Mechanisms of Compartmentalization of Decay in Living Trees

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When trees die, the decomposition and decay which releases CO_2 to the atmosphere and minerals to the soil is part of the normal cycle in undisturbed or unmanaged forests. Decay that develops in living trees, particularly in urban plantings and in forests being managed for timber production, becomes a serious problem. It is the single most destructive disease of trees and for many years has been described according to the heartrot concept (1,5).

THE HEARTROT CONCEPT

The basic assumption of the heartrot concept is that living sapwood at the outer margin of the trunk gradually matures and becomes nonliving heartwood at the core of mature trees. Exposure of the heartwood by wounding (by fire, storm damage, lumbering scars, or boring insects) leaves it open to attack by saprobic fungi which fruit, produce spores, and thus spread to other dead heartwood exposed by wounding. Simply stated, the heartrot is the decomposition of dead heartwood inside a living tree by saprobic organisms that gain entrance via wounds. Many plant pathologists have not considered heartrot to be a disease because the decay-causing agent is not interacting with living host tissue.

This simplistic concept has been challenged by later observations. In 1935, Hepting (6) reported that wounded young sweetgum trees without heartwood decayed as readily as wounded young oaks that had heartwood. Further, he observed that the decay was limited to wood formed before the wounding occurred. This led to the postulation that the susceptibility to decay of sapwood formed after wounding differed from that formed before wounding. When it was noted that a dark-colored tissue resembling heartwood was formed in response to wounding, the term "pathological heartwood" was coined to designate localized dark-colored tissue resembling heartwood that formed after wounding rather than by the normal aging process.

This was true in species such as sweetgum, not because localized discoloration was like heartwood, but because the cores of dark-colored wood also were wound-initiated discolorations (27). In sweetgum, larger wounds, such as broken tops or the simultaneous loss of several branches, gave rise to a uniform core of discolored wood, but small trunk wounds caused irregular regions of discolored wood. In both cases, the same wounds that initiated discoloration were the source of decay.

The patterns of decay and discoloration in the northern hardwoods—beech (*Fagus*), birch (*Betula*), and maple (*Acer*)—were established by Shigo (15–17, 22,24). Mature trees of those species do not form a heartwood. Wounded oaks (*Quercus*), which form a heartwood, discolor and decay in the same manner as trees without a heartwood. The results of tree-wounding experiments confirmed the following principles:

i, Wounds initiate discoloration and the process that leads to decay in living trees.
ii, Discoloration precedes decay in wood exposed by wounding.
iii, Discoloration and decay are limited to wood extant at the time of wounding.
iv, The amount of discolored and decayed wood is proportional to the number and severity of wounds.
v, Both sapwood and heartwood discolor as a result of processes initiated by wounding.
vi, The processes initiated by wounding involve both a response of the tree and of microorganisms.
vii, If both sapwood and heartwood are present at time of wounding, decay begins in discolored sapwood and spreads into discolored heartwood.
viii, If discolored wood is wounded, it does not discolor like heartwood.
ix, The electrical resistance of discolored wood is less than that of sapwood, and the electrical resistance of heartwood is greater than that of sapwood.
x, If decayed wood is wounded, discoloration and decay of freshly exposed wood is enhanced.

Thus, it has been clearly established that the presence of heartwood in the stem of a living tree is not essential for the decay process, but the discoloration process is.

Microorganisms other than decay fungi are active in wood during its discoloration. Bacteria and imperfect fungi have been considered pioneers in a succession of microorganisms (18,20,21) in which decay fungi follow. Decay fungi are in turn followed by many organisms that include not only bacteria and fungi, but also protozoa, nematodes, insects, birds, and mammals.

Of interest to the pathologist are the pioneers that interact with living tissue exposed by wounding and the fungi that decompose that tissue. It appears that both pioneer and decay fungi can interact with living sapwood, but only decay-causing fungi can induce discoloration (28). Other pioneers and decay fungi are dependent on the discoloration process that provides a nonliving substrate. Some of these dependent microorganisms invade through the open wound (17,27), while others may already be present as sparse populations in live sapwood, but cannot develop until the sapwood dies.

The growth of decay fungi is retarded during the discoloration process (28). This phenomenon is well known in the Bavendamm reaction on gallic acid medium used as an aid to identify decay fungi (2). Many fungi that cause decay in live trees cannot grow on this medium, but they darkly discolor it by secreting phenol oxidases. If such fungi are placed on live sapwood, the wood may also become darkly discolored. Hyphal growth is greatly retarded during this period. However, if the fungus is able to decolorize the discolored wood—that is, remove dark pigments—then abundant hyphal growth is achieved (28).

This pattern of color change is repeated in the live tree. Wood that is exposed by wounding becomes darkly discolored, and isolates of the pioneer bacteria and fungi are the predominant isolates. Then the wood becomes lighter in color, and the isolation frequencies for decay fungi begin to increase. Both the formation of dark wood and decolorization—the first steps of the decay process—are associated with the loss of phenols as components of the soluble dry matter (31). Phenols act as growth regulators of decay fungi, but not of pioneers. It was postulated that the initial phenol content is sufficient to inhibit decay fungi which must polymerize these substances by oxidation to remove the source of inhibition. Phenol-tolerant pioneers (28,29) flourish until the phenol content has been reduced to a level that permits decay fungi to use cell wall substances as food, at which time the pioneers are replaced. This phenomenon of selection on nondecay organisms by wood preservatives is well founded (3).

THE CODIT CONCEPT

Decay in living trees can now be explained by a model system called compartmentalization of decay in trees (acronym, CODIT) (23), and a concept of succession in which both pioneers and decayers can act as either pathogens or saprobes (28). The CODIT system explains the simple patterns of discoloration and decay observed in trees that lack heartwood and also the more complex patterns observed in trees that form heartwood and that have multiple wounds. The model is based on four "walls" that limit the spread of decay fungi and their pioneers. Wall 1 is a plugging component that limits the vertical spread. Wall 2 is an anatomical component that limits the spread parallel to rays. Wall 3 is a vital component that limits spread perpendicular to rays. Wall 4 is a differentiation component that limits spread into wood formed after wounding. Walls 1, 2, and 3 act in wood extant at the time of wounding; wall 4 forms only after wounding.

Three major factors appear to affect the decay fungi and their pioneers (28): a vitality factor, a preservative factor, and a solubility factor. The vitality factor of live sapwood prevents the growth of many pioneers and decay fungi. It varies with the species and individual tree and with the species and strain of inoculum, but is lost when live cells die. The preservative factor becomes operative during the conversion of live wood to discolored wood and it involves the formation of natural wood preservatives that reduce the high decay rates observed on rapidly killed sapwood. The preservative(s) favor tolerant pioneers and inhibits sensitive wood decay fungi. In the final stages, the importance of the preservative factor declines and that of the solubility factor increases as the dissolution of cell walls (the major source of nutrients in wood) proceeds and becomes limiting. Decay fungi, which have a superior capacity to dissolve cell wall substances, replace the pioneers that formerly were favored by the preservative factor.

Development of the CODIT system with its conceptual four walls and succession of microorganisms governed by major limiting factors has required postulation of the following molecular mechanisms to account for compartmentalization in the simplest case—a single wound in sapwood. A general response of plant tissue to injury and infection is a shift in oxidative metabolism from glycolysis and TCA cycle pathways to the acetate and pentose shunt shikimic acid pathways (8). Accumulating products of these alternate pathways may then act as growth inhibitors (a preservative factor) or help seal off the tissue (a solubility factor). Other consequences of a shift in oxidative metabolism are: loss of available energy as food reserves are converted to products of the acetate and shikimic acid pathways, stabilization of readily ionizable phenols owing to reduced polarity of their environment, increased randomness of chemical bonding in food sources generated by resonance forms of conversion products, loss of available nitrogen by precipitation of tanned proteins, and lowering of available oxygen by polyphenols.

Although large, dead tracheal cells make up the bulk of mass and volume, the metabolic shift occurs in the live parenchyma cells, which are the most abundant cells of sapwood (Table 1). The network of small, live cells react to protect the large, dead cells from decomposition after an injury. Years of study have established that products converted from food reserves stored in sapwood protect the cell wall substances of heartwood, and provide wood with a natural resistance to decay (11). In fact, the same shift in metabolism must accompany the death and lignification of the

large cells as they differentiate from cambial derivatives.

A biosynthetic change in constituents of the small live cells should be maximized in the rays, where such cells are concentrated CODIT system, wall 3 and in the last latewood, where conducting elements are smallest and least abundant (CODIT system, wall 3) (Fig. 1). Failure to generate enough plugging materials or tyloses in the remaining tissue (CODIT system, wall 1) most often leads to the failure of compartmentalization. Levels of preservative factors that are sufficient to prevent the spread of microbes in dense tissues may fail in more open tissues, and large columns of discolored and decayed wood may form.

Where walls 1, 2, and 3 fail in extant wood, the biosynthetic machinery of cambial derivatives usually succeeds. Cambial derivatives form a sheet of living tissue in which shifts in metabolic activity can produce profound changes in both anatomical structure and chemical composition. These changes maximize the reactivity of wall 3 and the density of wall 2, thus negating the inherent weakness of wall 1 (Fig. 2).

TABLE 1. Cellular composition of sweetgum sapwood

Cell type	Weight (%)	Volume (%)	Number (%)
Parenchymal	5	20	95
Tracheal	95	80	5

TABLE 2. Formation of phenols in response to injury of sugar maple sapwood (milligrams phenol per gram of moisture-free wood)[a]

Condition of wood	Acetone	Hot water	Total
Noninjured	0.7	1.8	2.5
Injured	1.2	2.3	3.5

[a] Mean of paired samples from three trees. Shavings of wood were taken from the blue-fluorescent zone (UV, 365 nm) associated with a wound and from equivalent nonfluorescent wood of the same tree (before formation of discolored wood), homogenized in acetone, boiled in water, and the phenol content was determined with Folin-Ciocolteau reagent.

Evidence is accumulating that metabolic activity shifts during compartmentalization. In 1967, Shain (12) characterized a zone in pine that surrounds wood infected by *Fomes annosus*. This "reaction zone" was resin-soaked and had an increased phenol content. Thus, normal soluble dry matter of pine sapwood was converted to products of the acetate and shikimic acid pathways as predicted by the general scheme of plant response to injury and infection. A similar zone in spruce was later described, where phenols accumulated in the reaction zone and resinous components were less prominent (13). The reaction zone described by Shain (12,13) corresponds in general to CODIT walls 1, 2, and 3.

For a decade, several collaborators and I have studied the response of sapwood to wounding and inoculation. We used maple for the basic experiments because of its simple structure and its wide range. Later we used other species to extend principles that were developed in the studies of maple.

The results of analyses following simple homogenization of wood exposed by wounding indicated that products of a shift in oxidative metabolism accumulated in sapwood before it became discolored (Table 2). Subsequent discoloration then resulted in a reduction of phenol content, which reached a minimum in decaying wood (31). Phenol content reached a maximum in the bright-colored tissue found at the margin of discolored wood and sapwood (26). Soluble dry matter of this marginal zone was double that of sapwood. The ratio of phenol to carbohydrate (wt:wt) in dry matter readily soluble in water increased from 1:10 in sapwood to 2:1 in marginal tissue, then decreased to 1:3 in discolored wood. Dry matter from both marginal and discolored wood retarded the growth of pioneer and decay fungi alike, although growth of the latter was retarded more. In culture, a ratio of gallic acid (the common water-soluble phenol of sapwood) to glucose of 1:10 allowed more growth of pioneer fungi than of decay fungi, but at 1:100, the decay fungi grew better than did the pioneers (28).

Phenols of the bright-colored marginal zone in maple were not the same as those of the sapwood. Sapwood contains phenols primarily of the tannin-type—both the hydrolyzable tannins built

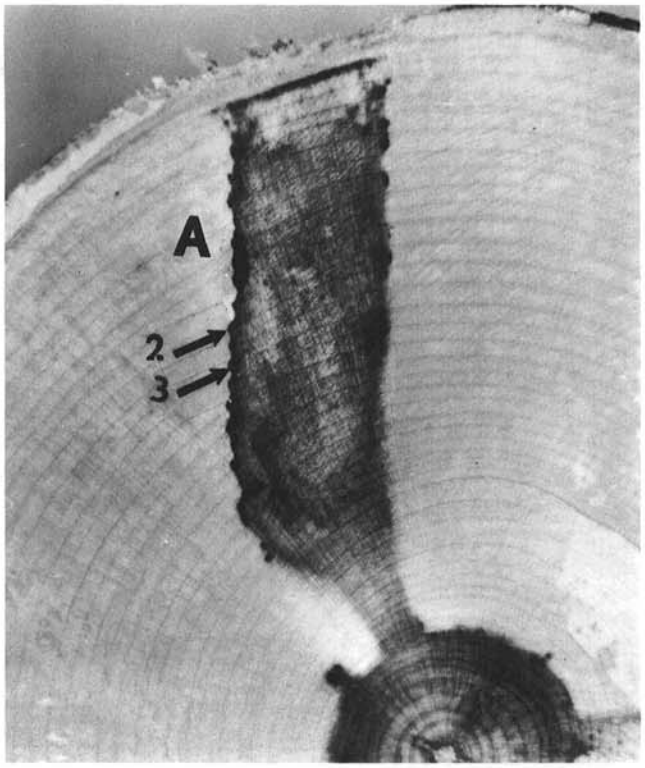

Fig. 1. Uneven edge of column of discolored wood (A); gross effect of greater effectiveness of a reaction zone formed in ray tissue (CODIT wall 3) and in latewood (CODIT wall 2).

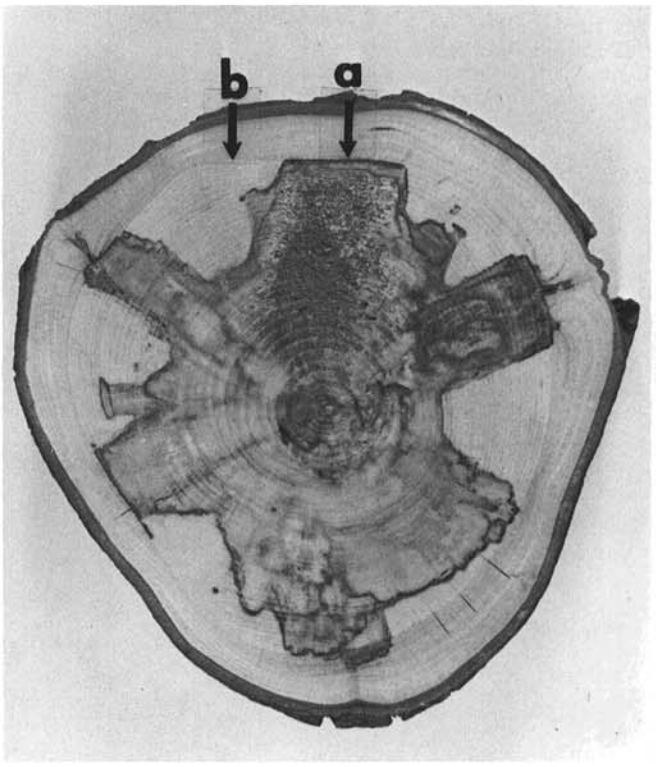

Fig. 2. Column of discolored and decayed wood 5 yr after multiple wounds were inflicted with a large screwdriver; a = barrier zone in contact with discolored wood, b = barrier zone not in contact.

on gallic acid residues and the condensed tannins built on catechin residues (30). Phenols of the marginal tissues were less polar, which suggests a relationship with the fatty products of the acetate pathway (26). The ultraviolet (UV) spectra and bathochromic shifts of these phenols when ionized suggested the presence of hydroxycinnamic acid derivatives. The phenols of the margin differed from tannins in chromatographic behavior and in susceptibility to aerial oxidation. However, further progress in their characterization requires a solution to the problem of sampling this narrow marginal zone (< 2 mm in width).

Given the working hypothesis that a reaction zone forms at the margin of columns of discolored wood and operates in a fixed structure as walls 1, 2, and 3 of the CODIT system, what regulates the effectiveness of compartmentalization? At present it appears that the genes of the tree are an important factor (25). The genes of the inoculum also appear to be important, as inferred from in vitro studies (28), and soon to be confirmed in vivo (2-yr studies nearing completion). Also, the normal response can be altered by chemical treatment of wounds (32) and by manipulating the inoculum in wounds (10). Detailed studies of mechanisms that regulate the compartmentalization process remain to be done.

The most effective barrier to the spread of discoloration and decay does not form in the wood extant at the time of wounding, but in the wood that grows afterward. This tissue, called a barrier zone, is not well understood. Sharon (14) described a "distinct tissue" in maple that corresponds to the "barrier wall" of Shigo (21). The gross features of a "barrier zone" were described by Shortle in sweetgum and yellow-poplar (27). The anatomical features of the barrier zone of sweetgum were then studied by Moore (9), who greatly expanded the pioneering work of Gerry (4). The storax secreted by resin canals in sweetgum wood formed after injury again supports the hypothesis for a shift in oxidative metabolism that results in products of the acetate pathway (β storesin) and the shikimic acid pathway (cinnamic acid). Both storax and cinnamic acid completely inhibited the growth of a decay fungus (28,29).

Studies in progress have shown that the wood formed adjacent to the wound at varying distances has fewer, shorter vessels and increased opacity to transmitted light. This zone has a higher than normal phenol content, and where it comes in contact with discolored wood, a very dark edge is formed (Fig. 2)—although the wood of the zone is not discolored.

Isoelectric focusing of isoenzymes of indole acetic acid oxidase (peroxidase) from differentiating cambial derivatives shows a loss of many isoenzymes within minutes after wounding. Compared to nonwounded controls, this loss is followed by changes in enzyme activity and levels of phenolic effectors of that activity during the next few weeks. Studies of the changes in the kinds and activities of enzymes that regulate levels of growth promotors and growth arrestors (7) as the barrier zone forms, may lead to further understanding of this process.

In general, where the barrier zone meets the tissue responsible for wound closure, it looks most like callus. Then, with increasing distance from the callus, the appearance of the zone grades back into that of normal wood—more quickly circumferentially than longitudinally. Thus, each wound has an elliptical barrier zone around it. It is not known how the size of the ellipse or the thickness of the zone varies with the size and severity of any given wound. It is known that ring shake develops between the barrier zone and the wood which was extant at the time of wounding (19).

SUMMARY

The heartrot concept has failed to explain the patterns of discoloration and decay observed in living trees. The CODIT system and a concept of succession, in which both pioneer and decay fungi may act as pathogen or saprobe, explain much more. Working hypotheses for the study of molecular mechanisms of compartmentalization can be deduced from these general schemes by reference to three well-established principles of plant and wood pathology: a shift in oxidative metabolism is associated with injury and infection, food reserves in sapwood converted to products of the acetate (resinous materials) and the shikimic acid pathways (phenols and other aromatic compounds) cause wood to become more decay resistant, and bacteria and nondecay fungi grow selectively in wood preserved against decay fungi. There is evidence to support these hypotheses, and more is being generated at this time. I can only hope that I have outlined new patterns of thinking about decay in trees that will lead to a better understanding of this serious tree disease problem.

LITERATURE CITED

1. BOYCE, J. S. 1961. Forest Pathology. McGraw-Hill, New York. 572 pp.
2. DAVIDSON, R. W., W. A. CAMPBELL, and D. J. BLAISDELL. 1938. Differentiation of wood-decaying fungi by their reaction on gallic or tannic acid medium. J. Agric. Res. 57:683-695.
3. DUNCAN, C. G., and F. J. DEVERALL. 1964. Degradation of wood preservatives by fungi. Appl. Microbiol. 12:57-62.
4. GERRY, E. 1921. American storax production: results of different methods of tapping red gum trees. J. For. 19:15-24.
5. HARTIG, R. 1894. Textbook of the Diseases of Trees. Translated by W. Somerville, revised and edited by H. M. Ward. Macmillan, New York. 331 pp.
6. HEPTING, G. H. 1935. Decay following fire in young Mississippi Delta hardwoods. U.S. Dep. Agric. Tech. Bull. 494. 32 pp.
7. HOYLE, M. C. 1974. The hypothetical case for methylene-oxindole as a plant-growth arrestor. pp. 659-664 in: R. L. Bieleski, A. R. Ferguson, and M. M. Cresswedl, eds. Mechanisms of regulation of plant growth. R. Soc. N.Z. Bull. 12. Wellington. 934 pp.
8. KUĆ, J. 1967. Shifts in oxidative metabolism during pathogenesis. pp. 183–202 in: C. J. Mirocha and I. Uritani, eds. The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction. Bruce Publ. Co., St. Paul, MN. 372 pp.
9. MOORE, K. E. 1978. Barrier zone formation in wounded stems of sweetgum (*Liquidambar styraciflua*). Can. J. For. Res. 8:389-397.
10. POTTLE, H. W., and A. L. SHIGO. 1975. Treatment of wounds on *Acer ruburm* with *Trichoderma harzianum*. Eur. J. For. Pathol. 5:274-279.
11. SCHEFFER, T. C., and E. B. COWLING. 1966. Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4:147-170.
12. SHAIN, L. 1967. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. Phytopathology 58:1493-1498.
13. SHAIN, L. 1971. The response of sapwood of Norway spruce to infection by *Fomes annosus*. Phytopathology 61:301-307.
14. SHARON, E. M. 1973. Some histological features of *Acer saccharum* wood after wounding. Can. J. For. Res. 3:83-89.
15. SHIGO, A. L. 1965. The pattern of decay and discoloration in northern hardwoods. Phytopathology 55:648-652.
16. SHIGO, A. L. 1965. Decay and discoloration in sprout red maple. Phytopathology 55:957-962.
17. SHIGO, A. L. 1966. Decay and discoloration following logging wounds on northern hardwoods. U.S. Dep. Agric. For. Serv. Res. Pap. NE-43. 23 pp.
18. SHIGO, A. L. 1967. Successions of organisms in discoloration and decay of wood. Int. Rev. For. Res. 2:237-299.
19. SHIGO, A. L. 1972. Ring and ray shakes associated with wounds in trees. Holzforschung 26:60-62.
20. SHIGO, A. L. 1975. Biology of decay and wood quality. pp. 1–15 in: W. Liese, ed. Biological Transformation of Wood by Microorganisms. Springer-Verlag, New York. 203 pp.
21. SHIGO, A. L., and W. E. HILLIS. 1973. Heartwood, discolored wood, and microorganisms in living trees. Annu. Rev. Phytopathol. 11:197-222.
22. SHIGO, A. L., and E. vH. LARSON. 1969. A photo guide to the patterns of discoloration and decay in living northern hardwood trees. U.S. Dep. Agric. For. Serv. Res. Pap. NE-127. 100 pp.
23. SHIGO, A. L., and H. MARX. 1977. Compartmentalization of decay in trees [CODIT]. U.S. Dep. Agric. Inf. Bull. 405. 73 pp.
24. SHIGO, A. L., and E. M. SHARON. 1968. Discoloration and decay in hardwoods following inoculations with hymenomycetes. Phytopathology 58:1493-1498.
25. SHIGO, A. L., W. C. SHORTLE, and P. W. GARRETT. 1977. Genetic control suggested in compartmentalization of discolored wood associated with tree wounds. For. Sci. 23:179-182.
26. SHORTLE, W. C. 1979. Compartmentalization of decay in red maple and hybrid poplar trees. Phytopathology 69:410-413.
27. SHORTLE, W. C., and E. B. COWLING. 1978. Development of discoloration, decay, and microorganisms following wounding of sweetgum and yellow-poplar trees. Phytopathology 68:609-616.

28. SHORTLE, W. C., and E. B. COWLING. 1978. Interaction of live sapwood and fungi commonly found in discolored wood and decayed wood. Phytopathology 68:617-623.
29. SHORTLE, W. C., T. A. TATTAR, and A. E. RICH. 1971. Effects of some phenolic compounds on the growth of *Phialophora melinii* and *Fomes connatus*. Phytopathology 61:552-555.
30. TATTAR, T. A., and A. E. RICH. 1973. Extractable phenols in clear, discolored, and decayed woody tissues and barks of sugar maple and red maple. Phytopathology 63:167-169.
31. TATTAR, T. A., W. C. SHORTLE, and A. E. RICH. 1971. Sequence of microorganisms and changes in constituents associated with discoloration and decay of sugar maple infected with *Fomes connatus*. Phytopathology 61:556-558.
32. WALTERS, R., and A. L. SHIGO. 1978. Discoloration and decay associated with paraformaldehyde-treated tapholes in sugar maple. Can. J. For. Res. 8:54-60.