PEANUT SCIENCE

VOLUME 29 JULY -DECEMBER 2002 NUMBER 2

Evaluation of *Arachis* Species for Resistance to Tomato Spotted Wilt Virus

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ABSTRACT

Tomato spotted wilt virus (TSWV) is an important plant pathogen with a wide host range, including the domesticated peanut (*Arachis hypogaea* L.). After initial outbreaks on peanut during the 1980s, the virus has spread to all peanut-producing states in the U.S. TSWV is transmitted by several species of thrips which are difficult to control with insecticides; therefore, control of TSWV most likely will come from selecting resistant genotypes in breeding programs. Although moderate levels of resistance have been discovered in *A. hypogaea*, complete virus resistance has not been found. Several *Arachis* species have desirable genes for plant resistances and tolerate many disease and insect pests better than the cultivated species. The objectives of this study were to (a) evaluate TSWV disease incidence and severity in accessions of *Arachis* species, and (b) compare levels of TSWV resistance in diploid species to selected *A. hypogaea* genotypes. In this study, 46 diploid *Arachis* spp. accessions were evaluated in the greenhouse by artificial inoculation tests for resistance to TSWV. Nine *Arachis* accessions were observed with no disease symptoms when TSWV isolate 10 was used as opposed to *A. hypogaea* lines that ranged from moderately to highly susceptible. Additional testing with more virulent isolates identified *A. diogoi* accession GKP 10602 and *A. correntina* accession GKP 9530 as highly resistant to the virus. These two accessions are being used as parents in crossing programs to incorporate TSWV resistance genes into *A. hypogaea*.

Key Words: Cultivar development, groundnut, TSWV, wild species.

Peanut (*Arachis hypogaea* L.) is a widely grown and important international crop plant. Peanuts are high in calories, contain about 25% protein, and produce high quality oil (Porter *et al.*, 1982). Many pathogens of peanut, including tomato spotted wilt virus (TSWV), reduce both quality and yield. TSWV was first reported by Brittlebank (1919) and is an important plant pathogen with an extensive host range of at least 800 plant species, including peanut (Cho *et al.*, 1989; Goldbach and Peters, 1996). The virus has spread across the southern U.S. after initial outbreaks on peanut in the 1980s. In some cases, disease incidence in the field has exceeded 60% (Culbreath *et al.*, 1996).

Infection by TSWV early in the growing season often results in stunting, wilting, and seedling death. These plants can provide an additional source of inoculum for thrips, allowing the virus to spread through the field. Infection later in the growing season usually causes destruction of the root system and poorly developed pods that are not marketable as edible seed. TSWV infection has been shown to lower both seed weight and numbers of seed produced per plant (Culbreath *et al.*, 1992).

Two species of thrips, *Frankliniella fusca* (Hinds) (tobacco thrips) and *F. occidentalis* (Pergande) (western flower thrips), are the primary vectors for TSWV in the U.S. *Frankliniella fusca* is more prevalent early in the growing season and *F. occidentalis* populations usually increase late in the season (Lowry *et al.*, 1995; Lynch and Mack, 1995; Reed and Sukamto, 1995). Suppression of TSWV infections in the field is very arduous because of the difficulties in properly controlling thrips, which are typically found in the leaf terminals and flowers (Wightman and Ranga Rao, 1994; Goldbach and Peters, 1996; Ullman *et al.*, 1997). Small numbers of thrips can result in high rates of pathogen spread (Ullman *et al.*, 1997). Spray applications are expensive and chemical drift damage to other crops can occur (Walkey, 1985). The use of pesticides may also increase shallow probing behavior and instigate epidemics in the field (German *et al.*, 1992). Thus, insecticide sprays have not consistently suppressed field infection of peanuts by TSWV.

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Peanut breeding has become a focus for control of TSWV because of the environmental and economic advantages of not applying pesticides to the crop. Field resistance has been reported for several cultivated lines of peanuts, including the recently released cultivar Georgia Green (Branch, 1996; Culbreath *et al.*, 2000). When *A. hypogaea* cultivars were artificially inoculated with TSWV, there were no significant differences observed for infection or disease severity (Hoffmann *et al.*, 1998) even though Southern Runner suppresses incidence and severity of spotted wilt in the field. High levels of resistance to TSWV in *A. hypogaea* germplasm have not been found and different sources of resistance need to be identified. A possible alternative source of TSWV resistance are the wild species of *Arachis*, where a precedent has been set by observations of high levels of resistance to many disease and insect pests (see Stalker and Moss, 1987; Stalker and Simpson, 1995). Several *Arachis* species also have multiple resistances to diseases or insect pests including tobacco thrips, corn earworm, and potato leafhopper (Stalker and Campbell, 1983; Stalker, 1992; Stalker and Simpson, 1995). Resistance found in *Arachis* species has resulted in germplasm releases with resistance to early leaf spot (Simpson *et al.*, 1993a; Stalker and Beute, 1993; Stalker *et al.*, 2002b), root-knot nematode (Simpson *et al.*, 1993a; Stalker *et al.*, 2002a), and several insects (Stalker and Lynch, 2002).

The objectives of this research were to (a) evaluate the disease incidence and severity in *Arachis* species accessions to identify TSWV-resistant genotypes, and (b) compare the levels of resistance in *Arachis* species to selected cultivars. The ultimate goal of this investigation is to identify new sources of resistance genes for TSWV and incorporate them into improved peanut cultivars.

Materials and Methods

Accessions were selected from the *Arachis* species inventory at North Carolina State Univ. to represent a large number of species in section *Arachis* because these taxa will hybridize with *A. hypogaea*. Twenty-one of the 27 species in section *Arachis* were represented in the initial evaluation, including *A. hypogaea* (Table 1). Seeds were germinated in vermiculite and planted in 10-cm pots containing a soil mix of sand, soil, and Scott's Metro Mix 220 (Scotts-Sierra Horticultural Products Co., Marysville, OH) in a 1:1:1 ratio. The pots were placed in an unshaded mist system in the greenhouse to provide a controlled and unstressed environment. The plants were misted daily every 30 min for a 30-sec interval from 8:00 AM to 6:00 PM. Plants were shaded 24 hr prior to inoculation by placing plants under a 40% shadecloth, and then moved out of the mist system onto a greenhouse bench immediately prior to inoculation with TSWV. Because of differences in germination and growth rates, plants were placed in inoculation groups based on maturity and all plants were inoculated at the two- to four-leaf stages.

The inoculation buffer for TSWV was 0.01M Tris with 0.01M Na_2SO_3 and 0.01% cysteine HCl added just before use. Buffers and grinding materials were maintained on ice during the inoculation procedure. Plants were dusted with silicon carbide (carborundum, 600-800 mesh). Symptomatic tissue was collected from infected plants and ground in the buffer with a cold mortar and pestle. For groups of fewer than 25 plants, approximately 2.0 g of symptomatic tissue was used to make the inoculum. For larger groups of 25 to 50 plants, between 3.0 g and 5.0 g of infected tissue was needed. After grinding the tissue, a small amount of silicon carbide was added to the suspension and mixed in with a cotton swab. The inoculum was immediately rubbed on the leaves with a cotton swab, and then the plants were rinsed

Table 1. Percent disease for *Arachis* accessions tested with TSWV isolate 10.

Species	Accession	PI no.	Plants inoculated	Infected plants[a]
			no.	%
A. cardenasii	36020 KSSc	475999	17	0.0
A. cardenasii	36018 KSSc	475997	5	0.0
A. correntina	9530 GKP	262808	7	0.0
A. correntina	19616 Sc	———	8	0.0
A. diogoi	10602 GKP	276235	8	0.0
A. diogoi	6330 VSGrCn	476044	11	0.0
A. diogoi	30106 KGPSc	468354	7	0.0
A. stenosperma	7377 VSMGeSv	497578	16	0.0
A. villosa	862 S	———	7	0.0
A. matiensis	30030 KG	468145	16	6.3
A. batizocoi	30082 KGBSPSc	468328	7	14.3
A. cardenasii	15101 ScBo	468372	14	14.3
A. duranensis	21763 ScVn	497262	13	15.4
A. duranensis	30071 KGBSPSc	475846	11	18.2
A. diogoi	30005 KG	468142	5	20.0
A. correntina	9548 GKP	262881	8	25.0
A. duranensis	30060 KGBSPSc	468197	12	25.0
A. glandulifera	30098 KGSSc	468341	8	25.0
A. kempff-mercadoi	30088 KGSSc	468333	8	25.0
A. subcoriacea	8916 VKSSv	———	8	25.0
A. batizocoi	9484 K	298639	7	28.6
A. correntina	30108 KGPSc	468356	7	28.6
A. stenosperma	410 HLK	338280	16	31.3
A. duranensis	30077 KGBSPSc	468323	6	33.3
A. hoehnei	9094 VpoBi	———	8	37.5
A. helodes	6331 VSGrCn	476045	10	40.0
A. valida	30011 KG	468154	5	40.0
A. herzogii	36029 KSSc	476008	12	41.7
A. cardenasii	36032 KSSc	476011	7	42.9
A. kempff-mercadoi	30085 KGBSPSc	468331	8	50.0
A. valida	9153 VpoBi	———	11	54.5
A. hypogaea	NC 7	565459	37	62.2
A. batizocoi	30079 KGBSPSc	468325	8	62.5
A. magna	30097 KGSSc	468340	8	62.5
A. cardenasii	10017 GKP	262141	6	66.7
A. villosa	22585 Bk	298636	6	66.7
A. hoehnei	9146 VPoBi	———	15	73.3
A. glandulifera	30100 KGSSc	468343	7	85.7
A. helodes	30031 KG	468146	7	85.7
A. magna	30092 KGSSc	468337	7	85.7
A. kuhlmannii	7639 VRGeSv	———	8	85.7
A. benensis	35006 KGSPSc	475878	8	100.0
A. benensis	860 Wi	———	6	100.0
A. ipaensis	30076 KGBSPSc	468322	6	100.0
A. kuhlmannii	30034 GK	468167	8	100.0
A. monticola	21769 ScVn	497261	7	100.0
A. valida	9157 VpoBi	———	7	100.0

[a]Percent of plants with systemic infection for respective accessions.

with tap water to prevent the inoculum from drying on the leaves.

Arachis Species Evaluations

Four virus isolates were originally collected in peanut fields during 1991 and maintained in the laboratory of Dr. J. Moyer, Dept. of Plant Pathology, North Carolina State Univ. In an initial experiment, 444 plants were inoculated between June and August 1996 with TSWV isolate 10. The inoculum source for this experiment was symptomatic *Nicotiana benthamiana* (D.) leaves. An additional group of 15 plants from several *Arachis* species plants was mock inoculated using noninfected *N. benthamiana* leaf tissue to distinguish mechanical damage from TSWV symptoms.

The plants were observed for a 6-wk period for symptom development. Observations included the number of days to onset of symptoms, development of systemic symptoms, and descriptions of the types of symptoms for each plant such as chlorosis, ringspots, necrosis, wilting, and plant death. Plants were classified as having systemic infection (rating = 1) or no systemic infection (rating = 0). Systemic infection was considered to be a susceptible response.

Nine accessions with no systemic symptoms (Table 2) were selected from the initial test with TSWV isolate 10 for further study. Cultivar NC 7 was used as a susceptible check. Plants were inoculated following the procedure described previously and using the same criteria to form inoculation groups. Observations included the number of days for onset of symptoms and for the development of systemic symptoms within 30 d after inoculation. Descriptions monitoring symptom development were recorded and plants were assigned a rating from 1 to 3 for statistical analysis where 1 = no symptoms; 2 = local lesion development and/or death of the inoculated leaves only; and 3 = systemic infection, indicating complete susceptibility to the virus.

Two hundred thirty-five plants representing five species—including the nine accessions 10602, 19616, 30106, 36018, 36020, 6330, 7377, 862, and 9530—were evaluated with TSWV isolates 26 and 22. Plants evaluated with isolate 26 were inoculated in seven groups, ranging in size from 10 to 25 plants, from 17 Jan. to 7 Feb. 1997. Plants evaluated with isolate 22 were inoculated in six groups, ranging in size from eight to 27 plants, from 22 Feb. to 27 June 1997. Seventy six plants from three species—including accessions 6330, 862, 9530, 10602, and 19616—were inoculated with isolate 11 between 22 May and 16 June 1997 in five groups that ranged in size from nine to 25 plants. Five plants from the cultivar NC 7 were included in each group as experimental controls.

Table 2. Comparison of mean ratings for nine *Arachis* accessions for TSWV isolates 22 and 26[a].

Accessions	Isolate 22	Isolate 26	Over isolates
Diploid Species			
A. cardenasii			
36018	1.29 abc	3.00 ef	2.50 cd
36020	1.04 ab	2.50 c-f	1.77 abc
Mean	1.17	2.75	1.96
A. correntina			
9530	1.89 a-d	2.05 acd	1.62 abc
19616	2.05 bcd	1.22 abc	1.64 abc
Mean	1.97	1.29	1.63
A. diogoi			
6330	1.81 a-d	1.45 a-d	1.63 abc
10602	1.18 ab	1.57 a-d	1.38 ab
30106	2.75 de	1.73 a-e	2.24 c
Mean	1.91	1.59	1.75
A. stenosperma			
7377	2.27cde	1.67 a-e	1.97 bc
A. villosa			
862	1.75 a-d	1.96 b-e	1.85 bc
Tetraploid Species			
A. hypogaea			
NC 7	2.79 e	3.00 ef	2.90 d

[a]Mean of all plants evaluated for the listed species accessions using 1 to 3 rating scale where 1 = no symptoms, 2 = local lesion development, death of inoculated leaves, or initial infection with plant recovery, and 3 = systemic infection. Means followed by the same letter within columns are not significantly different at $P \leq 0.05$.

Virus Assays

Biological assays were conducted using plants inoculated with isolates 22 and 26 to verify the presence of the virus in the plant. Each inoculated peanut plant was grouped with three young *N. benthamiana* plants at the four- to five-leaf stages. Inoculation buffer was prepared and all materials were kept on ice. Leaf material from each peanut plant was ground with mortar and pestle in buffer to prepare inoculum, which was then rubbed on the leaves of the three tobacco plants using a cotton swab. Forty-six plants inoculated with isolate 22 and 81 plants inoculated with isolate 26 were assayed using this method. Mortars and pestles were washed in 0.52% sodium hypochlorite solution and then rinsed in distilled water before being placed on ice to chill for the next inoculation. Tobacco plants were rinsed with tap water after each inoculation and then observed 2 wk later for symptom development. A negative result consisted of no infected tobacco plants, and any infected plant was considered to be a positive result.

A. hypogaea Evaluations

An evaluation to compare disease incidence in *Arachis* species with cultivated lines included plants from *Arachis* accessions 10602 and 30092 and from the cultivars NC 7, NC 9, and Georgia Green. Although NC 7 was used as a susceptible control in previous tests, it has shown some field resistance to TSWV. The cultivar NC 9 was included in this study as an additional control, as it is highly susceptible to TSWV (T.G. Isleib, pers. comm.). The Georgia Agric. Exp. Sta. released Georgia Green (*A. hypogaea* subsp. *hypogaea* var. *hypogaea*) in 1996. It is a runner market-type cultivar that originated from a cross of Southern Runner and Sunbelt Runner and is reported to have resistance to TSWV comparable to Georgia Browne and Southern Runner (Branch, 1996). Plants from each accession or cultivar were inocu-

lated with isolate 26 at the two- to three-leaf stages in two groups on 17 Oct. and 4 Nov. 1997 using the procedure described previously. Following inoculation, the plants were rinsed with tap water and placed on greenhouse benches with supplemental greenhouse lights on a 10/14-hr light/dark cycle. Plants were observed from 7 to 32 d after inoculation for the appearance of TSWV symptoms. Plants in this evaluation were rated on a scale of 1 to 3, as previously described.

Data Analysis

Data from the first test with TSWV 10 were subjected to chi-square analyses to detect variation between species, within species, and between accessions. The Yates correction was employed to account for the small sample sizes. Mean ratings were calculated and entries with a percent disease of 0.0% were selected for further study. Statistical analyses were completed for plants in the experiments with TSWV isolates 11, 22, and 26 using the PROC GLM procedure in the SAS System (SAS Inst., 1990). A total of 76 plants from inoculations with isolate 11, 108 plants from isolate 22, and 107 plants from the inoculations with isolate 26 were analyzed by making orthogonal contrast comparisons. To select accessions resistant to more than one virus isolate, data were combined from isolate 22 and 26 evaluations and analyzed using the PROC GLM procedure. Contrast statements were utilized to partition variation and to better characterize effects. Statistical analysis for the comparison study of *Arachis* species and cultivars was completed using the same statistical model as for the isolates 11, 22, and 26 evaluations. Fifty plants were analyzed in this data set and Fisher's protected LSD was used to make comparisons between *Arachis* species and cultivars.

Results and Discussion

TSWV Isolate 10

An initial study was undertaken with TSWV isolate 10 to evaluate a large number of *Arachis* species accessions because of the availability of the isolate and its use in similar screening programs in the Dept. of Plant Pathology, North Carolina State Univ. (K. Hoffman, unpubl. data). Accessions that were susceptible to this isolate could be considered highly susceptible and eliminated from further study. *Arachis* species exhibited a broad range of symptoms after inoculation of the leaves and symptoms varied both among species and accessions within species. The characteristic chlorotic spots or ringspots usually seen in TSWV-infected leaves were observed for 37 accessions. Other symptoms included wilting (11 accessions), defoliation (12 accessions), plant stunting (11 accessions), stunting of the emerging leaves (11 accessions), chlorotic streaks (eight accessions), wrinkled or misshapen leaves (nine accessions), vein streaking or banding (four accessions), and necrosis (20 accessions).

Accessions 862 (*A. villosa* Benth.) and 6330 (*A. diogoi* Hoehne) developed local lesions only, and no systemic symptoms. The time from inoculation to local lesion appearance averaged 8.2 d. Local lesion appearance on the inoculated leaves often preceded the development of systemic symptoms, but not in all cases. Susceptible plants developed systemic infection on the newly developing leaves and on the leaves above the inoculated leaves in an average of 13.6 d after inoculation. Eighteen *Arachis* accessions and cultivar NC 7 had plants that died from TSWV infection.

The development of chlorotic streaks was an unusual symptom, and for accessions 36020 (*A. cardenasii* Krapov. and W.C. Gregory) and 30106 (*A. diogoi*) the only symptom observed. The streaks appeared on the new leaves rather than on the inoculated leaves and preceded the development of chlorotic spots or ringspots for *Arachis* accessions 9484 (*A. batizocoi* Krapov. and W.C. Gregory), 30077 (*A. duranensis* Krapov. and W.C. Gregory), 30097 (*A. magna* Krapov., W.C. Gregory and C.E. Simpson), 9153 (*A. valida* Krapov. and W.C. Gregory), and the cultivar NC 7.

Another unexpected symptom was the appearance of tiny brown or purple spots or streaks along the leaf veins in accessions 410 (*A. stenosperma* Krapov. and W.C. Gregory), 9094 (*A. hoehnei* Krapov. and W.C. Gregory), 36029 (*A. herzogii* Krapov., W.C. Gregory and C.E. Simpson), and 9146 (*A. hoehnei*). This symptom only appeared on newly formed leaves and not on inoculated leaves. Vein streaking was accompanied by the development of additional symptoms and occurred with chlorotic spots or ringspots in all cases.

Significant differences were detected between the 21 evaluated species ($c^2_{calc.} = 125.16 > c^2_{.05, 20} = 31.4$). Also, both resistant and susceptible accessions were identified within *A. cardenasii* and *A. villosa*. This indicates a need to evaluate many accessions per species; and conclusions for a single entry cannot be extrapolated to represent an entire species. Fifteen *Arachis* accessions were more susceptible to TSWV isolate 10 than NC 7, while 31 accessions were more resistant (Table 1). Accessions 862, 6330, 7377, 9530, 10602, 19616, 30082, 30106, 36018, and 36020 did not develop systemic infections and were selected for additional evaluations with more virulent isolates of TSWV.

Evaluations with Additional Virus Isolates

Identification of resistant species accessions to several TSWV isolates should detect germplasm with greater potential for cultivar improvement. Thus, nine species accessions and the NC 7 control were challenged with TSWV isolates 26 and 22 (Table 2).

TSWV Isolate 26. The first symptoms appeared between 9 and 12 d after inoculation when accessions were inoculated with isolate 26. Symptoms included chlorotic spots or ringspots, necrosis, general chlorosis, defoliation, chlorotic streaking, and plant death. All NC 7 plants developed systemic infection, indicating that it was highly unlikely that plants tested in this study escaped inoculation.

The 107 inoculated plants were divided into seven groups that were considered to be blocks over time, with multiple observations per block. This test did not have the same number of plants per accession within or across each group because of nonuniform seed germination and the necessity to form groups based on plant maturity. Type III sums of squares were used to take the unbalance of the test into account. The group effect was not significant; however, highly significant differences were observed among species ($P = 0.0001$) (Table 2). The orthogonal contrast between the cultivar NC 7 and the diploid species accessions was highly significant ($P = 0.0001$), indicating a large difference be-

tween the response of *A. hypogaea* and the other *Arachis* species.

For TSWV isolate 26, accession 19616 [*A. correntina* (Burkart) Krapov. and W.C. Gregory] had the lowest level of disease, followed by accessions 9530 (*A. correntina*), 6330 (*A. diogoi*), and 10602 (*A. diogoi*) (Table 2). Accessions 7377 (*A. stenosperma*), 862 (*A. villosa*), and 30106 (*A. diogoi*) also had mean ratings less than 2.0, which suggests that the virus could not replicate and/or that virus movement was inhibited. Accession 36020 (*A. cardenasii*) had a mean rating of 2.5, which may indicate a low level of resistance; but the virus was able to move through the plant. All plants from accession 36018 (*A. cardenasii*) and the cultivar NC 7 developed systemic infection, indicating that these genotypes are highly susceptible to TSWV isolate 26.

TSWV Isolate 22. One hundred eight plants were inoculated with isolate 22. The first symptoms appeared 7 to 10 d after inoculation and included development of chlorotic spots or ringspots, plant stunting, chlorotic streaking on the leaves, defoliation, necrosis, and plant death. All control plants became infected, with two plants exhibiting local lesions only, and the remainder developing systemic infection.

Highly significant differences ($P = 0.0001$) were observed among species when isolate 22 was used. The accession within species effect also was significant ($P = 0.004$), indicating that generalizations about host plant resistance for TSWV cannot be made regarding individual species (Table 2). A significant difference in the response of NC 7 and other *Arachis* species ($P = 0.0001$) was observed. Accession 36020 (*A. cardenasii*) had the lowest level of disease, followed by accessions 10602 (*A. diogoi*) and 36018 (*A. cardenasii*) (Table 2). Accessions 862 (*A. villosa*), 6330 (*A. diogoi*), and 9530 (*A. correntina*) had mean ratings of 2.0 or less, which reflects a moderate to high level of resistance and no systemic infection. Accession 30106 (*A. diogoi*) and the control NC 7 are susceptible to isolate 22.

Biological Assay for the Presence of TSWV. A biological assay was conducted on 111 plants from the evaluations with TSWV 22 and 26 to verify the presence of the virus in plants (Table 3). Five of the 43 peanut plants assayed that had no apparent disease symptoms resulted in tester plants that became infected. This indicates an asymptomatic infection in some of the peanut plants. When the peanut plants had local lesions only, virus was transmitted to only four tobacco plants, but this suggests that some plants that recovered from initial infection were still infected with TSWV. The assay successfully detected virus in peanut plants with systemic infection in 84.6% of the inoculated tobacco testers (Table 3).

Table 3. Biological assay results of 111 peanut plants for infection with isolates 22 and 26.

Rating from TSWV testing[a]	Assay score			
	Testers not infected		Testers infected	
	no.	%	no.	%
1	38	88.4	5	11.6
2	38	90.5	4	9.5
3	4	15.4	22	84.6

[a]Rating from TSWV evaluations on 1 to 3 scale, with 1 = no infection, 2 = local lesions, and 3 = systemic infection.

TSWV Isolate 11. Plants from five diploid *Arachis* accessions (10602, 19616, 6330, 862, and 9530) and the NC 7 check were inoculated with TSWV isolate 11. In this evaluation, the first symptoms appeared 9 to 13 d after inoculation and included the development of chlorotic spots or ringspots, defoliation, necrosis, and plant death. Only plants from the cultivar NC 7 developed systemic infection, with the symptoms being severe. Death of the inoculated leaves was observed in two plants, one from accession 9530 and the other from accession 862. The remaining plants from *Arachis* species did not exhibit symptoms. Significant differences were detected and attributable to the susceptibility of NC 7 versus resistance in other accessions tested, and not due to variation among the diploid *Arachis* species. Thus, isolate 11 was not highly pathogenic on the *Arachis* accessions evaluated. The results demonstrate that testing with multiple isolates is needed when evaluating *Arachis* germplasm for TSWV resistance.

Analysis over Tests. In searching for resistance genes for TSWV, it would be desirable to identify accessions with resistance to multiple virus isolates. Although accessions performed differently in the tests with isolates 26 and 22, several accessions had low ratings in both evaluations. When data for the nine species accessions for the two virulent isolates 26 and 22 were combined, the effect of virus isolate was not significant. However, the species effect was highly significant ($P = 0.0001$); therefore, the species evaluated responded differently to TSWV infection. The accession within species effect also was significant ($P = 0.028$). The test by species interaction effect was significant ($P = 0.0014$), which was expected since the accessions changed rank order when different TSWV isolates were used. Orthogonal comparisons revealed a highly significant difference between the cultivar NC 7 and the *Arachis* species accessions ($P = 0.0001$) and among accessions of *A. diogoi* ($P = 0.022$). No difference was detected in NC 7 with the two virus isolates; therefore, the strain by species interaction could be attributed to variation in the responses between the diploid species ($P = 0.0007$) and depended on the virus isolate. Overall, accessions 10602 (*A. diogoi*), 9530 (*A. correntina*), 6330 (*A. diogoi*), and 19616 (*A. correntina*) had the lowest mean ratings across both tests (Table 2). These four accessions have potential for incorporating resistance to TSWV into the cultivated peanut.

Comparison of Disease Incidence for *A. hypogaea* Cultivars

Several cultivars have been reported to have partial resistance to TSWV, including Georgia Browne, Southern Runner, and Georgia Green (Culbreath *et al.*, 1994, 1996, 2000; Branch, 1996). To examine differences in disease incidence and severity among *Arachis* accessions and cultivars, 50 plants were inoculated with isolate 26. Symptoms began to appear after 14 d in the cultivated lines and after 21 d in *Arachis* species. Several types of responses were observed including plants with no symptoms, local lesions on inoculated leaves, death of the

inoculated leaves, and systemic symptoms. Differences among species were highly significant (P = 0.0001) with accession 10602 having the lowest level of disease, 30092 the highest level, and the *A. hypogaea* cultivars fell between these two extremes (Table 4). Although the three peanut cultivars were not significantly different, Georgia Green was somewhat more susceptible and also not significantly different from the susceptible species accession 30092. This was unexpected because this cultivar has been reported to suppress TSWV infection in the field (Branch, 1996), but Hoffmann *et al.* (1998) reported similar results with Southern Runner, which has moderate field resistance. These findings indicate that resistance to the virus *per se* is not the mechanism for disease suppression in peanut cultivars. Characterization of the mechanisms of resistance to TSWV in peanut has not been completed, but may result from differential primary infection rates.

Table 4. Mean ratings by accession and group for comparison of peanut cultivars for resistance to TSWV isolate 26.

Accession/cultivar	N	Mean rating[a]
30092	10	2.70 a
Georgia Green	10	2.30 ab
NC 7	10	2.00 b
NC 9	10	1.90 b
10602	10	1.30 c

[a]Means followed by the same letter are not significantly different at P ≤ 0.05 as tested by Fisher's protected LSD.

This investigation has indicated that high levels of TSWV resistance exist in several of the diploid *Arachis* species closely related to the cultivated peanut. Theoretically, the resistance can be transferred into *A. hypogaea* and then cultivars selected with high levels of TSWV resistance. However, when plants from 22 hexaploid or tetraploid breeding lines having either 10602 or 9530 in their pedigrees were evaluated by Lyerly (2000), plants of all entries had systemic TSWV infection. The lines had been self-pollinated for nine to 16 generations without selection and gene(s) conferring resistance likely were lost due to segregation. Future breeding programs will need to exercise selection during all stages of hybridization and selfing to ensure that highly resistant genotypes are recovered.

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