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Correlation of Isolability of the Oak Wilt Pathogen with Leaf Wilt and Vascular Water Flow Resistance

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ABSTRACT

Isolations and water flow-rate measurements made on short stem sections of young red oak seedlings inoculated with the oak wilt pathogen, *Ceratocystis fagacearum*, about 1 to 2 inches above the soil line, revealed that the oak wilt pathogen was isolable first near the inoculation site. As time after inoculation increased, the pathogen was isolated progressively farther up the stem until, when leaf symptoms became visible, it was present throughout the stem. At the onset of leaf symptoms, many but not all petioles contained the pathogen. The pathogen was never isolated from leaf blade homogenates or vessel washings from stem sections. The resistance to water flow through the vessels of the short stem sections was progressively less at greater distances up the stem from the inoculation site. Phytopathology 61: 1003-1005.

The oak wilt pathogen, *Ceratocystis fagacearum* (Bretz) Hunt, has been studied intensively, but no previous attempt has been made to correlate its growth and distribution from the point of inoculation with the development of symptoms of wilt disease and resistance to water flow through the stem in young greenhouse-grown red oaks (*Quercus borealis* Michx. S.). This study was designed to determine the location of the pathogen during pathogenesis of red oak seedlings, and to correlate this with leaf wilt and resistance to water flow in the vascular system of the tree.

MATERIALS AND METHODS.—Uniform, greenhouse-grown red oak seedlings, 1 to 2 years old (ca. 1 foot high and 0.25 inch in diameter), were inoculated with 2 ml of conidial suspension (more than 5,000,000 conidia/ml) of a single spore isolate of *C. fagacearum*. The suspension was added to a reservoir attached around the stem 1 to 2 inches above the soil line. Then three cuts about equidistant around the stem were made into the xylem with a small scalpel (5). Control trees were treated similarly with distilled water. At 3- or 4-day intervals, beginning 1 day after inoculation and continuing for about 20 days, four inoculated trees and four control trees were surface-sterilized with 95% ethanol and a 0.5% solution of sodium hypochlorite, and samples of stem and leaf tissue were removed.

One-eighth-inch cross sections of each stem were removed at 1-inch intervals from ⅛ inch above the inoculation site to the terminal. The 1-inch sections were used for liquid flow-rate measurements. The cross sections were peeled of bark and were plated on oak wilt identification agar (1).

One disc, 6 mm in diameter, was taken from each half of each leaf; no major leaf veins were included. The leaf discs from each tree were combined and put in a glass tube homogenizer; 1 ml of sterile distilled water was added. Then, with a teflon pestle, the tissue was disrupted enough for the tissue suspension to be pipetted. About 0.5 ml of each of the homogenates was plated out on oak wilt identification agar. The petioles were also plated out on oak wilt identification agar.

Water flow rates through the 1-inch serial cross sections from control and pathogen-inoculated trees were measured by timing the flow of sterile distilled water through the stem sections. To determine water flow rates, a volume of water equal to 6 times the fresh wt of each stem section was forced through the sections by 25 psi of N_2 gas. The fluid obtained through the stem sections was collected, and two 10-μliter portions and one 100-μliter portion of the fluid from each section of each inoculated and control red oak seedling were plated out on oak wilt identification agar.

All isolation plates were incubated at 24 C for 2 weeks before data were taken.

RESULTS.—As the time after inoculation increased, the pathogen was isolated from cross sections that were successively higher up the stem (Table 1). On the 12th day after inoculation, the pathogen was isolated from sections taken at 0.98 of the total distance up the stem length, but none of the trees had leaf wilt symptoms. However, 15 and 19 days after inoculation, trees did have leaf wilt symptoms. Control plants remained without symptoms.

The pathogen was not isolated from any petioles on the 1st day after inoculation. On the 5th day after inoculation, one plant had a petiole from which the pathogen was isolated. The pathogen was isolated from at least one petiole from each inoculated tree by the 8th day after inoculation. The pathogen was isolated throughout the stem and from all the petioles in two trees 12 days after inoculation, but no leaf symptoms developed in these trees. However, in one tree that had leaf symptoms (19 days after inoculation), the pathogen was isolated throughout the stem, but was isolated from only one of the petioles. The pathogen was isolated from some petioles of leaves with symptoms as well as from some petioles of symptomless leaves. Thus, it appears that isolability of the causal organism from a particular petiole is not correlated with the presence of leaf wilt symptoms on that particular leaf. However, this may be a consequence of the degree of pathogen development at the time the experimental plants were checked for isolability of the pathogen. Isolations from control plants always failed to yield the pathogen.

The validity of the isolation technique and the regu-

TABLE 1. The effect of time after inoculation on the proportion of the distance up the total stem length from which the oak wilt pathogen was isolated

Days after inoculation	Proportion of the distance up total stem length from which the pathogen was isolated[a]
1	0.23
5	0.63
8	0.93
12	0.98
15	1.00
19	1.00

[a] The number of isolations per plant depended upon height, as they were taken at inch intervals. Since the four inoculated seedlings at each time period had a total of about 50 inches of stem length, one topmost stem isolation that did not yield the pathogen would result in a proportion of 0.98.

lar progression of isolability of the pathogen from red oak seedlings has been repeatedly verified by inoculating red oak seedlings about halfway or more up the stem and then isolating from the whole stem. In such cases, the pathogen is isolated up and down the stem from the inoculation site. The distances varied with time after inoculation, but the distance at which the pathogen could be isolated above the inoculation site was almost always greater than the distance below the inoculation site.

The pathogen was never isolated from any of the homogenates of the leaf blade tissue. Since inhibition of oak wilt fungus growth by oak leaf phenols was considered a possibility, conidia were added to some similar homogenate, and oak wilt fungus growth occurred. This does not imply that the pathogen might not invade the leaf blade in very small amounts, for only a small per cent of the total leaf area was plated out. Also, it seems likely that in some cases the pathogen probably was in the leaf midrib, if not in the major leaf veins.

Average flow times through the 1-inch stem sections were compiled for quarter lengths of the stems (Table 2). The first quarter was considered to start ⅛ inch above the inoculation site. The data from the fourth quarter were not included in Table 2 because that quarter was mostly current growth (greenwood) that was generally collapsed; thus, flow-through time in the fourth quarter was either extremely long or indeterminant for both the inoculated and control tree sections.

Resistance to water flow showed a marked increase in the first and second quarter 8 days after inoculation. Fifteen days after inoculation, the flow through some of the stem sections had essentially stopped. There was no reduction in water flow rate in the sections taken from control trees.

Examination of flow data from individual trees showed that all trees that had leaf wilt symptoms also had markedly reduced flow rates. However, not all trees whose stem sections had greatly reduced flow rates had leaf wilt symptoms.

The pathogen was never isolated from any of the

TABLE 2. Time (sec) required for water passage through oak wilt-infected stem sections

Days after inoculation	Resistance to water flow[b]		
	1st Stem quarter[c]	2nd Stem quarter	3rd Stem quarter
1	69	53	58
5	66	63	69
8	380	670	67
12	241	937	276
15	Very high[d]	Very high[d]	Very high[d]
19	Very high[d]	Very high[d]	Very high[d]

[a] Control sections showed no reduced water-flow rate.

[b] The data from which this table was compiled was taken in time (in sec) required for water equal to 6 times the fresh wt of a particular stem section to pass through that particular stem section. The data in this table are the average of flow times for all 1-inch sections in a particular quarter length of all four infected plants harvested at that time.

[c] The resistance to water flow was tabulated on the basis of one-quarter lengths of the stem because the greenwood (4th stem quarter) of both inoculated and noninoculated trees was generally collapsed and gave very long or indeterminate flow times, and because averages based on linear distances would have differing numbers of sections upon which to calculate averages.

[d] Many sections had passed only a small fraction of the water after 1,200 or more sec, so testing was discontinued.

fluids washed through the stem sections. Thus, it appears that, if free-floating culturable propagules were present in the vascular stream, they were very few and possibly were readily trapped or screened out. However, the situation in larger trees may be different because their vessels are longer and bigger around.

The results reported here confirm those of several experiments with red oak and one with bur oak (using greenhouse plants of about the same size) performed at different times of the year in the greenhouse. However, in general, time required for symptom development in the other experiments was longer; and symptom development was less uniform.

DISCUSSION.—Length of time that the oak wilt pathogen has been present in a particular host-stem section is apparently correlated with increase in resistance to water flow through vessels of that stem section. Beckman et al. (3) reported reduced water flow through cuttings from inoculated large northern pin oak trees (*Q. ellipsoidalis* Hill) at 3 days before wilt. By the time severe wilt symptoms were present, the water flow was drastically reduced.

In another study, Kuntz et al. (8) reported that the oak wilt pathogen was often isolated from the entire tree (large northern pin oak) a week before, and consistently at the time of, initial symptoms. Also, in studies on oak-wilt-inoculated large bur oak trees, Parmeter et al. (9) reported that isolations from symptomless branches were negative, whereas isolations from branches with symptoms were positive.

Henry & Riker (7), studying the vertical distribution of the oak wilt fungus in black oak (*Q. velutina* Lam.), red oak (*Q. borealis* Michx. S.), bur oak (*Q. macrocarpa* Michx.), and white oak (*Q. alba* L.), state that the host species did not seem to influence the ver-

tical distribution of the pathogen. However, Bart & Young (2), isolating in increments up and down from the inoculation site on the trunks of oak-wilt-inoculated nursery-grown red oaks (*Q. borealis*) and white oaks (*Q. alba*), established distances moved based on ability to isolate the pathogen. Maximum upward movement was 174 inches in red oak and only 15 inches in white oak.

It must be concluded from this study that the causal organism is not present or is present at very low concentration in fluids that can be washed from these stems. The sparse and longitudinal development of hyphae in the vessels before severe wilt symptoms develop has been noted (4, 10). These facts, together with the ability to isolate the pathogen in an uninterrupted series up and down the stem from the inoculation site, causes me to contemplate this apparent growth when considering the vertical distribution of the pathogen. Spores have been shown to pass through the vessels of oak stem sections (2, 10). Bart & Young (2) reported little or no screening out of conidia from conidial suspension by vessels of white or red oak. If vertical distribution is by means of secondary spores, then perhaps the screening-out experiments and the attempts to culture fluid from the vessels is giving considerable bias to the larger vessels (the smaller vessels having much greater resistance to flow). Fergus & Wharton (4) reported that no fungal spores were found in examining more than 300 sections of stems and roots, which is consistent with my failure in obtaining cultures from vascular wash fluid.

The pathogen as reported herein was not found in leaf blade tissue; hence it was concluded that the pathogen was not present there in any appreciable quantity. Other researchers (6, 7) have reported a few sporadic successful isolations of the pathogen from leaf blade tissue. It appears that the pathogen usually makes no appreciable growth in the leaf blade.

The results of my attempts of isolation from petioles revealed that for any given leaf the pathogen may or may not be present at the time of initial symptom expression.

At the time when leaf symptoms are evident, the pathogen appears to be present throughout all or nearly all of the stem; and stem sections have markedly increased resistance to water flow. Although more data are necessary, it appears that the pathogen must be in a particular stem section 5 to 10 days under these experimental conditions for water flow through that section to be retarded.

Generally, in wilt diseases, it is comparatively difficult to relate the location of the pathogen to development and progression of the disease. However, for host-pathogen interaction studies to have appreciable significance, these relationships must be determined.

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