The Occurrence and Cause of Cavitation of American Elm Shoot Nodal Tissue Infected by Ceratocystis ulmi

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ABSTRACT

The cause and nature of cavities observed in infected shoot nodes and the relationship between this nodal cavitation and foliar wilt development were examined. Cavitation occurred in thin-walled pith parenchyma tissue that is highly sensitive to drying conditions. Cavitation was induced experimentally by infection with *C. ulmi*, by desiccation, and by exposure to hydrolyzing enzymes produced in vitro by *C. ulmi* followed by desiccation. Nodal cavitation is not essential to foliar wilting. Cavitated shoot nodes were never noted prior to foliar wilting, and shoots bearing wilted leaves did not always have "pocketed" nodes. Vessels bordering cavities remained functional. The presence within vessel elements of culture filtrate containing hydrolyzing enzymes did not interfere with dye movement into leaves.

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Cavitation within the nodes of American elm (*Ulmus americana* L.) shoots infected with *Ceratocystis ulmi* (Buism.) C. Moreau has not been previously reported, although cavities have been observed in medullary rays (8). In a recent study, however, cavities were observed within shoot nodes of infected 3- to 5-yr-old American elms. Concomitant physiological studies on these infected elms indicated that foliar wilt and chlorosis were associated with a reduction in the amount of water available to the leaves due to blockage of water-conducting elements, particularly within one-yr-old twigs, shoot nodes, and leaf nodes (6).

C. ulmi is known to produce cellulases (1) and pectinases (1, 4), but the effect of these hydrolytic enzymes on elm wood is not known. In one instance Holmes et al. (4) showed that *C. ulmi* can produce pectinolytic enzymes in vivo. Fluid extracted from the vascular elements of an infected elm prior to the appearance of leaf wilt produced a steady increase in fluidity of a 0.5% sodium polypectate solution. Dimond (2) suggested that pectic compounds exposed at pits of vessels are particularly vulnerable to hydrolyzing enzymes. Gagnon (3) noted the presence of lignin and pectin materials in vessel plugs and suggested that pectolytic enzyme action on vessel walls may be directly involved in pathogenesis.

The objectives of this study were (i) to identify the nodal tissue(s) involved in cavitation, (ii) to determine whether lysis caused by hydrolytic enzymes produced in vitro by *C. ulmi* can account for the cavitation, (iii) to determine whether water stress can account for cavitation, and (iv) to assess the relationship between tissue cavitation and the appearance of foliar symptoms.

MATERIALS AND METHODS.— Potted American elms 3-5 yr old were placed in growth chambers and inoculated with a *C. ulmi* composite spore suspension. The programming of the chamber environment, preparation of inoculum, and inoculation procedure have been previously described (6).

To obtain *C. ulmi* metabolites, a liquid medium was used which contained lg KH_2PO_4, 5g diammonium citrate, 5g pectin NF, and 20g dextrose/liter of distilled H_2O. Aliquots (200-ml) were dispensed into cotton-stoppered 500-ml Erlenmeyer flasks and autoclaved. Each flask was seeded with 5-ml portions of a turbid spore suspension (approximately 1×10^6 spores/ml) prepared from 18-day-old cultures of four *C. ulmi* isolates grown on potato-dextrose agar. Seeded flasks were placed on a shaker at 120 cycles/min. After incubation in darkness at room temperature for 6 days, the cultures were Millipore-filtered (0.22μm) into sterile flasks. One ml of merthiolate (1:5,000) was added per 100 ml of culture filtrate before filtering.

Viscometric assay was used to measure pectolytic and cellulolytic activity. Two-ml aliquots of culture filtrate were added to 4-ml aliquots of 1.5% pectin NF or methyl cellulose solutions. The mixtures were poured into Ostwald-Fenske viscosimeters, size 300, and incubated for 90 min at 30 C. The tests were replicated three times for each substrate. Culture filtrate, boiled for 10 min, served as a control. The percentage decrease in flow time was calculated according to Kelman and Cowling (5).

To study the effect of extracellular enzymes in elm nodal tissue, 1 ml of culture filtrate, boiled culture filtrate, or sterile distilled water was dispensed into sterile 1-ml and 5-ml beakers. Transverse and longitudinal sections of 55 shoot nodes and 23 leaf nodes were placed into the beakers. After 24 hr the sections were examined microscopically for tissue maceration, browning, and vessel blockage. Boiled culture filtrate and sterile distilled water served as controls.

The effect of culture filtrate on water movement within shoots was determined with steam-sterilized gravity-fed columns. Each column consisted of a funnel attached to 100 cm of polyethylene tubing. Sixty ml of culture filtrate, boiled culture filtrate, sterile 0.1% light green SF dye solution, or sterile distilled water were added asceptically to each column. Elm twigs were obtained from 5- to 7-yr-old potted American elm trees growing in a greenhouse. Twigs were excised within four wk after bud break. Following surface sterilization with 10% sodium hypochlorite solution, 1-, 2-, or 3-year-old foliated twigs were severed and attached to the columns containing culture filtrate or water. Each twig was then removed and attached to a dye-filled column prior to sectioning to obtain a pattern of dye distribution through functional water-conducting tracheal elements. After 24 or 48 hr, shoot and leaf nodes were sectioned and examined microscopically for tissue maceration, browning, and vessel blockage.

RESULTS.— The hydrolytic activity of culture filtrates was first determined. Increases in the fluidity of pectin and methyl cellulose solutions averaged 57 and 47%, respectively. These results are comparable to those of Beckman (1) who recorded fluidity increases of 60 and 45% in pectin and cellulose solutions, respectively, after the addition of culture filtrates from 6-day-old *C. ulmi* cultures. Both pectolytic and cellulolytic enzymes were therefore produced by *C. ulmi*.

When the twigs of infected elms were examined, cavities (Fig. 1) were found in approximately 25% of the shoot nodes of 17 trees. Foliar wilt always appeared in leaves distal to cavitated nodes. However, wilting often occurred in the absence of cavities. Coremia of *C. ulmi* were produced abundantly throughout all cavitated nodes on twigs that were sectioned longitudinally, placed with the cut surface uppermost, and incubated on potato-dextrose agar.

Transverse and longitudinal sections of shoot and leaf nodes were placed in boiled or unboiled culture filtrate or in sterile distilled water for 24 hr to determine whether cavitation could result from the hydrolytic action of enzymes produced by *C. ulmi*. Occlusion of vessel elements was not evident in tissue sections following any of the above treatments. Sections placed in water exhibited no tissue maceration, but browning of scattered pith cells occurred within shoot nodes. Sections placed in boiled culture filtrate also showed no tissue maceration, but browning of shoot nodal tissue was extensive. Shoot nodes placed in untreated culture filtrate developed extensive discoloration of the pith. Maceration of pith, cortical, and vascular parenchyma tissue occurred. Xylem tissue could be teased apart following such treatment, but individual tracheal elements remained intact. Maceration of pith tissue was restricted to shoot nodes and within precisely the same location in which cavitation had been observed in infected nodes (Fig. 1). Cells comprising the nodal pith tissue were easily separated by teasing with a probe, but no "pocketing" occurred until after the sections were desiccated by exposure to the air. Cavitation did not occur in leaf nodes.

Longitudinal sections of four shoot nodes from freshly-

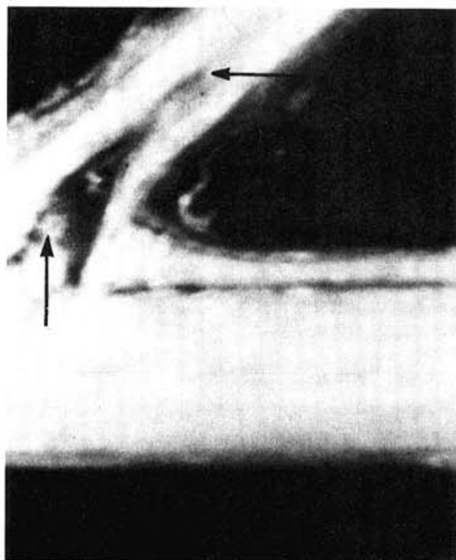

Fig. 1. Section of a diseased elm shoot node showing the cavity that develops (horizontal arrow) and the terminus of dye distribution (vertical arrow) distal to the cavitated region.

excised twigs were examined microscopically to see if the limitation of pith maceration to the "pocketed" area could be explained anatomically. It appeared that the tissues subject to cavitation are distinguishable by parenchyma cells of high water content. To determine how sensitive these cells are to drying, six additional shoot nodes were cut lengthwise and left exposed to the air. Within 30 min the nodal pith tissue had browned and a cavity had formed, apparently due to tissue desiccation.

Two twigs were excised from a healthy tree and placed on a laboratory table for 2 days until all leaves had wilted. No cavitation occurred in six shoot nodes examined, indicating that it does not immediately follow the wilting of uninfected shoot nodes. Three of six nodes examined after 4 days of exposure to drying conditions, however, did show browning and cavitation. The three cavitated shoot nodes were located at the basal end of the excised twig whereas the three healthy-appearing nodes were near the distal end.

To determine whether the presence of culture filtrate within vessel elements would result in cell wall break-down and contribute to interference of water movement to the leaves, six twigs were attached to gravity-fed columns filled with culture filtrate or boiled culture filtrate and left for 24 hr. The twigs were then attached to gravity-fed columns filled with light-green dye solution. Examination of leaf and shoot nodes showed that neither tracheal elements nor pith parenchyma cells were discolored nor was dye movement into the leaves obstructed.

DISCUSSION.—The findings indicate that nodal cavitation is not essential to foliar wilt, but that it is an additional symptom of the Dutch elm disease. Many shoot nodes of infected twigs were examined over a 2-yr period, but cavitated shoot nodes were never noted prior to foliar wilt. Nodal cavitation was always associated with foliar wilt, but many shoots bearing wilted leaves did not have cavitated nodes. Although dye penetration often ended at or near a cavitated node, passage of dye was also noted beyond the cavitated area (Fig. 1). Thus, uninterrupted water movement was found to occur through vessels bordering the cavitated zone.

Zudilin (8) observed cavities within medullary rays of infected elms and attributed their formation to the destruction of parenchymatous cell walls. In the present study, nodal cavitation occurred in a thin-walled parenchyma tissue highly sensitive to drying conditions. Cavitation of this tissue occurred as a result of desiccation alone, but was accelerated when desiccation followed maceration by hydrolyzing enzymes produced by *C. ulmi* in vitro. That healthy nodal tissues varied in susceptibility to cavitation was shown by subjecting excised twigs to drying. After 4 days of exposure, three shoot nodes located at the basal end of a twig were cavitated while three nodes at the distal end remained symptomless. It appears from these results that either the distal end had not desiccated sufficiently to produce cavitation or that the osmotic value and water retaining power of the more distal tissues may have been higher than that of the basal tissues.

Cavitation was noted within 2 days after initial leaf wilt on infected twigs but only after 4 days in artificially desiccated twigs. It is proposed that the apparent hastening of desiccation and cavitation within the nodes of infected twigs exhibiting foliar wilt, as compared to healthy twigs subjected to water stress, may be due to tissue maceration and changes in cell membrane permeability associated with fungal activity within the node. The fungus presumably had considerable time to develop both physically and physiologically and to colonize nodal tissue prior to symptoms since, as Pomerleau and Mehran (7) showed, fungal spores accumulate at shoot nodes shortly after introduction into elm trees. In addition, the present study has shown that this tissue is macerated by enzymes produced in vitro by *C. ulmi*.

These studies suggest that elm nodal tissue may provide not only an excellent site for beetle feeding and the concomitant introduction of spores of *C. ulmi* into vascular elements, but also that the nodal tissue provides an excellent environment for the development of *C. ulmi*. Nodal tissue therefore could serve as an infection court from which fluids and inoculum could be withdrawn into more distal tissues through the vascular system or as an energy base from which the fungus could make rapid entry into the vascular elements. It could also serve as an excellent source of inoculum for direct node-to-node transmission of the pathogen by adult beetles in the act of feeding. All of these factors may contribute significantly to the disease cycle.

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