Survey by ELISA of Thrips (Thysanoptera: Thripidae) Vectored Tomato Spotted Wilt Virus Distribution in Foliage and Flowers of Field-Infected Peanut[1]

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ABSTRACT

The foliage of individual field-infected peanut plants (*Arachis hypogaea* L.) was examined by enzyme-linked immunosorbent assay (ELISA) for the presence of tomato spotted wilt virus (TSWV). TSWV was detected commonly in terminals (folded quadrifoliates) and flowers, the vector's feeding and breeding niches. Reconstructions of the assayed plants demonstrated that the virus concentration varied from plant to plant and symptoms were significantly correlated to virus concentration in individual leaves and terminals. The virus was not distributed uniformly throughout individual plants but was concentrated in young, developing terminal tissue. The presence of symptoms was indicative of detectable virus 95% of the time.

Key Words: Insecta, *Frankliniella fusca, Frankliniella occidentalis*, tobacco thrips, western flower thrips, tospovirus.

In the last decade, tomato spotted wilt virus (TSWV) has become an economically important threat to the peanut producers in the southern United States. Disease problems have been reported in Alabama, Mississippi, Florida, and Georgia (Hagan *et al.*, 1987; Chamberlin *et al.*, 1992). TSWV was first found infecting Texas peanut in 1971 but may have been present as early as 1941 (Halliwell and Philley, 1974). The disease first reached epidemic proportions in Texas in the mid-1980s, with losses in neighboring Frio, Atascosa, and Wilson counties estimated in the millions of dollars in 1985 and 1986 (Mitchell and Smith, 1991). Epidemic levels were again evident in these counties during the 1991 and 1992 growing seasons with TSWV prevalence peaking in monitored peanut fields at 20.6 and 25.4%, respectively. Individual fields experienced difficulties in the 1993 and 1994 growing seasons.

TSWV is a tospovirus (German *et al.*, 1992), a group vectored by at least nine species of thrips (Smith and Barfield, 1982; Kobatake *et al.*, 1984; Palmer *et al.*, 1990; German *et al.*, 1992; Hunter *et al.*, 1995). Two of these, *Frankliniella fusca* Hinds (the tobacco thrips) and *Frankliniella occidentalis* Pergande (the western flower thrips), are the primary species associated with south Texas peanut (Mitchell *et al.*, 1990). Adult and larval thrips are found mainly in the terminals (folded quadrifoliates) and flowers of peanut. Terminals serve as the predominant site for oviposition and larval feeding (Smith and Sams, 1977). Larval thrips acquire the virus from feeding on infected tissue and transmit the virus as adults (Bald and Samuel, 1931) in a persistent manner (Cho *et al.*, 1988). Recent research by Ullman *et al.* (1993) and Wijcamp *et al.* (1993) demonstrated that TSWV is propagative within the thrips before transmission occurs. Wijcamp and Peters (1993) provided evidence that larvae can also transmit TSWV.

Symptoms can be prominent in TSWV infections, and expression of the virus can be highly variable on a single infected plant. In peanut, symptom expression may range from concentric ring patterns and mosaics in older leaves to distorted and stunted young leaves (Dyer, 1949). Plants may become stunted with time (Halliwell

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and Philley, 1974). Peanut in India suffered from axillary shoot proliferation and terminal bud necrosis in addition to chlorotic leaf speckling and distortion when infected by bud necrosis virus (BNV), a closely related tospovirus (Ghanekar *et al.*, 1979).

The relationship between virus infection and thrips feeding and breeding niches in terminals and flowers is not well understood, nor is the relationship between symptomatic tissue and virus titer. This study was conducted to characterize the relationship between TSWV seroactivity and symptomology, and the association of infection with thrips feeding and breeding niches in peanut. Reports of asymptomatic peanut that are ELISA (enzyme-linked immunosorbent assay) positive for TSWV (Culbreath *et al.*, 1991) indicate the relationships may be complex.

Materials and Methods

Plant Collection. Seventeen Florunner peanut plants expressing TSWV symptoms were collected from three irrigated south Texas fields in Frio and Atascosa counties. Relatively small plants (100 or fewer leaves) were selected for ease of handling and to preclude overly complex reconstructions. None of the plants were flowering. Collections were made during the 1990 growing season from July through October and the plants experienced the normal range of growing conditions and agronomic treatments. Two of the plants were collected 25 d after planting, three were collected 26 d after planting, two were collected 40 d after planting, two were collected 47 d after planting, one was collected 54 d after planting, two were collected 70 d after planting, two were collected 75 d after planting, and three were collected 97 d after planting.

Foliage Rating. Every leaflet (quadrifoliolate) of each open quadrifoliate leaf and each folded quadrifoliate terminal on every plant was numerically rated on a scale of 1 to 3 based on the amount of symptoms expressed. A rating of 1 indicated no visible symptoms (ringspots, speckles, yellow blotches, line patterns, or color breaks) on the adaxial leaf surface; a 2 represented a leaflet with less than 50% of the adaxial surface expressing symptoms; and a 3 corresponded to more than 50% of the adaxial surface expressing symptoms. An ordinal rating was obtained for each leaf and terminal by averaging leaflet values. Stunting and deformity of terminals was not used as a symptom because this could be caused by thrips feeding damage alone.

Foliage Processing. After ordinal ratings were recorded, all insects and foreign matter were removed. Entire leaves and terminals were individually weighed and triturated in extraction buffer at a 1:10 dilution with mortar and pestle as recommended by Agdia, Inc. (Elkhart, IN): extraction buffer = phosphate buffered saline-tween (PBST) [0.14M sodium chloride, 0.008M sodium phosphate-dibasic (anhydrous), 0.001M potassium phosphate (monobasic), 0.05% Tween-20, and 0.003M potassium chloride, pH 7.4] containing 0.01M sodium sulfite, 2% (w/v) polyvinylpyrrolidone (m.w. 40,000), 2% Tween-20, 4% (w/v) fresh egg white, and 0.003M sodium azide, pH 7.4 at a 1:10 (w/v) dilution. The macerated leaves were filtered through 5-cc syringes packed with spun polyester aquarium filter floss into 1.5-mL polypropylene microcentrifuge tubes, and stored at -20 C before assaying. All plants were refrigerated and processed within 10 d of collection.

Healthy controls for the ELISA consisted of leaves obtained from greenhouse-raised Florunner peanut, while diseased controls were leaves from field-infected symptomatic peanut previously responding positive for TSWV presence by ELISA. Both controls were processed in the same manner as the samples.

Foliage Assays. When the foliage from the plants had been processed, all leaves and terminals (757 samples) were subjected to double antibody sandwich (DAS) ELISA (Clark and Adams, 1977). Antisera was purchased from Agdia, Inc.. All buffer solutions and assay protocols were based on Agdia guidelines.

The processed foliage samples were thawed to room temperature and vortexed to resuspend the antigen. The suspension was transferred at 200 µL of sample per well to microassay plates (Falcon 3912, Becton-Dickinson, Oxnard, CA) previously coated with TSWV-L polyclonal IgG antisera suspended in a coating buffer (0.02M sodium carbonate, 0.04M sodium bicarbonate, and 0.003M sodium azide, pH 9.6) at a 1:1000 (v/v) dilution of 1 mg/mL stock. Each plate utilized eight wells of extraction buffer to serve as blanks for the plate reader, four to eight wells for diseased controls and eight wells for healthy controls, while the remaining wells were used for sample testing. Plates with samples were incubated either for 2 hr at room temperature or overnight at 4 C in a closed humid box.

When the sample incubation period had ended, each plate was washed three times with PBST and 200 µL of peroxidase-conjugated TSWV-L monoclonal IgG at a dilution of 1:200 in a diluent (Agdia MRS component diluted 1:5 with PBST) was added to each well. The plates were incubated as before and again rinsed three times with PBST. The substrate solution (400 µL of 30% hydrogen peroxide in 0.024M citric acid (anhydrous) and 0.052M sodium phosphate dibasic (anhydrous) with *o*-phenylene diamine dihydrochloride (OPD) added at 1 mg/mL just before use) was pipetted at 200 µL per well and incubated for 20 min at room temperature in a closed humid box to allow for color development. The reaction was terminated with the addition of 50 µL per well of 3M sulfuric acid. Absorbance values were measured at 492 nm with a Titertek Multiskan™ PLUS (ICN Biomedical, Costa Mesa, CA) plate reader.

Flower Collection. Since none of the plants used for foliage assays were flowering, flowers were collected from a field of Florunner peanut in Frio County on 21 Aug. 1991, 42 d after planting. TSWV prevalence was 2.12% based on transect counts in the field. Since peanut flowers do not always display symptoms of infection, 30 flowers were randomly collected from plants displaying foliar symptoms of TSWV, and 30 flowers were randomly collected from plants showing no symptoms. Flowers were collected into extraction buffer and returned to the laboratory where thrips were removed.

Flower Processing and Assays. Flower samples were processed using the same protocol as for the foliage samples. Each flower was ground at a 1:10 (w/v) dilution in extraction buffer (this averaged approximately 500 µL of extraction buffer per flower), filtered, and 200 µL of each flower sample were placed in microplate wells. Flowers for healthy controls were collected from asymptomatic Florunner peanut plants in Stephenville, TX where TSWV is rare. Diseased controls were the same as for foliage assays.

Plant Reconstructions and Virus Distribution Histo-

grams. At the completion of the assays, each plant was diagrammatically reconstructed to depict TSWV distribution throughout the individual plants. A computer-aided graphics design program (DesignCAD™, American Small Business Computers, Inc., Pryor, OK) was used for the reconstructions. Leaves expressing symptoms and/or a positive optical density (OD) reading were denoted with specific hatch markings. Healthy leaves were left blank unless they had expressed symptoms.

A graphical method previously described (Sutula *et al.*, 1986) was used to establish a positive-negative threshold. A frequency histogram was created from the optical density readings for all 757 foliage samples. Histograms were also produced for each plant to show the frequency at which certain OD readings occurred within a plant. The values were grouped in categories ranging from 0.00 to 4.00 with an interval width of 0.25, and any negative OD values were grouped with the zero readings at the origins of the graphs. A similar histogram was prepared from OD data from flower assays.

Effect of Storage on TSWV Detection in Infected Peanut Tissue. Peanut foliage in this study was stored in a refrigerator at 4 C up to 10 d before processing (maceration in grinding buffer) was complete. Processed foliage was then frozen at -20 C for up to 70 d before being subjected to ELISA. In order to determine if either type of storage affected the OD value, a portion of a group of detached peanut leaves and terminals infected with TSWV was subjected to ELISA immediately (day 1) and then refrigerated. The positive control for this ELISA was previously frozen tissue from symptomatic plants that had tested positive in other assays. The negative control for this and subsequent ELISAs was Florunner peanut raised in a greenhouse. Samples were removed from the refrigerator, processed, and subjected to ELISA at 3, 6, 8, 10, and 14 d.

Another group of infected leaves and terminals was processed, also on day 1, an initial ELISA conducted, and the aliquot divided into two groups. The first group was stored in 1.5-mL microfuge tubes in a freezer at -20 C. Samples from this group were removed and subjected to ELISA at 7, 14, 34, 42, 56, and 70 d. The second group was stored in 2-mL tubes in liquid nitrogen. These constituted the positive control for both frozen and refrigerated ELISA samples from day 3 forward. On each day an ELISA was conducted, eight wells of sample, eight wells of positive control, and eight wells of negative control were examined. The only exception was on day 6 of the refrigeration experiment, where just six wells of positive and negative control were assayed.

Statistical Analysis. The OD measurements obtained for each sample were combined into a data set for analysis with the Statistical Analysis System (SAS Institute, Inc., Cary, NC, vers. 6.03). Analyses of variance (ANOVAs) were conducted to determine if significant differences in the OD of open leaves or folded terminals occurred and if branch terminals or main plant stem terminals had significantly different OD readings. Comparisons were also made between mean branch OD readings by plant. A Pearson product-moment (r) test was conducted to determine if a correlation existed between the symptom rating and OD for each leaf. The tissue storage study was analyzed by ANOVA and TTEST to determine if storage in either the refrigerator or freezer affected ELISA detection of TSWV.

Results

Negative Threshold. A break between the optical density readings for all samples occurred at an OD of 0.250. All optical density readings less than or equal to the threshold were considered to be TSWV negative, and all readings greater than the threshold were considered to be TSWV positive. The histogram used to determine the TSWV negative threshold is presented in Fig. 1. The mean TSWV negative value (from the healthy controls) plus three standard deviations were calculated also, but found to be less conservative. The TSWV negative threshold for flowers was 0.1 OD units (Fig. 2). OD values for all positive controls exceeded the threshold value in all assays, while OD values for all negative controls were less than the threshold value in all assays.

Plant Histograms. Histograms of each plant's virus distribution could be categorized in one of two ways. Eleven plants had values that were bimodally distributed, showing clear distinctions between optical density readings of TSWV negative and TSWV positive tissue as represented by plant 13 (Fig. 3). Two plants demonstrated histograms with optical density values distributed continuously from the origin (plant 17, Fig. 4). Four plants displayed histograms with optical density values clustered near the origin (plant 12, Fig. 5).

Plant Reconstructions. The plant reconstructions demonstrate that the virus is not spread uniformly throughout the plant. Leaves and terminals were found to be representative of four categories: symptomatic and

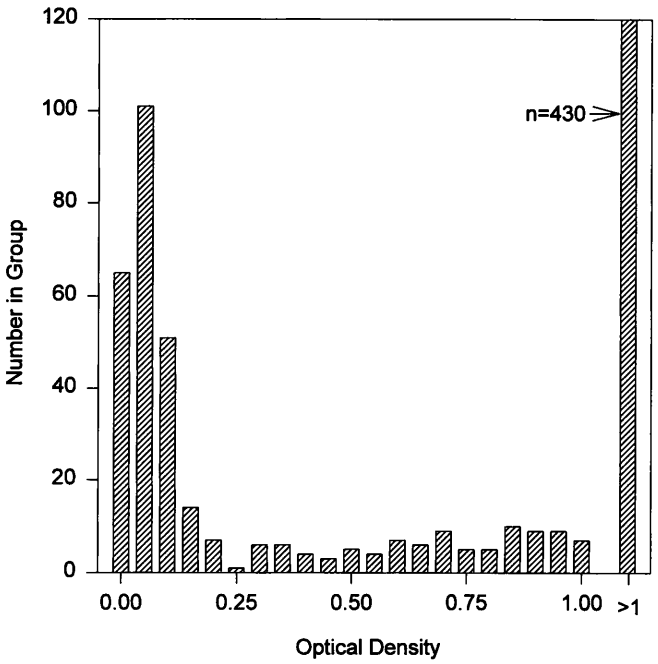

Fig. 1. Histogram of optical densities obtained from ELISA of peanut foliage. All 757 observations are included and the TSWV negative threshold at 0.25 OD units is demonstrated graphically. All optical density readings less than or equal to the threshold were considered to be TSWV negative, and all readings greater than the threshold were considered to be TSWV positive.

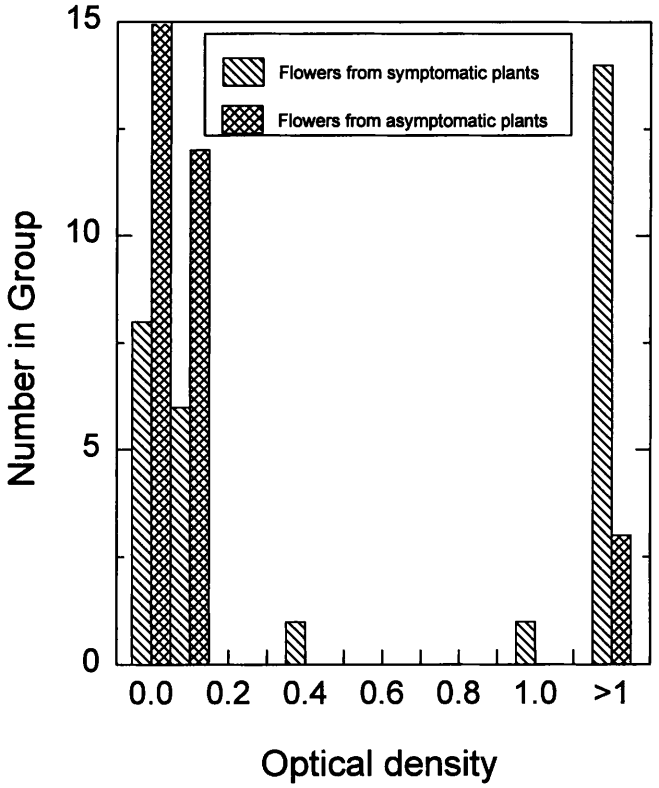

Fig. 2. Histogram of optical densities obtained from ELISA of peanut flowers. Thirty observations represent assays of flowers obtained from plants displaying TSWV symptoms and 30 observations represent assays of flowers obtained from plants not displaying symptoms. The TSWV negative threshold is 0.1 OD units. All optical density readings less than or equal to the threshold were considered to be TSWV negative, and all readings greater than the threshold were considered to be TSWV positive.

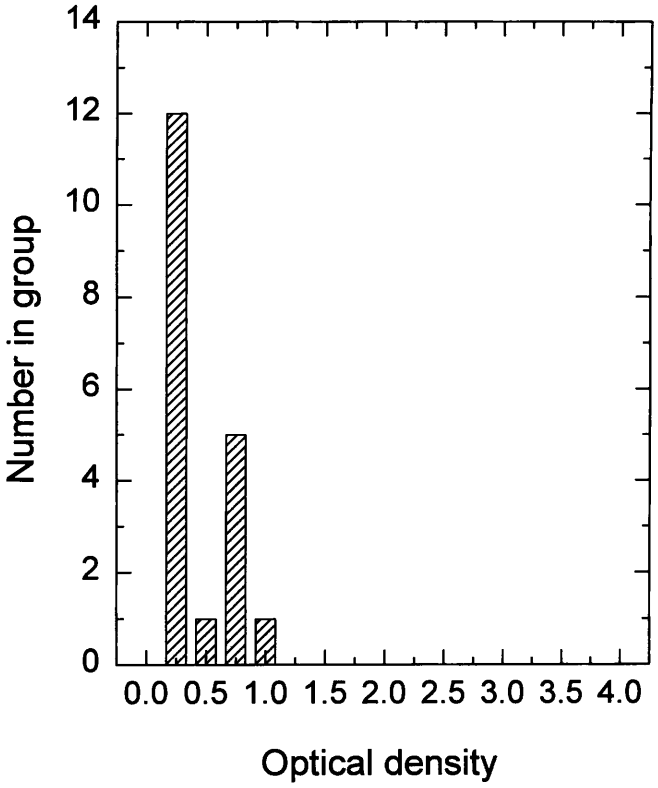

Fig. 3. Histogram of optical densities obtained from ELISA of foliage from plant 12. The values cluster near the origin of the graph, typical for two of the 17 plants assayed.

TSWV positive, asymptomatic but TSWV positive, asymptomatic and TSWV negative, or symptomatic but TSWV negative. Of the 471 symptomatic leaves and terminals assayed, 95.1% were positive by ELISA, or TSWV positive. Of the 286 asymptomatic leaves and terminals assayed, 78.7% were found to be TSWV negative by ELISA. Category percentages for terminals and leaves are found in Table 1. Total TSWV positive leaves equalled 58.3% and total TSWV positive terminals equalled 96.6%. The majority of terminals and young leaves were symptomatic and TSWV positive while older leaves were generally asymptomatic and TSWV negative. Determination of ELISA detectable virus by presence or absence of symptoms was accurate for 88.9% of the leaves and terminals.

Three representative plants are diagrammed to depict the within-plant distribution of TSWV in peanut. Plant 12 (Fig. 6) was small (19 leaves and terminals) and collected 54 d after planting. TSWV infection is concentrated mainly in the younger leaves and newly formed terminals. Plant 13 (Fig. 7) was larger (62 leaves and terminals), collected 47 d after planting. All symptomatic leaves on this plant were TSWV positive except for one. Plant 17 (Fig. 8) was the largest plant assayed (100 leaves and terminals) and collected 75 d after planting.

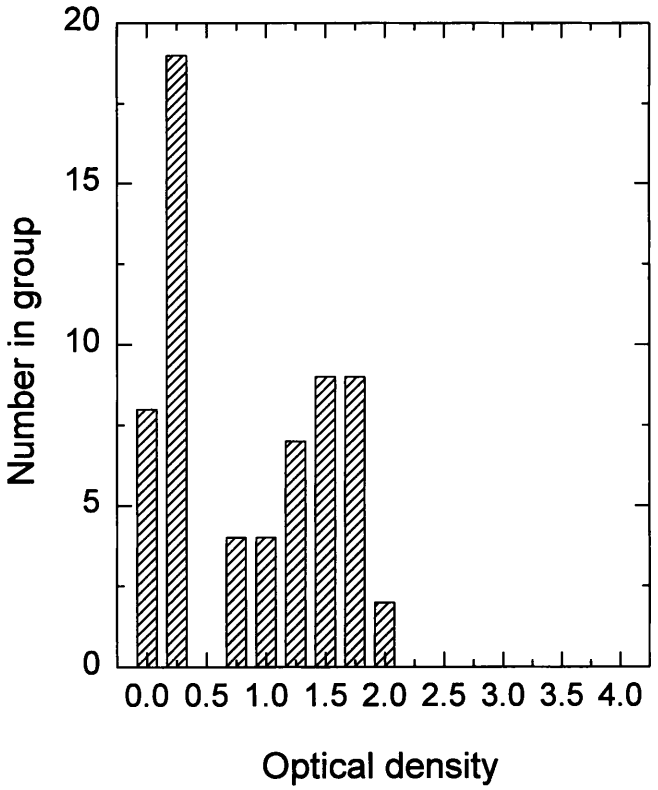

Fig. 4. Histogram of optical densities obtained from ELISA of foliage from plant 13. The values are bimodal and represent the type of distribution obtained from 11 of the 17 plants assayed.

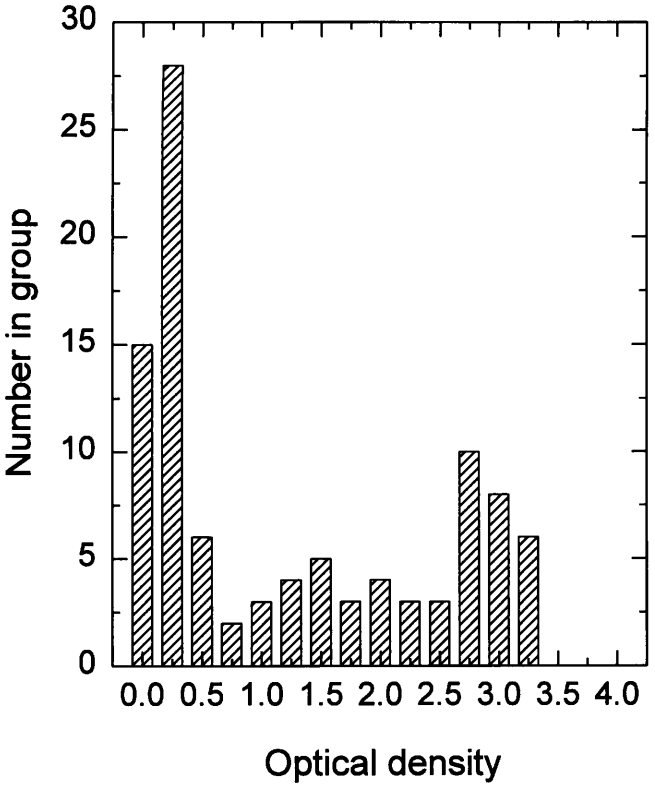

Fig. 5. Histogram of optical densities obtained from ELISA of foliage from plant 17. This demonstrates the continuous distribution of OD values typical for four of the 17 plants assayed.

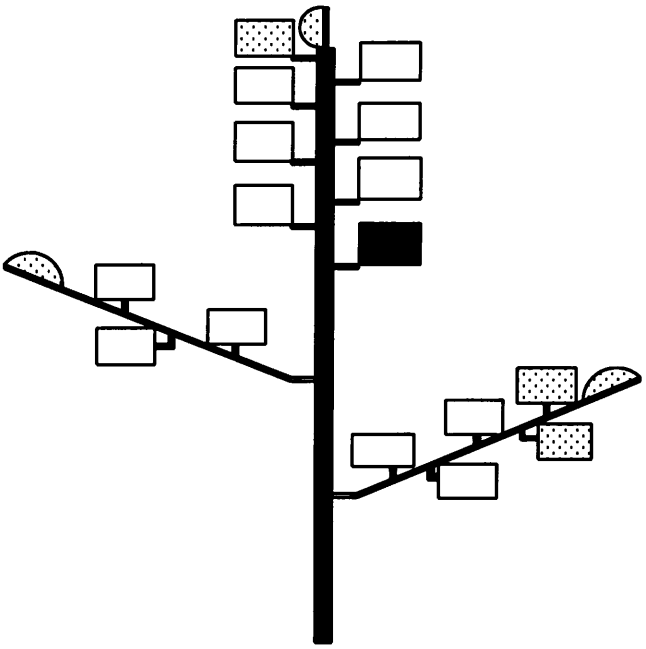

Fig. 6. Reconstruction of plant 12 demonstrating the location of foliage categories. Leaves and terminals are represented diagrammatically by squares and semicircles respectively. Hollow symbols indicate an asymptomatic visual rating with a negative ELISA response, solid symbols indicate an asymptomatic visual rating with a positive ELISA response, lined symbols indicate a symptomatic visual rating with a negative ELISA response, and dotted symbols indicate a symptomatic visual rating with a positive ELISA response. This plant was collected 54 d after planting.

Table 1. Count of leaves and terminals by infection categories. Percentages are derived by dividing the number in the table by the number tested.

	Visual assessment			
	Terminals[a]		Leaves[b]	
ELISA	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
	no.	no.	no.	no.
Positive	14 (7.9%)	157 (88.7%)	47 (8.1%)	291 (50.2%)
Negative	6 (3.4%)	0 (0%)	219 (37.8%)	23 (4.0%)

[a]177 terminals tested.
[b]580 leaves tested.

All four leaf types are present on this plant, including 12 asymptomatic, TSWV positive leaves and two asymptomatic, TSWV positive terminals. All but one of the 17 plants had at least one or more asymptomatic, TSWV positive leaves or terminals, and all but one of the plants had at least one asymptomatic, TSWV negative leaf.

Analytical Results from Foliage. Pearson product-moment (r) correlation coefficients showed a significant, positive correlation between expressed foliar symptoms and the OD recorded for each leaf and terminal by plant (Table 2). One-way ANOVAs were used to separate differences in OD readings between terminals and open leaves. The mean OD for terminals (1.79) on all plants was significantly higher than the mean OD for open

Table 2. Pearson product-moment (r) test results for correlation between expressed foliar symptoms and recorded optical density from ELISA assays.

Plant no.	N	Correlation coefficent	Probability
1	40	0.401	0.013
2	46	0.408	0.004
3	55	0.585	0.001
4	18	0.695	0.001
5	51	0.607	0.001
6	25	0.807	0.001
7	35	0.645	0.001
8	38	0.604	0.001
9	53	0.532	0.001
10	66	0.540	0.001
11	17	0.530	0.028
12	19	0.673	0.001
13	62	0.752	0.001
14	31	0.601	0.001
15	49	0.898	0.001
16	62	0.677	0.001
17	100	0.654	0.001

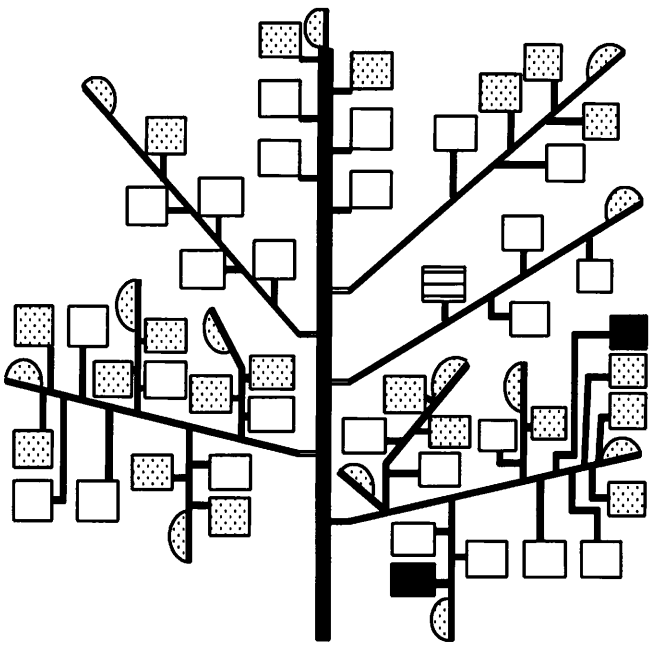

Fig. 7. Reconstruction of plant 13 demonstrating the arrangement of foliage categories. Leaves and terminals are represented diagrammatically by squares and semicircles, respectively. Hollow symbols indicate an asymptomatic visual rating with a negative ELISA response, solid symbols indicate an asymptomatic visual rating with a positive ELISA response, lined symbols indicate a symptomatic visual rating with a negative ELISA response, and dotted symbols indicate a symptomatic visual rating with a positive ELISA response. All symptomatic leaves on this plant were TSWV positive except for one, and two leaves that were asymptomatic had a positive ELISA response. This plant was collected 47 d after planting.

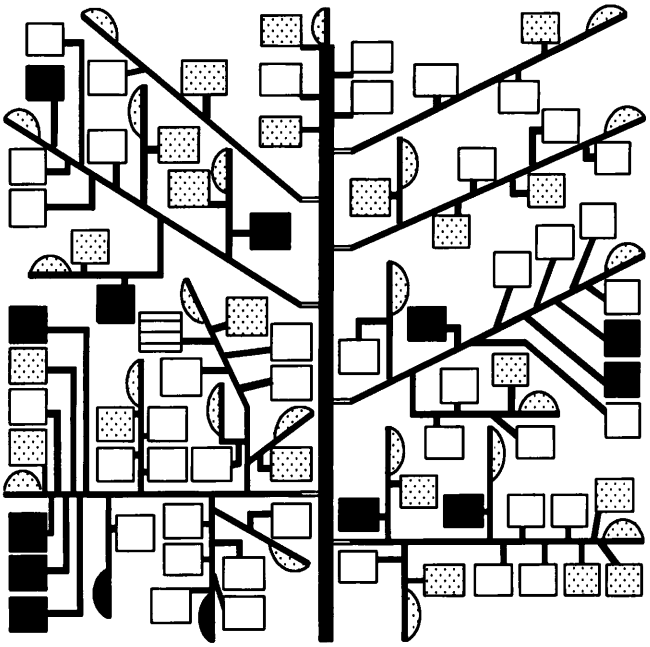

Fig. 8. Reconstruction of plant 17 demonstrating the arrangement of foliage categories. Leaves and terminals are represented diagrammatically by squares and semicircles, respectively. Hollow symbols indicate an asymptomatic visual rating with a negative ELISA response, solid symbols indicate an asymptomatic visual rating with a positive ELISA response, lined symbols indicate a symptomatic visual rating with a negative ELISA response, and dotted symbols indicate a symptomatic visual rating with a positive ELISA response. This plant was the largest assayed (100 leaves and terminals), and all four leaf types are present. This plant was collected 75 d after planting.

leaves (1.15) on each plant (Table 3, Model 1). However, when TSWV negative foliage was removed from the analysis, no difference was found between leaf OD and terminal OD (Table 3, Model 1A). The higher OD values of symptomatic, TSWV positive open leaves was statistically different from the OD values for asymptomatic, TSWV positive open leaves (Table 3, Model 2).

Mean OD values differed significantly among plants (Table 3, Model 3). Since the mean OD values for plants were different, the ODs between branches were compared by plant (Table 3, Model 4). Only two plants, 1 and 10, had significantly different values. The cause for the difference in plant 1 is easily explained, as the third branch contained only a single leaf with a small OD value. No reasonable explanation could be found for the differences in plant 10.

Analytical Results from Flowers. Sixteen of 30 flowers collected from peanut plants symptomatic for TSWV were positive by ELISA. The reaction was generally strong, with no gradation between TSWV positive and TSWV negative reactions (Fig. 2). Optical densities obtained from flowers collected on symptomless plants demonstrated a similar result. Three of the 30 flowers were TSWV positive and were likely taken from plants that had not yet begun to display disease symptoms. Alternatively, one or more may have had a contaminated thrips inside that was overlooked during examination before conducting the ELISA. A single first instar *Frankliniella* larva 300-500 μm long and contaminated with TSWV can yield an OD value of nearly 0.5 in an ELISA, while second instars and adults may generate values over 3 (Cho *et al.*, 1988, unpubl. data).

Refrigeration and Freezing of Tissue. Mean optical density values by day of ELISA are presented in Table 4 for refrigerated samples and Table 5 for frozen samples. Mean OD values for refrigerated samples were not significantly different from one another in an ANOVA ($F=0.01$; $df=5,42$; $P<0.05$), nor did the positive controls (excluding day 1, when no liquid nitrogen stored tissue was available) significantly differ from one another ($F=0.06$; $df=4,33$; $P<0.05$). Individual T-tests for days 3 to 14 comparing mean positive control OD with mean refrigerated sample OD showed no significant differences.

When mean OD values for frozen samples were analyzed via ANOVA, a significant difference occurred ($F=2.52$; $df=6,49$; $P<0.05$). Mean OD values for positive controls did not differ significantly ($F=1.00$; $df=5,42$; $P<0.05$) and, in individual T-tests conducted by date between positive control and frozen samples OD means,

Table 3. Analysis of variance results from comparisons of optical density.

Model[a]	Variate	F	DF	P>F
1	Foliage	47.7	1,756	0.001
1A	Foliage	0.21	1,523	0.651
2	Positive leaves	9.99	1,359	0.002
3	Plants	36.8	16,756	0.001
4	Branches			
	Plant 1	10.8	4,37	0.001
	Plant 2	2.1	6,45	0.082
	Plant 3	0.6	8,49	0.812
	Plant 4	0.3	5,17	0.890
	Plant 5	0.6	5,50	0.672
	Plant 6	0.5	5,24	0.744
	Plant 7	1.3	5,34	0.274
	Plant 8	1.0	4,34	0.443
	Plant 9	0.6	6,52	0.729
	Plant 10	2.3	6,65	0.048
	Plant 11	0.3	4,16	0.881
	Plant 12	0.8	2,18	0.472
	Plant 13	1.9	5,61	0.114
	Plant 14	0.5	5,30	0.742
	Plant 15	0.8	4,48	0.515
	Plant 16	1.6	5,61	0.182
	Plant 17	1.3	7,99	0.279

[a]Model 1 tests for differences in ELISA OD between all leaves and all terminals assayed in the study. Model 1A tests for differences between leaves and terminals when all negative foliage is removed from consideration. Model 2 tests for differences in OD values between symptomatic TSWV positive and asymptomatic TSWV positive leaves. Model 3 tests mean OD values between plants. Model 4 tests for OD differences by branch within each plant.

Table 4. Results of ELISA using refrigerated TSWV samples of peanut leaves.

Sample	Day	N	Mean	SE
			OD	
Positive control	1	8	-	-
Positive control	3	8	1.746	0.029
Positive control	6	6	1.730	0.034
Positive control	8	8	1.741	0.024
Positive control	10	8	1.745	0.030
Positive control	14	8	1.733	0.030
Negative control	1	8	0.018	0.007
Negative control	3	8	0.018	0.007
Negative control	6	6	0.008	0.005
Negative control	8	8	0.005	0.003
Negative control	10	8	0.015	0.006
Negative control	14	8	0.016	0.008
Refrigerator sample	1	8	1.742	0.025
Refrigerator sample	3	8	1.741	0.040
Refrigerator sample	6	8	1.746	0.022
Refrigerator sample	8	8	1.744	0.045
Refrigerator sample	10	8	1.748	0.030
Refrigerator sample	14	8	1.749	0.023

Table 5. Results of ELISA using frozen TSWV samples of peanut leaves.

Sample	Day	N	Mean	SE
			OD	
Positive control	1	8	-	-
Positive control	7	8	1.749	0.014
Positive control	14	8	1.733	0.030
Positive control	34	8	1.738	0.027
Positive control	42	8	1.698	0.022
Positive control	56	8	1.706	0.009
Positive control	70	8	1.713	0.008
Negative control	1	8	0.006	0.002
Negative control	7	8	0.010	0.005
Negative control	14	8	0.016	0.008
Negative control	34	8	0.009	0.003
Negative control	42	8	0.010	0.004
Negative control	56	8	0.006	0.002
Negative control	70	8	0.017	0.004
Freezer sample	1	8	1.742	0.025
Freezer sample	7	8	1.756	0.019
Freezer sample	14	8	1.733	0.016
Freezer sample	34	8	1.746	0.019
Freezer sample	42	8	1.679	0.023
Freezer sample	56	8	1.690	0.015
Freezer sample	70	8	1.688	0.022

no significant differences occurred. When highest and lowest OD values for the frozen samples (days 7 and 42) were compared in a T-test, the difference was significant ($t=2.57$; $df=14$; $P<0.05$). However, when the mean OD value of frozen samples from the ELISA conducted on day 1 was compared to those from the ELISA conducted on day 70, no difference was detected ($t=1.62$; $df=14$; $P<0.05$).

The largest difference found in frozen samples constituted only 4.6% [(1.756-1.679)/1.679] of the lowest OD mean. Apparently the difference at 0.05 was due to random error in the ELISA rather than degradation of the frozen sample since (a) the positive control means did not differ from one another, (b) the frozen sample means did not differ from the positive control means, and (c) the sample ELISA mean on day 1 did not differ from the sample ELISA mean on day 70.

Discussion

Peanut crops in south Texas may produce over 5 million thrips per acre in fields ranging in size from a few acres to over 100 acres (Mitchell and Smith, 1991). With this many potential vectors in a field during the growing season, identification of the microhabitats the insect vector can inhabit to acquire inocula from the plant host will help define the role of secondary spread in TSWV epidemics.

Studies of TSWV acquisition by thrips (reviewed by German *et al.*, 1992) have demonstrated virus-infected cells are variable in distribution in an infected leaf, depending on the host plant species, and that the distribution of virus infected cells within plant leaves can

contribute to the efficiency of virus aquisition by thrips (Ullman *et al.*, 1990). The results of our study also demonstrate that at least in peanut TSWV-infected leaves are not spread uniformly throughout plants as well. A similar lack of uniformity in whole-plant virus distribution was noted also in barley infected with barley yellow dwarf virus (BYDV), and variation was attributed to leaf age when infection occurred (Foxe and Rochow, 1975). Competent aphid vector species had no difficulty in acquiring or transmitting BYDV, but the inefficient ability of the vector species to acquire or transmit this virus was reduced due to the increasing age of the leaf and its subsequent virus content (Foxe and Rochow, 1975). Leaf location on a plant as well as leaf age was a factor in determining BYDV content in several cereal crops (Palmer *et al.*, 1990), where virus content rose and fell in a non-uniform fashion both within and between plants.

While TSWV is not uniformly spread throughout peanut plants, the virus does appear to be concentrated in the younger, newly formed leaves and developing terminal tissue. The unfolded terminal is the microhabitat where the larvae feed and develop (Smith and Sams, 1977). Hewings *et al.* (1990) reported a similar concentration trend in developing soybean tissue infected with soybean dwarf virus. Higher antigen concentration was observed in younger tissue when compared to older tissue, with the greatest concentration of viral antigens detected in aphid-inoculated leaves and the newly formed, expanding trifoliates of soybean.

Symptoms were shown to be significantly and positively correlated to virus presence in peanut foliage infected with TSWV, and 95% accurate in representing the presence of virus in a leaf or a terminal. Culbreath *et al.* (1992) also demonstrated a 95% correlation between visual diagnoses of symptomatic peanut plants and positive ELISA tests. The degree of symptom severity also reflects the optical density readings in foliage (Table 2). A similar trend has been demonstrated with *Chenopodium amaranticolor* infected by pea seed-borne mosaic virus (Ligat *et al.*, 1991). The number of lesions on *C. amaranticolor* diseased foliage increased with the amount of pea seed-borne mosaic virus present. Other research involving aphid vectors and persistent, circulative virus systems also has addressed the possibility of using symptomology to measure the virus content in a host and its availability to vectors. A definite relationship was determined to exist between the accessibility of potato virus Y and symptomology (Bradley, 1962), and the concentrations of three BYDV isolates were shown to follow the symptom severity in oats (Pereira and Lister, 1989). However, the accessibility of leaf roll virus and turnip latent virus in tobacco to aphids was not reflected by symptomology (MacKinnon, 1963).

All possible combinations of symptomatic or asymptomatic and TSWV positive or TSWV negative leaves or terminals were found except for symptomatic, TSWV negative terminals. A sequence of the TSWV infection process in the peanut canopy may be postulated with this information. Asymptomatic, TSWV negative leaves tend to be older, subtending the TSWV positive foliage on the stem or branch (Figs. 6-8), and more terminals than leaves were infected with TSWV (96.6 vs. 58.3%). Once the virus is systemic, disease occurs in leaves as they develop from terminals and the ringspotting and deformation symptoms are expressed. Nearly all subsequent leaves formed from this branch are TSWV positive and symptomatic. Only 14 asymptomatic, infected terminals were found during the study and many of these were small and deformed, which may have masked the common ringspotting symptoms. From these deformed terminals, a leaf probably would not have developed, resulting in bud necrosis, loss of apical dominance, and subsequent development of the bushy appearance described for BNV (Ghanekar *et al.*, 1979) as more branches were initiated by the plant to compensate. If symptomatic leaves are a product of diseased terminals, then asymptomatic TSWV negative leaves are only those leaves present at the time of systemic infection and represent a static group. Continuous gradations of OD distributions in the plant histograms as shown in Fig. 4 would then be the result of falling TSWV levels in leaves, rather than a dynamic rising and falling pattern. The majority of histograms show that OD values in an infected plant are in two distinct groups (Fig. 3). If rising and falling patterns of virus infection were the norm, then the majority of histograms should show gradations in OD. The symptomatic, TSWV negative leaves indicate that total loss of virus below the ELISA detection threshold can occur in a leaf. The cause of asymptomatic, TSWV positive leaves is uncertain. Most peanut plants (16/17) had at least one representative of this group. Given the information above, these are not likely to be leaves developing an infection. They may, however, represent the site of inoculation by the thrips vector, either while still a terminal or while a young, open leaf. The fact that asymptomatic, TSWV positive leaves had lower OD values and presumably less TSWV than symptomatic, TSWV positive leaves supports this idea. If symptoms must be generated from a terminal and since TSWV takes more than a week to develop symptoms in peanut foliage, the inoculated terminal would have developed into a mature, diseased leaf without bearing symptoms.

The concentration of TSWV in developing peanut terminals represents an important adaptation of the virus in that this is the major feeding niche of larvae and the only plant life stage that can acquire the virus and develop into vectors. The efficiency of TSWV acquisition as well as transmission to and from this site remains to be determined. Further, the role of flowers in either transmission or acquisition must be elucidated. Relatively few larvae were recovered from flower samples in this study even though adults commonly were found. Because peanut flowers are ephemeral, lasting only 1 d, they must be colonized when they open in the morning and abandoned late in the day as they shrivel and dry. Larval thrips are relatively immobile and would be less likely to colonize such a site than leaf terminals [although Lynch *et al.* (1984) commonly found them in flowers during their insecticide study]. It is unlikely that flowers play a major role in TSWV acquisition. The presence of TSWV in flowers may be simply because they are a rapidly growing portion of the plant, like a terminal; and,

because of the large number of adults they attract, flowers may prove to be more important as a site of peanut plant inoculation than this study indicated.

The presence of TSWV symptoms in peanut plants and the assumption of their accuracy in determining infection is the basis for sampling in the entomology research and management program concerned with this disease in Texas. Taken as a whole, positive or negative symptoms were 88.9% accurate in determining the presence of TSWV in foliage when confirmed by ELISA, and positive symptoms alone were 95% accurate in determining the presence of ELISA detectable amounts of TSWV. If, however, symptoms are generated only in new leaves, then accuracy in visually detecting new infections may be reduced in older plants that have slowed terminal production.

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