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HEARTWOOD, DISCOLORED WOOD, AND MICROORGANISMS IN LIVING TREES

Alex L. Shigo and W. E. Hillis

Northeastern Forest Experiment Station, U.S.D.A. Forest Service, Durham, New Hampshire, and Forest Products Laboratory, Division of Applied Chemistry, Commonwealth Scientific and Industrial Research Organization, South Melbourne, Victoria, Australia

The state of our knowledge of plant pathology is reflected by the terms we use (120). Consider some of the terms used to describe wood altered by processes associated with aging and injury of living trees: heartwood, wound heartwood, pathological heartwood, traumatic heartwood, false heartwood, precocious heartwood, blackheart, brownheart, red heart, blue butt, mineral streak, mineral stain, woundwood, discolored wood, wound-initiated discolored wood, wetwood, ripewood, reaction zone, protection wood, and even true wood. Indeed, there is confusion!

The main visible change observed in wood of trees is change of color. This can be the result of processes associated with aging (heartwood), injury (discolored wood), or both. However there are more important characteristics than color for the sapwood altered by these processes. The factors that initiate the formation of heartwood, discolored wood, and extractives (which are largely responsible for color) are different.

This is the major reason for the confusion in understanding a situation when color alone is the basis for distinguishing the type of tissue under study. When injury-altered tissues are considered as age-altered tissues, and the role of microorganisms in the processes are not considered, it is impossible to interpret the situation accurately. The confusion is further compounded when the injury processes occur in tissues already altered by aging. Clarification of these processes obviously is needed.

We will discuss in this review those processes in living trees that are associated with colored wood, in the hope of clarifying them so that future research will be more accurately oriented and the opportunity to bring these changes under our control will be improved. We will consider and contrast two types of wood, which we will refer to as "heartwood" and "discolored wood."

Heartwood occurs in mature specimens of most, but not all, tree species. It is formed in a more or less regular manner in individual trees, and is not normally associated with detectable injury. Discolored wood occurs in many species, but it is distributed irregularly among and within trees, often not being prominent in most individuals of the species, and it is usually associated clearly with mechanical or biological damage.

In view of the complexity of the situation and the volume of the literature, we will consider only the *major* points, and only a portion of the published work is cited because of space limitations.

Formation of Wood

Herbaceous and woody plants have points of both similarity and dissimilarity in their life cycles (167). In almost all cases, trees are larger than herbaceous plants, and can live longer and grow larger in mass than any other organisms on earth. It is remarkable that they can achieve this despite their inability to move away from destructive forces as other organisms can. It would be rare indeed for trees to live even a short time without receiving wounds or damage from external agencies, in addition to those received from the natural shedding of branches.

Because wounds were probably common to trees as they evolved, the survival of trees as tall perennial plants depended in part on their development, through natural selection, of effective systems for protection (as with heartwood) and repair of wounds (resulting in many cases in discolored wood). Despite the different habits or appearance, and the wood anatomy of the Gymnosperms (cone-bearing trees, mostly evergreens) and the Angiosperms (flowering broad-leaved hardwood trees with spreading branches, often deciduous) the wood-formation processes are similar.

A series of biological processes results in the formation of wood. The thin-walled early-wood portion of a growth ring is produced during the period of terminal growth of the tree. The fiber (or tracheid) diameter is regulated by hormones produced by the foliage and transmitted to the developing fibers in the cambium. The fiber wall thickness appears to be genetically controlled, but also is dependent on the amount of available photosynthate. The interaction of the two physiological processes results in the type of fiber produced, and also ultimately the amount of primary metabolites in the sapwood and the amount of extractives in the heartwood or injured wood.

When the lignification of the fiber wall (composed largely of cellulose microfibrils packed in a matrix) and the middle lamella is completed, the cell dies. Capillaries existing in the secondary wall occupy a significant volume, estimated to be 25% in *Pinus resinosa* green sapwood (9). These capillaries probably have a diameter between 16 and 60 $\times$ 10^{-10} meters, and collapse when the wood dries (75).

In contrast to fibers and tracheids, the transverse and longitudinal parenchyma (about 7% in conifers and 17% or more in deciduous hardwoods) can

remain viable for many years. In *Tamarix aphylla* sapwood, however, the fibers retain their living protoplasts for the same period as the parenchyma and ray cells (36). The shape of the nuclei of living ray parenchyma cells in the sapwood of several species of Gymnosperms and Angiosperms changes and they eventually disintegrate (45, 64). They lose organelles (36), their vitality (115), nitrogen-containing compounds (106), starch (69), and their ability to consume oxygen (65) as their distance from the cambial region increases. The amount of sugars and biotin and pyridoxine can decrease abruptly at the heartwood boundary (27, 181).

The sapwood vessels in Angiosperms occasionally contain tyloses; but as heartwood and discolored wood form, they usually appear in much greater numbers in many species, so that movement of liquids and perhaps microorganisms is blocked. Aspiration of the pits in conifer tracheids as heartwood forms has a similar effect on liquid movement.

To meet different physiological needs throughout the year, primary metabolites are stored in the sapwood in the form of starch or fats, according to the type of tree (68). The amounts vary according to prior needs and current demands, and are not uniform across the sapwood. The distribution of photosynthates within a tree can be considered a system of competing metabolic sinks, which are constantly changing in size according to the needs of a particular zone at a particular time. There is little information about the dynamic translocation of carbohydrate through the rays of the sapwood.

Formation of Heartwood

The sapwood of the trunk, branches, and roots of many—but not all—uninjured trees changes abruptly in appearance and function after a certain age. This interior core is "heartwood," of which one definition is: "The inner layers of wood which, in the growing tree, have ceased to contain living cells and in which the reserve materials (e.g. starch) have been removed or converted into heartwood substances. It is generally darker in color than sapwood, though not always clearly differentiated" (84).

The proportion, and even the existence, of heartwood in a mature tree varies within the family, genus, and even species (25, 76). Within a species, under normal circumstances, the amount and rate of heartwood formation varies to a lesser extent with tree age (66), growth rate, environment, and silviculture practice (154). In some genera (such as *Eucalyptus*) and in some species, the age of the sapwood transformed to heartwood is remarkably constant (25), and possibly is mainly genetically determined. It has been observed with some species that heartwood formation commences at some distance above ground level (25, 166) and that the proportion of heartwood in some species remains greatest at this level. Sometimes, however, heartwood may never form, as in *Alstonia scholaris* (19). Living cells 115 years old have been found in *Acer saccharum* (50).

The periphery of the approximately conical-shaped central core of heartwood often undulates vertically and horizontally and can cut across parts of annual rings as abrupt tongues.

Transition of sapwood to normal heartwood is initiated by internal processes rather than by external conditions. Once it begins, the process continues so that frequently the number of sapwood rings remain more or less constant during the life of the tree. There are also species in which heartwood formation is initiated after many years, but thereafter more than one ring of sapwood is transformed annually to heartwood.

The little available information indicates that the area of sapwood relative to heartwood is greater in the trunk than in the roots and branches of similar size, but the ratio can vary with species.

Usually heartwood contains less moisture than sapwood, and the decrease can be abrupt and considerable, as in *Picea glauca* which has 136–162% moisture in the sapwood and 47–48% in the heartwood (22). Some *Pinus* species show similar decreases (25). On the other hand, the heartwood of some hardwood species contains more moisture than the sapwood (74, 177, 178). Analyses of the gas in the heartwood of a number of trees show, in addition to nitrogen, a large proportion of carbon dioxide and a small amount of oxygen (25, 85, 110, 111).

Freshly cut cross-sections of many trees reveal a transition, intermediate, or white zone surrounding the heartwood. Usually the transition zone is less than 1 cm wide, and the width can increase with tree height and with season. There are also intermediate zones of up to 15 cm, as reported in *Sloanea woollsii* (19). The zone is paler in color, clearly distinguishable from the sapwood, with a moisture content sometimes even lower than that of the heartwood (25, 179), increased amounts of nicotinic acid amide, biotin, pyridoxine (181, 182), and in some cases protein nitrogen (182). In pine species the amount of extractives is low (52).

In contrast to reports of the absence of organelles at the heartwood boundary mentioned previously, the amount of organelles in the transition zone of *Cryptomeria japonica* has been found to be only decreased (118). Furthermore, respiration and the activity of the malate and glucose-6-phosphate dehydrogenases in this zone in *Pinus radiata* is higher than in the surrounding sapwood during the dormant season (L. Shain & J. F. G. Mackay, unpublished data). When slowly dried, the boundary of the sapwood-transition zone of some species (e.g. eucalypts) can become very dark colored (W. E. Hillis, unpublished data), and this is probably due to localized increases of phenol oxidases. Increases in peroxidase activity in the "pre-heartwood" zone of *Larix europea* and *P. sylvestris* have been reported (95).

In some species the transition zone has not been recognized or does not exist, so some properties cannot be allocated precisely to the transition zone or to the heartwood periphery. Some of the recent results contradict those of earlier workers (45, 65). Cytological studies of ring-porous hardwoods have

shown an increase in vitality in the parenchymous cells (80), and in respiration (180) at the heartwood periphery. Extractives are formed mainly in the radial parenchyma, but the longitudinal parenchyma can also form them. Peroxidase activity increased markedly at the periphery of the heartwood (27, 95, 172), as did the activities of amylase (78), phenol oxidases, malate dehydrogenase, etc. (182). Recent studies showed that tyloses form before extractives in *Eucalyptus* and *Nothofagus* species (V. Nečesaný, unpublished), and that calcium oxalate crystals are not found in the cells containing polyphenols (G. Scurfield, unpublished). The chemical reactivity of cellulose in Douglas-fir trees, as shown by the accessibility of hydroxyl groups, revealed a marked maximum at the sapwood-heartwood boundary. The carbonyl index, moisture content, and extractives content also showed maxima at the same point, indicating increased biochemical activity in this region (20).

The transformation of sapwood to heartwood is accompanied by necrosis of the xylem parenchyma, although some enzymic activity may be found in the heartwood. Phenol-oxidizing enzymes have been reported in the heartwood of *Pinus lambertiana* (159), and the two found in *P. radiata* heartwood were probably of host origin (137). Other major differences from sapwood can include aspiration of the pits in Gymnosperms (53), formation of tyloses in Angiosperms, or gum when the pit aperture is less than 10μ (18). Although starch is absent from heartwood, small amounts of free sugar may be found (74). The nitrogen content is lower (106), and the pH higher than in sapwood (74). Fatty and resinous materials that are stored in sapwood of some trees instead of starch, are changed in composition as heartwood forms (62, 109). The most noticeable change is the formation of nonstructural material, (extractives), sometimes in amounts exceeding 30% of the total wood, which increases the density, color, durability, and many other properties of the wood (68, 74, 76).

Extractives accumulate in the lumen, or occlude or encrust pits and walls (7, 38, 91, 92). In some species, phenolic substances can diffuse from the ray parenchyma cells into the cell walls and into fiber lumens (38). The capillaries of the cell wall are wide enough to accommodate the molecules of some extractives (75). There is a good deal of indirect evidence for the presence of extractives in cell walls of the heartwood of different species (162, 170) (W. E. Hillis, unpublished data). New techniques, such as the use of gas-liquid chromatography and microspectrophotometry, make it possible for very small amounts of most extractives to be estimated or detected in small parts of tissues. These and other techniques will enable the relative amounts of extractives in the cell wall and lumen to be determined (10, 92). Toxic components probably convey greater durability if present in the cell wall than in the lumen.

The amounts of polyphenols formed in cultures of *Juniperus communis* (23), rose (26), and tea (40) were dependent on the sugar concentration.

ese findings support an earlier proposal (69), that the amount of polyphe-
s in heartwood is related to the amount of carbohydrate reaching the
ndary.

A large number of secondary compounds has been identified in heartwood
). The composition varies according to the family, genus, and species to
h an extent that sometimes species can be identified from their heartwood
ractives, although there are cases where there is variation in composition within
hin a species (71). The composition of extractives in the sapwood often differs
ers from that in the heartwood of the same tree (74). The existence of
ractives in heartwood (and in discolored wood) is possibly concerned with
evolution of species that can resist insect predators and microorganisms
).

A large portion of published data supports the view that the extractives are
med at the heartwood periphery from carbohydrate (76). Other views on
rtwood formation have been expressed (158). Recent data give further
dence in support of *in situ* formation (L. Shain & W. E. Hillis, unpub-
ed information), although it has been proposed that the factors control-
the amount and composition of heartwood extractives are incorporated in
he ray cells during the early stages of their development (61).

The wide range of colors seen in heartwood is due largely to extractives. The
ount in heartwoods of similar age of some species is partly genetically
trolled (44), but a fast growth rate can lower the amount normally
med (76), and even the soil type can affect the color (117).

Usually the amount of extractives increases from the pith to the heartwood
iphery, and this is generally considered to be due to the larger amount
med at the time of heartwood formation. There are cases of a uniform
ribution of extractives across the heartwood of Scots pine (30). The work
Anderson et al (3), Rudman (128), and others has shown that the toxicity
of extractives in the interior of heartwood to fungi and insects can de-
ase on aging (93, 132). This could be due to enzymic oxidation (99,
), free radical reactions, polymerization, hydrolysis, and changes caused
acid and microbiological degradation. Extractives have widely differing
ic or repellent properties to different wood-destroying microorganisms
7, 132). Even mildly toxic components present in adequate amounts can
fer durability (58).

chanism of the Formation of Heartwood and Extractives

abrupt change of sapwood to heartwood reveals the existence of an ac-
situation. It is generally considered that the change is a DNA-coded age
effect that can be influenced by environment. However, the erratic and
lulating heartwood boundaries that cross over several growth rings in
ny trees indicate that more study is required to define the trigger that sets
the changes.

The differences between sapwood and heartwood already pointed out pin-

point the narrow transition zone (when present) or the heartwood periphery as dynamic zones in the living tree. Chattaway (19) suggested that heartwood formation must be preceded by a period of increased metabolic activity. Other workers suggested that both the Krebs cycle and pentose shunt enzymes were affected (66, 70, 72, 74, 182).

Although some direct evidence of increased metabolic activity has been found at the heartwood periphery (95, 105, 180), most studies have been unsuccessful in this regard. Hirai (77) produced evidence that heartwood formation takes place mainly when cambial growth ceases, so that the frequently reported lack of evidence of activity at the heartwood periphery could be due to collection of samples at inappropriate periods. The recent work of L. Shain & J. F. G. Mackay (unpublished data) has shown that increases in respiration and the activity of malate and glucose-6-phosphate dehydrogenases in the transition zone of *P. radiata* are seasonal, maximum amounts occurring in the dormant period of tree growth. The factor that initiates these increases in activity requires consideration.

Many studies have shown that ethylene acts as a regulatory hormone (14, 119) in a variety of physiological changes occurring at many stages in the ontogeny of plants. It can play an important role in the regulation of cellular metabolism, which is related not only to morphological changes, but also to basic cell processes. Very small amounts (1–5 ppm and smaller) of ethylene effectively trigger a wide range of events according to the tissue involved (12, 14, 119). Because of the ready production of ethylene on injury of many tissues and their sensitivity to it, experimental work in this area is fraught with difficulties (119). The considerable data collected on ethylene in studies of vegetative tissues point to a probable pattern of events that lead to heartwood and extractives formation.

Ethylene is produced by the transition zone surrounding the heartwood of *Pinus radiata* (the peak of production taking place in the dormant period) and in larger amounts than the adjacent sapwood (L. Shain & W. E. Hillis, unpublished data). In *P. radiata,* the transition zone contains very small amounts of polyphenols and a lower moisture content than the heartwood. The transition zone of *Eucalyptus tereticornis* also produces more ethylene than the sapwood, but in this species the transition zone contains more polyphenols than the sapwood, and has a moisture content similar to that of sapwood and heartwood (W. E. Hillis, unpublished data). Cell suspension or callus cultures of different plants, including sycamore, also release ethylene; and a very sharp peak of production occurs in the latter after 10–14 days of culture (113) or toward the end of the growing phase of cell cultures (96).

The factor initiating ethylene production by injured, diseased, or senescing plant tissues has not been established (119). There is an absolute need for oxygen (102) and, at low oxygen concentration, sensitivity of the tissue to ethylene is decreased. Once the threshold value is exceeded, the system can produce ethylene autocatalytically. However, the system does not seem to be fully

ly activated until a certain physiological age is reached (119). Carbon dioxide is competitive at the receptor site of the ethylene, particularly at low ygen levels (13, 103).

It has been found that polyphenol formation increases in *Sirex-Amylostern* lesions within 24 hours of the increases of ethylene and respiration 35). Heartwood polyphenols have been formed in fresh blocks of *P. radiata* sapwood stored in a container continuously ventilated with humidified air containing ethylene (5 ppm), but not in air containing carbon dioxide 7.5%) (L. Shain & W. E. Hillis, unpublished data). The composition of polyphenols, however, more closely resembled that from injured wood than from heartwood of this species.

The factors initiating dehydration of the transition zone or heartwood in many species are unknown. However, moisture stress may be important for the stimulation of ethylene production (113, 114). It is noteworthy that, in *P. radiata* blocks used for in vitro polyphenol synthesis, the latter were med predominantly in the partially desiccated zone near the surface. The mation of pinosylvins in *P. resinosa* cultures (88) or of heartwood in stem tions of *Fagus sylvatica* (183) (which has been attributed to dehydration) y have been caused directly by ethylene.

Ethylene increases, in a short period, RNA and protein synthesis (104) led the activity or the *de novo* synthesis of a number of enzymes. Those so reported include phenylalanine ammonia lyase (PAL) (82, 122), polynol oxidase (156), α-amylase (86), cellulase (1), and particularly peroxie (83, 123). Several cases are known in which ethylene increases the rate of respiration (81, 121). The increase in respiration of the transition zone lative to the surrounding sapwood) in *Pinus radiata* increases (L. Shain & F. G. Mackay, unpublished data) over a similar period to the increase in ylene content (L. Shain & W. E. Hillis, unpublished data). So far, there no specific data showing that ethylene enhances the activity of enzymes of he Krebs cycle and pentose shunt, such as those found to increase in the transition zone in the dormant period of *P. radiata* (L. Shain & J. F. G. ckay, unpublished data).

The amount of ethylene present has been related to the concentrations of a polyphenol formed in carrot tissues (17). The amount of polyphenol formed reduced by carbon dioxide. Also, treatment with natural and synthetic auxins produced the characteristic polyphenol in carrot (17), and other polynols in the cell and callus suspension cultures of other plants (8, 26,).

An association between auxin and formation of heartwood and extractives has been considered (74, 182). Hormones, metabolites, etc. could be more readily available for heartwood formation after periods of active growth in the cambium. Several workers have shown that natural and synthetic auxins initiate ate, stimulate, and prolong ethylene production in higher plants (46, 79, , 131). Recent work indicates, however, that the balance between auxin- and ethylene is more important than absolute amounts of either (14, 103). The overall effect of auxin in heartwood formation requires further study. A very high concentration of carbon dioxide has also been reported to be conducive to the formation of heartwood polyphenols in *Acacia mearnsii* (15).

Although the most detailed studies on ethylene were concerned with the production of polyphenols, it is well established that ethylene promotes an increased production of rubber in *Hevea braziliensis* (2) and carbohydrate gum in *Prunus* spp. (W. E. Hillis, unpublished data).

In summary, evidence indicates that ethylene plays a key role in the formation of extractives, which are largely responsible for the color of heartwood. Whether the initiation of ethylene formation is triggered by water stress, which has been suggested as a key factor in heartwood formation (129, 183), remains to be determined. Heartwood extractives are formed at the heartwood periphery or in the transition zone during the dormant season, from translocated or stored carbohydrate. Peroxidase, whose activity increases at the heartwood periphery (95, 172), and the phenol oxidases in the heartwood (27, 89), can cause darkening of the tissues after exposure to air.

The role played by ethylene in the formation of discolored wood requires determination. It is notable that different trees of *P. radiata* respond differently to *Sirex-Amylostereum* damage in the formation of ethylene, polyphenols, and discolored wood. The ethylene appears to result from host-parasite interaction. It is known that some fungi produce ethylene; and whether these produce discolored wood—in contrast to those that do not form ethylene—requires further study. It should be noted, however, that discolored wood can have a different composition from that of heartwood in the same tree.

Formation of Discolored Wood

The major conditions in heartwood formation—cell death, depletion of nutrients, deposits in cells with darkening of tissues—also occur in formation of discolored wood, but there are other processes too.

Though discoloration is a condition of the wood, the color is a poor indicator of the changes that have occurred (144). Attention should be focussed on the events that follow wounding, rather than on one minor condition—discoloration—of these important events. Although this minor condition has served as the focus for so many studies, it will be treated here within the broad context of the events that follow wounding.

The many events that occur from wounding to total decomposition of tissues are continuous over time, and actually it is not possible to separate them. But, for the sake of clarifying the events that follow wounding and putting discoloration and decay in proper perspective, the sequence of events in a model system are separated into three major stages (143) (See Table 1).

Stage I includes all processes associated with host response to wounding, in which both the tree and the environment are considered. Slight discoloration may occur in the xylem as a result of chemical processes, including those involving formation of phenols and other components, and oxidation resulting from exposure to air (37, 47, 90, 98, 160).

Stage II includes those events that occur when microorganisms surmount chemical protection barriers and invade the xylem. These pioneer invad- are usually, but not always, bacteria and nonhymenomycetous fungi (139,), 141, 142, 144, 146). The discoloration of the wood is intensified as a ult of interactions between invading microorganisms and living xylem cells 14). There is now also a host response to invasion (133).

Stage III includes the events that occur when decay microorganisms, espe- lly hymenomycetes, invade and degrade the cell-wall substances. The mi- organisms compete among themselves; all cells in the xylem are now dead. er the pioneer decay microorganisms invade the wood, many other micro- anisms—phycomycetes, actinomycetes, myxomycetes, and nematodes— ow and compete for the remaining portions of the tissues (51, 140, 146).

ble 1 The Sequence of Events in the Decomposition of Wood

	Stage I	Stage II	Stage III	
	Host response to wounding	Infection & invasion by pioneer microorganisms	Decay processes	
WOUND	>			> DECOMPOSITION
	Tree and abiotic environment	Tree, abiotic environment, and microorganisms	Interactions among microorganisms as they digest cell-wall substances	

When trees are vigorous and the wounds are not severe, the processes stop Stage I. As the vigor of the tree decreases, or as more severe wounds are icted, the processes may go on to Stage II before they stop. When the or of the tree is low, the wounds are severe, and the aggressiveness of the croorganisms is strong, the processes may go to Stage III.

In summary, there are several stages following the many wounds inflicted trees before they reach maturity. A host response to wounding always urs—Stage I; infection and invasion of xylem by microorganisms that sur- unt the protective barriers of the tree and a host response to invasion oc- most of the time—Stage II; and some of the time decay follows—Stage While these events are occurring, the tree is growing, branches are dying, l other wounds are occurring.

PES OF WOUNDS There are two basic types of wounds: those that expose marily the axis of the stem or root of trees via broken branches, broken s, and broken roots; and those that expose primarily the xylem immediately under the bark by means of mechanical wounds, animal wounds, fire unds, etc.

The most common type is the branch wound. Branches die for many rea- s. When they die or when they are broken off, air and microorganisms y quickly or gradually enter the center of the tree and the growth layers in trunk that extend into the branch. A broken top or broken root exposes

similar tissues. Most branch wounds heal and prevent exposure of the trunk to air and microorganisms, but healing may be complete only after some of the trunk tissues have been altered. One of the minor conditions of the altered tissues in Stage I is discoloration. More discoloration is associated with branch wounds than with any other type of wound, and it is usually in the center of the tree.

The severity of the wound and the vigor of the host affect the rate and effectiveness of the tree's response to the wound (116, 161). Wounds that break the bark, but injure the cambium and xylem only slightly, usually heal rapidly (21, 143), although it has been found that the "wound heartwood" of *Pinus sylvestris* forms with the beginning of cambial activity and terminates in winter (100). The processes can stop in Stage I, and some discoloration may be associated with the wound.

It is noteworthy that when the sapwood is deeply penetrated by a wound, a pale-colored transition zone (similar to that around heartwood) surrounds the discolored wood in a number of species (133, 144). A similar zone has been observed around the discolored wood of lesions resulting from attack on *P. radiata* by the *Sirex-Amylostereum* complex (136), and on *Picea abies* by *Fomes annosus* (134). Thus, with some species at least, there is a visual similarity between the formation of discolored wood and heartwood, and further examination may show the existence of the transition zone to be more widespread.

HOST RESPONSE TO WOUNDING The response to wounding in herbaceous and woody plants is similar in principle: a chemical protective response occurs, and tissues darken (127). Most woody plants survive after wounding because the protective response is effective most of the time. However, in some cases, the tree may be so low in vigor, or the conditions for invasion by microorganisms may be so favorable because of inoculum quantity, environmental conditions, and severity of wound, that invasion occurs rapidly. Between the extremes of no invasion and rapid invasion are all degrees of effectiveness of host protection and aggressiveness of microorganisms. Also, between the extremes there are all degrees and gradations of color changes in the wood.

In general, the living sapwood cells show a dynamic response, and discolored wood containing extractives is formed in a zone several millimeters wide around the area containing microorganisms (133). Heartwood shows a passive response. When the protection processes in Stage I function effectively, the xylem altered by host response to wounding is indeed a protective wood that resists invasion by microorganisms, and "protection wood" (43, 63, 87) is an accurate term for these tissues.

The extractives formed in "protection wood" or "reaction zone," as in the lesions from *Fomes annosus* (133, 135), *Sirex noctilio-Amylostereum areolatum* (73), etc., can be different from those of the heartwood and even those of lesions in different trees of the same species. The extractives can play a

nificant part in enabling the living tree to hinder the extension of the dam-ed zone.

ECTION AND INVASION PROCESSES Propagules of many microorganisms carried to moist fresh wound surfaces by wind, rain, snow, animals, man, d insects (4, 143). Saprophytes infecting wound surfaces may prevent the ablishment of other more aggressive microorganisms that can invade (11, , 34, 49, 107, 125).

When severe wounds are inflicted and conditions are favorable, invasion microorganisms occurs. The principal pioneer microorganisms that invade unds are usually bacteria and nonhymenomycetous fungi (5, 6, 31, 97, 1, 140, 143, 151). There is some indication that they can utilize or detox- the chemicals formed in Stage I (149, 163, 164). As the pioneers enter, living cells distal to the wound at the margins of the lesion continue to ct (133, 144), but the response is more to the invasion of microorganisms n to the wound (133). Here the term "reaction zone" is an accurate one 33), describing the margin of the lesion where the interactions are occur-g between tree and microorganism. Shain (133, 134) has given details on events occurring in the reaction zone.

The pioneer invaders in some cases—such as root rots—are hymenomy-es (150, 169, 173). Bacteria and yeasts are intimately associated with ne of the pioneer nonhymenomycetous filamentous fungi (101, 140, 143). e bacteria may be aerobes (24), facultative anaerobes (24), or obligate aerobes (145, 157). The ray parenchyma cells are usually the first to be aded by pioneer invaders. These cells contain a high concentration of nu-ents, and they are also the cells that show the first signs of darkening as ir contents break away from the cell wall and begin to degenerate (138, 0). Some microorganisms produce polyphenol oxidases (150) that in-ase oxidation of the phenols (90, 150), leading to further darkening of the ues.

As the tissues die and discolor in Stage II, pH increases, minerals accumu-e, and moisture increases (50, 54, 142). Although these changes are ac-mpanied by intensification of discoloration in most species, the changes in ne species occur without a color change, a condition commonly called etwood" (32, 59, 94, 168, 174). Bacteria are commonly associated with twood (16, 59).

CAY PROCESSES The processes that result in decay follow discoloration , 140, 141, 143), but there are great differences between discoloration pro-ses and decay processes, especially in rate. A large column of discolor-on does not indicate that a large column of decay will follow (64, 142). e rate of decay depends on the aggressiveness of microorganisms.

The columns or lesions of discoloration and decay advance most rapidly ove and below the wound, but towards the center of the tree in species of *er* that have no colored core of age-altered wood. In species of *Quercus*

that have a colored core of age-altered wood, the columns of discoloration and decay advance most rapidly above and below the wound along the sap-wood-heartwood boundary that was present at the time of wounding (146). When severe wounds occur, however, the entire column of wood present at the time of wounding may discolor and decay (64).

SUCCESSION OF MICROORGANISMS Microorganisms that inhabit wood in living trees have the greatest survival advantage when they attack wounds in a sequential manner. Each invading microorganism exerts its specific force against the dynamic protective barriers formed by the wounded tree. The pioneer microorganisms first alter the substrate to their advantage and then digest the cell contents. As the pioneers advance, the substrate is altered further to the advantage of other organisms that follow—a *succession* (5, 6, 31, 34, 51, 97, 101, 107, 140, 163).

A good account of microorganisms associated with heartwood in *Thuja plicata* was given by Eades & Alexander (28) and by Findlay & Pettifor (39). Dark and light heartwood occur in this species; the dark heartwood contained nondecay fungi, but no organisms were found in the light heartwood. Findlay & Pettifor concluded that the fungi were responsible for the dark heartwood and its reduced strength and specific gravity. The toxicity of dark heartwood is also low (48). Consequently it would be more correctly defined as discolored wood. The hyphae they observed in the cells was shown by Roff (126) to be due to nonhymenomycetous fungi, and thus the conclusion of Findlay & Pettifor was supported that the fungus in the "dark heartwood" probably was not a decay fungus.

Findlay & Pettifor also reported (39) results from laboratory tests showing that test blocks of dark heartwood were susceptible to attack by certain hymenomycetous fungi such as *Coniophora cerebella,* whereas it was with great difficulty that fungi could be induced to grow at all over the light-colored heartwood. The results indicated that the pioneer nonhymenomycetous fungi altered the wood to the advantage of *C. cerebella.* Similar results with *Acer saccharum* suggested that the pioneer microorganisms attack wood altered as a result of host response to injury, and the alterations are to the advantage of hymenomycetes (142). Tissues in Stage I may have evolved as effective deterents to invasion by hymenomycetes. The bacteria and nonhymenomycetous fungi then probably adapted to the new substrate.

COMPARTMENTALIZATION As resistant as the tissues may be in Stage I, under certain conditions some microorganisms are able to surmount the chemical protective barriers and invade. At this time the tree forms a second line of defense and restricts the path of the invaders.

One of the first mechanical barriers to form in tissues after wounding is plugged vessels (124, 138). In those species capable of doing so, tyloses are formed; and in the other species the vessels are plugged with a gummy material (138, 160). These plugs begin to form in *Acer rubrum* a few days after

'ounding (124). The most dramatic anatomical response to wounding oc-
urs when the uninjured cambium around the wound begins to form cells
ıat are different from those formed normally (138, 147). The severity of
ıe wound determines the extent of the reaction. When wounds occur during
ıe time when the cambium is not forming cells, the barrier wall begins to
ɔrm as soon as cambial activity resumes (147).

After injury, the active living cambium, even well away from the wound,
ɔrms cells with thicker walls (138, 147). These cells in *Acer* are similar to
ıte wood (138). The ray cells have thicker walls, and the cells are more
ɔunded. Vessel production is retarded (147). These and other changes ac-
ount for a barrier wall separating tissues formed after wounding from those
resent at the time of wounding. As subsequent tissues and annual growth
ıyers form, they remain separated from the compartmentalized injured tis-
ıes. The living ray parenchyma acts as a barrier wall tangential to the sides
f the injury.

These changes also occur in tropical species that have no distinctive
rowth rings. A different behavior is shown by eucalypts (152), in which the
ambium shortly after injury in the growing season goes from the induction
f multiple cambial divisions to the production of anomalous parenchyma,
'hich give rise to roundish patches of thin-walled cells that develop into la-
unae with layers of tissue. Eventually the cambium resumes normal xylem
ɔrmation, but the tissues in the lacunae break down, and the region is filled
'ith polyphenols ("kino"). In most eucalypts kino has a markedly different
ɔmposition from xylem polyphenols (68, 70).

When the invading microorganisms spread, they do so along the path of
:ast resistance, vertically through the compartmentalized tissues (143, 146).
ıpparently this is the weakest link to the compartment. The rims or margins
f the compartments remain intact throughout the life of the tree. At the
ortion of the column distal to the wounds—the reaction zone—the pioneer
ıngi are still present in most cases (133, 142, 144).

When a tree is wounded another time, another barrier wall or compart-
ıent begins to form and to envelop the inner compartments (143, 146). This
rocess is similar to having a pipe slide over other smaller pipes. The wood
etween the rim of the last compartment and the new compartment includes
ıe tissues invaded by microorganisms. The degree of tissue alteration or the
ate of invasion of each new compartment may be different, and different
ıicroorganisms may be involved (140, 142). This explains the ring rots
ommonly found in trees; they are compartments between the barrier walls
140, 142). This also explains the darker streaks of tissues within altered
eartwood.

The barrier wall formed by the cambium after wounding functions effec-
:vely most of the time in compartmentalizing the injury and microorganisms.
'he wall acts as a partition between wood formed before and after wound-
ıg. The wall is the major site of partitioning of growth rings, termed
shake," which occurs when other pressures are exerted, such as drying pro-

cesses, growth of microorganisms (112, 147, 171), etc. Shakes are associated with wounds, but not all wounds form shakes (147).

If part of the cambium of eucalypts and some other trees containing heartwood is killed, the region between that part and the heartwood remains as "included sapwood" when the adjacent sapwood is converted to heartwood during subsequent growth of the tree (68). These areas can subsequently discolor and decay. Included sapwood is frequently seen in trees that have been scarred by fire (146).

Differences Between Heartwood and Discolored Wood, and Means of Recognition

When living sapwood cells encounter different stimuli, they usually respond differently when forming colored wood. The latter can be heartwood when the oldest tissues are affected first, or different types of discolored wood when often the youngest tissues are affected first. The differences between sapwood-heartwood and sapwood-discolored wood are mainly in the amount and distribution of inorganic elements, the pH, the amount and composition of extractives, and moisture content.

EXTRACTIVES Normal heartwood has a similar color throughout the cross-section of a log, and a chemical composition that is in almost all cases constant for a particular species. In injured and discolored wood, the amount of extractives is higher than in the sapwood, amorphous deposits of melanistic substances are more abundant than in heartwood (55), and the extractable materials in these tissues frequently differ qualitatively from each other (74, 76, 135). Discolored woods of the same species, and apparently resulting from the same cause, can contain different ratios of components (73).

Components in discolored wood can be different from those found in heartwood, as in *Prunus* species affected by *Trametes versicolor* (60) and *Stereum purpureum* (67). The cellular inclusions in histological examinations have been defined as tannins, deposits, etc.—in most cases without consideration of their variable composition, which can be different even a few cells apart (92). Aside from the confusion caused by the theories regarding their biogenesis, such loose terminology overlooks their difference in properties to invading organisms, either by presenting physical barriers such as gums or by forming toxic components. Certain types of wounds in certain species show a stimulation of the synthesis and accumulation of materials inhibitory to decay fungi; in other species the discolored wood surrounding the wounds is no more decay-resistant than the sapwood of that particular species (57). The age of the sapwood that has given rise to discolored wood may also influence the ability of the latter to resist decay fungi. It should be noted, however, that the ability of the sapwood to form discolored wood containing toxic components may be linked with an ability to produce heartwood resistant to decay fungi (57).

When heartwood is wounded, it can discolor, although in dark heartwood he changes are difficult to see. Kondo (89) pointed out that heartwood contains enzyme systems that can function after injury, and oxidase enzymes ave been reported in the heartwood of *Pinus* spp. (137). The brown stain f hemlock heartwood can be controlled with an enzyme inhibitor (35). Discolored heartwood has been associated also with galleries of insects (155) nd with microorganisms (175, 176).

The pioneer invaders of wood—bacteria and nonhymenomycetous fungi—re often ignored in studies of heartwood. The heartwood altered by microorganisms is then considered—on the basis of color—to be another type of eartwood.

When heartwood is wounded, microorganisms can invade (142, 146); but hen discolored tissues in Stage I that have resulted from host response to ounding are wounded again, even when they are in heartwood, they seldom iscolor further (142). This indicates that tissues in Stage I are more a protective tissue than unaltered heartwood.

Much useful information has been gained from in vitro tests about the relative durability of sapwood, discolored wood, and heartwood from the same ree, and between different trees and species. Some of these conclusions may eed to be modified when more becomes known about the complexities of nction of specific substrate molecules and their relationship to gene and nzyme activation. These aspects are not usually considered in agar plate and ood-block tests.

INORGANIC ELEMENTS The main mineral constituents of wood are salts of lcium, potassium, and magnesium, but many other elements are present in inor amounts. The acid radicals are carbonates, phosphates, silicates, sultes, and oxalates—and probably the acidic groups of components of the ll wall. The inorganic material occurs scattered throughout the cell wall or accumulations in the form of crystals, either deposits in the cells or intrallular large lumps.

The relative amounts of ash in the sapwood and heartwood show great riation between species, sometimes even in the same species. Usually the nount of ash is higher in the sapwood than in the heartwood, but the nount of certain elements is selectively different. During the transformation the sapwood of *Robinia pseudoacacia* and *Maclura pomifera* to heartood, the ash fell about 30%, but the amount of phosphorus dropped about %, and calcium 24% and 41% respectively, but the other elements reained constant (55). Magnesium and manganese tended to be concentrated the heartwood of pine, unlike calcium which steadily decreased with the cceeding growth rings (41).

An increase in inorganic components in discolored tissues of sapwood has en reported by many workers. These accumulations are commonly calcium rbonate, but other components can be present. The significant aspect of iscolored wood is that the amount of inorganic material is higher than that

in the surrounding tissue. Discolored wood of sugar maple had 6 times more ash than normal sapwood; however, there was a 9-fold increase in calcium but a 56% reduction in potassium (E. L. Ellis, unpublished data). There can also be an increase in manganese with this species (148). The differences in these three aspects in shagbark hickory are much greater (29).

The situation can be more complex. Hart (55) found in the discolored wood of *Robinia pseudoacacia* a 136% increase in ash as compared with normal sapwood, when potassium increased 61%, calcium 100%, and magnesium 168%, but phosphorus decreased 35%. A similar pattern was observed with *Maclura pomifera.* The minerals in the stained wood of *Acer saccharum* are not removed by water, and this behavior may be due to combination with the polyphenols (N. Levitin, unpublished). It is interesting that, whereas the accumulation of calcium salts in aging cells of plants is well documented (167), the increase observed in discolored wood has not so far been observed in heartwood.

The discolored woods of *Quercus alba, Maclura pomifera, Robinia pseudoacacia, Juglans nigra,* and *Acer saccharum* had higher pH, moisture, and ash contents than uninjured sapwood (56). The deeper the stain of discolored wood of *A. saccharum,* the higher the pH and mineral content (50; N. Levitin, unpublished). As the wound that resulted in the surrounding discolored wood of sugar maple is approached, there is a general increase in moisture (144), pH, and ash (144, 163, 164). The pH of the discolored wood of *Picea abies* (134) and *Quercus rubra* (130, 142, 171) is also higher near the wound. The pH of discolored wood of many species is above 6, even as high as 9 (50, 144), whereas that of sapwood and, in particular, heartwood in the same stem is below the pH of discolored wood, usually below pH 5.5.

Valuable use has recently been made of the content of inorganic materials and of developments in electrical techniques to detect discoloration and decay in living trees. The technique enables a quantitative and objective assessment of discolored wood and heartwood.

The changes in concentration of ions in wood are in direct relation to resistance to a pulsed electric current (153, 165). As tissues die, discolor, and decay, the resistance to a pulsed electric current decreases, as long as the moisture content of the wood being measured remains above the fiber saturation point (165). The decrease in resistance is related to the increase in mobile ions (such as potassium) in dying tissues, leading to discolored wood (165). Resistance to a pulsed electric current throughout unaltered and uninfected heartwood in *Quercus* spp. is higher than that of sapwood (165). The electrical apparatus indicates the differences between age-altered high-resistance (12–60 thousand ohms) tissues and injury-altered low-resistance (1–20 thousand ohms) tissues. The measurements indicate accurately the degree of degradation of injury-altered tissues (153, 165) in living trees. With this method, numbers can be put on the model system to show that, as the tissues go from Stages I to II to III, the resistance to a pulsed electric current decreases steadily.

nmercial Importance of Distinguishing Between irtwood and Discolored Wood

he need for understanding processes associated with color changes in d has increased in the last decade; it will increase much more in the future. The demand for high-quality wood—indeed all wood—is increasing ughout the world.

roperties associated with change in color are important economically because they affect wood quality and utilization. Darkened age-altered wood often yields high-value products. The adoption of multiple-use forestry practices will lead to a higher incidence of injury in trees, but in this case the darkened ened wood resulting from injury, and inhabited by microorganisms, has little or no value for quality products.

echniques are required (such as the above electrical technique) that will make possible the ready detection and quantification of the biggest loss of wood in the forests of today and of the future, and ultimately to assist in control of such losses. Investigations into the formation of colored wood—heartwood or discolored wood—must be made so that the maximum amount of he most suitable wood can be obtained to meet the world's expanding ds.

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BIOLOGICAL ACTIVITIES OF VOLATILE FUNGAL METABOLITES

❖3572

S. A. Hutchinson
Department of Botany, The University, Glasgow, Scotland

The authors of the last two reviews (46, 62) of this topic have questioned whether it is worthwhile to base a discussion on "volatility" at all. Outside the cell the distinction between volatility and nonvolatility is only qualitative, in the magnitude of the vapor pressures in particular conditions; within the cell solutions the distinction serves no purpose. They agree, however, that in practice it has become a stimulating focus for thoughts and that this has led to useful new work and knowledge. Six attributes seem to affect this closely:

(*a*) The ability to approach an organism through the gas phase may be particularly relevant for that majority of fungi that develop part of their mycelium and their entire reproductive structure in air above wet or liquid substrates (46).

(*b*) Many lipophilic compounds produced directly into the air and almost insoluble in water may, even at some distance from a donor source, accumulate faster in the plasma membrane of an acceptor cell if the transfer takes place via the gas phase instead of via the liquid phase (46).

(*c*) During movement in the gas phase, metabolites will be exposed only to gaseous and physical inactivating or stimulating factors. Those diffusing in complex liquid solutions are likely to be exposed to more concentrated chemical inactivating or stimulating factors, and their movements will be limited by discontinuities in water films (46, 62).

(*d*) Because the substances are active as gases, they are likely to be relatively simple molecules; with modern methods their identification is probably less of a problem than their measurement and control; this leads to a common experimental approach, particularly for their chemistry (62).

(*e*) The very low concentrations in which some of the identified ones are active suggests a comparison with antibiotics, growth factors, and vitamins, all areas of knowledge in which a loosely defined concept has promoted inquiry and discovery (62).

(*f*) Perhaps the most important practical point; that their volatility leads to impermanence in particular situations, and to risks of escape from observation (62).