Apparent Sap Flow Velocity in Peanut Genotypes Susceptible and Resistant to *Verticillium dahliae*

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ABSTRACT

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The thermoelectric (heat pulse) method, which is used for measuring apparent sap flow velocities (AS_v) in living plants, was studied to determine whether it could be used to detect sap flow differences between peanut (*Arachis hypogaea*) genotypes susceptible and resistant to *Verticillium dahliae*. Two peanut genotypes differing in disease sensitivity (susceptible cultivar Tamnut 74 and resistant cultivar PI 295233) were evaluated in two greenhouse experiments by inoculating plants with 0.5 ml of conidial suspension (5×10^5 conidia per milliliter) of *V. dahliae* 44 days after planting. Both genotypes grown with and without *V. dahliae* were compared for differences in AS_v at 43, 47, 50, 57, 64, 71, 78, 84, 91, and 98 days after planting. Plant water loss was monitored periodically throughout the study. At harvest (105 days after planting), disease index and biomass (vegetative and reproductive) production were also determined. Significant differences in genotype AS_v response to infection by *V. dahliae* were found in both experiments, with significant temporal differences for each genotype. Significant reduction in AS_v occurred in inoculated PI 295233 (47–50 days after planting), but were less than 10% at 71 days after planting. Tamnut 74 showed consistent AS_v reductions 57–98 days after planting, but not earlier. Differential genotype response to *V. dahliae* were also reflected in the plant water loss, biomass production, and disease index data. The thermoelectric method effectively detected the difference between peanut genotypes susceptible and resistant to *V. dahliae*: significant differences in AS_v were found among treatments in both experiments, and coefficients of variation were low.

Additional key words: disease, groundnut.

Verticillium wilt disease, caused by *Verticillium dahliae* Kleb., causes significant loss in plant vigor and reproduction capacity of some genotypes of peanut (*Arachis hypogaea* L.) (10), while other genotypes are considered resistant to the disease (3). Physiological reasons for this variation are unknown. Heald (6) reported that the water-conducting system ceases to function in mature infected plants. Some reports have suggested that the vascular system becomes occluded (1), giving rise to such symptoms as leaf epinasty, flaccidity, stunting, and eventual dehydration of infected plants. Vascular injury or occlusion due to the disease could affect sap flow and other physiological processes. Tyloses formed in tomato plants resistant and susceptible to *Fusarium* with more vessels containing tyloses in the former than in the latter (8). Impeding of water conductivity in the vascular system of wilted plants has been studied with the aid of various dyes (5,9,11,15). However, these methods are destructive and do not provide a measure of the apparent sap flow velocity in the vascular system. Measurements of sap (xylem) movement may be useful in detecting differences between Verticillium-susceptible and -resistant peanut genotypes and may provide more information on the nature and control of the disease.

Of the methods used for determining the apparent sap flow velocity (AS_v) in the xylem of living plants, the thermoelectric (heat pulse) method has been widely adopted (12) because it is simple and accurate compared to other methods (13). Stone and Shirazi (14) have analyzed the theory of the thermoelectric methods and have shown that the method can be used in such a manner that preserves AS_v and true sap velocity phase relationship. This requires only a single point measurement per analysis. A recently developed thermoelectric sensor that is nonintrusive and nondestructive has been shown to provide rapid, accurate, and repeatable AS_v measurements in intact plant stems (4). In field tests, the improved thermoelectric sensor detected significant differences in AS_v between irrigated peanut plants grown in wide (1 m) and narrow (0.3 m) spacings. It also showed differences in AS_v among cotton (*Gossypium* spp.), prickly sida (*Sida spinosa* L.), Palmer pigweed (*Amaranthus palmerii* S. Wats.), and cottonwood (*Populus deltoides.* Marsh.) plants.

No previous attempts to measure the apparent sap flow velocity in relation to wilt diseases have been reported. Therefore, the objectives of our study were: to determine the effectiveness and reliability of the thermoelectric method for detecting the difference between a Verticillium-susceptible and -resistant peanut genotype, and to determine the physiological significance of peanut AS_v in relation to plant water loss and biomass production of infected and uninfected plants.

MATERIALS AND METHODS

Peanut seeds of the genotypes Tamnut 74 (TAM) and PI 295233 (P33) (which are resistant and susceptible, respectively, to *Verticillium dahliae*) were dusted with 50% captan and germinated in the dark at 30±1 C for 48 hr on Whatman #1 filter paper (12.5 cm) moistened with distilled water. Seedlings with uniform radicles were transferred to the greenhouse on 18 January 1984 and planted in 17.8 × 16.5 cm (diameter) pots, one seedling per pot. Each pot contained 3.0±0.2 kg of a mixture of sand, soil, and peat (5:4:1, v/v/v), with a 1-cm coarse sand casing layer. Greenhouse environmental conditions during the day and night were maintained at 30±2 C and 27±2 C, respectively, with 56±10% relative humidity and 0.4 m/sec horizontal wind velocity at plant height. Thirteen days after planting, 16 seedlings of each genotype were selected for uniformity and independently randomized in two 4 × 4 latin square designs denoted as north (N) and south (S) experiments. Border plants surrounded both N and S. Twenty-one days after planting, each seedling was fertilized with 50 ml of 0.2% NH_4NO_3 and 125 ml of half-strength Hoagland's solution (7). Forty-four days after planting, the crowns of four plants of each genotype in each Latin square were inoculated by using a syringe fitted with a 23-gauge hypodermic needle (10) to inject 0.5 ml of a conidial suspension (5×10^5 conidia per milliliter) of *V. dahliae*. Plants in the S experiment were inoculated first. Sterile water was used in uninoculated controls. All plants were well watered throughout the study.

Physiological measurements included AS_v and the rate of plant water loss (T_r). Thermoelectric probes (4) were used to measure AS_v in petioles of fully developed terminal leaflets of the second fully expanded leaf below the terminal primordia on the main stem. Measurements of AS_v were made between 1130 and 1300 hours apparent solar time 1 day prior to inoculation (43 days after planting), 3 and 6 days after inoculation (47 and 50 days after planting) and weekly thereafter to 99 days after planting. Periodic determinations of T_r were made by weighing each pot in the morning and evening, subtracting the daily water loss from a similarly shaded and moist bare pot, and dividing the difference in weight by the time interval (units, grams per hour).

Plants were harvested 105 days after planting, and the severity of Verticillium wilt (disease index) on each plant was determined by using a subjective 1–5 scale described by Melouk et al (10). The total number of pegs and pods per plant were counted and dry weights of root, shoot, pegs, and pods were determined after drying for 8 days at 30±5 C in the greenhouse. Data were analyzed by standard procedures for analysis of variance, and those for N and S were pooled when appropriate.

RESULTS AND DISCUSSION

Petiolar AS_v values for each measurement day are presented in Table 1. Although some variability in treatment AS_v responses was observed between N and S, similar trends are evident. The AS_v of inoculated (I) P33 was significantly lower ($P < 0.05$) than uninoculated (UI) P33 on 3–6 days after inoculation, indicating a strong inital sensitivity and response to inoculation with *V. dahliae*. Symptoms of leaf epinasty in P33-I were observed about 13 days after inoculation, but subsequently disappeared about 33 days after inoculation. By 40 days after inoculation and thereafter, there were no significant differences in AS_v between P33-I and P33-UI. Conversely, significant differences between TAM-I and TAM-UI were observed about 13 to 27 days after inoculation and thereafter. Visible disease symptoms in TAM-I were not observed until 33 days after inoculation. By 54 days after inoculation, the effects of *V. dahliae* were severe enough to cause stunting, wilting, leaf chlorosis, and partial defoliation in TAM-I. Significant AS_v differences among treatments and low coefficients of variation values (Table 1) indicate that the thermoelectric method can be useful in detecting differences between Verticillium-susceptible and -resistant peanut genotypes. The advantage of repeated measurements on the same plants was also realized.

The relative difference in Verticillium wilt resistance of TAM and P33 is indicated from an examination of Fig. 1, which shows the average [(N+S)/2] percent reduction in AS_v for both genotypes. The AS_v in P33-I as compared to P33-UI was reduced by 10% to 34% between 3 and 20 days after inoculation, respectively, compared to less than 7% reduction in TAM-I during the same time interval. Initially TAM appeared to be more resistant to *V. dahliae* than P33. However, 27 days after inoculation, reductions in AS_v for TAM-I ranged from 31 to 39%, whereas P33-I showed about 10% reduction in AS_v during this period. Apparently P33 was hypersensitive to *V. dahliae* initially, but resistance to the

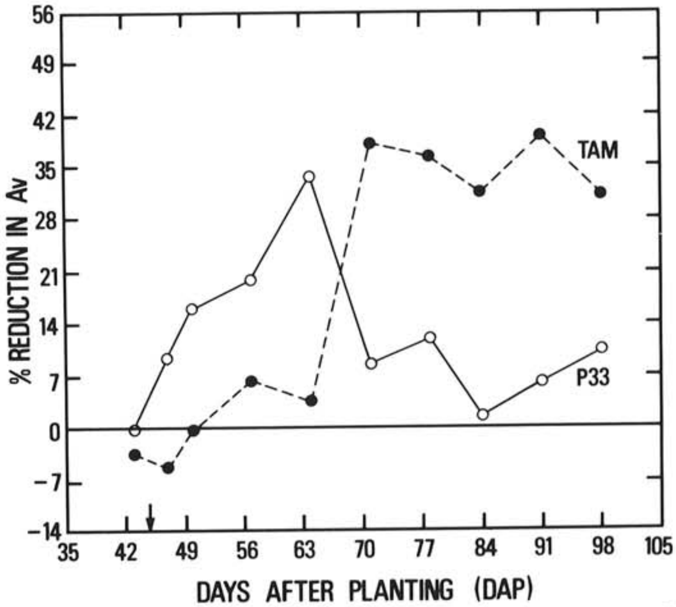

Fig. 1. Percent reduction in apparent sap flow velocity (AS_v) in petioles of inoculated peanut genotypes Tamnut 74 (TAM) and PI 295233 (P33) which are resistant and susceptible, respectively, to *Verticillium dahliae*. Data are presented as the means of two similar experiments (each a 4×4 Latin square design, one designated "north" and the other "south") on each measurement day. Arrow indicates day of inoculation.

TABLE 1. Apparent sap flow velocities (AS_v, cm/min) in peitoles of inoculated (I) and uninoculated (UI) peanut genotypes Tamnut 74 (TAM) and PI 295233 (P33) (which are resistant and susceptible, respectively, to *Verticilium dahliae*) measured over time in north and south experiments[v]

DAP[w]→ DAI[x]→	43 −1	47 3	50 6	57 13	64 20	71 27	78 33	84 40	91 47	98 54
North experiment										
P33-UI	0.91 b[y]	0.87 ab	1.01 a	0.93 ab	1.02 a	0.96 a	0.97 a	0.78 b	0.87 b	0.75 b
P33-I	0.91 b	0.84 b	0.75 b	0.70 c	0.66 c	0.84 b	0.91 a	0.79 b	0.81 b	0.65 c
TAM-UI	0.91 b	0.94 a	1.13 a	1.02 a	0.79 b	1.03 a	1.06 a	0.96 a	0.80 a	1.00 a
TAM-I	0.98 b	0.94 a	1.00 a	0.90 b	0.67 c	0.56 c	0.55 c	0.57 c	0.68 c	0.52 d
C.V. (%)	11.8	12.7	20.8	18.4	19.5	21.5	29.0	23.8	14.1	4.6
South experiment										
P33-UI	0.79 b	1.06 a	1.06 a	1.18 a	0.87 b	0.94 ab	1.11 a	1.01 ab	0.86 b	0.78 a
P33-I	0.82 b	0.83 b	0.90 b	1.01 b	0.58 c	0.89 b	0.91 b	0.95 b	0.82 b	0.73 a
TAM-UI	1.05 a	0.91 a	0.91 b	0.85 c	1.02 a	0.97 a	1.07 a	1.06 a	1.01 a	0.79 a
TAM-I	1.04 a	0.99 a	0.96 b	0.84 c	0.96 ab	0.67 c	0.80 c	0.65 c	0.54 c	0.58 b
C.V. (%)[z]	20.7	12.7	17.7	18.2	19.6	13.2	12.6	12.9	14.6	22.4

[v] North and south experiments are two similar 4 × 4 Latin square designed blocks which were run simultaneously.

[w] DAP, days after planting.

[x] DAI, days after inoculation.

[y] Means followed by different letters in the same colum of each experiment are significantly different ($P \leq 0.05$).

[z] Coefficient of variation.

disease developed later. The low reduction percentages of AS_v in TAM from 3 to 20 days after inoculation suggest that this genotype had a delayed response to *V. dahliae* infection without subsequent recovery and, thus, little if any resistance developed. Similar findings of interference with water conductance, as indicated by tracing of dyes, in pathogen-induced wilts were obtained by other investigators (5,9,11,15); however, the technique described in our research provided useful data for comparing the apparent sap flow velocity in peanut genotypes susceptible and resistant to *V. dahliae*.

Levels of T_r and AS_v have been shown to be correlated in trees (2), cotton (*Gossypium hirsutum* L. 'Stoneville 7A') (14), and in soybean and sunflower (12). Likewise, in this study, linear regression analysis of pooled N and S data revealed that AS_v and T_r were highly correlated for both genotypes (Fig. 2). Slope (B) comparisons indicated that more water was lost per centimeter of sap movement in P33 (B = 0.68 g/cm) than in TAM (B = 0.36 g/cm). At high AS_v ($\geq$1.0 cm/min), P33 lost about 18% more water than TAM. Greater T_r in P33 may explain its strong initial sensitivity and response to inoculation with *V. dahliae*. This contrasts with findings by Hall et al (5) who reported that transpiration rate, as indicated by stomatal resistance, decreased in Verticillium-wilt-affected chrysanthemum. At low AS_v ($\leq$0.5 cm/min), appreciable T_r occurred (~10 g/hr), which indicates that the vascular system was functional in both infected peanut genotypes. Direct microscopic examinations (×50, without the use of dye) of cross and longitudinal sections of petioles of inoculated peanut genotypes revealed discoloration of the vascular bundles, but no occlusion was evident. Discolored portions of vascular strands in TAM-I did, however, appear to have less structural integrity (i.e., were partially collapsed) than P33-I, which may explain its greater reduction in AS_v and T_r during later stages of disease development (27–54 days after inoculation).

Comparisons of biomass (mean dry weight) production and disease index (Table 2) showed differential genotypic responses to infection by *V. dahliae*. Shoot and root dry weights of P33-I were significantly lower than those of P33-UI, but dry weights of pegs and pods, pod number, and disease index were not significantly different. Early AS_v reductions in P33-I may have contributed to lower shoot and root dry weights. All biomass production parameters, except root dry weight, were significantly different between TAM-I and TAM-UI, but pod dry weight was reduced more (38.2%) by *V. dahliae* than any other parameter. Dry weights of shoots, roots, pegs, and pods were significantly lower in TAM-I compared to P33-I. Root and shoot dry weights of P33-UI were significantly higher than TAM-UI, but pod number was significantly lower.

In summary, measurements of AS_v showed significant differences between TAM and P33 responses to infection by *V. dahliae*, and coefficient of variation values were low. These data indicated P33 was more resistant to *V. dahliae* than was TAM. Biomass production parameters and disease index data showed similar results, but provided little information on the nature of the disease as it affects sap movement. The thermoelectric method provided evidence of a functional vascular system in infected plants of both TAM and P33 because reduced AS_v and T_r were not low enough to preclude sap flow. Therefore, the thermoelectric method needs closer examination for its potential use as a screening method to identify Verticillium wilt-resistant peanut genotypes.

TABLE 2. Biomass production parameters and disease index ratings of inoculated (I) and uninoculated (UI) peanut genotypes Tamnut 74 (TAM) and PI 295233 (P33). Values shown are means of north and south experiments[x]

	Shoot dry weight (g)	Root dry weight (g)	Peg dry weight (g)	Pod dry weight (g)	Pod number	Disease index
P33-UI	19.6 a[y]	3.4 a	0.075 a	10.7 ab	13.4 b	1.0 a
P33-I	15.8 b	2.5 b	0.056 a	12.0 a	13.1 b	1.4 a
TAM-UI	11.1 c	1.5 c	0.041 a	13.6 a	19.4 a	1.0 a
TAM-I	8.5 d	1.1 c	0.032 b	8.4 b	13.6 b	4.0 b
C.V. (%)[z]	22.1	6.2	0.4	21.2	37.8	4.0

[x] North and south experiments are two similar 4×4 Latin square designed blocks which were run simultaneously.

[y] Means followed by different letters in the same column are significantly different ($P \leq 0.05$).

[z] Coefficient of variation.

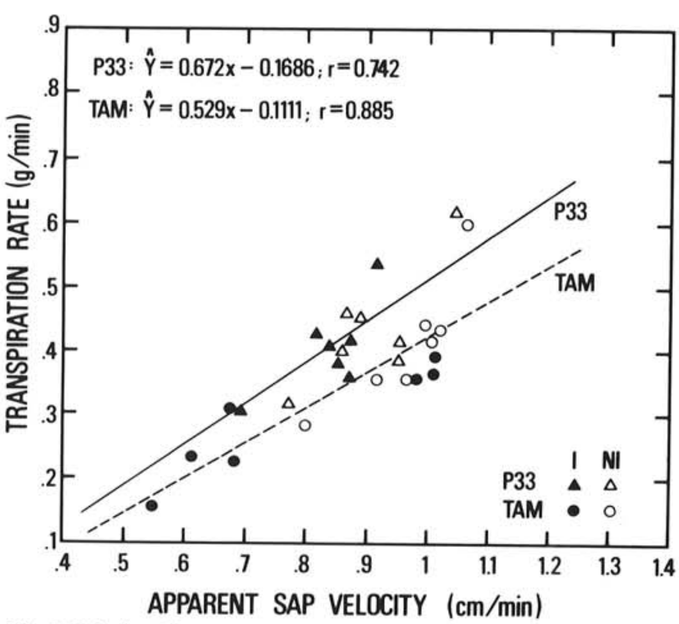

Fig. 2. Relationship between apparent petiolar sap flow velocity and rate of plant water loss (T_r) for Tamnut 74 (TAM) and PI 295233 (P33) which are resistant and susceptible, respectively, to *Verticillium dahliae*. Data are presented as the means of two similar experiments (each a 4×4 Latin square design, one designated "north" and the other "south").

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