Reaction of Peanut Cultivars to Tomato Spotted Wilt Virus (TSWV) Under Field Conditions and Their Response to Mechanical Inoculation by TSWV Under Greenhouse Conditions

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ABSTRACT

Spotted wilt virus disease caused by *Tomato spotted wilt tospovirus* (TSWV) has become a limiting factor in peanut (*Arachis hypogaea* L.) production in the Southwest region of the U.S. Selected peanut cultivars (Okrun, Southwest Runner, Tamrun-98, Tamrun-96, Georgia Green, Tamrun OL-02, Tamrun OL-01, and Georgia Hi Oleic) were evaluated for reaction to TSWV in field plots in 2001, 2002, and 2003. Field plots were located near Pearsall, TX, where TSWV is endemic. In 2001, at 72 days post planting (DPP), incidence of TSWV was significantly ($P < 0.05$) higher in Southwest runner, Okrun, and Tamrun-98 than the other cultivars except Tamrun OL-02 in the test. In 2002, disease incidence of TSWV was low and thus only one reading was taken at 135 DPP where the cultivars Georgia Green and Georgia Hi Oleic exhibited the least incidence of spotted wilt. In 2003, at 58 DPP, Georgia Green, and Georgia Hi Oleic showed the lowest incidence of spotted wilt compared with other cultivars. The response of the eight peanut cultivars to TSWV following mechanical inoculation with TSWV at three plant ages was evaluated under greenhouse conditions. Disease severity index (DSI) and plant growth parameters such as root length, root volume, plant height, and fresh weight were determined. ELISA values (as indicative of the relative presence of TSWV), and relative water content were also determined to show the impact of TSWV infections in inoculated plants. Reductions in most plant growth parameters were observed when plants were inoculated with TSWV at five days post-planting, than twelve and nineteen days post-planting inoculation. Positive values of correlation coefficient (CC) between DSI and root volume or root length in the cultivars Georgia Hi Oleic, Georgia Green, Tamrun-96 and Tamrun OL-01 indicate resistance or tolerance to mechanical inoculation with TSWV. Negative values of CC between DSI and root length or root volume in the cultivars Tamrun-98, Tamrun OL-02, Southwest Runner, and Okrun provide evidence for their susceptibility to TSWV. Comparison of results obtained from the field with those from greenhouse evaluations suggest that Georgia Hi Oleic, Georgia Green, Tamrun-96 and Tamrun OL-01 cultivars are useful as potential tools for management of TSWV in peanut for the Southwestern United States.

Key Words: Groundnut, disease management.

Spotted wilt disease caused by TSWV is a serious disease in peanut in the United States (Black *et al.,* 1986; Culbreath *et al.,* 1990; Garcia *et al.,* 2000; Hagan *et al.,* 1990). In 1971, TSWV was first reported infecting peanut in Texas (Halliwel and Philley, 1974). During 1985, 1986, 1990, and 1991, several epidemics of TSWV occurred on peanut in southwest Texas, with yield reductions approaching 95% (Black 1987; Black *et al.,* 1986). In 1985 and 1986, losses in Texas were estimated in the millions of dollars (Mitchell and Smith, 1991). From 1989 throughout the mid 1990's in Georgia, the incidence of spotted wilt increased greatly in peanut and other crops. (Culbreath *et al.,* 1990; 1991; 1992). TSWV has a wide host range of more than 1,000 plant species in at least 92 families where both monocotyledons and dicotyledons have been reported to host the virus (Peters, 1998). Symptoms in peanut due to TSWV include yellowing, necrosis, chlorotic ring spots of various sizes and shapes, and stunting (Kokalis-Burelle *et al.,* 1997; Sherwood and Melouk, 1995; Simpson *et al.,* 2003). TSWV infection in peanut causes reduction in pod number and size. Kernels of infected plants may also be reduced in size or become malformed, and discolored (Sherwood and Melouk, 1995).

TSWV is transmitted by at least seven thrips species belonging to the insect Order *Thysanoptera*. The tobacco thrips, *Frankliniella fusca* (Hinds), western flower thrips, *Frankliniella occidentalis* (Pergande) are two predominant species in the peanut-producing states that transmit TSWV (Mitchell and Smith, 1991; Mulder *et al.,* 1991; 2001; Todd *et al.,* 1990). TSWV is acquired by immature thrips feeding on infected host plants

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(German *et al.,* 1991; Goldback and Peters, 1996), and is retained in the gut of thrips throughout the molting, pupation, and adult stages in a persistent manner. TSWV replicates within the vector *F. occidentalis*, allowing the virus to be transmitted for long periods of time and increasing the vector's ability to infest many healthy peanut plants within the field (Wijkamp *et al.,* 1993).

Controlling the vector chemically seems to have no or little impact in reducing disease incidence, and other alternative means of managing this disease are needed (Culbreath *et al.,* 2003). Host resistance is the most effective means of reducing the incidence of spotted wilt disease in most peanut-producing states (Culbreath *et al.,* 1996; 1999; 2000; Wells *et al.,* 2002). Several peanut cultivars have been reported to have moderate resistance and/or tolerance to TSWV infection (Culbreath *et al.,* 2003). Southern Runner was the first cultivar reported to possess partial resistance to multiple soilborne diseases and foliar diseases, including TSWV (Culbreath *et al.,* 2003). Georgia Browne, Georgia Green, ViruGard, UF MDR 98, C-99R, AnduII, NC V-11, and Norden are peanut cultivars reported to have moderate resistance to TSWV (Culbreath *et al.,* 1996; 1999; 2000; Wells *et al.,* 2002). Tamrun-96 is a runner-type cultivar developed by Texas A&M University, reported to have moderate resistance to TSWV (Smith *et al.,* 1998).

Recently, several breeding lines have shown greater field resistance to TSWV in the eastern U. S. (Lyerly *et al.,* 2002; Mandal *et al.,* 2001). Research on host resistance to TSWV in western peanut growing areas is needed. Therefore, the objectives of this research are: 1) to evaluate the reaction of eight peanut cultivars to TSWV under field conditions in the southwest, and 2) to evaluate the response of these cultivars to mechanical inoculation with TSWV under greenhouse conditions.

Materials and Methods

Reaction of Peanut Cultivars to TSWV in Field Plots. During the 2001, 2002, and 2003 growing seasons, peanut cultivars Tamrun-98, Tamrun-96, Southwest Runner, Georgia Green, Tamrun OL-01, Georgia Hi Oleic, Tamrun OL-02 and Okrun were planted in field plots near Pearsall, TX, where TSWV disease is endemic. In all growing seasons, field plots consisted of eight cultivars (treatments) arranged in a randomized complete block design with four replications. In each replicate, cultivars were planted in two rows 6 m long with row spacing of 0.91 m. Number of plants per plot was between 120–130 which was lower than commercial fields to promote higher incidence of spotted wilt disease (Culbreath *et al.,* 1999), and to facilitate observation of symptoms in individual plant. Plants with symptoms on one or more leaflets were considered symptomatic (Culbreath *et al.,* 1999). Positive diagnosis of TSWV was confirmed by sampling two symptomatic leaves and performing enzyme-liked immuneosorbant assay (ELISA) test (Clark and Adam, 1977) using a commercial kit obtained from Agdia, Inc. (Elkhart, IN). Reactions of ELISA were measured with an ELISA-plate reader (Microplate Automated Reader EL 310, Bio-Tek Instruments, Winooski, VT) at 490 nm. A sample was considered positive for TSWV if the A490 reading was greater than twice the average negative control.

Area under disease progress curve (AUDPC) was calculated for each plot as described by Shaner and Finney (1977) using time in days after planting and disease incidence. Chlorothalonil or other recommended fungicides were applied on a 14-day schedule for managing leaf spot fungal diseases.

All data were subjected to statistical analysis of variance (SAS Inst., 1985). Duncan's multiple-range test (MRT) was used for means comparison of peanut cultivars (Steel *et al.,* 1997).

Response of Peanut Genotypes Mechanically Inoculated with TSWV Under Greenhouse Conditions. The greenhouse experiment was designed as an $8 \times 3 \times 2$ factorial arrangement of treatments (8 genotypes $\times$ 3 inoculation times $\times$ 2 inoculation treatments) in a randomized complete block design (RCBD) with four replications. Seeds were germinated in trays lined with water-saturated- filter paper (Whatman #1). Each germinating seed was planted in a polyvinyl chloride (PVC) tube (10 cm inside diameter and 76 cm in length) filled with fritted clay as described by Ketring (1984). Twenty four hours prior to inoculation plants were covered with paper (Mandal *et al.,* 2001). Plants at 5, 12, and 19 days post planting were dusted with 320 grit carborundum (Fisher Scientific, New Jersey USA) and mechanically inoculated with TSWV. Inoculum was prepared by grinding 5g of TSWV-infected leaves of red tasselflower (*Emilia sochifolia* L.) or jimsonweed (*Datura stramonium* L.) in 10 ml. of 0.05 M potassium phosphate buffer (pH 7.0) containing 0.2% of 2-mercaptoethanole and 1% Celite (Fisher Scientific, New Jersey USA). Plants inoculated with buffer containing 0.2% of 2-mercaptoethanole and 1% Celite served as controls. All plants were maintained in the greenhouse, watered daily, and fertilized weekly with a solution

Table 1. Reaction of peanut cultivars to Tomato Spotted Wilt Virus in field plots at Pearsall, TX, in 2001.

Entry	No. of symptomatic plants/plot at (x) days post planting			
	x =30	x =44	x =58	x =72
Okrun	2.0 ab[1]	6.8 b	15.8 a	39.3 abc
Southwest runner	2.3 ab	4.8 bc	17.0 a	47.3 a
Tamrun 96	2.8 ab	7.3 b	16.5 a	25.5 cd
Georgia Green	2.0 ab	4.3 c	9.5 b	25.5 cd
Tamrun 98	1.0 b	7.0 b	15.0 a	44.5 ab
Tamrun OL01	4.3 a	9.8 a	17.3 a	28.8 cd
Tamrun OL02	2.0 ab	7.3 b	17.0 a	31.0 bcd
Georgia Hi Oleic	1.8 ab	4.3 c	9.3 b	19.8 d

[1]Means within columns, followed by same letter are not significantly different [P < 0.05, Duncan's New Multiple Rang Test].

containing 2 g/L of a fertilizer (20-20-20). Plants were examined daily for symptoms. Three weeks after each inoculation, plants were lifted from the tubes, and root length (cm), root volume (cm^3), plant height (cm), fresh weight (g), relative water content, and values from ELISA from leaves were determined. Values of ELISA were used to indicate the relative presence of TSWV, similar to research reported by Hunger et al (1991). Root volume was determined by water displacement method (Heinzman and Eilrich, 1977; Ketring, 1984). Relative water content (RWC) of foliage was determined following a standard method according to Erikson *et al* (1991). Disease severity index (DSI) of TSWV symptoms was scored for each plant using a 1–4 scale where 1=no symptoms, 2=only inoculated leaves displaying symptoms, 3= systemic symptoms (mosaic and / or mottled), yellowing, top chlorosis, and / or leaf distortion, and 4= severe necrosis, stunting, and / or dead plant.

Results and Discussion

Field Experiments. In the 2001 growing season, TSWV-like symptoms were first observed at 30 days post-planting (DPP) in all peanut genotypes tested. Leaves from symptomatic plants were collected and tested for the presence of TSWV by ELISA, where values ranged from 0.28 to 1.33. TSWV disease incidence in 2001, in all peanut cultivars increased with time (Table 1). Disease incidence of spotted wilt at 72 DPP was higher (P < 0.05) in Southwest Runner, Okrun, and Tamrun 98 than the other cultivars (Table 1). During the 2002 growing season, disease incidence of TSWV was low and thus only one reading was taken at 135 DPP where the cultivars Georgia Green and Georgia Hi Oleic exhibited the least TSWV incidence (Table 2). In the 2003 growing season, TSWV infection was observed at 30 DPP. At 58 DPP, Tamrun-96, Georgia Green, and Georgia Hi Oleic showed the lowest disease incidence compared with other peanut cultivars (Table 3).

Table 2. Reaction of peanut cultivars to Tomato Spotted Wilt Virus in field plots at Pearsall, TX, in 2002.

Entry	No.of symptomatic plants at 135 days post planting
Okrun	10.5 abc[1]
Southwest runner	12.8 ab
Tamrun 96	13.5 a
Georgia Green	7.5 c
Tamrun 98	14.0 a
Tamrun OL01	13.8 a
Tamrun OL02	12.8 ab
Georgia Hi Oleic	8.8 bc

[1]Means within columns, followed by same letter are not significantly different [P < 0.05, Duncan's New Multiple Rang Test].

During the 2001 growing season, AUDPC values of spotted wilt of Georgia Hi Oleic, Georgia Green, and Tamrun-96 were 162, 206 and 212, respectively, which were lower than those of Okrun, Tamrun-98, and Southwest Runner (310, 341, and 372, respectively). Similarly, in the 2003 growing season, the AUDPC of Georgia Hi Oleic, Georgia Green, and Tamrun-96 were 120, 125 and 187, respectively, which were lower than those of Southwest Runner, Tamrun-98, and Okrun (208, 215 and 230, respectively). During the three growing seasons, disease incidence of TSWV varied between the peanut cultivars tested. Georgia Green, Georgia Hi Oleic, and Tamrun-96 cultivars

Table 3. Reaction of peanut cultivars to Tomato Spotted Wilt Virus in field plots at Pearsall, TX, in 2003.

Entry	No. of symptomatic plants/plot at (x) days post planting		
	x = 30	x = 44	x =58
Okrun	4.3 ab[1]	9.7 ab	16.7 a
Southwest Runner	2.3 b	8.7 ab	16.3 a
Tamrun 96	4.0 ab	7.7 abc	13.3 ab
Georgia Green	2.7 b	4.0 c	10.0 b
Tamrun 98	3.3 ab	8.7 ab	16.7 a
Tamrun OL01	6.7 a	11.0 a	18.0 a
Tamrun OL02	2.3 b	7.7 abc	16.3 a
Georgia Hi Oleic	1.7 b	4.3 bc	10.0 b

[1]Means within columns, followed by same letter are not significantly different [P < 0.05, Duncan's New Multiple Rang Test].

Table 4. Disease severity index (DSI) [1] of mechanically inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of inoculation at (x) days post planting					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	1.00 a A[2]	2.70bc B	1.00 aA	2.50bcB	1.00 aA	2.10aB
Georgia Green	1.00aA	2.60bB	1.00 aA	2.20aB	1.00 aA	2.30abB
Okrun	1.00aA	2.55bB	1.00 aA	2.50bcB	1.00 aA	2.50cB
Southwest Runner	1.00aA	2.80bcB	1.00 aA	2.26abB	1.00 aA	2.15abB
Tamrun 96	1.00aA	2.20aB	1.00 aA	2.30abcB	1.00 aA	2.25abB
Tamrun 98	1.00aA	2.98cB	1.00 aA	2.60dB	1.00 aA	2.40bcB
Tamrun OL 01	1.00aA	2.70bcB	1.00 aA	2.55cdB	1.00 aA	2.15abB
Tamrun OL 02	1.00 aA	2.55bB	1.00 aA	2.55cdB	1.00 aA	2.10aB

[1]DSI where, 1 = no symptoms, 2 = Only inoculated leaves were symptomatic; 3 = Systemic symptoms (Mosaic, Mottle, Chlorosis, Yellowing, or leaf distortion), and 4 = Severe symptoms consisting of necrosis, stunting, leaf deformations, death of plant.

[2]Means with the same capital letter, within rows at each inoculation time, and means in the same columns, followed by the same small letters are not significantly different from each other (LSMEANS; $P < 0.05$).

exhibited greater resistance to TSWV compared to the other cultivars tested.

The resistance of Georgia Green to TSWV observed in our plots in 2001, 2002, and 2003 corroborated with the partial resistance reported by other researchers (Culbreath *et al.,* 1996). In addition to other moderate resistant cultivars, Georgia Green has been accepted as a cultivar with moderate level of resistance to TSWV by growers in Southeastern peanut producing areas (Culbreath *et al.,* 2003). In field evaluations conducted in Georgia and Florida, Georgia Green exhibited on average 31 to 59% TSWV disease incidence during the 1997 and 1998 growing seasons, respectively (Culbreath *et al.,* 2000). In a related study it was reported that Georgia Green had the lowest incidence of TSWV compared to other peanut genotypes evaluated (Culbreath *et al.,* 1996). Tamrun-96, a peanut cultivar released by Texas A&M University, is reported as being moderately resistant to TSWV (Smith *et al.,* 1998). However, it exhibited susceptibility to TSWV, when evaluated in field plots in Georgia and Florida (Culbreath *et al.,* 1996). Georgia Hi Oleic cultivar was released recently and displayed some resistance to TSWV under field conditions. Tamrun OL-01 cultivar, recently released by Texas A&M University, was reported to have a moderate tolerance to TSWV, southern blight (*Sclerotium rolfsii* Sacc.), and Sclerotinia blight (*Sclerotinia minor* Jagger) (Simpson *et al.,* 2003). In our study, Georgia Green, Georgia Hi Oleic, and Tamrun-96 exhibited moderate resistance to TSWV in field plots near Pearsall, TX. These results agree with results reported on moderate resistance of other cultivars to TSWV when evaluated under field conditions (Culbreath *et al.,* 1996; 1999; 2000). The mechanisms that are responsible for differences in disease incidence of TSWV among peanut cultivars have not yet been characterized (Culbreath *et al.,* 1996; Lyerly *et al.,* 2002).

Response of Peanut Cultivars to Mechanical Inoculation by TSWV Under Greenhouse Conditions.

Disease Severity. More than 90% of TSWV-inoculated peanut plants grown in the greenhouse, of all peanut cultivars expressed foliar symptoms. Plants inoculated with buffer only (Controls) did not exhibit TSWV symptoms. Expression of TSWV on inoculated peanut cultivars is shown in Table 4. The first inoculation at 5 DPP, revealed that Tamrun-96 had significantly lower DSI values compared to the other cultivars. However, Tamrun-98 was the most susceptible to TSWV compared to other cultivars. The second inoculation, at 12 DPP, showed that Georgia Green, Southwest Runner, and Tamrun-96 had the lowest DSI values when compared to other cultivars while Tamrun-98 was the most susceptible to TSWV (Table 4). When plants were inoculated with TSWV at 19 DPP, Okrun had significantly higher DSI value than other cultivars with the exception of Tamrun-98 (Table 4).

It was reported that Georgia Green did not express the observed level of field resistance to TSWV following mechanical inoculation. The resistance of Georgia Green in the field might be effective only against thrips-borne inoculum, and might have broken down against the relatively high dosage applied in a mechanical inoculation (Mandal *et al.,* 2002). Tamrun-96 evaluated in field plots in Georgia and Florida has exhibited susceptibility to TSWV. In contrast, in our study Tamrun-96 grown in Texas plots exhibited a moderate level of resistance to TSWV. Differences between these

Table 5. ELISA values* of mechanically-inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of inoculation at (x) days post planting					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	0.02[1] A	0.69 B	0.02 A	0.67 B	0.02 A	0.58 B
Georgia Green	0.02 A	0.78 B	0.02 A	0.73 B	0.02 A	0.85 B
Okrun	0.02 A	0.71 B	0.02 A	0.67 B	0.02 A	0.64 B
Southwest Runner	0.02 A	0.76 B	0.02 A	0.70 B	0.02 A	0.53 B
Tamrun 96	0.02 A	0.54 B	0.02 A	0.78 B	0.02 A	0.60 B
Tamrun 98	0.02 A	0.70 B	0.02 A	0.60 B	0.02 A	0.92 B
Tamrun OL 01	0.02 A	0.65 B	0.02 A	0.77 B	0.02 A	0.72 B
Tamrun OL 02	0.02 A	0.57 B	0.02 A	0.53 B	0.02 A	0.86 B

*Values represent the absorbance (i.e. optical density) measured at 490 nm.

[1]Means with the same letter, within each row for each inoculation date, are not significantly different from each other (LSMEANS; $P < 0.05$).

studies might be due to variation between the isolates of TSWV in Southwest and in the Southeast.

Detection of TSWV. Significant differences were observed in all inoculated plants compared with non-inoculated controls at all three mechanical inoculation times under greenhouse conditions. Readings of ELISA in inoculated planted showed variations (Table 5). TSWV was not detected in non-inoculated plants (Table 5); however, no differences were observed in ELISA values among the various cultivars irrespective of time of inoculation.

Root Length. Root lengths of inoculated plants and non-inoculated controls are presented in Table 6. Inoculation at 5 DPP, resulted in a 17% reduction in root length of Tamrun-98 which was significant ($P < 0.05$) from Georgia Hi Oleic, Georgia Green and Tamrun OL-01 (Table 6). At 12 DPP, the root length of Tamrun-96 was not affected, while other cultivars were reduced significantly (Table 6). At 19 DPP inoculation, the greatest reduction in root length compared to non-inoculated plants, was observed on Okrun, Tamrun OL-01, and Tamrun OL-02. Generally, at 12 DPP most inoculated plants compared to non-inoculated peanuts showed significant reductions in root length, but the percent reduction among cultivars was not significant at the $P < 0.05$ level (Table 6).

Root Volume. When non-inoculated controls and inoculated plants were compared for root volume, significant differences were revealed at all three inoculation times (Table 7). However, percentages of reduction in root volume varied among cultivars. At 5 DPP, inoculation of Southwest Runner with TSWV resulted in 48% reduction in root volume, which was significant ($P < 0.05$) from Georgia Hi Oleic, Georgia Green, Okrun, Tamrun-96, and Tamrun OL-02 (Table 7). When inoculation was performed at 12 DPP, Tamrun-96 showed the least reduction (22%) in root volume which was

Table 6. Root length (cm) of mechanically-inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of inoculation at (x) days post planting					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	60.9 A[1]	57.1 A (6a)	67.5 A	60.9 B (10a)	81.5 A	78.7 A (3a)
Georgia Green	54.6 A	52.1 A (5ab)	63.1 A	54.8 B (13a)	76.5 A	74.5 A (3a)
Okrun	55.9 A	49.1 B (11bc)	61.4 A	54.7 B (11a)	76.9 A	70.2 B (9a)
Southwest Runner	57.6 A	48.6 B (16bc)	64.1 A	56.4 B (12a)	78.3 A	74.8 A (4a)
Tamrun 96	54.9 A	48.9 B (11abc)	58.8 A	56.9 A (3a)	76.5 A	73.0 A (5a)
Tamrun 98	54.1 A	44.7 B (17c)	61.5 A	52.6 B (14a)	73.2 A	71.3 A (3a)
Tamrun OL 01	57.5 A	52.7 A (8ab)	63.1 A	54.4 B (14a)	79.8 A	73.2 B (8a)
Tamrun OL 02	55.7 A	50.6 A (9bc)	57.7 A	51.8 B (10a)	77.2 A	67.5 B (13a)

[1]Means with the same capital letter, within rows at each inoculation time. Values in parenthesis preceding the lower case letter (s) represent the reduction (%) in root length as compared with the controls. Means in the same columns in parenthesis, followed by the same small letter are not significantly different from each other (LSMEANS; $P < 0.05$).

Table 7. Root volume (cm^3) of mechanically-inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of Inoculation at (x) days post planting					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	19.6 A[1]	15.3 B (22a)	23.1A	15.3 B(34ab)	31.9A	24.7 B(23ab)
Georgia Green	20.5 A	14.6 B (29ab)	22.5A	16.9 B(25ab)	29.3A	20.7 B(29ab)
Okrun	17.3 A	13.3 B (23a)	19.6A	13.6 B(31ab)	26.5A	22.6 B(15a)
Southwest Runner	19.9 A	10.4 B (48c)	23.0A	15.0 B(35ab)	28.9A	23.1 B(20ab)
Tamrun 96	17.3 A	12.0 B (31ab)	20.4A	15.9 B(22a)	28.5A	20.1 B(29ab)
Tamrun 98	19.1 A	10.8 B (43bc)	20.9A	12.5 B(40b)	27.8A	21.0 B(24ab)
Tamrun OL 01	18.8 A	11.4 B (39bc)	21.7A	12.6 B(40b)	29.1A	20.2 B(31b)
Tamrun OL 02	18.9 A	12.9 B (28ab)	20.0A	12.0 B(40b)	25.4A	17.6 B(31b)

[1]Means with the same capital letter, within rows at each inoculation time. Values in parenthesis preceding the lower case letter (s) represent the reduction (%) in root volume as compared with the controls. Means in the same columns in parenthesis, followed by the same small letter are not significantly different from each other (LSMEANS; $P < 0.05$).

significant ($P < 0.05$) from Tamrun-98 and Tamrun OL-01 (Table 7). Least reduction (15%) in root volume was observed in Okrun at 19 DPP inoculation, which was significant ($P < 0.05$) from Tamrun OL 02 only (Table 7).

Plant Height. When comparing non-inoculated controls and inoculated plants at 5 DPP, plant height of Tamrun OL 01 was reduced the least (3%), which was significant ($P < 0.05$) from Tamrun-98 only (Table 8). At 12 DPP inoculation, plant height of Georgia Hi Oleic was least affected (13%) which was significant ($P < 0.05$) from Tamrun-98, Tamrun OL-01, and Tamrun OL-02 (Table 8). At 19 DPP inoculation, the percentage reduction of plant height was not significant ($P < 0.05$) among the cultivars (Table 8).

Plant Fresh Weight. Fresh weights of peanut cultivars of TSWV-mechanically-inoculated and non-inoculated controls were presented in Table 9. Inoculations at 5 DPP showed that Okrun had the least reduction (17%) of fresh weight which was significant ($P < 0.05$) from Southwest Runner. When plants were inoculated at 12 DPP, Tamrun OL-01 exhibited the most reduction (36%) in fresh weight, which was significant ($P < 0.05$) from Georgia Hi Oleic, Georgia Green, Okrun, and Tamrun-96 (Table 9). When plants inoculated at 19 DPP, the least reduction (17%) in fresh weight occurred on Tamrun-96 which was significant ($P < 0.05$) from the other cultivars (Table 9). Generally, across all three times of inoculation, significant reductions in fresh weight were observed in all cultivars except Okrun when inoculated at 5 DPP (Table 9).

Relative Water Content. When peanut cultivars were inoculated at 5 DPP, significant differences in relative water content (RWC) were observed in Georgia Green and Southwest Runner compared to non-inoculated plants (Table 10). At 12 and 19 DPP inoculation, no significant differences in

Table 8. Plant height (cm) of mechanically-inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of Inoculation at (x) days post inoculation					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	13.2 A[1]	11.7 B (11ab)	13.5A	11.7 B (13a)	14.9A	13.7 A (8a)
Georgia Green	08.4A	07.6 A (9ab)	08.5A	07.1B(16ab)	08.7A	07.5 A (14a)
Okrun	11.6A	10.4 A (10ab)	12.2A	10.5B(14ab)	14.0A	12.3 B (12a)
Southwest Runner	11.1A	10.0 A (10ab)	13.0A	11.1B(15ab)	13.9A	11.3 B (19a)
Tamrun 96	11.7A	10.6 A (9ab)	13.3A	11.1B(16ab)	14.1A	12.3 B (13a)
Tamrun 98	11.1A	8.9 B (20b)	12.2A	09.2 B (25b)	13.1A	10.8 B (18a)
Tamrun OL 01	12.1A	11.4 A (3a)	13.3A	10.4 B (22b)	14.0A	12.1 B (14a)
Tamrun OL 02	12.1A	11.1 A (8ab)	13.4A	10.4 B (22b)	14.3A	12.2 B (15a)

[1]Means with the same capital letter, within rows at each inoculation time. Values in parenthesis preceding the lower case letter (s) represent the reduction (%) in plant height as compared with the controls. Means in the same columns in parenthesis, followed by the same small letter are not significantly different from each other (LSMEANS; $P < 0.05$).

Table 9. Plant fresh weight (g) of mechanically-inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of Inoculation at (x) days post inoculation					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	26.3 A[1]	20.9B (21ab)	35.3 A	26.1 B (26a)	50.5 A	37.5B (26ab)
Georgia Green	25.0 A	18.5 B(26ab)	33.5 A	26.7 B (20a)	44.1 A	34.0B (23ab)
Okrun	21.5 A	17.9 A (17a)	28.8 A	21.6B (25ab)	41.0 A	34.0B (17ab)
Southwest Runner	26.9 A	15.8 B (41b)	35.3 A	24.6B (30ab)	48.9 A	37.3B (24ab)
Tamrun 96	23.8 A	17.1 B(28ab)	31.7 A	24.4B (23ab)	41.1 A	34.0 B (17a)
Tamrun 98	24.2 A	15.8 B(35ab)	32.1 A	21.4B (33ab)	45.7 A	33.6B (26ab)
Tamrun OL 01	25.4 A	16.1 B (37b)	32.9 A	21.1 B (36b)	44.6 A	33.5B (25ab)
Tamrun OL 02	23.9 A	16.7 B (30b)	29.9 A	20.0B (23ab)	42.4 A	29.5B (30b)

[1]Means with the same capital letter, within rows at each inoculation time. Values in parenthesis preceding the lower case letter (s) represent the reduction (%) in plant fresh weight as compared with the controls. Means in the same columns in parenthesis, followed by the same small letter are not significantly different from each other (LSMEANS; $P < 0.05$).

RWC were observed between treated and untreated plants with the exception of Okrun at the12 DPP inoculation, and Tamrun-96 at the 19 DPP inoculation (Table 10).

Positive correlations were obtained between DSI and ELISA values among most inoculated cultivars (Table 11). Correlation coefficient between DSI and root length, root volume, and RWC varied across cultivars and times of inoculation. The positive correlation coefficient values between DSI and root volume or root length in the cultivars Georgia Hi Oleic, Georgia Green, Tamrun 96, and Tamrun OL01 imply resistance or tolerance to mechanical inoculations with TSWV (Table 11). The negative correlation coefficients between DSI and root length or root volume in the cultivars Tamrun-98 and Tamrun OL-02, Southwest Runner, and Okrun imply lack of resistance or tolerance to mechanical inoculations with TSWV (Table 11). Also, the positive correlation coefficient values between DSI and RWC in the cultivars Georgia Hi Oleic, Georgia Green, Tamrun-96, and Tamrun OL-01, imply resistance or tolerance to mechanical inoculations with TSWV (Table 11).

Under controlled conditions, Pereira *et al.* (1995), reported the response of Florunner and Southern Runner against TSWV when seven quantitative growth parameters (e.g. main stem length, the two cotyledonary stem lengths, the two primary stem lengths, pod and peg number, canopy fresh weight, and root fresh weight) were determined to elucidate the impact of mechanical inoculation on these cultivars at two growth stages. No significant differences were observed among the two cultivars. Hoffmann *et al.* (1998) reported that mechanical inoculation of TSWV alone or co-inoculation with *Peanut mottle potyvirus* (PMV) to six peanut cultivars (GK7, NC7, VC1, Florunner, Southern Runner, and Spanco) did not reveal any significant differences in disease progress. Mandal

Table 10. Relative water content (RWC %) of mechanically-inoculated peanut cultivars with Tomato Spotted Wilt Virus under greenhouse conditions.

	Time of Inoculation at (x) days post inoculation					
	x= 5		x= 12		x= 19	
Entry	Control	Inoculated	Control	Inoculated	Control	Inoculated
Georgia Hi Oleic	91ab A[1]	91b A	90ab A	91b A	90cd A	88ab A
Georgia Green	92b A	88ab B	87ab A	87a A	89bcd A	86a A
Okrun	91ab A	90b A	92b A	86a B	89bcd A	87ab A
Southwest Runner	90ab A	86a B	89ab A	87a A	88abc A	86a A
Tamrun 96	88a A	88ab A	89ab A	87a A	89bcd A	85a B
Tamrun 98	92ab A	94ab A	90ab A	91b A	92d A	90b A
Tamrun OL 01	89ab A	88ab A	88ab A	87a A	86ab A	86a A
Tamrun OL 02	89ab A	90b A	87a A	88a A	85a A	88ab A

[1]Means with the same capital letter, within rows at each inoculation time, and means in the same columns, followed by the same small letter (s) are not significantly different from each other (LSMEANS; $P < 0.05$).

Table 11. Correlation coefficient between Tomato Spotted Wilt Virus disease severity index and plant root length and root volume, relative water content (RWC) and ELISA readings in peanut cultivars grown under greenhouse conditions.

Entry	Inoculation Time at DPP[1]	Root Length	Root Volume	RWC	ELISA
Georgia Hi Oleic	5	0.236	0.199	0.23	0.42 [c]
	12	0.355 [b]	0.643 [d]	0.30 [b]	0.29
	19	0.250 [a]	0.151	0.02	0.51 [d]
Georgia Green	5	0.009	0.184	0.14	0.48 [b]
	12	0.128	0.149	0.10	0.55 [d]
	19	0.749	0.587 [d]	0.38 [b]	0.44 [c]
Tamrun 96	5	0.397 [c]	0.476 [c]	0.28 [a]	0.46 [c]
	12	0.097	0.171	0.07	0.24
	19	0.359 [b]	0.429 [c]	0.16	0.52 [d]
Tamrun OL 01	5	0.263 [a]	0.275 [a]	0.57 [d]	0.28 [a]
	12	0.145	0.308 [b]	0.05	0.69 [d]
	19	0.433 [c]	0.325 [b]	0.50 [d]	0.64 [d]
Tamrun OL 02	5	−0.172	−0.684	0.06	0.42 [c]
	12	−0.253	−0.397 [c]	−0.22	−0.36 [b]
	19	0.123	−0.262 [a]	−0.01	0.6 [d]
Tamrun 98	5	0.237	0.014	−0.13	−0.16
	12	−0.109	−0.312 [b]	−0.36 [b]	0.33 [b]
	19	−0.008	0.137	0.52 [d]	0.09
Southwest Runner	5	−0.601 [d]	−0.698 [d]	−0.39 [c]	0.63 [d]
	12	−0.164	−0.162	0.06	0.74 [d]
	19	−0.077	−0.125	−0.16	0.52 [d]
Okrun	5	0.137	0.171	−0.10	0.31 [b]
	12	0.137	0.118	0.39 [c]	−0.02
	19	−0.458 [c]	−0.454 [c]	0.26 [a]	0.58 [d]

[a]Significant at $P < 0.1$.
[b]Significant at $P < 0.05$.
[c]Significant at $P < 0.01$.
[d]Significant at $P < 0.001$.
1 = Inoculation time at (X) days post planting (DPP)

et al. (2002) studied the reaction of three peanut cultivars and one breeding line against an isolate of TSWV. Their data were limited only to detection and distribution of the virus on these inoculated plants at two temperature regimes, based on ELISA. In this study, we obtained a detailed account of plant growth parameters that included; root length, root volume, plant height, fresh and dry weight. Disease severity index, relative water content, and virus titer were also evaluated on the eight cultivars inoculated mechanically at three time intervals.

Screening for plant resistance to viral agents via mechanical inoculation can provide useful information on genetic variations within the peanut germplasm. The identification of such genetic variations is important in breeding efforts to develop genotypes with resistance to TSWV. Mechanical inoculation, to screen for resistance under controlled conditions can be performed throughout the year, regardless of seasonal variations. However, it has the disadvantage of excluding the role of the vector in determining the observed reaction.

Screening peanut genotypes under field conditions has the advantage of identifying inherent viral resistance, and possibly vector preference and/or genotypes resistant to the thrips that can also provide indirect strategies for disease management to TSWV. However, the disadvantages of field screening include; limiting the evaluation to the growing season, variation of TSWV pressure from one location to another and yearly variation. In addition, the field environment with its complex biotic and abiotic agents may interfere either positively or negatively with identification of resistance. Our data of the field and greenhouse evaluations indicate the potential usefulness of the peanut cultivars Georgia Green, Tamrun-96, and Georgia Hi Oleic for managing TSWV in peanut production in Southwestern United States.

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