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Utilizing immunoassays to determine systemic tomato spotted wilt virus infection for elucidating field resistance in peanut

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Abstract

Tomato spotted wilt disease was compared in three peanut cultivars, SunOleic 95R, Southern Runner (S. Runner) and DP-1, at two planting dates (April and May) and row spacings (7.6 and 15.2 cm) in a 2-year study in Florida. Tomato spotted wilt virus (TSWV) was detected directly using an enzyme-linked immuno-sorbent assay (ELISA) in both leaf and root crown tissues throughout the growing season to determine the timing and percentage of infected plants. Under low disease pressure, in harvested samples, more April-planted peanuts were infected with TSWV than May-planted. TSWV infection was not affected by plant spacings regardless of disease pressure. Under low disease pressure, from 60 days after planting (DAP), the most susceptible cultivar, SunOleic 95R, had a significantly higher percentage of plants with TSWV compared to the more resistant cultivars. Under high disease pressure, TSWV infection was detected earlier (30 DAP), and there was a clear separation of cultivars, with SunOleic 95R showing the highest infection (75%) followed by S. Runner (55%) and then DP-1 (20%). A higher incidence of TSWV in root crowns compared to leaves was observed for all cultivars. A delayed accumulation of TSWV in a cultivar was a reliable indicator of resistance. The field resistance manifested by peanut may be due to factors that decrease TSWV systemic spread resulting in slower TSWV accumulation in root crowns.

Keywords: *Arachis hypogaea* L.; Groundnut; Tospovirus; TSWV resistance

1. Introduction

Spotted wilt, caused by tomato spotted wilt virus (TSWV) (Bunyaviridae, *Tospovirus*) is a major disease that severely impacts peanut (*Arachis hypogaea* L.) production in the US. Tomato spotted wilt virus was first detected in the southeastern US in Georgia tobacco fields in 1986 (Culbreath et al., 1991a), and continues to be a severe problem which limits peanut yields (Hagan et al., 1990; Culbreath et al., 1992c, 1996; Goldbach and Peters, 1994). TSWV is transmitted to plants by several species of thrips (Thysanoptera), namely western flower thrips [*Frankliniella occidentalis* (Pergande)], tobacco thrips, [*F. fusca* (Hinds)], *F. bispinosa* (Morgan), and possibly others (Todd et al., 1996; Webb et al., 1997). The polyphagous feeding behavior of the thrips vectors and the wide plant host range of TSWV make it difficult to control (Ullman et al., 1997).

Host-plant resistance is the most valuable long-term strategy for TSWV control, and is a main objective of a majority of peanut breeding programs. Traditional breeding has resulted in peanut cultivars with increased spotted wilt field resistance being released. Florida MDR98, C-99R, ViruGard and Georgia Green have moderate levels of field resistance to spotted wilt and currently are the main cultivars used for peanut

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production in the southeastern US (Gorbet and Shokes, 1999). More recently, new peanut cultivars with better field resistance have been released, namely DP-1, Hull, Andru II, ANorden, and Carver (Culbreath et al., 1999b; Gorbet, 2003). The importance of cultivar selection in spotted wilt management has been well documented (Kumar et al., 1995; Brown et al., 1999). However, because none of these cultivars have levels of TSWV resistance approaching immunity, spotted wilt can still significantly reduce peanut yields. Culbreath et al. (2003) concluded that peanut field resistance is not due to a decrease in attractiveness to thrips vectors, vector reproduction, or injury to the plant from thrips feeding. Also, to date, there are no published reports examining TSWV resistance among peanut genotypes to determine genetic factors responsible for their natural resistance. Therefore, the mechanism controlling field resistance to TSWV and spotted wilt among peanut cultivars remains unknown.

Along with cultivar selection, cultural practices have proven effective in minimizing losses due to spotted wilt in peanut (Brown et al., 1999). Several critical factors affect virus severity, including planting date and within-row spacing between plants. Planting peanuts in April has resulted in higher yield losses due to TSWV infection than planting later in the season (Mitchell et al., 1991; Brown et al., 1999; Culbreath et al., 2000; McKeown et al., 2001). Variability of thrips populations and higher temperatures for fast plant establishment have been suggested as possible reasons that plantings are less affected by TSWV (Todd et al., 1995; Culbreath et al., 2003). However, conclusive evidence for this effect has not been obtained (Culbreath et al., 2003). Peanuts planted on 7.6 cm within-row spacing had higher pod yields than those planted on 15.2 or 31 cm row spacings (Gorbet and Shokes, 1994). The higher plant population in the narrower spacing may result in a reduction of the percent of plants infected (Culbreath et al., 2003).

Spotted wilt in peanut displays a wide array of symptoms that range from minor spotting on leaves to severe plant stunting (Halliwell and Philley, 1974; Culbreath et al., 1992b, c). Plant wilting and stunting have been correlated with pod yield loss (Culbreath et al., 1992b). Below ground portions, especially the root crown, are also affected by spotted wilt and with severe infestations can result in plant death (Culbreath et al., 1991b). Standard detection of spotted wilt in the field is through a visual disease intensity rating that corresponds to both incidence and severity (Wells et al., 2002). However, peanuts can be asymptomatic, yet still contain TSWV. Immunoassays of peanut leaf and root tissues from plants not displaying visual symptoms have detected the presence of TSWV (Culbreath et al., 1992a). Consequently, the relationship between TSWV infection and symptom development is complex and not well understood (Culbreath et al., 1991b). There have been no studies that we are aware of that have evaluated peanut genotype response and field management practices using TSWV detection by immunoassays to determine the timing and percent of TSWV infection and how they relate to disease intensity ratings (DIR). Also, determining the location and movement of TSWV within a genotype seasonally has not been documented in peanut and may provide clues to factors responsible for field resistance. Our objectives were to: (1) examine TSWV immunoassays for evaluating the effects of peanut cultivar, planting date, and within-row plant spacing to determine the timing of TSWV infection and how that relates to resulting pod yield and tomato spotted wilt severity as determined by DIR at the end of the growing season, and (2) examine the timing and percentage of TSWV infection that occur during the season in specific peanut tissues among cultivars displaying variable levels of TSWV resistance to gain an understanding of mechanisms that may be operating in spotted wilt field resistance.

2. Materials and methods

2.1. Plant materials and plot design

Field studies were conducted in 1998 and 1999 at the North Florida Research and Education Center (NFREC), Marianna, FL. Genotypes with variable levels of spotted wilt field resistance were chosen for examination and included the peanut cultivars, SunOleic 95R (highly susceptible), Southern Runner (S. Runner) (moderately resistant), and the University of Florida line, F86 × 43-1-1-1-1-1-b2-B (more resistant), which has since been released as cultivar DP-1 (Gorbet, 2003). Designated levels of spotted wilt field susceptibility/resistance for each genotype were based on previous field trials (Culbreath et al., 1992c, 1999a, b).

A randomized complete block plot design, with a split-plot arrangement of treatments was used in both years. Planting date (April or May) was the main plot and genotypes and within-row spacing (7.6 or 15.2 cm) were the subplots. Plots were two rows, 1.8 m wide and 6.1 m long, each row 0.9 m apart, with four replications. Naturally occurring thrips populations were used for TSWV infection, although thrips species infesting plants were not identified. Plots were maintained according to commercial peanut production practices for the region with fertilizer, herbicide, fungicide and insecticide applied as recommended by the University of Florida extension guidelines. All plots were irrigated as needed.

2.2. Sampling and pod yield

Plants in each plot were evaluated for spotted wilt using a disease intensity rating (DIR) system that

represented the percentage of linear row with severe symptoms of spotted wilt as determined visually immediately prior to digging. A 1–10 rating scale was used, with 1 being no spotted wilt symptoms and 10 being 100% severe symptoms with plants dying. Each genotype was dug and inverted at optimum maturity and partially dried in the wind-row for 3–4 days before mechanical harvesting. Pods and seed were artificially dried to ~6% moisture for future processing. Pod yield data were collected from entire plots and are presented as kg/ha.

2.3. TSWV analysis

Plants were analyzed immunologically for TSWV at 30, 60 and 100 days after planting (DAP) and at harvest in 1998, and at 30, 60, and 120 DAP and at harvest in 1999. To determine the timing of TSWV infection, young, terminal leaves (Kresta et al., 1995) were collected from four plants initially chosen at random in each plot at planting and at each of the prescribed dates. To determine the location of TSWV within specific plant tissues, four randomly selected plants at each of the prescribed dates were destructively sampled. Leaf tissue collected from the repetitively sampled plants, as well as the newly developed terminal leaves and the root crowns of the destructively sampled plants, were kept at −80 °C until analysis.

A double antibody sandwiched enzyme-linked immuno-sorbent assay (DAS-ELISA) kit for TSWV (Agdia Incorporated, Elkhart, IN, USA) was used for analysis according to the manufacturer's instructions. Briefly, a 96-well ELISA plate was coated with 100 μl of 1/1000 dilution TSWV antibody and stored at 4 °C overnight. Plates were then washed three times with washing solution. Plant tissue (0.1 g) was ground in a 1.5 ml microcentrifuge tube (Fisher Scientific, Pittsburgh, PA, USA) with 1 ml general extraction buffer. Wells were loaded with 100 μl of this sample solution and plates were incubated overnight at 4 °C. After incubation, plates were washed three times with washing solution and 100 μl of 1/1000 diluted enzyme conjugate was loaded per well and incubated at room temperature for 4 h. Plates were washed again and 100 μl of *Para*-nitro phenol phosphate solution was loaded per well for the final reaction. The ELISA reaction was terminated after 30 min by adding 50 μl of 3 M sodium hydroxide. Absorbance was measured at 405 nm with an automated microplate reader (Model 550, Bio-RAD, Hercules, CA, USA). Two replications were made on each sample, and averages were used for evaluation. Symptomatic plants field-infected with TSWV earlier in the season were collected and used as positive controls and plants grown in a greenhouse were used as negative controls. A reading three times higher than the negative control constituted a positive reading for the presence of TSWV.

2.4. Statistical analysis

Percent infection responses of plants sampled throughout the season were analyzed using repeated measures analysis of variance (ANOVA) (PROC GLM, repeated, SAS Institute, 2001) and means were separated using the REGWQ test. DIR and yield data were analyzed using PROC GLM (SAS Institute, 2001). Regression equations were produced using PROC REG (SAS Institute, 2001) and regression coefficients were separated by non-overlap of 95% confidence intervals.

3. Results

3.1. TSWV infection-1998—low disease pressure

Young leaves were sampled throughout the season to determine the timing and percent of TSWV infection as detected by ELISA among genotypes, planting date, and row spacing (Fig. 1). TSWV was not detected in young leaves early in the season (30 DAP). However, at 60 DAP, 100 DAP, and at harvest, leaf samples showed differences in infection among genotypes (Fig. 1a). SunOleic 95R showed higher infection than S. Runner and DP-1 beginning at 60 DAP ($P = 0.014$). The higher TSWV infection detected in SunOleic 95R increased dramatically (from less than 20% to more than 60%) compared with the more resistant genotypes (18%) at 100 DAP ($P < 0.001$), and remained significantly higher at harvest ($P = 0.002$). There were no statistical differences in TSWV infection between leaves removed from S. Runner and DP-1 for any sampling period, as both remained low.

For planting date, only harvest samples showed that April-planted peanuts had higher levels of infection than May-planted peanuts ($P < 0.001$) (Fig. 1b). In this study, TSWV infection was not significantly different between plant spacings at any sampling date in 1998 ($P > 0.05$) (Fig. 1c).

3.2. TSWV infection-1999—high disease pressure

TSWV infection was detected by ELISA in all genotypes at the earliest sampling date (30 DAP), where SunOleic 95R and S. Runner had higher infection than DP-1 ($P = 0.046$) (Fig. 2a). By 60 DAP, there was a clear separation of genotypes with SunOleic 95R showing the highest infection (75%) followed by S. Runner (55%) and then DP-1 (20%) ($P < 0.001$). At 120 DAP, SunOleic 95R and S. Runner showed significantly higher infection than DP-1 ($P < 0.001$). However, at harvest, no differences among genotypes were found because >95% of the plants sampled from all genotypes contained TSWV in their leaves ($P = 0.4219$).

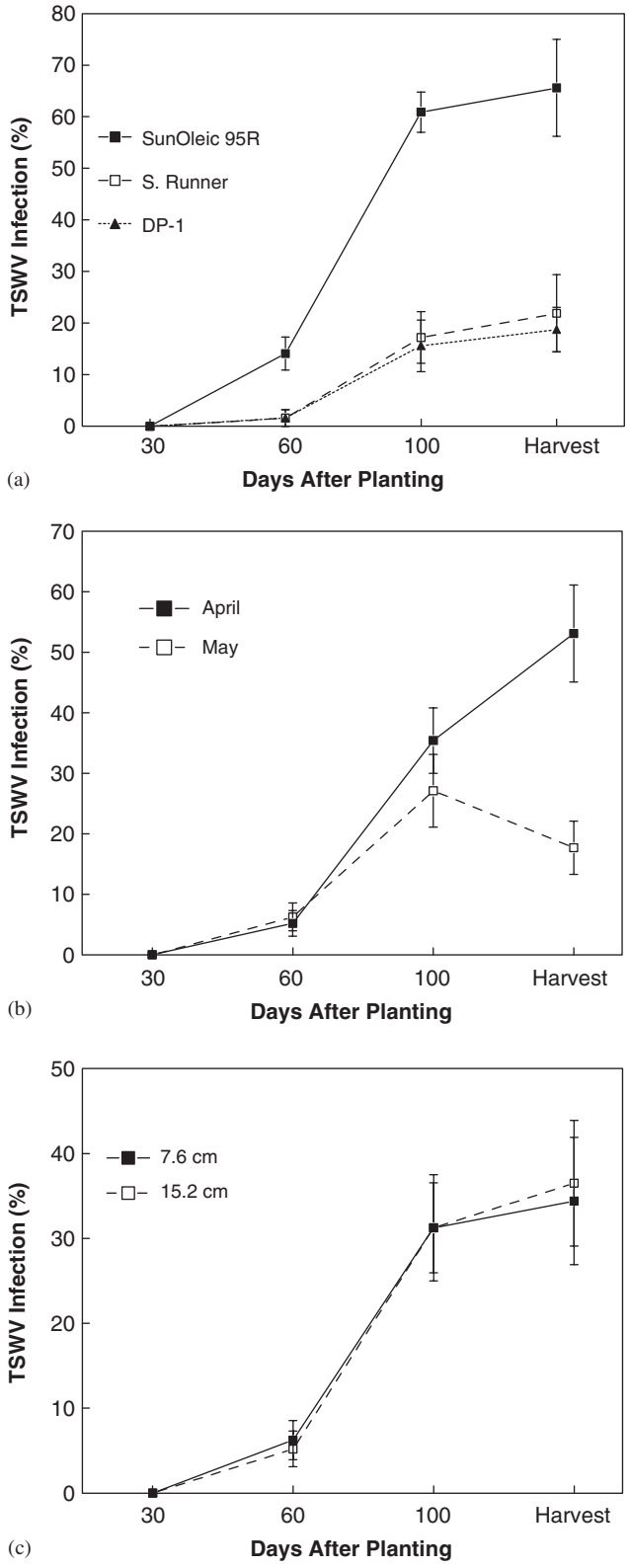

Fig. 1. TSWV infection detected by ELISA in young peanut leaf tissue collected at different DAP from: (a) SunOleic 95R, S. Runner and DP-1 plants, (b) peanuts planted in April and May, or (c) peanuts planted in 7.6 or 15.2 cm spacing, 1998, Marianna, FL. Error bars refer to SE.

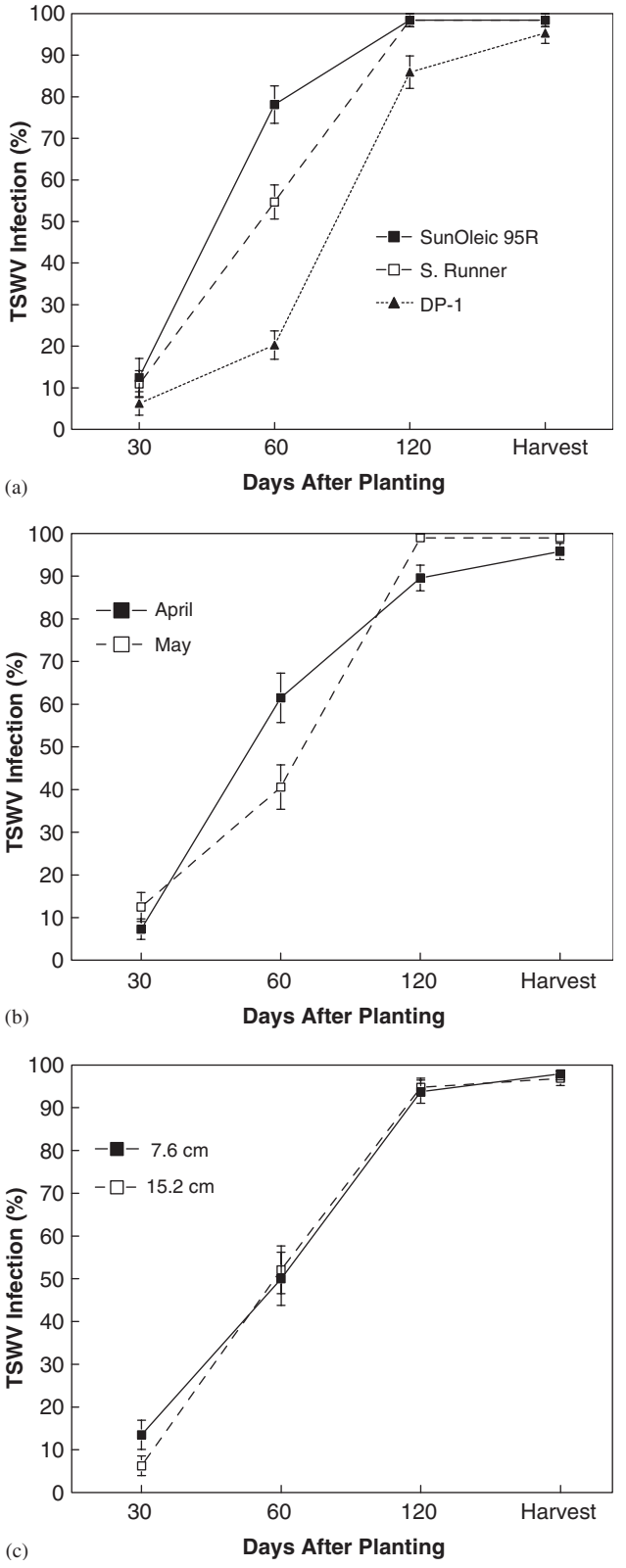

Fig. 2. TSWV infection detected by ELISA in young peanut leaf tissue collected at different DAP from: (a) SunOleic 95R, S. Runner and DP-1 plants, (b) peanuts planted in April and May, or (c) peanuts planted in 7.6 or 15.2 cm spacing, 1999, Marianna, FL. Error bars refer to SE.

April- and May-planted peanuts showed no differences in infection at 30 DAP ($P = 0.3429$) or at harvest ($P = 0.3910$). However, April-planted peanuts had higher levels of TSWV infection at 60 DAP ($P < 0.001$), while May had higher levels at 120 DAP ($P = 0.026$) (Fig. 2b). Virus infection was different between planting dates at the 120 DAP sample because of relatively low infection in DP-1 plants in April ($75.0 \pm 4.7\%$); this difference however did not produce a significant planting date by genotype interaction ($P = 0.1988$). Planting date had a significant effect earlier in the season in 1999 because of earlier and higher TSWV pressure compared with 1998. Plant spacing was only significant at the 30 DAP sampling, where plants 7.6 cm apart had higher infection than plants 15.2 cm apart ($P = 0.046$) (Fig. 2c).

3.3. Location of TSWV in plant tissues

This experiment was conducted to investigate how systemic spread of TSWV in genotypes may indicate reasons for their differences in resistance. In 1998, leaves from 30 DAP samples did not contain TSWV. The 60 DAP samples showed relatively low infection rates in root crown and leaf tissue that were not significantly different for the three genotypes ($P > 0.370$) (Fig. 3a–c). However, at 100 DAP differences in infection between tissue types in SunOleic 95R ($P = 0.008$) (Fig. 3a) and S. Runner ($P = 0.003$) (Fig. 3b) became evident where far more infection was detected in root crowns than leaves. There was no difference in virus infection between root crown and leaf tissue in DP-1 at 100 DAP ($P = 0.293$) (Fig 3c.). However, by harvest, all genotypes had much higher infection detected in root crowns than leaves ($P < 0.003$) (Fig. 3a–c).

Regression analysis was used to compare TSWV infection among genotypes in both leaves and root crowns during the 1998 season. Comparison of the regression coefficients (slopes) showed that infection rose more substantially in both leaves (Fig. 4a) and root crowns (Fig. 4b) throughout the season for the susceptible SunOleic 95R (leaf 24.4, root 34.1) than for moderately resistant S. Runner (leaf 4.1, root 23.8) or the most resistant DP-1 (leaf 7.2, root 21.6).

In 1999, all genotypes had TSWV in samples taken at 30 DAP, but there were no differences between leaves and root crowns for the two resistant genotypes at this early sampling stage. However, for SunOleic 95R infection was already higher for root crowns than leaves ($P = 0.0442$) (Fig. 5a–c). Infection by the 60 DAP sampling date was >80% in plant tissues taken from SunOleic 95R and over 40% in S. Runner (Fig. 5a and b), with root crowns having higher infection rates than leaves ($P < 0.0001$ and $P < 0.0280$, respectively). There also was a difference in virus infection between plant tissue samples for DP-1 at 60 DAP ($P = 0.0408$) with more infection found in root crowns (Fig. 5c). TSWV infection in the 120 DAP and harvest samples was >80% in both plant tissue types (all genotypes $P > 0.08$) (Fig. 5a–c).

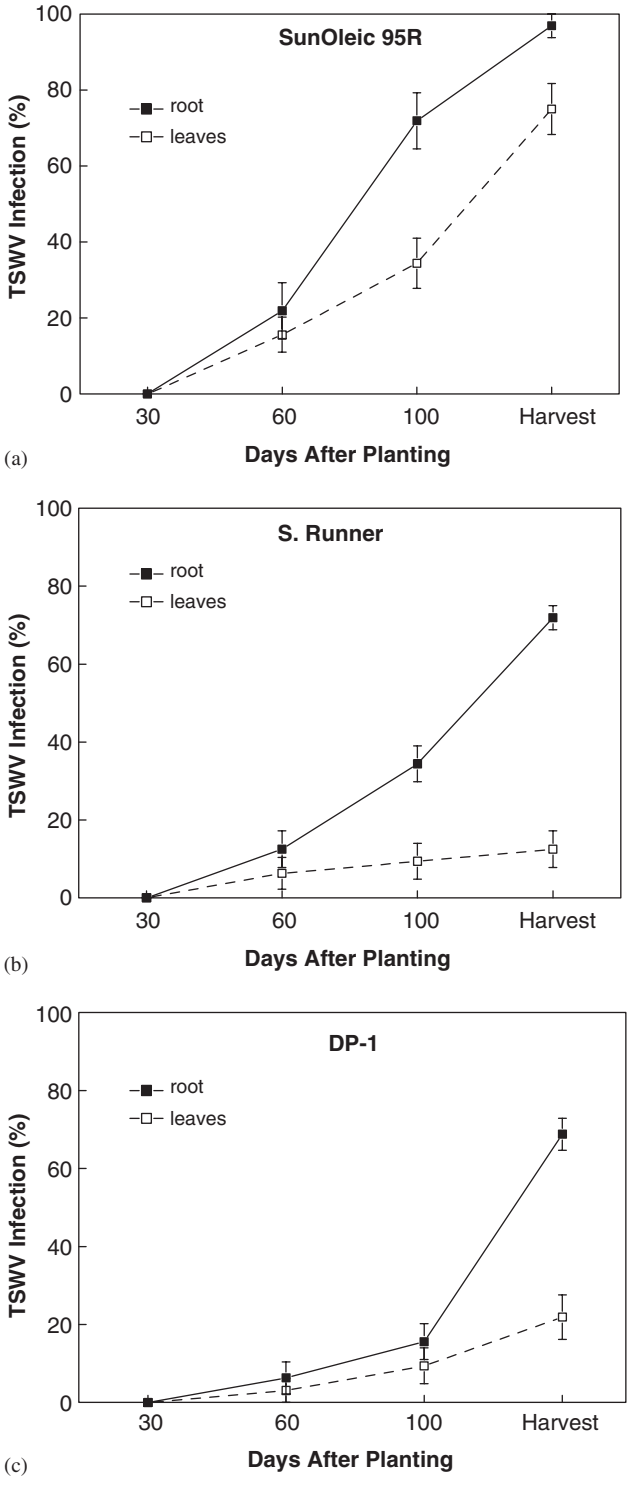

Fig. 3. TSWV infection detected by ELISA in young peanut leaf and root crown tissue collected at different DAP from: (a) SunOleic 95R, (b) S. Runner and (c) DP-1 plants, 1998, Marianna, FL. Error bars refer to SE.

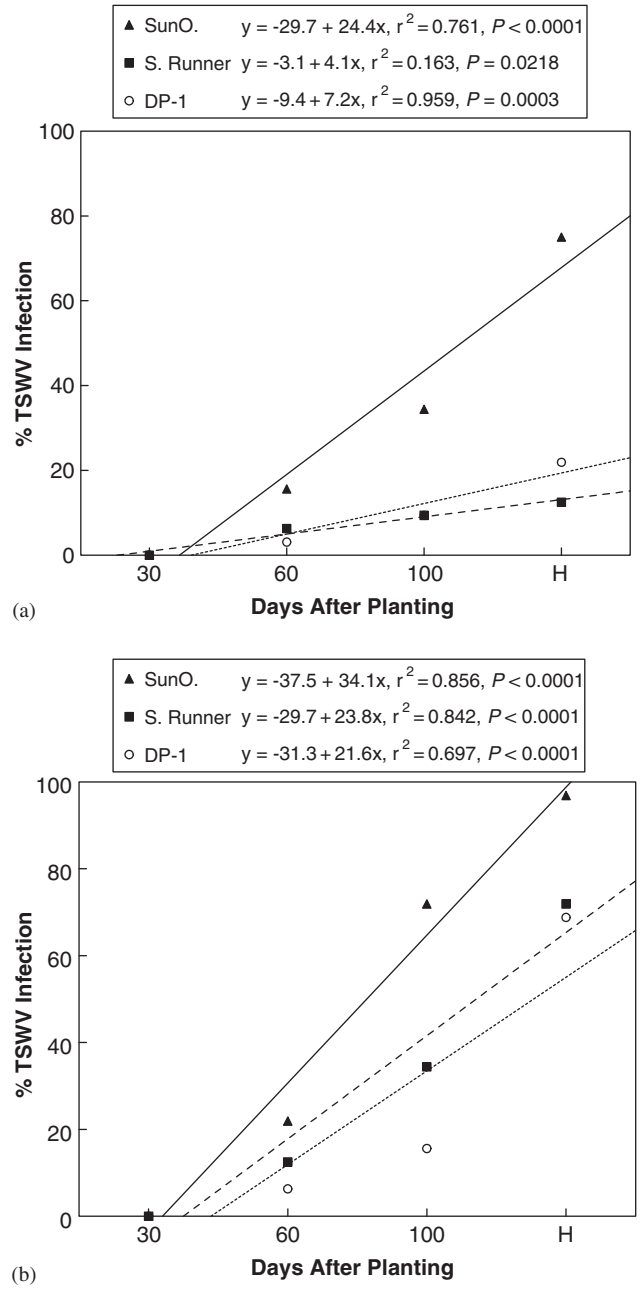

Fig. 4. Regression equations describing the relationship between virus infection in young leaf tissue (a) or root crown tissue (b) and sampling dates for SunOleic 95R (SunO.), S. Runner, and DP-1 peanut plants, 1998, Marianna, FL.

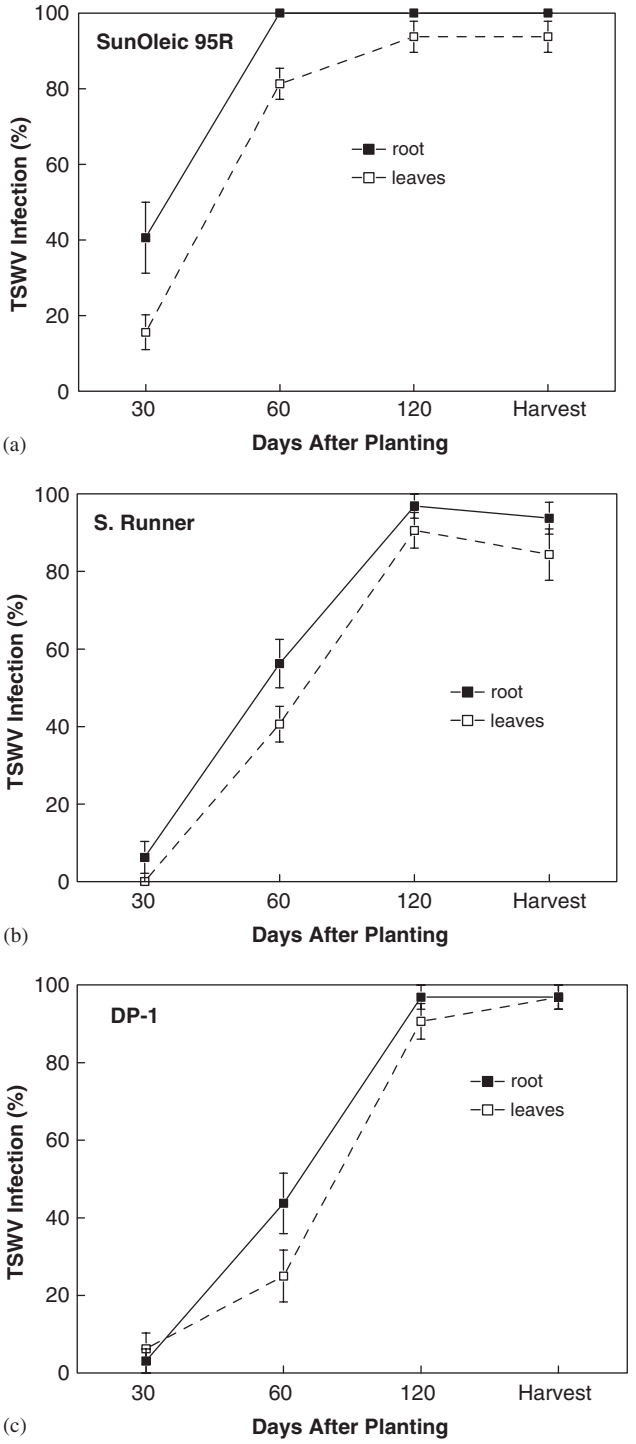

Fig. 5. TSWV infection detected by ELISA in young peanut leaf and root crown tissue collected at different DAP from: (a) SunOleic 95R, (b) S. Runner and (c) DP-1 plants, 1999, Marianna, FL. Error bars refer to SE.

Regression analysis showed that infection detected in leaf tissue increased at a similar rate across genotypes (Fig. 6a). However, in root crown tissue, SunOleic 95R had a slower increase in infection than DP-1 (17.8 vs. 33.4, respectively) (Fig. 6b). S. Runner's regression coefficient was intermediate. This opposite trend compared to 1998 was a result of initially detecting higher TSWV infection in SunOleic 95R and therefore the increase in infection to 100% was not as steep.

3.4. DIR

There was lower spotted wilt incidence in 1998 as measured by the DIR (4.05 ± 0.34) than in 1999 (5.14 ± 0.29, $P < 0.0001$). Despite the lower disease

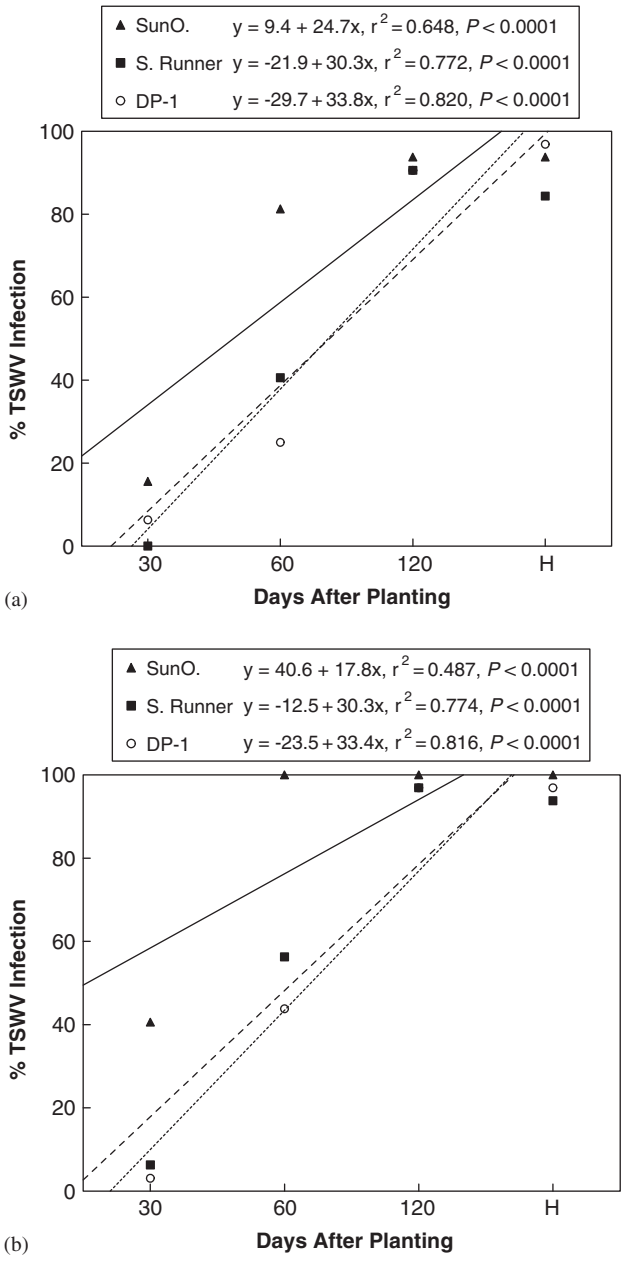

Fig. 6. Regression equations describing the relationship between virus infection in young leaf tissue (a) or root crown tissue (b) and sampling dates for SunOleic 95R (SunO.), S. Runner, and DP-1 peanut plants, 1999, Marianna, FL.

pressure in 1998, differences in the DIR among genotypes were observed. SunOleic 95R had a higher DIR (7.2±0.21) than S. Runner (2.8±0.16), which was higher than DP-1 (2.2±0.09) ($P<0.001$). A significant planting date by genotype interaction ($P<0.001$) was detected because in both April- and May-planted peanuts, SunOleic 95R plants had higher DIR compared to the other genotypes (April: SunOleic 95R 6.7±0.3, S. Runner 3.2±0.21, DP-1 2.3±0.13, $P<0.0001$; May: SunOleic 95R 7.7±0.16, S. Runner 2.4±0.18, DP-1 2.1±0.11, $P<0.0001$). However, DIR was similar for both April- (4.0±0.42) and May-planted peanuts (4.0±0.54, $P=0.9142$), and this was not different for the genotypes (data not shown). Peanut plants in the wider 15.2 cm plant spacing had a higher DIR than those in the narrower 7.6 cm spacing (4.4±0.49 vs. 3.8±0.46, respectively, $P=0.015$).

Table 1
Regression coefficients and statistics between %TSWV infection and DIR in 1998 and 1999

Year	Dependent variable	Equation	r^2	P
1998	60 DAP			
	Leaves	$y=0.15+2.0x$	0.153	0.0591
	Root crowns	$y=2.9+2.6x$	0.137	0.0745
	100 DAP			
	Leaves	$y=-0.8+4.6x$	0.323	0.0038
	Root crowns	$y=2.3+9.5x$	0.603	<0.0001
1999	60 DAP			
	Leaves	$y=2.7+9.0x$	0.381	0.0013
	Root crowns	$y=28.8+7.4x$	0.236	0.0160

The ranking of genotypes by DIR results in 1999 were similar to 1998: SunOleic 95R 7.1±0.36 > S. Runner 5.0±0.34 > DP-1 3.3±0.32, $P<0.001$). The DIR was higher in April- (6.1±0.40) than in May-planted peanuts (4.1±0.32, $P=0.009$). Since in these experiments DIR was measured according to plant appearance at the end of the season, the planting date effect may be evident only under high disease pressure, as was the case in 1999. As in 1998, plants in the wider 15.2 cm plant spacing had a higher DIR than those in the narrower 7.6 cm spacing (5.6±0.42 vs. 4.6±0.4, respectively, $P=0.0112$).

Linear regression analysis was used to document relationships between TSWV infection of leaves and root crowns at particular sampling points with the end-of-season DIR. In 1998, virus infection detected at 60 DAP and 100 DAP was used. At 60 DAP, equations for both leaves and root crowns were not significant (Table 1). However, samples taken at 100 DAP provided significant regressions for both tissues. In 1999, samples taken at 60 DAP produced significant regressions for both leaves and root crowns (Table 1). Therefore, ELISA detection at particular points in the season may predict end-of season plant severity.

3.5. Pod yield

Peanut pod yield (kg/ha) was higher in 1998 (8121.0±394.2) than in 1999 (4086.4±523.7, $P<0.001$). Pod yield was higher in May-planted than in April-planted peanuts in both years ($P<0.01$) (Fig. 7a and b). Although disease pressure was different in 1998 and 1999, a similar genotypic effect was observed. Plants

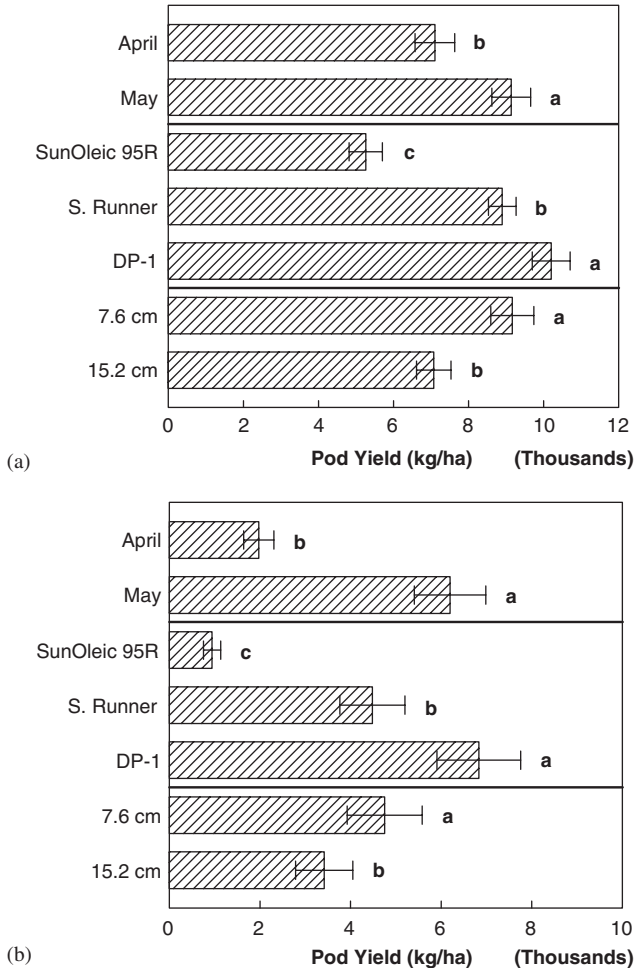

Fig. 7. Peanut pod yield from 1998 (a) or 1999 (b) plots showing comparisons (error bars = SE) between planting date, among genotypes, and between plant spacing, Marianna, FL. Bars within each group followed by the same letter are not significantly different ($P > 0.5$, REGWQ test).

of DP-1 produced higher pod yields than S. Runner plants, which had a higher pod yield than SunOleic 95R plants ($P < 0.0001$). An interaction between planting date and genotype was significant in both years ($P < 0.005$). Peanuts planted in April produced similar pod yields from DP-1 and S. Runner compared to the lower yield produced by SunOleic 95R plants, whereas S. Runner had an intermediate pod yield in May-planted peanuts. Plant spacing effects on pod yield were also observed in both years. Narrow plant spacing produced higher pod yields than the wider plant spacing ($P < 0.003$) (Fig. 7a and b).

4. Discussion

The most important factor in the control of tomato spotted wilt in peanut is the cultivar planted. Research related to TSWV resistance in peanut has focused almost exclusively on screening genotypes for the best field performance (Culbreath et al., 1996; McKeown et al., 2001; Wells et al., 2002). However, both the genetics and mechanisms involved in TSWV resistance in peanut are unknown and under-explored. There is limited understanding regarding the spread of TSWV throughout the peanut plant and its relationship to resistance. Symptomatic development of tomato spotted wilt following systemic infections has been described (Mandal et al., 2001, 2002). However, plants that lack symptoms, a major indicator of resistance, may still be infected with TSWV (Culbreath et al., 1992a). It is believed that after initial local leaf infection following thrips feeding, TSWV moves to the roots and then back up to the growing point. Such virus movement leads to an earlier detection in the root crown, and later detection in the newly developed leaves (Kresta et al., 1995).

Results from the present work indicate that the more susceptible genotypes have a higher percentage of infected plants as determined by the presence of TSWV in their newly developed leaves early in the season compared to the more resistant genotypes. This difference is manifested throughout the growing season unless disease pressure is extremely high. Then at the time of harvest there are detectable levels in leaves of all genotypes and so differences are not as clear. It appears from our work that field resistance in peanut is related to delayed virus accumulation in the root crown and subsequent transportation back to the growing points. Therefore, the longer period between root crown and leaf infection suggests a greater restriction of long distance movement of TSWV in those genotypes displaying higher field resistance.

Planting date has been shown to influence spotted wilt epidemics. Results from ELISA detection of TSWV and DIR indicated that peanuts planted earlier in the season developed more infection than those planted later in the season (Mitchell et al., 1991; Brown et al., 1999; McKeown et al., 2001). It has been suggested that higher temperatures in May compared to April may allow for faster and better establishment of peanut plants, thereby resulting in lower susceptibility to TSWV infection in the field (Culbreath et al., 2003). In our study, ELISA results suggested that more plants were infected with TSWV in the April-planted peanuts, although this difference was not significant until late in the season. Higher pressure from spotted wilt early in the season has been shown to negate the beneficial results of planting early. In 1999, both DIR and ELISA results indicated that later planting produced less diseased plants.

For the cultural management practice of plant spacing, the DIR indicated that wider plant spacing (lower plant population) resulted in higher spotted wilt incidence, whereas direct virus detection showed no difference between plant spacings. Previous reports

concluded that there was a positive correlation between spotted wilt incidence and plant population in Florida (Gorbet and Shokes, 1994), and that spacing effects are more consistent as spotted wilt incidence increases in severity (Brown et al., 1999). The reason for this difference is unknown. It has been speculated that higher plant spacing reduces the percentage of infected plants in a field (Culbreath et al., 2003).

Pod yields reflected DIR, as the susceptible genotype (SunOleic 95R) had the lowest yield, while the moderately resistant genotype's (S. Runner) yield was similar to the more resistant genotype's (DP-1) yield under mild disease pressure. However, a difference in yield between the moderately resistant genotype and more resistant genotype was expressed under higher disease pressure.

Linear regression analysis used to determine relationships between TSWV infection of leaves and root crowns with DIR showed that ELISA detection of TSWV at particular points in the season can predict end-of season plant severity. The severity of virus infection and disease pressure for a particular season will determine which sampling time serves as the best predictor.

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