Water Relations in Cotton Plants Infected with *Phymatotrichum*

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Journal Series Paper 3576 of the Arizona Agricultural Experiment Station, Tucson.
This study was supported by a grant from USDA/SEA Competitive Grants Program.
Accepted for publication 4 August 1982.

ABSTRACT

Olsen, M. W., Misaghi, I. J., Goldstein, D., and Hine, R. B. 1983. Water relations in cotton plants infected with *Phymatotrichum*. Phytopathology 73:213-216.

Water relations in cotton plants infected with *Phymatotrichum omnivorum* were studied to determine the mechanism of wilt development. Relationships between leaf water and osmotic potentials, relative water contents, and diffusive resistances of leaves from diseased and water-stressed healthy plants were similar. Rates of recovery from wilting, measured as increases in relative water content with time in both diseased and water-stressed healthy plants, also were identical. No significant differences were found in root dry weights of healthy and diseased plants, indicating that wilting is not a consequence of reduced root area. As upper leaves of diseased plants began to wilt, resistances to water flow in roots and lower stems of diseased plants increased significantly over those of healthy plants. However, resistance to water flow in petioles of diseased plants was unchanged. These results show that wilting in *Phymatotrichum*-infected cotton plants is probably the consequence of increased resistance to water flow in roots.

Phymatotrichum omnivorum is a soilborne fungus that causes root rot of more than 2,300 species of dicotyledonous plants in the southwestern United States and Mexico. In Arizona, the disease occurs in cotton plants from late June through September. Initial symptoms of the disease in cotton include wilting of upper leaves, rotting of the lower tap roots, and root xylem discoloration. When upper leaves first become flaccid and develop visible wilt, root decay is limited to the lower tap roots, and secondary roots appear healthy. Root decay progresses rapidly, and within two to three days after development of the initial symptoms, plants wilt permanently and die. Rapid wilting at high temperatures is a symptom of *Phymatotrichum* infection of other host plants. The cause of wilting in *Phymatotrichum*-infected cotton plants is unknown.

Wilting in plants infected with wilt or root rot pathogens has been attributed to factors such as reduction of water uptake by plants due to decreased root area or destruction of cortical tissue, increased resistance to water flow through xylem elements, or changes in leaf cell permeability resulting from dysfunction in osmotic regulation (1,4–6,12,14). The objective of this study was to determine whether one or more of these mechanisms is responsible for wilting in *Phymatotrichum*-infected cotton plants.

MATERIALS AND METHODS

Biological materials. For controlled environment studies, cotton plants (*Gossypium hirsutum* L. var. Delta Pine) were grown in autoclaved field soil in pots 15 cm in diameter in growth chambers at 30 C and 14 hr of fluorescent and incandescent light daily (225 μE $m^{-2}sec^{-1}$). Plants were fertilized at emergence with Osmocote 14-14-14 (Sierra Chemical Co., Milpitas, CA 95035) and watered as needed with distilled water with an automatic drip system. In field studies, 2- to 3-mo-old cotton plants (*G. hirsutum* L. var. Delta Pine) grown at Marana, AZ, were used.

Inoculum preparation and inoculation of plants were done according to Perez (10) with modifications. Cultures of *P. omnivorum*, isolated from diseased cotton plants, were maintained on potato-dextrose agar at 28 C. About 15 g of sorghum seeds that had been soaked in water overnight were put into 10-cm petri dishes and autoclaved for 2 hr on each of two consecutive days. Sterile sorghum seeds were inoculated with agar plugs from 10-day-old cultures and incubated at 28 C until seeds were fully colonized. The colonized seeds were then cut into 1-cm blocks. Blocks were incubated for an additional 3 days to allow mycelial coverage of cut surfaces. For plant inoculations, a test tube (1.5 cm in diameter) was inserted into the soil to a depth of 10 cm and about 2 cm from the base of 1-wk-old cotton plants. Plants were inoculated 1–2 wk later with two or three colonized sorghum seed blocks placed in holes formed by the removal of the test tubes. Equal amounts of sterile sorghum seeds were used for control plants.

Root weights. Two-week-old cotton plants, grown in growth chambers, were grouped into 10 pairs so that plants in each pair were of equal size. In each pair, one plant was inoculated with *P. omnivorum* according to the procedure described above. When inoculated plants began to wilt, pairs of plants were submerged in water, and soil was gently washed from the roots. Roots were then excised from the plants at soil level, dried at 65 C for 48 hr, and weighed. Results were analyzed statistically using Student's *t*-test for matched pairs.

Leaf water relations. Studies were done on pairs of plants, one infected and one healthy, of approximately the same physiological age, grown in growth chambers. Wilting was induced in healthy plants by withholding water. Leaf diffusive resistances were measured in the topmost, fully expanded leaves at various stages of wilt development, using a LI-COR diffusive resistance meter, model LI 60 (LI-COR, Inc., Lincoln, NE) 3–4 hr after plants were illuminated. Sampling and calibrations were according to the procedures described by Kanemasu et al (7). Total leaf water potential was then determined with a pressure bomb (PMS Instrument Co., Corvallis, OR) following diffusive resistance measurements.

Osmotic potential of leaves was measured with thermocouple psychrometers (JRD Merrill Specialty Co., Logan, UT), using the freeze-thaw method as described by Brown and Van Haveren (2). One-centimeter disks were cut from leaves of healthy and diseased plants at different stages of wilting. Ten to 12 disks were frozen in liquid nitrogen either immediately after measurement of total leaf water potential or after bringing disks to turgidity by floating them on water. Disks brought to turgidity before freezing were used to compare osmotic potentials of tissues at different relative water contents at turgid cell volumes (3,4). Disks frozen immediately after total water potential measurements were used to compare total water potential with osmotic potential.

Leaf disks also were used to measure relative water content (RWC). Three disks from leaves of healthy and diseased plants at different stages of wilting were put into sealed vials and kept cool.

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For fresh weight (FW) determinations, disks were weighed in the tared vials to avoid errors due to water loss. Disks were floated 4 hr at 20 C in the dark to obtain turgid weights (TW), then dried at 60 C for 48 hr to obtain dry weights (DW). The percent RWC was determined as $(FW - DW)/(TW - DW) \times 100$.

The ability of leaf tissue from both infected and water-stressed healthy plants to recover from wilting was determined by measuring RWC of leaf disks after they were floated on water. FWs of five disks from each of three leaves from diseased and water-stressed healthy plants were recorded at zero time. Disks were then floated on sterile, distilled water at 20 C in the dark. At timed intervals, disks were gently blotted dry and reweighed. TW of leaf disks was determined after 4 hr. Disks were dried at 60 C for 48 hr to determine the DW.

Resistance to water flow. The rates of water flow through roots, lower stems, and petioles were calculated in field-grown healthy and diseased plants at initial stages of wilting, using a pressure chamber modeled after that of Mees and Weatherley (9). Field-grown infected plants, with the topmost leaves showing incipient wilt, were collected at Marana, AZ. Control plants were collected outside diseased areas but from within the same field. Care was taken to keep the tap roots intact. Stems were cut at the sixth leaf node and the entire root system was placed in degassed water in the chamber. Flow rates of water from the cut ends of stems were measured under 1 bar of pressure as milliliters per second over a 30-min period, after an initial 10-min adjustment time. Stems were then cut off at soil level, and rates of water flow through the lower stem segments were measured in the same manner. Rates of water flow through roots were calculated by subtracting the flow rates through lower stems with roots attached from the flow rates through lower stem segments alone. Petioles were cut from wilted leaves of diseased plants or from nonwilted leaves at the corresponding leaf nodes of healthy plants. Rates of water flow through petioles were measured in a pressure bomb chamber at 2 bars of pressure for 15 min after a 5-min adjustment time.

Resistance to water flow (R) is equal to $\Delta\Psi/F$ where $\Delta\Psi$ represents the difference between total water potentials of the water in the reservoir inside the chamber and the water collected at the cut end of the stem, and F represents the water flux. In this study, $\Delta\Psi$ was considered equal to the chamber pressure because differences in osmotic potentials between water in the reservoir and water pushed through the roots, stems, or petioles were too small to be significant. Flux was calculated as rate of water flow per leaf area (6). Leaf areas were measured with an automatic area meter (type AAM 5, Hayashi Denko Col., Ltd., Tokyo, Japan) from excised leaves or from leaf tracings cut from paper. Resistance values for healthy and diseased plants were analyzed statistically using Student's *t*-test.

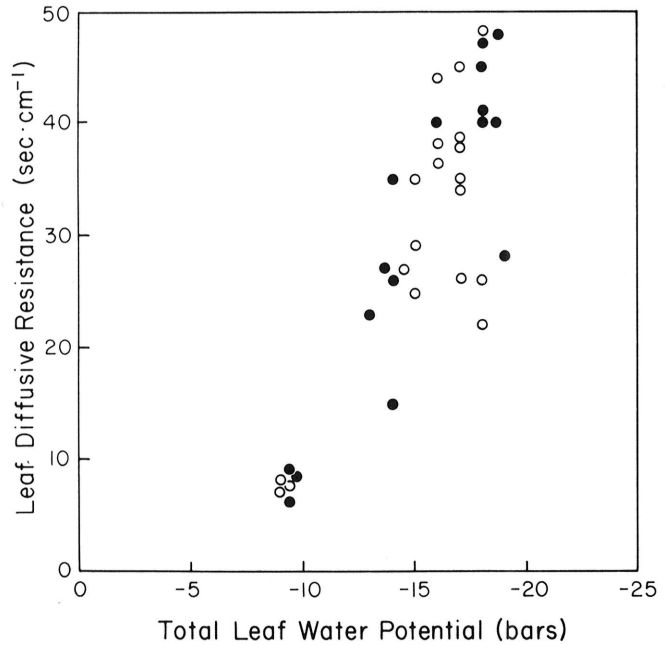

Fig. 1. Leaf diffusive resistance as a function of total leaf water potential (Ψ) in leaves of healthy (o) and diseased (●) plants. Each point represents the diffusive resistance and Ψ values taken from one leaf; the regression equation for diffusive resistance in healthy plants is $-3.32\,(\Psi) - 20.79$, with $r = -0.80$, and in diseased plants, $-3.54\,(\Psi) - 23.08$, with $r = -0.89$.

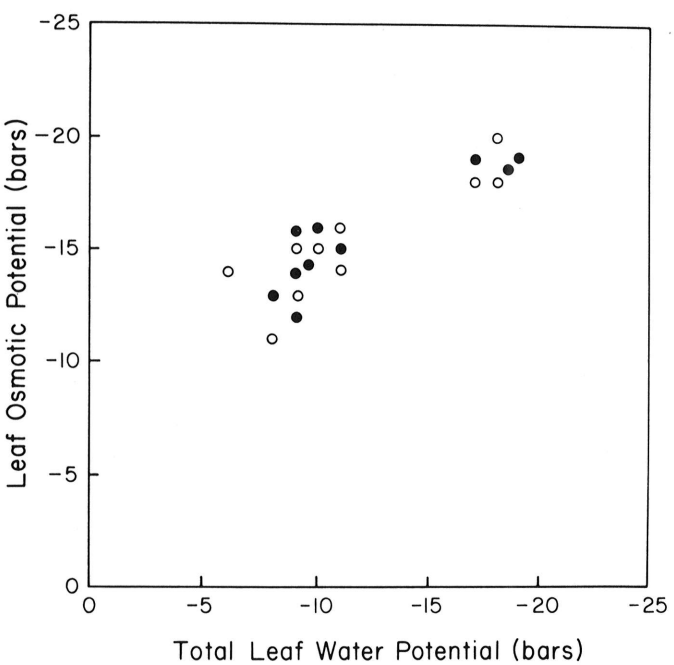

Fig. 2. Total leaf water potential (Ψ) as a function of osmotic potential (Ψo) in leaves of healthy (o) and diseased (●) plants. Each point represents Ψo and Ψ values taken from one leaf; the regression equation for Ψ in healthy plants is $1.43\,(\Psi o) + 10.35$, with $r = 0.87$, and in diseased plants, $1.56\,(\Psi o) + 12.36$, with $r = 0.89$.

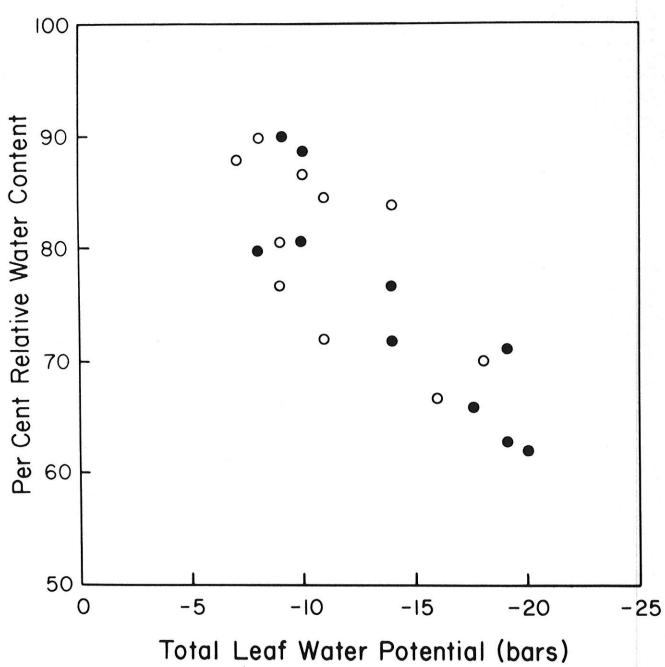

Fig. 3. Total water potential (Ψ) as a function of percent relative water content (% RWC) in leaves of healthy (o) and diseased (●) plants. Each point represents % RWC and Ψ values of one leaf; the regression equation for Ψ in healthy plants is 0.32 (% RWC) -36.57, with $r = 0.71$, and in diseased plants, 0.42 (% RWC) -45.52, with $r = 0.90$.

RESULTS

Inoculations. Plants began to wilt 5–10 days after inoculation. The percent infection of inoculated plants varied from 0 to 85 in different trials in the growth chambers. The reason(s) for observed variations in disease incidence among inoculation trials is not known. The fungus was reisolated from decayed root tissue of diseased plants but was not isolated from discolored xylem in advance of decayed cortical tissue.

Root weight. No statistically significant differences were found in root dry weights of 10 matched pairs of plants (one diseased and one healthy), using Student's *t*-test for matched pairs.

Leaf water relations. Changes in leaf diffusive resistance in response to changes in total leaf water potential were similar in healthy and diseased plants (Fig. 1). The relations between total leaf water potential and osmotic potential (Fig. 2) and between total leaf water potential and percent RWC (Fig. 3) were also similar in healthy and diseased plants. Because osmotic potentials vary with cell volumes (3,4), the relationship between percent RWC and osmotic potential was determined using osmotic potentials of turgid leaf disks. Osmotic potentials of leaves from healthy and diseased plants did not change appreciably with changes in percent RWC (Table 1). Leaf tissue from water-stressed healthy and diseased plants regained turgor at essentially the same rate (Fig. 4).

Resistance to water flow. Resistance to water flow in diseased plants, compared to that in healthy plants, increased significantly at early stages of wilting (Table 2). Resistances in roots and lower stems of diseased plants increased from 40 times to infinite times (no measureable water flow at 1 bar of pressure) over those of healthy plants. The differences in resistance values of healthy and diseased plants were statistically significant for roots at $P = 0.025$ and for stems at $P = 0.01$. Resistance values for petioles of healthy and diseased plants were not significantly different.

DISCUSSION

Results of this study suggest that increased resistance to water flow in roots may be the major cause of wilting in cotton plants infected with *P. omnivorum*. Root resistance, which increased significantly at early stages of wilting, may cause reduction in water flow and rapid wilting of plants in the field where transpiration rates are very high. Resistance to water flow also increased in lower stems of infected plants, but the increases were not large enough to contribute appreciably to wilting. Resistance to water flow in petioles was not altered in diseased plants. Wilted leaves from both healthy and diseased plants, cut at the basal end of the petiole and put into water, regained turgor rapidly.

Patterns of changes in leaf diffusive resistance as a function of leaf water potential were similar in water-stressed healthy and diseased plants, suggesting that disease-induced wilting may not be caused by altered transpiration rates brought about by changes in stomatal behavior. Stomates closed as fast in diseased plants as in water-stressed healthy plants. Therefore, loss of leaf turgor is not a result of toxin-induced opening of stomates such as that reported for *Fusicoccum amygdali* (8). Wilting is probably not due to altered leaf cell permeability caused by toxin or by cell-wall-degrading enzymes, because no significant differences were found in osmotic potential as a function of total leaf water potential or RWC in leaves from water-stressed healthy and *Phymatotrichum*-infected cotton plants at the onset of wilting. In diseases in which cell membranes are altered during pathogenesis, such as *Pseudomonas syringae* on tobacco (4), the osmotic potential of cells is increased due to changes in cell walls or cell membranes. Cell turgor is lost and wilting results. However, the osmotic potential of leaf tissue from cotton plants infected with *P. omnivorum*, like that of water-stressed healthy plants, decreased linearly with decreasing leaf water content.

Factors contributing to increased resistance to water flow in roots and stems of *Phymatotrichum*-infected cotton plants are not known. Increased resistance to water flow in other host-parasite systems has been attributed to plugging of xylem elements by fungal structures (16) or by high-molecular-weight substances produced by the pathogen directly (13–15) or as a result of enzymatic degradation of host cell walls (11). Fungal structures were not observed above infected sites in xylem elements of roots and stems of *Phymatotrichum*-infected cotton plants. However, xylem elements in roots and lower stems of infected plants were

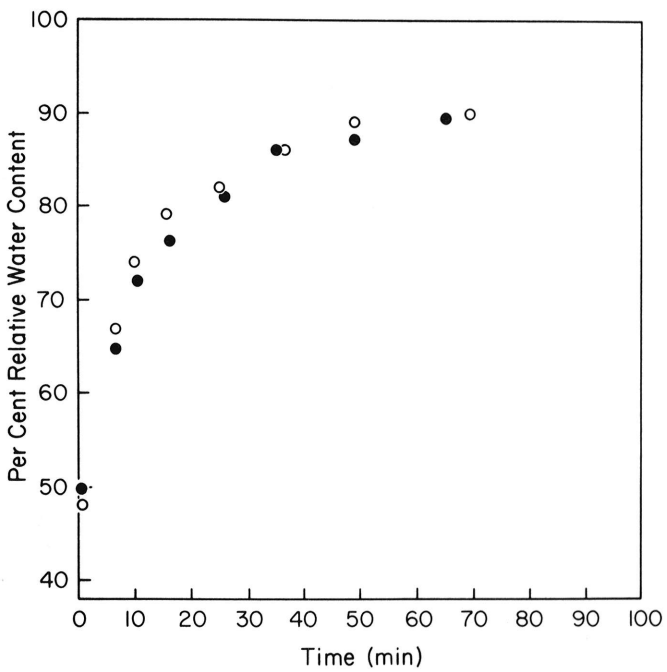

Fig. 4. Relative water content as a function of time required for disks from wilted leaves of water-stressed healthy (o) and diseased (●) plants to regain turgor. Each point represents an average of five measurements in three replications.

TABLE 1. Comparisons of percent relative water content (% RWC) and leaf osmotic potential (ψo) at turgid cell volumes of leaves from healthy and diseased plants[a]

Plant	Leaf condition	% RWC	ψo (bars)
Healthy	Wilted	83.2 ± 3.5	−10.8 ± 2.3
	Nonwilted	88.8 ± 2.7	−10.8 ± 2.1
Diseased	Wilted	77.2 ± 4.1	−9.6 ± 1.0
	Nonwilted	89.8 ± 1.3	−10.6 ± 1.3

[a] Each value represents the average of five measurements taken from five individual leaves. No significant differences were found at $P = 0.05$ in % RWC between wilted leaves of healthy and diseased plants nor between nonwilted leaves of healthy and diseased plants.

TABLE 2. Average values for resistance to water flow ([bars · sec · cm^{-1}] ($\times 10^5$) in petioles, lower stems, and roots of field-grown healthy and diseased plants

Plant	Resistance values[a]		
	Petiole	Lower stem	Root
Healthy	0.35 ± .08	0.45 ± 0.06	2.6 ± 1.2
Diseased	0.34 ± .07	10.8 ± 11.7	294 ± 351[b]

[a] Significant differences in resistance values of healthy and diseased plants were found in roots at $P = 0.025$ and in lower stems at $P = 0.01$, but no significant difference was found in petioles.

[b] Calculation of the average value for root resistance in diseased plants does not include infinite resistance (no measureable water flow through roots at a pressure of 1 bar) found in four of the 14 plants tested; therefore, the average resistance value in diseased roots is actually much higher than indicated.

discolored and seemed to be occluded with unknown materials. Although it is tempting to correlate resistance to water flow in roots and lower stems of infected plants with xylem discoloration and occlusion, no direct correlation was found between the amounts of xylem discoloration and increased resistance to water flow. Occlusion and darkening of xylem elements in roots and stems above infected sites may be the result of accumulation of products of enzymatic breakdown of cell walls or of degradation of root cells as the lower tap root is decayed. Because the magnitudes of resistances to water flow were also variable among roots and lower stems of infected plants, increased resistance could be partially due to disruption of water movement across the cortex and not solely due to the occlusion of xylem elements in upper portions of the roots. Death of cortical cells may result in reduction of water flow from the soil-root interface to the pericycle. Reduced water uptake and transport, particularly when combined with high transpiration rates, could result in wilting of upper leaves during early stages of infection. The mechanism of increased root resistance to water flow in *Phymatotrichum*-infected plants is being studied.

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