Effect of Preinoculation and Postinoculation Water Stress on the Severity of Phytophthora Root Rot in Processing Tomatoes

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ABSTRACT

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The effect of water stress imposed either before or after inoculation of processing tomatoes on the severity of Phytophthora root rot was evaluated under controlled conditions in greenhouse experiments. Three-week-old tomato seedlings or plants in early flowering stages were either inoculated with zoospores of *Phytophthora parasitica* or not inoculated. Plants either were stressed by withholding water until leaf water potentials reached −9 bars before or after inoculation or were watered regularly to maintain leaf water potentials above −4 bars. Severity of symptoms on roots, length of brown roots, and numbers of colonies of *P. parasitica* recovered from roots were greater in inoculated seedlings that were water-stressed either before or after inoculation. In the absence of water stress, older flowering plants were more resistant to infection than seedlings. Preinoculation water stress increased disease severity in flowering plants, whereas postinoculation water stress did not affect disease.

Additional keywords: *Lycopersicon esculentum*, water potential

The development of Phytophthora root rot in processing tomatoes is influenced greatly by irrigation schedule, with disease developing faster and earlier with more frequent and prolonged irrigation (18). Saturated soil conditions associated with irrigation affect *Phytophthora* species directly by enhancing inoculum formation and dispersal (2,6,9). In addition, saturated soil conditions have been shown to predispose alfalfa and rhododendron to Phytophthora root rot (3,12,14). The lack of water, or water stress, has also been shown to predispose some hosts to soilborne pathogens, including *Phytophthora* species (3,5,17,21,23). In the example of Phytophthora root rot of safflower, a susceptible cultivar exposed to preinoculation water stress had greater disease than inoculated plants that were not stressed in both greenhouse and field experiments (5,6). Both the very high and low extremes in water status that occur routinely in agricultural fields may significantly predispose plants to attack by *Phytophthora* species.

Drought conditions can occur in irrigated fields of processing tomato, and the possibility exists that water stress may affect the severity of Phytophthora root rot in them. The objective of this research was to examine the influence of water stress imposed at two growth stages, either before or after inoculation, on the susceptibility of a processing tomato variety to Phytophthora root rot under controlled conditions. A preliminary report on part of this work has been published (1).

MATERIALS AND METHODS

Inoculum was prepared by culturing *Phytophthora parasitica* Dastur for 1 wk at room temperature in petri plates containing V-8 agar (200 ml of V-8 juice, 800 ml of distilled water, 2 g of $CaCO_3$, 15 g of agar). Sporangia were obtained by cutting agar cultures into pieces and incubating the pieces in a shallow layer of sterile distilled water in petri plates at room temperature for 72 hr. Sporangia were then induced to release zoospores by chilling at 6 C for 1 hr and rewarming to room temperature for 1 hr. Zoospores were filtered through cheesecloth, quantified with a hemacytometer, and diluted to appropriate concentrations for each experiment. Five isolates obtained by baiting from soil (9) in different tomato fields were combined in equal proportions and used for inoculum in all experiments (18).

The effect of both preinoculation and postinoculation water stress on the severity of Phytophthora root rot in processing tomatoes was evaluated in growth chamber and greenhouse experiments. A completely randomized experimental design with a 2 × 2 factorial arrangement of treatments was used. Experiments were repeated three times. In one set of experiments, the tomato cultivar Ferry Morse 6203 was planted into 10.2-cm-diameter pots containing sieved (1 cm mesh) U.C. potting mix (15). Pots were watered daily, and seedlings were thinned to 10 per pot after emergence. Greenhouse temperatures ranged from 22 C at night to 28 C during the day. Each individual pot was an experimental unit and was replicated four times. All plants in all experiments were fertilized with half-strength Hoagland's solution on a biweekly basis (7). Two weeks after emergence, plants were transferred to a growth chamber having 14-hr periods of incandescent and fluorescent light at 960 ft-c and temperatures of 27 C during the day and 21 C at night. When the plants were 3–4 wk old, one-half of the pots were inoculated with 1.25×10^5 zoospores per pot of *P. parasitica* by application of 25 ml of suspension to the soil surface. After the inoculum had penetrated the soil, all seedlings were given a 3-hr saturation period; pots were placed in individual containers of water, and soil was saturated from below until the surface was wet.

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Plants were water-stressed by withholding water until leaf water potentials (ψ_l), measured with a pressure chamber (model 3005, Soil Moisture Equipment, Santa Barbara, CA), were less than −9 bars, either before or after inoculation. The ψ_l values reported were measured during light periods at the end of stress treatments and therefore are minimum values experienced by the plants. The youngest fully expanded leaflet on one randomly selected plant in each pot was used for ψ_l measurements. Plants stressed before inoculation were watered before zoospores were added to soil. To obtain postinoculation stress, water was withheld after inoculation and saturation, and stress was relieved by watering when desired ψ_l values were observed. Plants were watered daily except when stress was induced. Soil samples were removed from roots at the end of the water stress period and from well-watered controls, and soil water content was determined gravimetrically. At harvest (9–14 days after inoculation), the incidence of damping-off and the severity of symptoms on roots were evaluated on a scale of 0–4, where 0 = no root necrosis; 1 = 25% or less of all lateral roots necrotic, no taproot necrosis; 2 = 26–50% of all lateral roots necrotic, 50% or less of taproot necrotic; 3 = 51–75% of all lateral roots necrotic, 51–75% of taproot necrotic; and 4 = greater than 75% of all lateral and taproots necrotic and/or plant damped-off.

Table 1. Influence of preinoculation water stress and *Phytophthora parasitica* on root length and disease severity in seedlings of processing tomatoes grown in Yolo fine sandy loam[a]

Treatment	ψ_l[b] (bars)	Severity of symptoms on roots[c]	Root length[d] Total (cm)	Root length[d] Brown (cm)	*P. parasitica* colonies/cm of root[e]
Noninoculated	−2.1	0	1,386.7	0	0
Noninoculated	−8.6	0	1,197.7	0	0
P. parasitica	−1.9	1.0	934.4	96.9	0.49
P. parasitica	−9.6	3.0	629.9	144.6	3.70
Contrast comparisons			**Contrast estimates**[f]		
Interaction of inoculum by water stress	1.20 ns	−1.40*	115.54 ns	−47.62*	−3.21**
Inoculum vs. no inoculum	0.44 ns	−2.30***	510.04***	−120.79***	−2.09***
Water stress vs. no water stress	7.12***	−0.70**	246.72*	−23.81*	−1.60**
Effect of water stress in inoculated plants	7.70***	2.0***	−304.5*	47.70**	3.21**

[a] Water stress imposed by withholding water for 5 days before inoculation with 1.25×10^5 zoospores per pot.
[b] Measured just before watering and inoculation.
[c] Rated on a scale of 0–4.
[d] Determined by line intersect method.
[e] Determined by plating roots on PARP medium.
[f] Treatment sums of squares partitioned into single degree of freedom orthogonal contrasts. Estimates significant at * = $P \leqslant 0.05$, ** = $P \leqslant 0.01$, *** = $P \leqslant 0.001$; ns = not significant.

Table 2. Influence of postinoculation water stress and *Phytophthora parasitica* on root length and disease severity in seedlings of processing tomatoes grown in Yolo fine sandy loam[a]

Treatment	ψ_l[b] (bars)	Severity of symptoms on roots[c]	Root length[d] Total (cm)	Root length[d] Brown (cm)	*P. parasitica* colonies/cm of root[e]
Noninoculated	−1.9	0	1,487.1	0	0
Noninoculated	−11.7	0	1,566.7	0	0
P. parasitica	−1.7	2.0	966.6	69.2	0.65
P. parasitica	−10.6	2.7	929.2	134.1	3.48
Contrast comparisons			**Contrast estimates**[f]		
Interaction of inoculum by water stress	−0.90 ns	−0.70*	146.98 ns	−64.92 ns	−2.83**
Inoculum vs. no inoculum	−0.63*	−2.35***	563.95***	−101.62***	−2.06***
Water stress vs. no water stress	9.37***	−0.35*	−6.05 ns	−32.46 ns	−1.41**
Effect of water stress in inoculated plants	−8.90***	0.70**	−67.40 ns	64.90*	2.83***

[a] Water stress imposed by withholding water for 6 days after inoculation with 1.25×10^5 zoospores per pot.
[b] Measured just before watering and after inoculation.
[c] Rated on a scale of 0–4.
[d] Determined by line intersect method.
[e] Determined by plating roots on PARP medium.
[f] Treatment sums of squares partitioned into single degree of freedom orthogonal contrasts. Estimates significant at * = $P \leqslant 0.5$, ** = $P \leqslant 0.01$, *** = $P \leqslant 0.001$; ns = not significant.

In a second set of experiments, tomato seeds were planted in 175 cm^3 of sieved Yolo fine sandy loam in plastic cups and thinned to one plant per cup after emergence. Plastic cups were perforated on the bottom to allow adequate drainage. Treatments identical to those described above were applied, but each cup containing one seedling was used as an experimental unit and was replicated five times. At harvest, the entire root system was removed from soil in each cup and roots were elutriated with a semiautomatic elutriator (4). Total plant root length and the length of visibly browned roots were determined for each sample, using Tenant's modification of the Newman line-intersect technique (16,22). Severity of symptoms on entire root systems was also rated visually on the 0–4 scale. One-quarter of the roots from each plant were rinsed in sterile distilled water, blotted dry, and placed on PARP medium (10). Numbers of colonies (cluster of mycelium growing from a root segment) of *P. parasitica* emerging from roots on the agar were counted microscopically after 24 hr.

Experiments similar to those described above were conducted with older tomato plants at the early flowering stage. Seeds were planted in 17.8-cm-diameter pots filled with sieved U.C. potting mix; seedlings were thinned to one per pot after emergence. Each pot was an experimental unit and was replicated five times. In contrast to the other experiments, plants were maintained in a greenhouse during the entire experiment, 10^6 zoospores per plant were added at inoculation (41–53 days after planting), and all plants were given a 20-hr saturation period after inoculation. At harvest (14 days after inoculation), roots were washed and rated for symptom severity on the 0–4 scale. Fresh weight of roots was determined after excess water was removed by blotting.

Data were tested for normality and homogeneity of variance before analysis of variance and correlation analysis using Statistical Analysis Systems packages (SAS Institute, Inc., Cary, NC). Percentage data were transformed by means of an arcsin transformation. Treatment sums of squares were partitioned into single degree of freedom orthogonal contrasts. All results shown are representative from one of three repeat experiments conducted.

RESULTS

In all experiments, ψ_l in plants not water-stressed remained above −4.2 bars, whereas plants subjected to water stress either before or after inoculation had lower ψ_l values (Tables 1–4). Soil matric potentials in either U.C. mix or Yolo fine sandy loam ranged from −10 to −80 millibars in well-watered controls, whereas soil matric potentials were below −5 bars at the end of water stress periods. All stressed plants regained turgor promptly after rewatering.

In experiments with 3-wk-old seedlings in potting mix, the severity of symptoms on roots of inoculated plants and the incidence of damping-off were greater in water-stressed than in nonstressed plants when water stress was imposed either before or after inoculation (*data not shown*). Both preinoculation and postinoculation water stress also increased root rot development in experiments with 3-wk-old seedlings in Yolo fine sandy loam (Tables 1 and 2). Water stress increased the severity of symptoms on roots of inoculated plants, and the inoculum × water stress interaction effect was significant in experiments where water stress was imposed either before or after inoculation (Tables 1 and 2). Root length was reduced by either inoculation or water stress alone in plants given a preinoculation water stress period, although the interaction effect was not significant (Table 1). Root length was reduced in inoculated plants that were also water-stressed before inoculation (Table 1). Water stress after inoculation with *P. parasitica* did not reduce root length, and only the inoculum effect on root length was significant (Table 2). Lengths of discolored or brown roots and numbers of colonies of *P. parasitica* recovered from roots were greater in roots that were inoculated and water-stressed (Tables 1 and 2). A highly significant ($P < 0.0001$) and negative correlation was found between total root length and the severity of symptoms on roots of individual seedlings from experiments where water stress was imposed either before ($r = -0.85$) or after ($r = -0.87$) inoculation. The overall severity of symptoms on roots of plants water-stressed before or after inoculation was also positively correlated with both the length of brown roots ($r = 0.87$ and $r = 0.92$) and the numbers of colonies of *P. parasitica* isolated on a selective medium ($r = 0.78$ and $r = 0.84$). Noninoculated seedlings did not develop any symptoms of root rot or damping-off.

Tomato plants beginning to flower (three to seven flowers per plant) appeared to be more resistant to infection by *P. parasitica* than younger seedlings, since they required a higher zoospore inoculum level (10^6 per plant) and a 20-hr saturation period for significant disease development. Preliminary studies with lower inoculum densities and briefer saturation periods did not result in high levels of disease. Flowering plants subjected to water stress before inoculation had greater symptoms of root rot and lower root fresh weights than plants inoculated but not water-stressed, and the inoculum × water stress interaction effects were highly significant (Table 3). Water stress in inoculated plants had a significant effect on the severity of symptoms on roots. In contrast, water stress after inoculation did not increase the symptoms of Phytophthora root rot or reduce fresh weight of roots, and the inoculum × water stress interaction effect was not significant for these parameters (Table 4). Only the inoculum main effect was significant for severity of symptoms on roots in plants given a postinoculation water stress period. Significant ($P<0.01$) and negative relationships were found between the severity of symptoms on roots and root fresh weight in experiments where plants were given a water stress period either before ($r = -0.57$) or after ($r = -0.52$) inoculation. No disease symptoms were observed in noninoculated plants.

Table 3. Influence of preinoculation water stress on severity of Phytophthora root rot and fresh weight of roots of processing tomatoes during early flowering[a]

Treatment	ψ_l[b] (bars)	Severity of symptoms on roots[c]	Fresh weight of roots (g)
Noninoculated	−4.2	0	19.5
Noninoculated	−11.9	0	29.1
P. parasitica	−3.9	1.2	19.1
P. parasitica	−11.9	2.6	9.0
Contrast comparisons		**Contrast estimates**[d]	
Interaction of inoculum by water stress	0.34 ns	−1.40***	19.66***
Inoculum vs. no inoculum	−0.13 ns	−1.90***	10.27***
Water stress vs. no water stress	7.85***	−0.70***	0.21 ns
Effect of water stress in inoculated plants	8.00***	1.40**	−10.10*

[a] Water stress imposed by withholding water for 6 days before inoculation with 1.0×10^6 zoospores per pot.
[b] Measured just before watering and inoculation.
[c] Rated on a scale of 0–4.
[d] Treatment sums of squares partitioned into single degree of freedom orthogonal contrasts. Estimates significant at * = $P \leqslant 0.5$, ** = $P \leqslant 0.01$, *** = $P \leqslant 0.001$; ns = not significant.

Table 4. Influence of postinoculation water stress on severity of Phytophthora root rot and fresh weight of roots of processing tomatoes during early flowering[a]

Treatment	ψ_l[b] (bars)	Severity of symptoms on roots[c]	Fresh weight of roots (g)
Noninoculated	−3.7	0	23.2
Noninoculated	−10.9	0	24.2
P. parasitica	−4.0	1.2	18.9
P. parasitica	−11.0	1.6	19.3
Contrast comparisons		**Contrast estimates**[d]	
Interaction of inoculum by water stress	−0.20 ns	−0.40 ns	0.66 ns
Inoculum vs. no inoculum	0.22 ns	−1.40 ***	4.55 ns
Water stress vs. no water stress	7.14***	−0.20 ns	−0.71 ns
Effect of water stress in inoculated plants	7.00**	0.40 ns	0.40 ns

[a] Water stress imposed by withholding water for 9 days after inoculation with 1.0×10^6 zoospores per pot.
[b] Measured just before watering and after inoculation.
[c] Rated on a scale of 0–4.
[d] Treatment sums of squares partitioned into single degree of freedom orthogonal contrasts. Estimates significant at * = $P \leqslant 0.05$, ** = $P \leqslant 0.01$, *** = $P \leqslant 0.001$; ns = not significant.

DISCUSSION

Water stress imposed before inoculation of processing tomato seedlings with zoospores of *P. parasitica* significantly increased all measures of disease examined (Table 1). Since water stress imposed before inoculation resulted in an increase in disease severity, this is clearly an example of host predisposition to disease (19). Disease severity also was increased in seedlings when water stress was imposed after inoculation (Table 2). However, while all other measures of disease severity increased significantly, postinoculation stress did not increase the impact of *P. parasitica* on root length (Table 2). *P. parasitica* has been shown to grow on agar media at water potentials as low as −40 bars (21) and is probably capable of growth in roots at low water potentials. It is also possible that water stress increased the ability of *P. parasitica* to colonize roots from already established infection sites. However, water was withheld during 6 of 12 days after inoculation, creating conditions for much of the experiment that were too dry for zoospore release and dispersal. Therefore, a reduction in secondary cycles of infection may have reduced the net impact of disease on root length when water stress was imposed after inoculation.

More mature tomato plants that were in the early stages of flowering were somewhat more resistant than seedlings to infection by *P. parasitica*. Symptom severity on roots was low in the absence of water stress even though high levels of inoculum and saturated soil conditions were used on older plants (Tables 3 and 4). Evidently, physiological changes in host resistance occurred as plants matured. However, preinoculation water stress in older plants increased subsequent symptom severity and the impact of disease on root fresh weights significantly (Table 3). Water stress before inoculation may have changed roots such that the initial establishment of infection sites on mature plant roots was enhanced. However, mature plant roots were not plated on agar to determine if this phenomenon occurred. Unlike younger tomato seedlings, postinoculation water stress had little effect on disease severity in more mature plants (Table 4). In similar experiments with safflower, postinoculation water stress also did not enhance disease development in plants inoculated with *P. cryptogea* Pethybridge & Lafferty (5). In our experiments, there was also less time for postinoculation stress effects to be manifested, since plants were harvested 6 days after the water stress was relieved.

Because preinoculation water stress increased disease severity in seedlings and more mature tomato plants, physiological events occurring before inoculation may have compromised resistance mechanisms. Water stress at the levels found to predispose tomato can have negative effects on many physiological processes, including expansive growth and net photosynthesis, and can increase root:shoot ratios (8,20). In no case in our experiments did water stress significantly reduce final root length or fresh weight in noninoculated plants, and in some experiments, root length and fresh weight in noninoculated plants were significantly greater in water-stressed than in nonstressed plants (Tables 2 and 3).

Because water stress induces such a variety of physiological changes, it is difficult to clearly hypothesize mechanisms of predisposition to *P. parasitica*. In a number of plants, including tomato, water stress can damage plant membranes and cause an increase in the release of amino acids and reducing sugars from roots (11). Increased root exudation could have resulted in increased attraction of motile zoospores to roots, as has been demonstrated for salt-stressed and flood-stressed chrysanthemum and alfalfa roots (12,13). On the other hand, osmotic adjustment in root tissues and/or an increase in the allocation of carbon to water-stressed roots may have provided a stimulus for increased growth and colonization of tomato roots by *P. parasitica*, both at the time of initial infection and after infection sites were established. The physiological mechanisms involved in stress predisposition are not well understood for any disease example, and more research is needed on the mechanisms of water stress predisposition to disease.

Knowledge that seedlings and more mature tomato plants are predisposed to more severe Phytophthora root rot by water stress has practical value. Even though the minimal level of water stress required for the predisposition response to occur was not determined in our study, the ψ_l values used can occur in the field. Incorporation of water stress periods into greenhouse and field screening programs may prove useful in identifying forms of genetic resistance to Phytophthora root rot that are stable under water stress conditions. Disease management strategies for Phytophthora root rot can be improved in irrigated tomato-growing areas by manipulation of irrigation schedules to avoid not only significant levels of soil saturation but also periods of water stress during the seedling and early flowering stages of plant growth. While irrigation on a less frequent schedule can reduce disease development in infested fields (18), it is also important to avoid water stress.

LITERATURE CITED

1. Beagle-Ristaino, J., and Duniway, J. M. 1986. Effect of water stress on the severity of Phytophthora root rot in tomato. (Abstr.) Phytopathology 76:1124.
2. Bernhardt, E. A., and Grogan, R. G. 1982. Effect of soil matric potential on the formation and indirect germination of sporangia of *Phytophthora parasitica*, *Phytophthora capsici*, and *Phytophthora cryptogea*. Phytopathology 72:507-511.
3. Blaker, N. S., and MacDonald, J. D. 1981. Predisposing effects of soil moisture extremes on the susceptibility of rhododendron to Phytophthora root and crown rot. Phytopathology 71:831-834.
4. Byrd, D. W., Barker, K. R., Ferris, H., Nusbaum, C. J., Griffin, W., Small, R. H., and Stone, C. A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.
5. Duniway, J. M. 1977. Predisposing effect of water stress on the severity of Phytophthora root rot in safflower. Phytopathology 67:884-889.
6. Duniway, J. M., and Gordon, T. R. 1986. Water relations and pathogen activity in soil. Pages 119-137 in: Water, Fungi and Plants. P. G. Ayers and L. Boddy, eds. Cambridge University Press, London.
7. Hoagland, D. R., and Arnon, D. I. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 32 pp.
8. Hsaio, T. C. 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24:519-570.
9. Ioannou, N., and Grogan, R. G. 1984. Water requirements for sporangium formation by *Phytophthora parasitica* in relation to bioassay in soil. Plant Dis. 68:1043-1048.
10. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
11. Katznelson, H., Rouatt, J. W., and Payne, T. M. B. 1954. Liberation of amino acids by plant roots in relation to desiccation. Nature 174:1110-1111.
12. Kuan, T. L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.
13. MacDonald, J. D. 1982. Effect of salinity stress on the development of Phytophthora root rot of chrysanthemum. Phytopathology 72:214-219.
14. MacDonald, J. D. 1982. Role of environmental stress in the development of Phytophthora root rots. J. Arboric. 8:217-223.
15. Matkin, O. A., and Chandler, P. A. 1957. The U.C. type soil mixes. Pages 68-85 in: The U.C. System for Producing Healthy Container-Grown Plants. K. F. Baker, ed. Calif. Agric. Exp. Stn. Man. 23.
16. Newman, E. I. 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3:139-145.
17. Papendick, R. I., and Cook, R. J. 1974. Plant water stress and the development of Fusarium foot rot in wheat subject to different cultural practices. Phytopathology 64:358-363.
18. Ristaino, J. B., Duniway, J. M., and Marois, J. J. 1988. Influence of frequency and duration of furrow irrigation on the development of Phytophthora root rot and yield in processing tomatoes. Phytopathology 78:1701-1706.
19. Schoeneweiss, D. F. 1975. Predisposition, stress, and plant disease. Annu. Rev. Phytopathol. 13:193-211.
20. Sharp, R. E., and Davies, W. J. 1979. Solute regulation and growth by roots and shoots of water stressed maize plants. Planta 147:43-49.
21. Sommers, L. E., Harris, R. F., Dalton, F. N., and Gardner, W. R. 1970. Water potential relations of three root-infecting *Phytophthora* species. Phytopathology 60:932-934.
22. Tennant, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63:995-1001.
23. Zimmer, D. E., and Urie, A. L. 1967. Influence of irrigation and soil infestation with strains of *Phytophthora drechsleri* on root rot resistance of safflower. Phytopathology 57:1056-1059.