivar Georgia Green) following mechanical inoculation with *Tomato spotted wilt virus*. Ringspot symptoms at 30 to 37°C (above) and mosaic symptoms at 25 to 30°C (below).

sap and then declined at higher dilutions (Figs. 4 and 5). ELISA reactions were discernible up to 10^{-4} dilution for all the genotypes at lower temperature and only for Georgia Runner but not for Georgia Green, C-99R, and C11-2-39 at higher temperature.

Temperature by genotype by dilution interaction on the ELISA values was nonsignificant, whereas interactions of genotype by dilution, temperature by dilution, and temperature by genotype were significant. Comparison of the trends of TSWV level in the genotypes averaged over temperature regimes is presented in Figure 4. At quadratic component of regression line (Fig. 4), breeding line, C11-2-39 had a significantly lower trend in ELISA values than the three cultivars, which had similar trends among dilution levels of sap. The effect of temperature on the level of TSWV averaged over genotype is shown in Figure 5, which showed that there was a significantly different trend at higher temperature regime than at lower temperature regime over the dilution level of 10^{-4}.

The genotype by temperature LS means of ELISA values are shown in Table 2. At both temperature regimes, C11-2-39 had a significantly lower level of TSWV than did C-99R, Georgia Green, and Georgia Runner. C-99R at the lower temperature regime and C-99R and Georgia Green at the higher temperature regime had an intermediate level of TSWV. Georgia Runner had the highest level of TSWV at both temperature regimes. Comparison of TSWV levels at the two temperature regimes showed that only C-99R and Georgia Green had significantly lower levels of TSWV at the higher temperature regime than at the lower temperature regime.

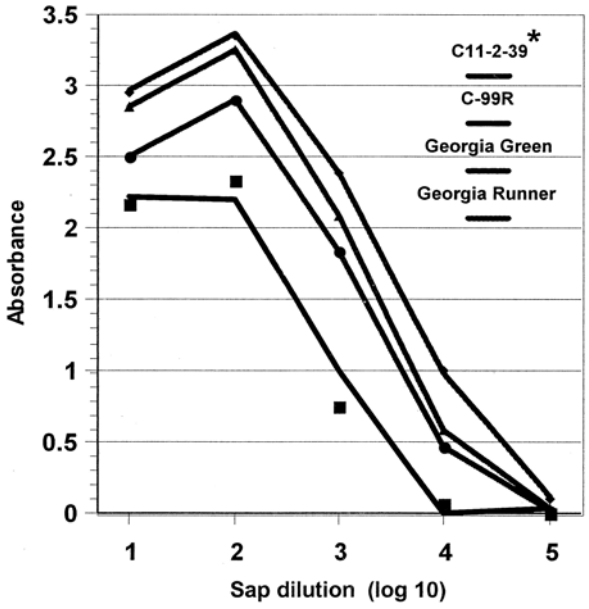

Fig. 4. The trends of relative levels of *Tomato spotted wilt virus* nucleocapsid protein antigen as determined by enzyme-linked immunosorbent assay in the peanut cultivars Georgia Runner, Georgia Green, and C-99R and the breeding line C11-2-39 at 10 to 15 days postinoculation. Absorbance values were averaged over the two temperature regimes. * indicates that C11-2-39 was significantly different from the three peanut cultivars at the quadratic component ($P < 0.01$). Lines represent predicted values and the points represent ANOVA means.

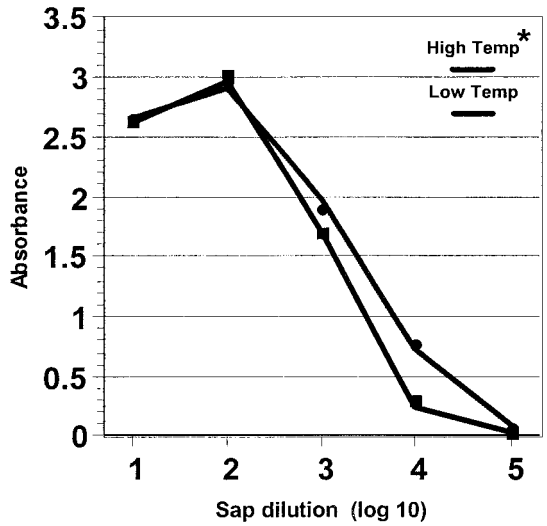

Fig. 5. Effects of temperature on the level of *Tomato spotted wilt virus* nucleocapsid protein antigen as determined by enzyme-linked immunosorbent assay at various dilutions of sap from infected plants averaged over four genotypes. * indicates that two lines were significantly different at the cubic component ($P < 0.05$). Lines represent predicted values and the points represent ANOVA means.

DISCUSSION

The aim of this study was to apply a highly efficient mechanical inoculation procedure to determine the reaction of selected peanut genotypes whose field response to natural infection of TSWV is known. Mechanical inoculation of Georgia Runner, Georgia Green, C-99R, and C11-2-39 resulted in differential disease responses in terms of percent transmission, localized and systemic infection, and viral distribution and titer.

Both Georgia Runner and Georgia Green were more susceptible than C-99R and C11-2-39, as evident by the proportion of systemically infected plants (Fig. 1). In field studies, Georgia Runner and Georgia Green showed 46 to 89.6% and 31.4 to 58.8% final disease incidence, respectively, in Georgia and Florida during 1997 to 1998 (5). Culbreath et al. (4) studied disease progress of TSWV in peanut cultivars Florunner and Southern Runner and found that the final incidence of TSWV was lower and disease progress was slower in Southern Runner than in Florunner. Disease response of Georgia Green and Southern Runner was similar in the field showing lower disease incidence (6). However, mechanical inoculation of Georgia Green in the present study did not result in a resistant response as was observed under field conditions. The observed field resis-

Table 2. Interaction between temperatures and genotypes on the level of *Tomato spotted wilt virus* as determined by enzyme-linked immunosorbent assay

	Absorbance[x]	
Genotypes	**High temp**	**Low temp**
C11-2-39	1.064 a[y]	1.058 a
C-99R	1.399 b	1.673 b*[z]
Georgia Green	1.547 b	1.959 c**
Georgia Runner	1.971 c	1.932 c

[x] LS means (averaged over five dilutions) from ANOVA where the cubic effects of dilution were determined. LSD = 0.256 for genotype × temperature.

[y] Means with the same letter in the column are not significantly different at $P = 0.05$.

[z] *, ** denote significant difference between two temperatures at $P < 0.05$ and $P < 0.01$, respectively.

tance in Georgia Green may be effective against thrips-borne inoculum and may have broken down against the relatively larger dosage administered by mechanical inoculation. Hoffmann et al. (10) reported that mechanical inoculation to seven peanut cultivars, GK7, NC7, VC1, Florunner, Southern Runner, and Spanco, did not result in significant differences in disease progress.

The breeding line, C11-2-39, produced the lowest percentage of systemically infected plants compared with the three cultivars tested in the present study. In field studies, C11-2-39 showed significantly lower incidence (11.9%) of TSWV in comparison to Georgia Runner, Georgia Green, and C-99R (5). C11-2-39 in the present study showed a temperature-dependent disease response. Ringspot symptoms were observed on the inoculated leaves at higher temperature but not at lower temperature. At both temperatures, C11-2-39 produced systemically infected plants, but at the higher temperature there was a significantly lower percentage of systemically infected plants than at the lower temperature (Fig. 2). When C11-2-39 plants that produced localized ringspot symptoms were analyzed by ELISA for the distribution of TSWV in different plant parts, TSWV was present only in the inoculated leaves showing ring spots but not in the newly developed quadrifoliates or terminals or roots (Table 1). ELISA data indicated that the localized infection of C11-2-39 was due to restriction of long distance movement of TSWV at the site of inoculation. Reddy et al. (15) also observed that resistance to *Peanut bud necrosis virus* in the wild *Arachis* species was due to a block in systemic movement of the virus.

Analysis of the level of TSWV by ELISA showed that C11-2-39, which showed the lowest incidence of TSWV by mechanical inoculation, also had the lowest level of TSWV compared to Georgia Runner, Georgia Green, and C-99R (Fig. 4, Table 2). Although the trends of TSWV level averaged over two temperature regimes were indistinguishable among the cultivars (Fig. 4), some distinction in level of TSWV was seen when data were analyzed separately at each temperature regime. For example, C-99R showed a lower level of TSWV than Georgia Runner and Georgia Green at the lower temperature, and C-99R and Georgia Green showed a lower level of TSWV than Georgia Runner at the higher temperature regime (Table 2).

The higher temperature regime resulted in a lower percentage of systemically infected plants (Fig. 1) compared to that at the lower temperature. More localized ringspot symptoms on C11-2-39 and more ringspot symptoms on all systemically infected genotypes were observed at higher temperature. The level of TSWV also dropped at the higher temperature in all the genotypes over the cubic component of the regression line (Fig. 5). Viral movement was restricted at the higher temperature at early (in case of C11-2-39 that showed localized infection) and late (in the cases of C11-2-39 and C-99R that showed systemic infection) stages of infection. The above observations suggest that the manifestation of resistant response can be correlated to higher temperature.

Based on transmission, symptomatology, virus-distribution, and relative levels of TSWV, Georgia Runner and Georgia Green were categorized as low, C-99R as medium, and C11-2-39 as high with respect to relative resistance to TSWV infection by mechanical inoculation. To our knowledge, this is the first report where the response of peanut genotypes to mechanical inoculation of TSWV could be delineated into resistant and susceptible phenotypes. The major difference between the present and previous studies (10,14) was the selection of genotypes for mechanical inoculation. In the present study, the genotypes selected were shown to have distinct differences in their response to field epidemics of TSWV (5,6). C11-2-39 is found to have the highest level of field resistance, followed by C99R and Georgia Green, while Georgia Runner is highly susceptible. Excluding the breeding line C11-2-39, of all the currently available peanut cultivars, C-99R is reported to have the highest level of field resistance to TSWV (5,8). While the reactions of Georgia Runner, C-99R, and C11-2-39 to mechanical inoculation correlated with their respective field performances, Georgia Green, which has field resistance to TSWV, could not be distinguished from TSWV-susceptible cultivar Georgia Runner under identical experimental conditions. A similar phenomenon was observed in the case of two other peanut cultivars. Southern Runner has a similar level of field resistance to Georgia Green, and Florunner is similar to Georgia Runner in its susceptibility to TSWV under field conditions. However, Southern Runner was found to be as susceptible as Florunner to mechanical inoculation by TSWV (10,14).

We have previously optimized a mechanical inoculation procedure for TSWV to peanut (11). The present study was an attempt to validate the inoculation procedure, which resulted in the identification of reference standards that may be of potential use for inclusion in screening of peanut germ plasm and plant introductions by mechanical inoculation. While screening of peanut germ plasm for TSWV resistance will have to include evaluation under field conditions involving multiple locations, the mechanical inoculation–based screening should facilitate a rapid identification of promising germ plasm before it is taken further into field testing.

ACKNOWLEDGMENTS

We are thankful to Kippy Lewis for technical assistance and Ben Mullinix for statistical analysis. Financial support including a postdoctoral fellowship to B. Mandal was provided by USDA-CSREES Special Grant (99-34412-7415). Financial support in part was also provided by the Georgia Agricultural Commodity Commission for Peanut and the University of Georgia Cultivar Development Committee.

LITERATURE CITED

1. Brown, S., Todd, J., Culbreath, A., Baldwin, J., Beasley, J., and Pappu, H. 2000. Tomato spotted wilt of peanut: Identifying and avoiding high-risk situations. Univ. Ga. Coop. Ext. Serv. Bull. 1165. p. 11.
2. Brown, S. L., Todd, J. W., and Culbreath, A. K. 1996. Effect of selected cultural practices to tomato spotted wilt virus and populations of thrips vectors in peanuts. Acta Hortic. 431:491-498.
3. Culbreath, A. K., Todd, J. W., Brown, S. L., Baldwin, J. A., and Pappu, H. R. 1999. A genetic and cultural "package" for management of tomato spotted wilt virus in peanut. Biol. Cultural Tests Control Plant Dis. 14:1-8.
4. Culbreath, A. K., Todd, J. W., Demski, J. W., and Chamberlin, J. R. 1992. Disease progress of spotted wilt in peanut cultivars Florunner and Southern Runner. Phytopathology 82:766-771.
5. Culbreath, A. K., Todd, J. W., Gorbet, D. W., Brown, S. L., Baldwin, J. A., Pappu, H. R., Holbrook, C. C., and Shokes, F. M. 1999. Response of early, medium and late peanut breeding lines to field epidemics of tomato spotted wilt. Peanut Sci. 26:100-106.
6. Culbreath, A. K., Todd, J. W., Gorbet, D. W., Brown, S. L., Baldwin, J., Pappu, H. R., and Shokes, F. M. 2000. Reaction of peanut cultivars to spotted wilt. Peanut Sci. 27:35-39.
7. Dubern, J., Huguenot, C., and Dollet, M. 1987. Tomato spotted wilt virus in Senegal? (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 19:30.
8. Gorbet, D. W., Shokes, F. M., Culbreath, A. K., Todd, J. W., and Whitty, E. B. 1999. C-99R - A new multiple disease resistant peanut cultivar. Marianna N. Fla. Res. Educ. Center Res. Rep. 99-2.
9. Halliwell, R. S., and Philley, G. 1974. Spotted wilt of peanut in Texas. Plant Dis. Rep. 58:23-25.
10. Hoffmann, K., Geske, S. M., and Moyer, J. W. 1998. Pathogenesis of tomato spotted wilt virus in peanut plants dually infected with peanut mottle virus. Plant Dis. 82:610-614.
11. Mandal, B., Pappu, H. R., and Culbreath, A. K. 2001. Factors affecting mechanical transmission of *Tomato spotted wilt virus* to peanut (*Arachis hypogaea*). Plant Dis. 85:1259-1263.
12. Moyer, J. W. 1999. *Tospoviruses* (*Bunyaviridae*). Pages 1803-1807 in: Encyclopedia of Virology. A. Granoff and R. G. Webster, eds. Academic Press, San Diego, CA.
13. Nome, S. F., Giorda, L. M., Truol de Izaurralde, G., and Laguna, I. G. 1985. Necrosis del borte del mani (*Arachis hypogaea* L.) causada por el virus de la marchitez manchada del tomate (tomato spotted wilt virus) en Argentina. IDIA 433-436:29-33.
14. Pereira, M. J. 1993. Tomato spotted wilt virus in peanut (*Arachis hypogaea* L.): Screening technique and assessment of genetic resistance levels. Ph.D. thesis. University of Florida, Gainesville.
15. Reddy, A. S., Reddy, L. J., Mallikarjuna, N., Abdurahman, M. D., Reddy, Y. V., Bramel, P. J., and Reddy D. V. R. 2000. Identification of resistance to Peanut bud necrosis virus (PBN) in wild *Arachis* germplasm. Ann. Appl. Biol. 137:135-139.

16. Sherwood, J. L., German, T. L. Moyer, J. W., Ullman, D. E., and Whitfield, A. E. 2000. Tomato spotted wilt. Pages 1030-1031 in: Encyclopedia of Plant Pathology. O. C. Maloy and T. D. Murray, eds. John Wiley & Sons, New York.
17. Sutula, C. L., Gillett, J. M., Morrissey, S. M., and Ramsdell, C. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70:722-726.
18. Ullman, D. E., Sherwood, J. L., and German, T. L. 1997. Thrips as vectors of plant pathogens. Pages 539-564 in: Thrips as Crop Pests. T. Lewis, ed. CAB International, Wallingford, UK.
19. Woodward, J. L. 2001. 2000 Georgia plant disease loss estimates. Univ. Ga. Coop. Ext. Serv. Publ. Path 01-001.