

Compartmentalization of American Elm Tissues Infected by *Ceratocystis ulmi*

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ABSTRACT

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American elm trees compartmentalized tissues infected by *Ceratocystis ulmi* where the cambium was not killed. After infection, the cambium that was not killed formed a barrier zone that separated infected xylem from newly developing healthy xylem. The barrier zones in the sections studied were tangential sheets of axial parenchyma bridging swollen ray parenchyma cells. Recovery of an elm tree after infection may depend on its ability to compartmentalize infected wood to small volumes rapidly and to generate new healthy tissues.

Why do some American elm trees (*Ulmus americana* L.) live for many years after numerous infections by the Dutch elm disease fungus, *Ceratocystis ulmi* (Buism.) C. Moreau, while others die (1)? We explored this question by studying Dutch elm disease from the viewpoint of CODIT, a model for the compartmentalization of decay in trees (10,11). The two-part CODIT model includes 1) walling off to small volumes injured and infected wood within wood present at the time of injury and infection, and 2) separating such injured and infected wood from healthy wood that forms subsequently (10,11). Wood walled off in part 1 may discolor and die and not function for transport or storage. After injury, a nonconducting protective zone, the barrier zone (part 2), is formed by the cambium that remains alive at a distance from the injury (6,7,9,12). The barrier zone has been characterized in other tree species as a nonconducting zone consisting of abnormal amounts of axial parenchyma and swollen ray parenchyma (6,7,9,12). The barrier zone protects the cambium from further injury.

Christine Buisman described barrier zones in infected elms in 1935 (3). Walter Banfield gave additional information on this zone in 1968: "This band effectively walls off the vessels of the new sheath

from the invaded vessels of the old sheath" (1). Similar zones that wall off infections of *C. fagacearum* (Bretz) Hunt in oaks have been described (5,8). Jones et al (4) indicated that *C. ulmi* was compartmentalized in *U. procera*.

Trees survive injury and infection as long as they can wall off the injured and infected tissues to small volumes and then rapidly generate new healthy tissues at a position away from those infected. Buisman (3), Banfield (1), and Jones et al (4) gave information to support this idea, but they did not incorporate the information into the Dutch elm disease etiology. We give further information on barrier zones and compartmentalization in American elm and discuss how this new information, when integrated with older, sound information, enhances understanding of the etiology of Dutch elm disease.

MATERIALS AND METHODS

Twenty American elm trees more than 50 yr old and 20 elms about 20 yr old in varying stages of Dutch elm disease and located in the vicinity of Durham, NH, were studied. The large trees were cut by others as part of routine sanitation programs. Galleries of *Scolytus multistriatus* were seen in the bark, cambium, and outer sapwood of most of these trees. The smaller trees were dug for root observations. Isolation of *C. ulmi* confirmed Dutch elm disease in three additional large trees in the early stage of disease. Trees were cut from June to August.

Disks were cut from all parts of the trees to examine patterns of discolored wood associated with infections. The disks were sanded, and selected areas were shaved with a razor blade and viewed under a stereomicroscope.

Other areas were selected for fixation

and subsequent sectioning. Blocks of stem and branch wood of at least 1 cm³ were fixed in 5% formaldehyde. Transverse, radial, and tangential sections 8–15 μm thick were cut on a sledge microtome and stained with either 0.05% toluidine blue O or 0.5% aqueous safranin and Fast Green. Potassium iodide (2% IKI) staining for starch was used to determine the distribution of parenchyma in some sections.

Blocks (1.5 × 1.5 × 2 cm) were cut from several portions of the disks, and the transverse surface was placed in aster blue dye, as outlined by Mulhern et al (7), to test upward penetration of the dye.

RESULTS

The patterns of discolored wood associated with *C. ulmi* infections could be explained by the CODIT model (Figs. 1 and 2). This interpretation was most obvious in large branches and trunks where the cambium remained alive after the pathogen had spread in the xylem, which indicated that in some cases, the pathogen did spread great distances in the xylem without killing the cambium. The cambium then responded to the infection. When small, noncoalescing portions of the earlywood in a growth ring were infected, discoloration spread inward to the preceding growth ring only slightly (Fig. 1). Because of these intermittent, or spotty, infections, transport to living cells inward was not disrupted, and the ray parenchyma remained alive and free from discoloration.

When infections in the earlywood coalesced to form a brown band, discoloration spread into the older growth ring (Fig. 1). The brown band isolated the ray parenchyma in the older growth rings, and they became discolored and died.

Intricate patterns of discolored wood were associated with multiple infections that had been walled off over many years (Fig. 2). The discoloration did not spread laterally beyond the portion of the growth ring infected. The patterns of discolored wood did not differ from those in trees that have sustained numerous mechanical wounds over many years (10–12).

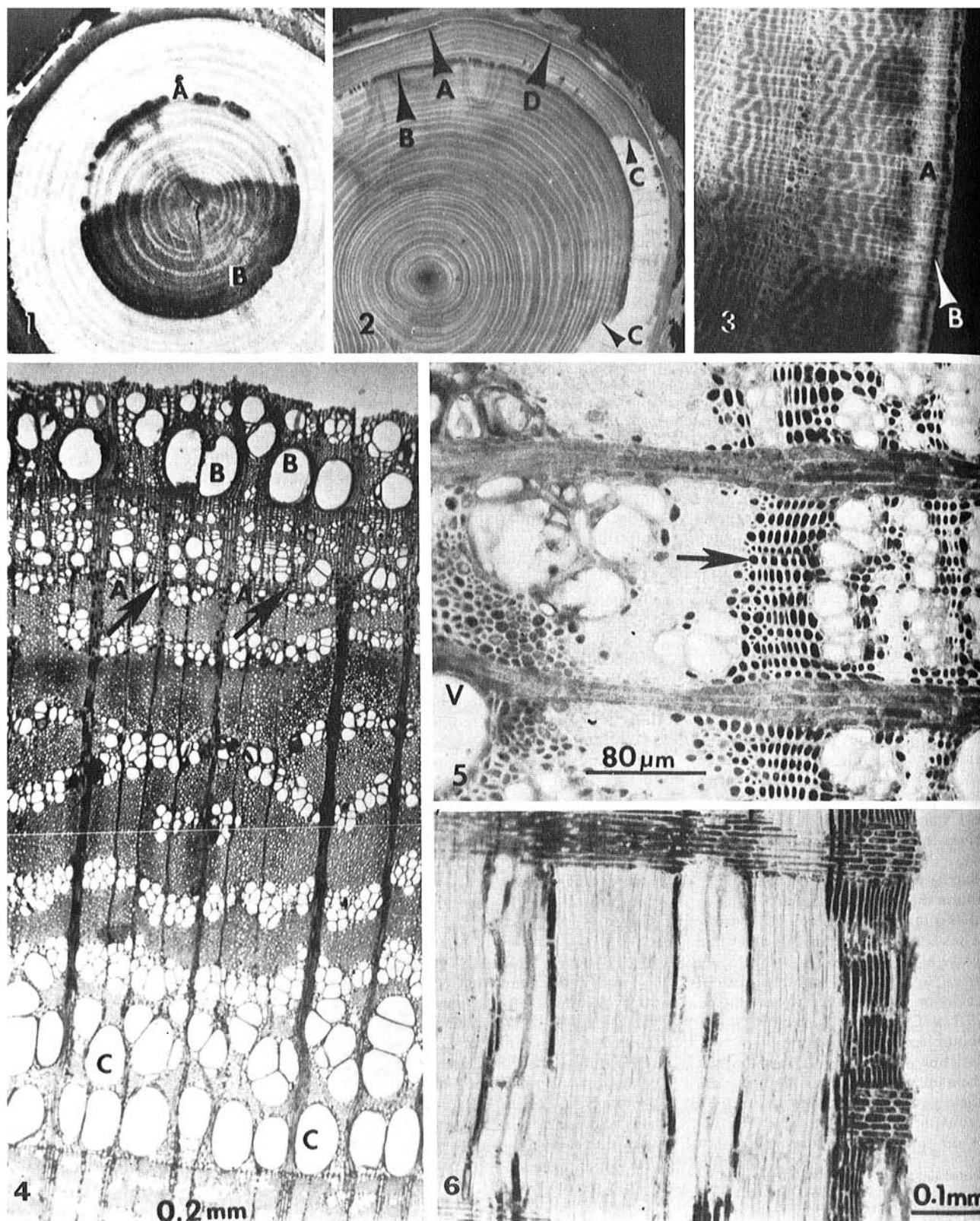
In large dead and dying branches, and in trunks of dying trees, a band of nondiscolored wood often appeared to separate inner discolored wood from the

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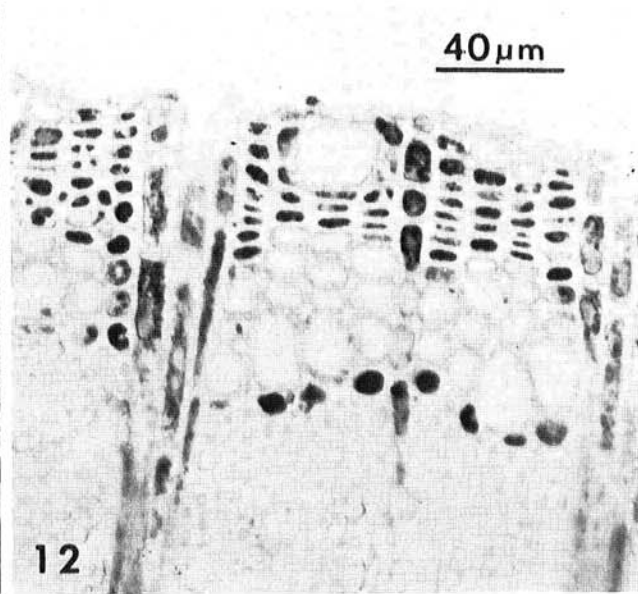
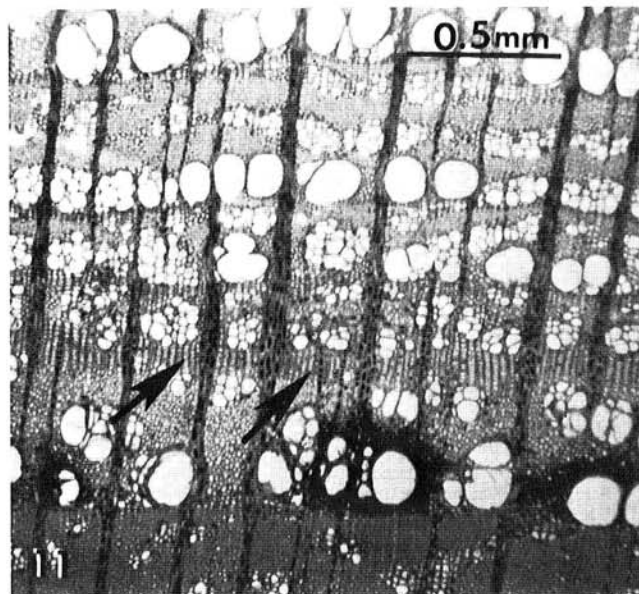
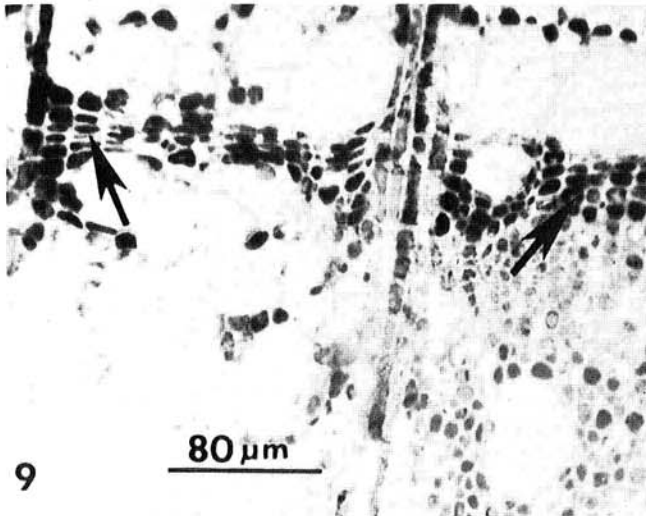
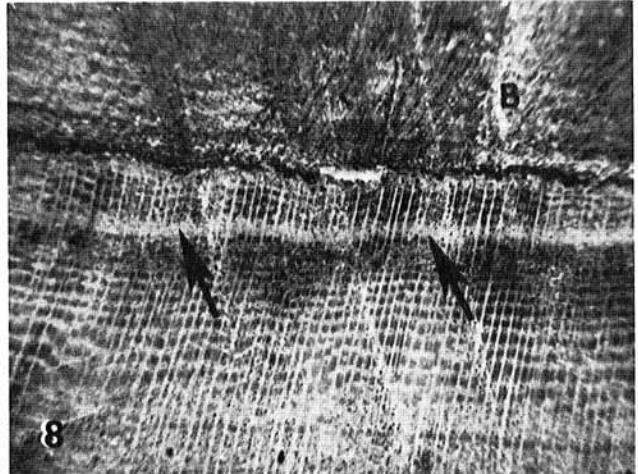
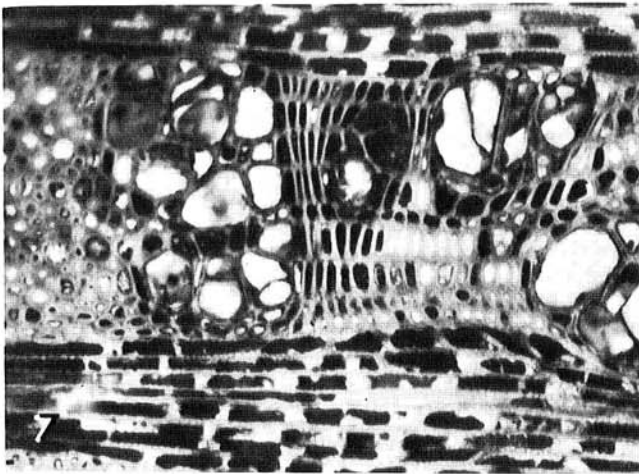


Figs. 1-6. (1) Discolored wood associated with noncoalescent (A) and coalescent (B) infections. Discolored wood developed inward behind the coalesced infections (B). The discolored wood was dead wood that no longer served for storage. (2) Compartmentalized discolored wood associated with several old infections. Where the infected wood coalesced laterally in 1978 (arrow A), the discolored wood developed inward to the old barrier zone (arrow B). Lateral spread of discolored wood ended abruptly where the barrier zones ended (arrows C). A nondiscolored zone was in the 1978 growth ring (arrow D). (3) A nondiscolored band including the barrier zone in the 1978 growth ring (A). The 1979 growth ring was infected (spring vessels, B), and the branch died. (4) A section from a dying branch similar to that shown in Fig. 3. A barrier zone formed near the end of the 1978 growth ring (arrows A). The 1979 growth ring was infected soon after it began to develop (B). Note spring vessels of the 1978 growth ring (C). (5) Barrier zone with wide band of axial parenchyma rich in starch (arrow). Transverse section stained with potassium iodide. At lower left, discolored tissue is associated with large vessel (V). (6) A radial section showing the axial parenchyma joining the ray parenchyma. Fusiform initials have divided to produce the axial parenchyma. This barrier zone was located in the most recent growth ring next to the cambial zone.

inner bark (Fig. 2). A closer look at this band showed that it was in the latter part of the previous year's growth ring (here, 1978) and separated discolored wood in the previous year's growth ring from a thin band of discolored wood in the

current growth ring (Fig. 3). Examination under a microscope showed that the nondiscolored band had characteristics of a barrier zone (Figs. 4, 5, and 7) similar to the zones reported by Buisman (3) and Banfield (1).

Staining with potassium iodide showed clearly that the zones were composed of an abundance of axial, starch-containing parenchyma and swollen ray parenchyma (Figs. 5, 6, and 12). Few vessels were in the zone. Dye did not penetrate the zone



Figs. 7–12. (7) Axial parenchyma bridging two swollen rays (transverse section, toluidine blue stain, magnification same as in Fig. 12). (8) The light band (arrows) in the last growth ring was not penetrated by a dye when the opposite face was placed in the dye. Note bark (B) crack along the cambial zone. (9) A "barrier zone" in a root (arrows). (10) These trees have had dead and dying branches for more than 5 yr. Many side shoots continue to form on them. (11) A barrier zone midway in growth ring (arrows). Uninfected growth rings formed after the barrier zone (transverse section, toluidine blue stain). (12) A barrier zone that formed at the end of the growth ring. Starch in the axial parenchyma stained with potassium iodide.

(Fig. 8). The barrier zone acted as a tangential shield between infected xylem and healthy cambium.

Barrier zones were not obvious in most of the roots examined, although discolored areas were localized. The normal root wood parenchyma may act in compartmentalization. Some extra tangential rows of axial parenchyma were formed, similar to those of the stem barrier zones, and appeared to be boundaries to root wood discolored by the fungus (Fig. 9).

The zones were most common in the latter half of the growth rings. In several large branches and trunks that had recovered from several older infections, the barrier zones were midway in the growth ring, and no infection or barrier zone formed the following year (Fig. 11). Some barrier zones formed at the end of the growth rings (Fig. 12).

In branches and trunks that died in 1979, a barrier zone formed late in the 1978 growth ring (Fig. 4). It was difficult to determine with certainty, but a new barrier zone appeared to start forming in 1979 soon after the large earlywood vessels began to form.

DISCUSSION

C. ulmi causes wilting of leaves and death of cambium, which can lead to death of trees. Many microorganisms kill cambium in twigs, branches, and trunks, but the trees rarely die. For example, white oaks commonly survive after multiple infections by *C. fagacearum* (13) by rapidly walling off the pathogen (5,8). Compartmentalization is a major defense system for trees, but to stay alive, the trees must be able to activate the system rapidly after injury and to generate new, healthy tissues away from those infected.

Apparently, the compartmentalizing and tissue-generating capacity of American elms varies greatly. Although the complexity of many interacting factors in Dutch elm disease—aggressiveness of the fungi, beetle populations, weather conditions, etc.—cannot be discounted, nonetheless some trees die rapidly while others survive after multiple infections over many years.

The infection may kill cambium in one area, but farther away, the cambium may still be alive. This is where compart-

mentalization starts. Trees cannot prevent beetles from wounding them, they cannot prevent *C. ulmi* from growing into dying tissues and vessels, but they can limit the spread of the pathogen, especially in secondary tissues.

Barrier zones were examined in all elms. The barrier zones were most pronounced in trunks and in large dying branches that had many new side shoots (Fig. 10). No barrier zones were found in dead or dying branches.

The position of the barrier zone in the growth ring may be crucial. If the zone is midway in the ring, the tree has time to generate new, healthy xylem during the growth season. Another barrier zone can form the following year after a new infection, and the tree remains alive if it again generates new xylem. If the zone is at the end of the growth ring, however, and another infection followed by a new barrier zone begins to form at the beginning of the next ring, the injury may overwhelm the tree, and it may die. A tree may also die from one infection if the fungus spreads rapidly in the vessels and no barrier zone forms to keep the infection from spreading to the cambium. Or a late-season infection not followed by a barrier zone can allow the fungus to spread rapidly into the vessels of the following year.

A tree either dies very soon after the first infection or a seesaw action begins between parasite and host; that is, infection followed by compartmentalization and generation of new, healthy tissues. If infection exceeds tissues compartmentalized and new tissues generated, the tree begins to die. If compartmentalization and generation exceed infection, the tree will continue to live.

How long it will live depends on energy reserves. A great amount of energy is probably required to produce a barrier zone. The many layers of parenchyma that contain starch initially form at the expense of fibers and vessels. As barrier zones associated with older infections wall off cells that store nutrients, the tree takes more and more "risks" as it forms new barrier zones. Most of the water-conducting system of an elm is restricted to the current growth ring (14), but ray parenchyma extending into several sapwood rings still maintains a nutrient

storage capacity. The fact that American elms usually produce a heavy seed crop, and that this occurs before the new growth rings begin to develop (2), is an additional drain on energy reserves from the previous year. As the leaves develop, the earlywood large vessels begin to form, which also requires energy stored from the previous year. This is when the first emerging beetles attack a tree for feeding and breeding. Their brood galleries can injure the phloem, thus disrupting downward transport. The new infection causes the leaves to wilt, and no new carbohydrate is formed. As a result, the cambium is killed and the tree dies.

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