Stomatal Resistance of Red Oak Seedlings Infected by Ceratocystis fagacearum

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ABSTRACT

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The diffusive resistance of individual leaves of northern red oak seedlings was measured with a diffusion porometer during the development of oak wilt. Stomatal resistance increased sharply approximately 3 days before visible symptoms became evident. Diffusion porometry provided a quick and nondestructive method for predicting this increase in diffusive resistance and subsequent symptom development.

Additional key words: Quercus rubra, oak wilt, water stress.

Vascular pathogens generally cause a reduction in the transpiration rate and leaf water potential of infected plants when foliar symptoms first appear (11, 12). However, in the oak wilt disease there have been indications that water stress develops sooner: both the movement of ^{86}Rb in the transpiration stream (3) and diurnal changes in stem radius due to tissue hydration (9) decreased 3-5 days before the initiation of foliar symptoms. Also, Gregory (6) reported that the transpiration rate of infected burr oaks decreased prior to wilting. However, because the behavior of all leaves on a diseased plant is not synchronous, it is difficult, especially in large trees, to determine the initial action of the pathogen on the path of water movement and the exact site(s) of dysfunction within the plant. To examine these phenomena more precisely, we studied the relationship between water stress and visible symptom expression on individual leaves of red oak seedlings.

MATERIALS AND METHODS

Acorns of northern red oak, *Quercus rubra* L., were stratified for 3-4 months at 4 C in moist peat moss and then were planted in 10-cm diameter pots containing sterile field soil. The seedlings were grown in a controlled environment chamber (15 hours per day at 660 lx, 26 C, and 40-100% RH). All experiments, which were repeated three times, were begun when seedlings selected for uniformity were 28-32 days old.

Seedlings were inoculated with a conidial suspension (1.2×10^6 conidia/ml) obtained from 8-day-old potato-dextrose agar cultures of *Ceratocystis fagacearum* (Bretz) Hunt. Each seedling was wound-inoculated with a 0.635-mm (23-gauge) hypodermic needle with a drop of inoculum (2×10^4 conidia per drop) at each of two places on the lower stem (5).

Stomatal resistance (R_s) to diffusion of water vapor from leaves was determined with a diffusion porometer (8). The stomatal resistances of the abaxial surfaces of three or four leaves from each seedling were measured daily 2.5-4.0 hours after watering. All measurements were taken from the apical half of each leaf (1). Readings were continued from the time of inoculation until foliar wilt developed. Visible symptoms consisted of leaf droop and a slight curling and discoloration at the apical leaf margins. When the stomatal resistance of each leaf on inoculated plants abruptly rose above its previously established level, the leaf was considered to be under water stress. Resistance values for healthy, control leaves had a standard error which ranged from 0.5 to 0.7 seconds cm^{-1} among experiments.

RESULTS

In a representative experiment in which 24 seedlings were stem-inoculated, the average R_s of all 87 leaves from infected seedlings increased sharply from days 7 through 10 after inoculation (Fig. 1). Initial symptoms first appeared 10 days after inoculation on 5% of these leaves. Different leaves developed symptoms from 1 to 6 days after the increase in their R_s (3.2-day average). However, a frequency distribution showed that 86% of these leaves developed symptoms within 2-4 days after an increase in their R_s and nearly 40% developed symptoms on the third day after the increase (Fig. 2).

The time at which R_s increased varied among individual leaves even on the same seedling. For example, R_s increases for four leaves of a single seedling occurred on days 6, 7, 8, and 11 after inoculation (Fig. 3). Symptoms developed on the first three leaves on day 10, and on the fourth leaf on day 13.

DISCUSSION

Infection of red oak seedlings caused an abrupt

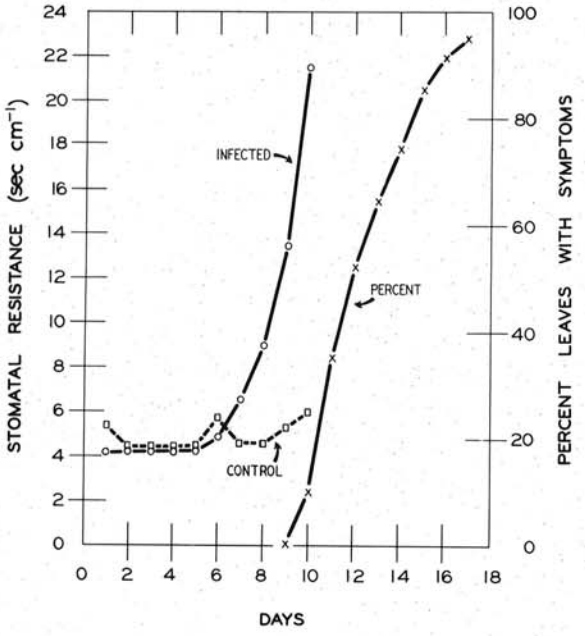

Fig. 1. Average stomatal resistance of 43 healthy (control) and 87 *Ceratocystis fagacearum*-infected leaves from red oak seedlings, and the percentage of infected leaves first showing visible symptoms on different days following inoculation.

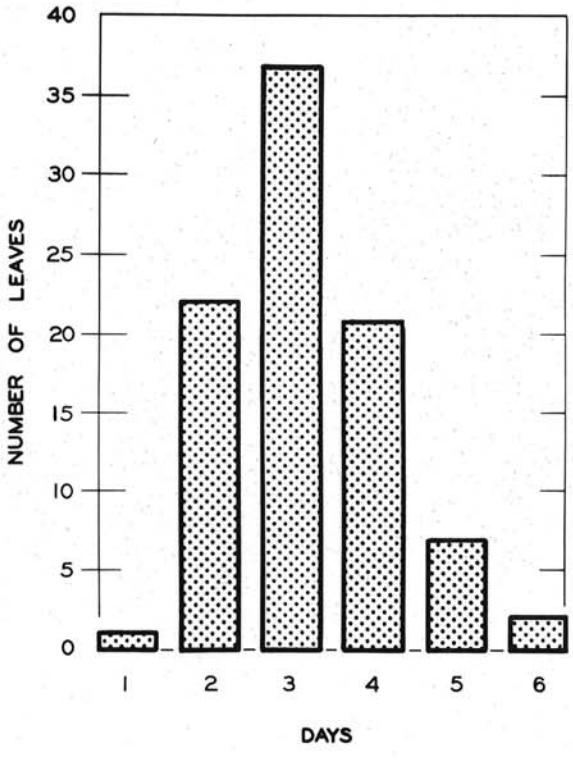

Fig. 2. Frequency distribution of the time required for symptoms of oak wilt (caused by *Ceratocystis fagacearum*) to develop on 87 individual red oak leaves after the initial increase in stomatal resistance.

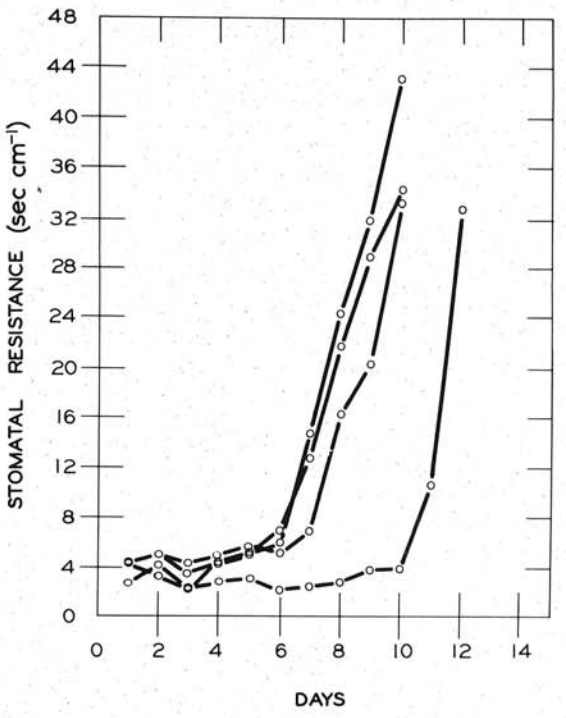

Fig. 3. Stomatal resistances of leaves on a single, representative red oak seedling following inoculation with *Ceratocystis fagacearum*. Symptoms appeared 1 day after the last resistance measurement.

increase in the resistance to diffusion of water vapor from leaves approximately 3 days before they developed visible symptoms. The reason(s) for this increase is not known. Several possibilities exist. For example, in mature oaks, foliar wilt has been attributed to the formation of gums and tyloses in the water-conducting vessels of stems and branches (3). In seedlings, water and dyes moved readily around partial stem girdles (D. Pengelly, *personal communication*). Therefore, if mechanical disruptions are important, they probably must occur generally throughout the stem to account for the asynchronous increase in leaf R_s observed with many infected seedlings. Indeed, initial observations of seedlings during the onset of the R_s increase (7-8 days after inoculation) have shown that portions of the vascular system, particularly on the sides of the inoculations, were partially filled with gums and debris (S. Jutte, *personal communication*). The R_s increase might also result from a localized obstruction in the petiole or even the leaf blade itself, as reported for Verticillium wilt of chrysanthemum (7), or from the action of a toxin-inducing stomatal closure (2, 4, 10, 13). Further studies are in progress to clarify this matter.

The onset of water stress, as indicated by the increased R_s, was sufficiently predictable of subsequent foliar wilt that a diffusion porometer can be usefully employed in various studies on water deficits of infected plants, especially in the field, providing certain precautions are taken (8). The nondestructive measurements can be made rapidly and repeatedly on individual leaves.

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