Evidence for Xylem Dysfunction by Embolization in Dutch Elm Disease

Dennis Newbanks, Arjen Bosch, and Martin H. Zimmermann

Harvard University, Cabot Foundation, Petersham, MA 01366.
Accepted for publication 5 January 1983.

ABSTRACT

Newbanks, D., Bosch, A., and Zimmermann, M. H. 1983. Evidence for xylem dysfunction by embolization in Dutch elm disease. Phytopathology 73:1060-1063.

Conducting vessels of elm seedlings were stained by dye uptake from a vacuum container during transpiration. Nonconducting vessels remained unstained or their walls were only stained where in direct contact with conducting vessels. The staining pattern of the vessel network was analyzed by shuttle microscopy over distances of 1,000 transverse sections, spaced 0.1 mm. Nonconducting vessels in infected stems were thus identified before any other visible effect, such as entry of hyphae or gums, could be detected.

Additional key words: vessel length.

Symptom development in American elms infected by *Ceratocystis ulmi* is considered to be largely the result of the interruption of xylem water movement from the roots to the leaves (1,4). Fungal growth, phytotoxic fungal metabolites, and formation of gums and tyloses by the host plant have all been implicated as factors contributing to this xylem dysfunction (5,7). The cause of this dysfunction is uncertain, however, and all of these factors may contribute to some extent.

Elms are ring-porous trees in which water conduction is almost entirely restricted to the large, very efficient earlywood vessels of the most recent growth ring. Their size and superficial location makes these vessels extremely vulnerable to injury (4). In healthy elms, embolization is a normal event and may be considered the first step of heartwood formation. It can be induced by wounding, winter freezing, and moisture stress and also simply by aging (8). It is, therefore, reasonable to assume that embolization may contribute to the interruption of water movement in diseased elms (10). D. M. Sylvia found that dye ascents in trees with Dutch elm disease left an unstained zone of wood between the vascular discoloration and the functional xylem (*unpublished data*). However, the presence of embolized vessels is difficult to demonstrate due to the normal xylem tension. Functional vessels become air-filled when wounded, but they may be refilled when they come into contact with liquid at atmospheric pressure. Therefore, measurement of conductivity or determination of the functional xylem area by dye perfusion of a piece of stem from an injured plant in a laboratory may produce results that do not represent the condition of the intact plant in nature.

The objectives of our research were to develop techniques that would allow the detection of embolized vessels and to determine whether embolization is a cause of xylem dysfunction in *C. ulmi*-infected American elms (*Ulmus americana* L.).

MATERIALS AND METHODS

Plant material. Potted American elm seedlings used in all experiments were approximately 1 m in height and 6–10 mm in diameter at 10 cm above the soil line. The plants were grown in a peat, perlite, and sand (1:1:1) mixture, fertilized semimonthly, and maintained in a greenhouse.

Inoculation. A single isolate of *C. ulmi* (Buism.) C. Moreau from an infected American elm branch was grown in shake culture (Tchernoff-Zentmyer medium) (3) for 10 days, then seeded on potato-dextrose agar. After 10 days, 10 ml of distilled water was added to the plates, stirred gently, and filtered through cheesecloth. The resulting spore suspension, containing 4.8×10^6 spores per milliliter, was used as inoculum.

All plants were wounded by inserting a cut-off, 3-mm-long, 20-gauge hypodermic needle into the main stem approximately 10

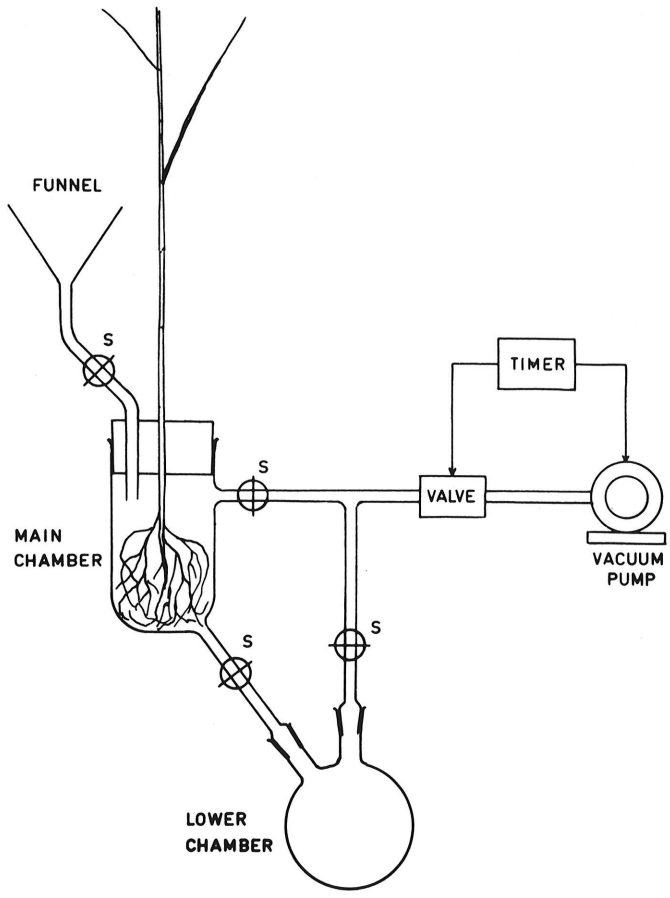

Fig. 1. Experimental design for dye ascents into elm seedlings. The root system takes up liquid from the main chamber, which can be drained into a lower chamber or refilled from a funnel without releasing the vacuum. The experiment can be run for long periods by operating the vacuum pump periodically (eg, 1 min every half hour) by an electronic timer. The timer also shuts the line between the pump and the chambers shortly before the pump turns off and opens it shortly after the pump runs again. S = stopcocks.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

cm above the soil line. Three plants were inoculated by inserting a similar needle into each wound and injecting *C. ulmi* spore suspension by pressurizing the attached syringe. The two control plants received distilled water in place of inoculum.

Dye ascents. Periodic acid Schiff's reagent (PAS) (2) was used for all dye ascents. The cut stem or the roots of intact plants were placed in periodic acid, vacuum infiltrated, and allowed to take up the acid for 1 hr. They were then rinsed in distilled water and placed in Schiff's reagent until dye was visible in the uppermost leaves (usually after 1–2 hr). All dye ascents were conducted under laboratory conditions with supplemental lighting and circulating fans to increase transpiration.

Dye ascents into cut stems. A 1-cm-diameter twig was cut from a naturally infected American elm tree; the cut end was trimmed with a razor blade and vacuum infiltrated, and a PAS dye ascent was conducted under atmospheric pressure. Similar dye ascents were conducted under vacuum on three elm seedling stems.

Dye ascents into intact stems. PAS dye ascents were conducted on seedlings 4, 14, and 23 days after inoculation (seedlings A, B, and C, respectively) and on two control seedlings 9 and 11 days after injection of water. The seedlings were removed from their pots and their roots washed gently of all soil. The entire root system was then placed in a vacuum chamber with the stem extending through the top (Fig. 1). Air was evacuated from the chambers to 0.1 or less atmospheric (10 kPa) pressure. This reduced pressure was maintained by running the vacuum pump periodically by an electronic timer, which also closed the vacuum line shortly before the pump was turned off and opened it again shortly after the pump was turned on. The double chamber arrangement allowed vacuum to be maintained even during the draining or refilling of the main chamber that contained the root system. When the dye was visible in the uppermost leaves of the plant, the main chamber was drained of Schiff's reagent, rinsed with distilled water, and again drained. Vacuum was maintained in the chamber for 24 hr before the plant was removed. A 10-cm stem segment was cut from each of the three inoculated and two control seedlings (from 2 cm below to 8 cm above the injection wound), sectioned, photographed, and analyzed.

Cinematographic analysis. All five 10-cm stem segments were cut into 50-μm sections using a sliding microtome. Every other section was then graded through alcohol and xylol, and mounted in Permount. Preliminary studies had shown that alcohol and xylol treatment of stem sections resulted in no detectable degradation of vessel occlusions. Each series of 1,000 sections was photographed sequentially using a shuttle microscope (11). The films were then analyzed with a 16-mm Vanguard analyzer and the vessel networks were reconstructed in the form of three-dimensional drawings.

Vessel length. Of the four American elm seedlings used in this experiment, seedlings 1, 2, and 3 were about 1 m tall and seedling 4 was about 1.5 m tall. Approximate maximum vessel length was first determined by finding the minimum length of stem through which a stream of air could not be forced. A stem segment somewhat longer than the maximum vessel length was then cut from each seedling beginning 10 cm above the soil line. The upper end was trimmed with a razor blade, and a piece of rubber tubing was attached. The tubing was filled with distilled water and vacuum infiltrated for 1

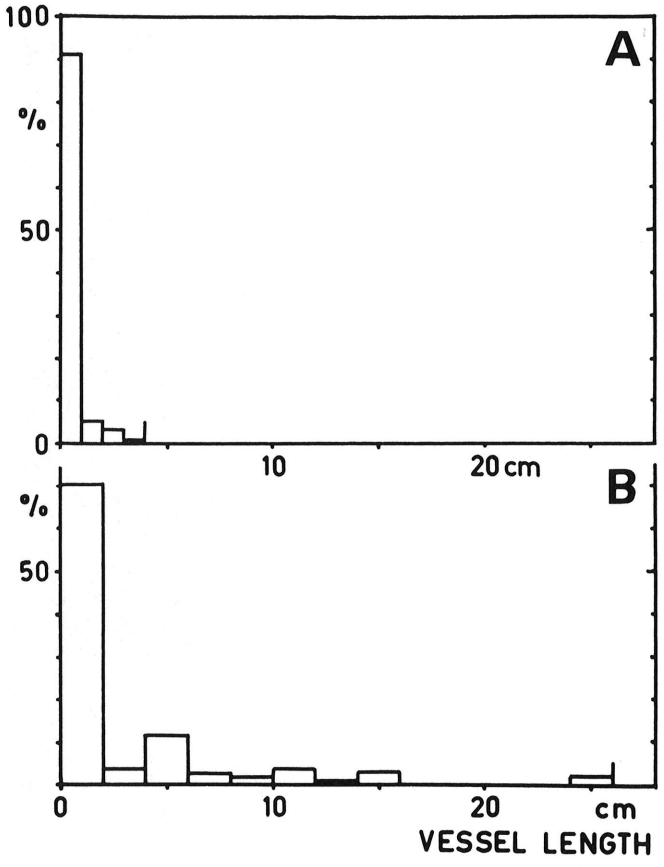

Fig. 2. Vessel length distribution in the stems of two elm seedlings. **A,** Seedling (about 1 m tall) had a maximum vessel length of 4 cm. **B,** Seedling (about 1.5 m tall) had a maximum vessel length of 26 cm (indicated by the small vertical line at far right). Percentages are per transverse sectional area.

Fig. 3. Vessel network of part of the stem of an elm seedling (control), reconstructed by cinematographic analysis. Broken vessels at 1.6 cm on the axial scale show the injection injury. The dye was taken up from a container under reduced pressure. Black areas show vessels stained with periodic acid Schiff's reagent. Injured and, therefore, air-blocked vessels did not conduct dye; their walls are intermittently stained only at points of contact with functioning vessels. Arrow indicates staining from contact with dye-carrying small tracheary elements that are not shown in the diagram. The axial scale is about 10 times foreshortened compared with the horizontal scale.

min. A dilute latex paint suspension was then applied while the stem segments were held upright for 5 days, allowing latex particles to flow into the cut stems by gravity. The stems were cut into 1-cm lengths, their ends were examined, and paint-filled vessels were counted. Vessel length distribution was calculated from these counts (9).

RESULTS

Vessel length distribution. Maximum vessel length in seedlings 1 and 2 was less than 5 cm. Seedling 3 had a maximum vessel length of less than 10 cm; 88% of the vessels were less than 5 cm long. Seedling 4 (1.5 m tall) had a maximum vessel length of 26 cm, and 77% of the vessels were less than 2 cm. Figure 2 shows the vessel length distribution in seedlings 1 and 4.

Cut stem dye ascent. PAS dye, taken up from atmospheric pressure into the cut twig of a naturally infected tree and ascending through it, resulted in intermittent or partial staining of wounded vessels. These vessels were air-blocked due to wounding. The fact that they were not stained along parts of their length indicates that they had not been normally conducting but that their staining was an artifact. Dye had partially refilled these wounded and, therefore, embolized vessels by capillarity through their contacts with nonwounded conducting vessels and rays. Vessels that had been severed when the stem was cut and had thus been embolized also showed partial dye staining beginning at their cut ends.

Dye ascents into cut elm stems under vacuum were unsuccessful. The plants were unable to take up dye against vacuum (reduced pressure) and wilted rapidly. This is not surprising; the principle has been known for many years (6). Water uptake into cut stems is via cell walls, uninjured tracheids, and small vessels. If these pathways are poorly developed as in ring-porous trees, a cut stem cannot take up enough water to support transpiration, although this is possible with many other plants. Dye was, therefore, introduced into the intact root system.

Intact control plants treated under vacuum. PAS dye ascents in the two wounded uninoculated elms resulted in the complete staining of all uninjured, conducting xylem vessels whereas all wounded (ie, embolized) vessels remained unstained throughout their lengths, except where they were in direct contact with parallel stained (functional) vessels (Fig. 3). This contact staining of the air-blocked vessels never extended beyond the areas of contact. However, dye movement through small tracheary elements occasionally resulted in contact staining of wounded vessels as well (Fig. 3).

Intact inoculated plants treated under vacuum. PAS dye ascents of seedlings A, B, and C infected by *C. ulmi* resulted in staining of wounded vessels only at points where they were in contact with dyed, conducting xylem vessels (Fig. 4). Vessels ending below the wound, beginning above the wound, or passing in close proximity to the wound were either completely stained, completely unstained, or stained only at contact points with dyed vessels. This pattern of staining was found without exception in all three infected seedlings.

Isolations. *C. ulmi* was isolated from all three inoculated seedlings 8–10 cm above the inoculation sites. Microscope survey of the 1,000 transverse sections of seedling A (collected 4 days after inoculation) showed no evidence of hyphae or occlusions in vessels. Seedling B (collected 14 days after inoculation) showed hyphae in unstained vessels, but neither hyphae nor occlusions were found in dye-conducting vessels. Occlusions were visible in many unstained vessels to some extent. Seedling C (collected 23 days after inoculation) was noteworthy in that all stem vessels that had been present at the time of inoculation were nonconducting and contained various amounts of hyphae and occlusions. Dye movement was restricted to vessels formed after the injury.

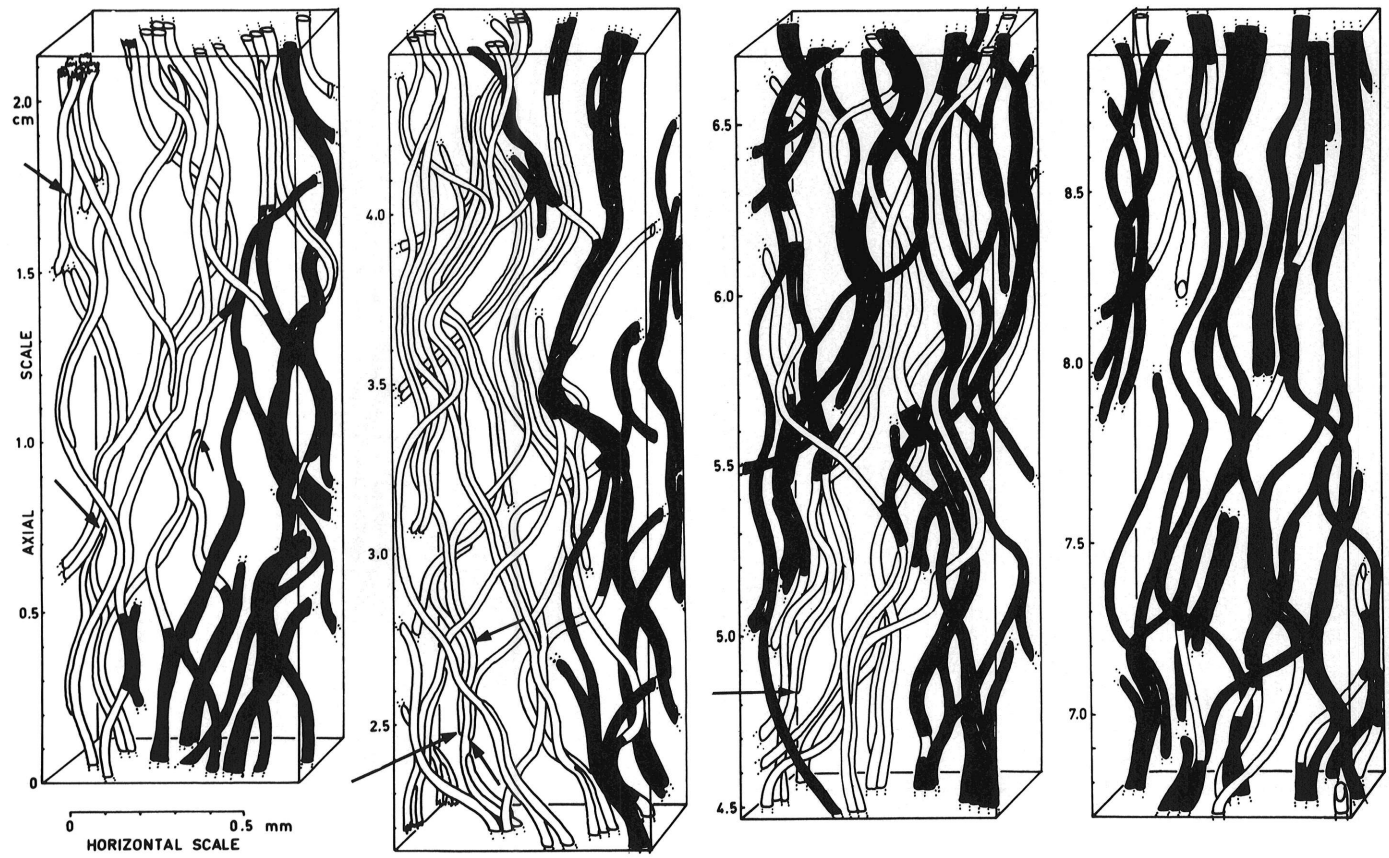

Fig. 4. Reconstruction of an 8.9-cm-long section of the vessel network of elm seedling A, collected 4 days after inoculation. Broken vessels at about 2.1 cm on the axial scale indicate the inoculation injury. Dye-stained vessels are black. Vessels that were injured by the infection puncture are blank. Arrows indicate tips of vessels that were entirely outside the mechanical injury area but were not conducting. These vessels were not functional and presumably vapor-blocked. The axial scale is foreshortened about 10 times compared with the horizontal scales.

DISCUSSION

Vessel length distribution in the stems of north temperate trees was discussed in detail by Zimmermann and Jeje (9). Ring-porous species have very wide and also very long vessels (up to many meters). Although elm was not discussed by Zimmermann and Jeje (9), we know from unpublished measurements that the vessel length distribution in stems of mature elm trees is not much different from those of oak and ash. However, vessels of seedlings are much narrower and much shorter than those of larger trees.

PAS dye ascents under atmospheric pressure led to intermittent staining of wounded vessels between contact points with adjacent dye-conducting vessels. Partial refilling of severed vessels by capillarity was also evident. These artifacts were caused by cutting the stem and bringing it into contact with the dye solution under atmospheric pressure, thus relieving xylem tension. PAS dye ascent through roots under vacuum allowed these problems to be overcome. It is very likely that the PAS treatment killed the roots, but this would not have affected our experiments.

Staining of nonfunctional vessels via contact points with functional vessels was clearly evident. In individual stem sections, contact staining could not be distinguished from staining due to dye conduction. The microtome sections show stained or unstained cell walls, but looking at single sections does not indicate whether the wall was stained by dye conduction through the vessel lumen or by secondary spreading through the wall alone. This information can only be obtained by thorough analysis. A vessel stained along its entire length probably was stained by conduction of the dye solution. If a vessel is not stained except for contact staining, however, it cannot be conducting. Whenever we encountered stain along short vessel sections, it was always in contact with conducting vessels (Figs. 3 and 4). It is, therefore, apparent that conclusions concerning the nature of xylem dysfunction cannot be based on individual stem sections or short series of sections.

Control and inoculated seedlings showed similar patterns of staining of wounded vessels. In inoculated plants this same pattern of staining was also seen in nonfunctional, but unwounded, vessels, ie, those the entire length of which was located above or below the inoculation site but which were directly connected to wounded, inoculated vessel (Fig. 4). The similarity of the patterns in inoculated and control plants suggests that embolization is a factor for vessel dysfunction in this experimental system. Enzymatic degradation of vessel walls may cause vapor blockage; fungal hyphae do not have to enter a vessel to accomplish this (10). This means that the xylem may become nonfunctional before any other form of plugging becomes evident (eg, seedling A, Fig. 4) and that plugging may indeed be a secondary phenomenon. If occlusions were the primary cause of cessation of water conduction, dye would intermittently occur between occlusions as it moved through adjacent functional and partly occluded vessels along the existing pressure gradient. Our preliminary findings indicate that this may be the case in *Verticillium*-infected sugar maples (*Acer saccharum* L.) in which such intermittent staining occurred and fungal hyphae were found in these vessels. These hyphae may have entered vessels without breaking the stressed water columns and thus without admitting air.

LITERATURE CITED

1. Beckman, C. H. 1980. Pathogenesis. Page 16 in: Compendium of Elm Diseases. R. J. Stipes and R. J. Campana, eds. Am. Phytopathol. Soc., St. Paul, MN.
2. Chayen, J., Bitensky, L., Butcher, R. G., and Poulter, L. W. 1969. Pages 74-77 in: A Guide to Practical Histochemistry. Lippincott Co., Philadelphia, PA.
3. Holmes, F. W. 1965. Virulence in *Ceratocystis ulmi*. Neth. J. Plant Pathol. 71:97-112.
4. Huber, B. 1956. Die Saftströme der Pflanzen. Springer-Verlag, Berlin. 126 pp.
5. Stipes, R. J., and Campana, R. J. (eds.). 1981. Compendium of Elm Diseases. Am. Phytopathol. Soc., St. Paul, MN. 96 pp.
6. Ursprung, A. 1913. Ueber die Bedeutung der Kohäsion für das Saftsteigen. Ber. Dtsch. Bot. Ges. 31:401-412.
7. VanAlfen, N. K., and MacHardy, W. E. 1978. Symptoms and host-pathogen interactions. Pages 20-25 in: Dutch Elm Disease: Perspectives after 60 Years. W. A. Sinclair and R. J. Campana, eds. Cornell Univ., Agric. Exp. Stn. Search (Agric.) 8(5):1-52.
8. Zimmermann, M. H., and Brown, C. L. 1971. Trees: Structure and Function. Springer-Verlag, New York.
9. Zimmermann, M. H., and Jeje, A. A. 1981. Vessel-length distribution in stems of some American woody plants. Can. J. Bot. 59:1882-1892.
10. Zimmermann, M. H., and McDonough, J. 1978. Dysfunction in the flow of food. Pages 117-140 in: Plant Disease: An Advanced Treatise. Vol. 3. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
11. Zimmermann, M. H., and Tomlinson, P. B. 1966. Analysis of complex vascular systems: Optical shuttle method. Science 152:72-73.