Influence of Dutch Elm Disease on Resistance to Water Flow Through Roots of American Elm

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ABSTRACT

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Changes in root resistance to water flow in American elm seedlings inoculated with *Ceratocystis ulmi* were investigated. In comparison with noninoculated controls, the rate of exudation from cut stumps of inoculated plants subjected to 2 bars pressure was reduced before foliar symptoms appeared in intact plants. Specific conductance of xylem exudate from infected elm roots was also less than that from roots of healthy plants. The water potential of aboveground parts of diseased elms appeared to be more closely associated with alterations in root resistance than with the development of vascular discoloration in stems. Dutch elm disease also had a negative influence on root growth, thereby reducing the amount of new absorptive surface.

Additional key words: Dutch elm disease, *Ulmus americana,* water relations, *Ceratocystis ulmi.*

Thermodynamically, the water status of plant tissue frequently is described in terms of potential or free energy. Water transport within a plant is a function of free energy gradients and is determined by the total resistance to water movement in the soil-plant-air continuum. The influence of pathogenesis in altering water potential gradients is not well understood. The water relations of diseased plants, particularly as affected by vascular wilt diseases, have been reviewed by Dimond (5, 6), Talboys (27), and Duniway (7).

Since the movement of water through a plant system is determined, in part, by the sum of the resistances encountered in the pathway, and since resistance to water movement is greatest in the intervening tissue between the root epidermis and the xylem (26), appreciable changes in water transport can result from changes in the resistance of roots to water flow.

In previous studies on the water relations of diseased plants, the resistance to water flow through root tissue has often been neglected or assumed constant. Basipetal movement of the Dutch elm disease organism, *Ceratocystis ulmi* (Buism.) C. Moreau, in inoculated elms is well documented (1, 2, 3, 18). Colonization of root tissue by the fungus may be an important aspect of the host-parasite interaction. This study was designed to investigate the influence of Dutch elm disease on the absorption and transport of water through the root systems of American elm seedlings.

MATERIALS AND METHODS

Four-year-old American elm seedlings (*Ulmus americana* L.), 61-76 cm in height, grown in the nursery from seed were transferred to continuously-aerated nutrient solution (14) in a greenhouse under supplemental illumination (12,900 lux combined incandescent, cool-white fluorescent light from 0600 to 2100 hours). Greenhouse temperatures ranged from 19-31 C, while relative humidity was 40-50% during the day and 70-90% at night. Nutrient solutions were changed biweekly, and solution volume was kept constant by adding distilled water daily. Four elm seedlings were placed in each of eight, 10-liter plastic containers fitted with plywood covers. After 1 month, serum vial caps were affixed to each stem using the technique described by Gregory (9), and three randomly selected seedlings in each container were inoculated with 2 ml of a suspension containing 10^6 conidia per ml of *C. ulmi*. The remaining seedling in each container served as a control.

At 2, 5, 8, 11, 15, 19, 25, and 29 days after inoculation, all four seedlings in one randomly selected container were severed 2 cm above the root collar. Water potential measurements were obtained on the uppermost 38 cm of each shoot using the pressure chamber technique (25). The stems were then divided into 10 equal sections and the presence or absence of vascular discoloration at the cut distal surface of each section was noted. One 2.54-cm segment from the top of each stem section was placed on potato-dextrose agar. The presence of *C. ulmi* was determined for each segment after 7-10 days incubation at 24 C in the dark.

At the time of each water potential determination, detopped root systems were placed in a chamber which was used to force water through root tissue at a constant pressure (19). A pressure-tight seal was made around each stem and nitrogen pressure of 2 bars was applied to the enclosed root system, which was bathed in nonaerated distilled, deionized water. This procedure has been used by Mees and Weatherley (16, 17) and by O'Leary (20) to measure root permeability. Exudate was collected in a

reservoir at the top of each stump after 1.0 hour. After measuring rate of exudation, the salt content of xylem sap was estimated by measuring the specific conductance of the exudate with a conductivity bridge calibrated with KCl solutions of known concentration. After completing the measurements of water flow, 1-cm sections of fibrous root tissue were assayed for *C. ulmi* as described previously for stem tissue. Because the fibrous root segments were massively contaminated with bacteria, the procedure was changed after 2 weeks so that five small chips were sampled from the most distal woody portion of each root system. Each root system was then dried at 105 C for 48 hours and weighed.

RESULTS AND DISCUSSION

Dutch elm disease decreased the rate of water flow through the root systems of inoculated plants (Fig. 1). Within 8 days after inoculation, the movement of water from root systems of diseased seedlings was only half that from noninoculated controls. This decline continued throughout the experiment, and, after 1 month, the rate of water transport through inoculated elm roots was only 7% of that from corresponding control plants.

Since water absorption depends chiefly on the size and permeability of the root system, the presence of *C. ulmi* might be expected to interfere with one or both of these factors. We were able to isolate the fungus regularly from xylem tissue immediately below the root collar of woody elm roots. To test the effect of the disease on root growth, we looked at the dry weight of root systems from inoculated plants over the duration of the study. Assuming a positive relationship between root surface area and dry weight, the data (Table 1) show no significant increase in root growth after the 2nd week. Thus, as the Dutch elm disease fungus becomes established in the roots of inoculated seedlings, root growth is depressed and subsequent absorption and transport of water and nutrients declines.

In the 1st week after inoculation, a period during which the root systems of inoculated plants grew substantially (Table 1), the rate of exudation from detopped roots of diseased elms decreased appreciably (Fig. 1). The failure of inoculated seedlings to produce new root tissue could not account for any decrease in water absorption at this time. Apparently, the fungus directly affects the uptake efficiency of roots of inoculated plants. It is not uncommon to find histopathologic change associated with the root systems of diseased plants. Krause and Wilson (12) reported direct penetration of peritracheal parenchyma cells of American elm by *C. ulmi*. Perhaps a similar condition occurs in the root tissue, thereby causing root efficiency to decrease.

To test the effect of Dutch elm disease on root permeability to solutes, we compared the specific conductance of xylem exudate collected from the cut stumps of inoculated and noninoculated seedlings. While the conductivity of control plants remained relatively constant (Fig. 2), the conductivity of *C. ulmi*-infected plants declined steadily. After 4 weeks, specific conductance of exudate from diseased roots was less than half that of the controls. The permeability of living

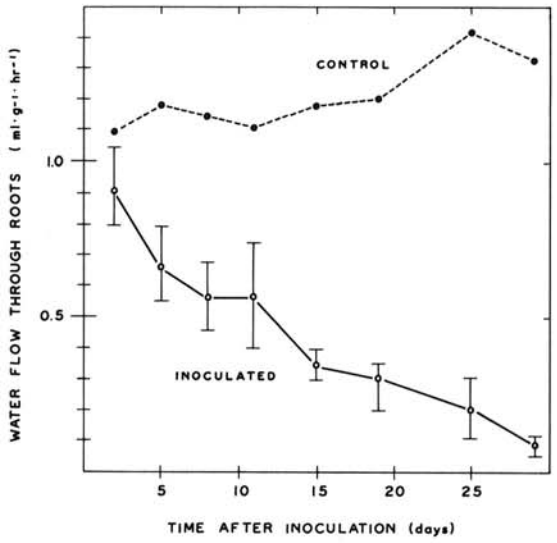

Fig. 1. Rate of water flow through detopped root systems of 4-year-old American elm seedlings after inoculation with Dutch elm disease. Data are volumes collected per unit dry weight of root tissue per hour. Each value for inoculated plants is the mean of three seedlings; each control value represents a single determination. Each bar represents the range of values observed.

TABLE 1. Influence of Dutch elm disease on root growth, shoot water potential, fungal distribution, and vascular discoloration of 4-year-old American elm seedlings[a]

Time after inoculation (days)	Root dry weight (g)	Shoot water potential[b] (bars)	*Ceratocystis ulmi* distribution[c] (%)	Vascular discoloration[c] (%)
5	2.0 y	−8.90 x	13 x	30 x
11	2.8 z	−10.52 x	58 y	40 y
19	3.3 z	−17.76 y	95 z	95 z
29	3.3 z	−22.88 z	90 z	100 z

[a]Data are means of six replicates. Means followed by different letters in the same column are significantly different, $P = 0.05$, by Student's *t*-test.

[b]Measurements made with a modification of the Scholander technique.

[c]Individual stems were divided into 10 equal sections before determining the presence or absence of vascular discoloration and then into 2.54-cm segments for measuring the presence or absence of *C. ulmi*. Values are the percentage of sections from which *C. ulmi* was cultured and the percentage of sections in which vascular browning was observed.

membranes is materially affected by several factors including temperature, aeration, and pH, which act either indirectly by affecting metabolism or directly by modifying membrane structure (11). The presence of *C. ulmi* in root tissue of diseased plants may alter host metabolism by releasing toxic by-products [as proposed by Feldman et al. (8), Kerling (10), Salemink et al. (24), and Zentmyer (30)], or by directly modifying membrane structure, as indicated earlier. The presence of foreign substances in the xylem also may affect ion transport as a result of binding and ionic competition.

Some indication of the sensitivity of root tissue to invasion by *C. ulmi* can be seen in Table 1. During the first 2 weeks after inoculation, no appreciable change was observed in the water potential of diseased shoots. However, during this same period, the flow of water was reduced about 40% as compared with the controls. These data suggest that the physiological response to Dutch elm disease becomes manifested first in the root tissue of inoculated plants. Although water flow and specific conductance are reduced during the first few days after inoculation, these alterations are not sufficient to influence shoot water potential until some time later in the disease syndrome. However, as changes in root resistance continue, shoot water potential gradually declines (becomes more negative) (Table 1).

Table 1 also shows the percentage of stem sections from which *C. ulmi* was cultured as well as the percentage of sections in which vascular browning was observed. Characteristic Dutch elm disease symptoms (foliar wilt and vascular discoloration) were observed between 5 and 11 days after inoculation, and thus were no different in this water culture study than in similar studies where plants were grown in solid media (22).

While the distribution of *C. ulmi* and the extent of vascular discoloration significantly increased in the first 11 days after inoculation, the water potential of diseased shoots remained essentially unchanged (Table 1). If the wilt symptoms in Dutch elm disease result primarily from mechanical blockage of xylem elements in the stem tissue of infected plants (4, 13, 21, 23, 29), a closer relationship between vascular discoloration and the development of an internal plant-water deficit might be expected. Although there are several possible explanations why such a relationship does not exist (15, 28), the data from this study show that there is a large, previously unconsidered, change in root resistance of inoculated plants. Thus, in early stages of the host-parasite interaction, pathological changes in root tissue caused by *C. ulmi* may be a very important factor in determining subsequent symptom expression in aerial parts of diseased elms.

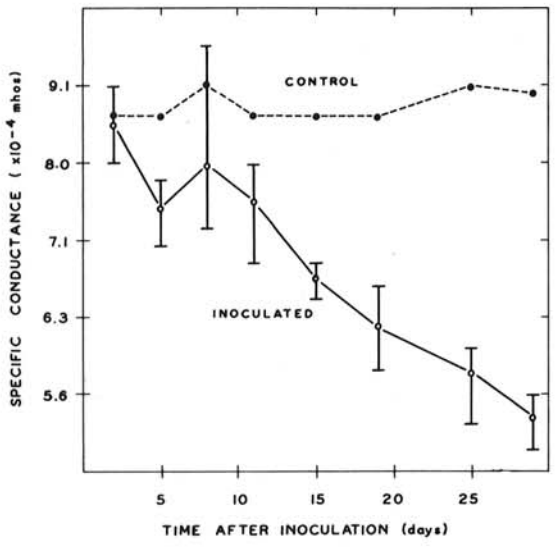

Fig. 2. Specific conductance of exudate collected from detopped root systems of 4-year-old American elm seedlings at various time intervals after inoculation with *Ceratocystis ulmi*. Each value for inoculated plants is the mean of three seedlings; each control value represents a single determination. Each bar represents the range of values observed.

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