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ANATOMICAL CHANGES IN WOOD CELL WALLS ATTACKED BY FUNGI AND BACTERIA

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INTRODUCTION

A numer of species of fungi live in or on wood, and as a result of their growth anatomical changes may be induced in substrate cell walls. These changes occur in several patterns that can be readily distinguished from one another, and that apparently result from quantitative or qualitative differences in the enzyme complement of the organisms in each group. The patterns resulting from the morphological effects of fungus action tend to correlate closely with taxonomic groupings based upon the morphology of the fungal thallus. As similarities of action within the groupings must be based upon similarities of the enzymes involved, differences in the effects produced by a given group of organisms in different species of wood must indicate structural or chemical differences between the woods. Therefore, study of the effects of fungus action in wood should be of interest to persons concerned with wood structure and composition, as well as to those dealing with fungus physiology and mycological taxonomy.

The terminology applied in the present paper to the various layers of the wood cell wall is that proposed by Kerr & Bailey (Bailey & Kerr, 1935; Kerr & Bailey, 1934), as alternative terminologies have caused considerable controversy (Meier, 1957; Nečesaný, 1957; Wardrop & Dadswell, 1957). In addition, I have followed the convention according to which layers of the secondary wall are assigned numbers, a simplification used extensively since its adoption by Meeuse (1942). Accordingly, a typical cell would consist of middle lamella (ML), the isotropic, intercellular substance; primary wall (P), the cambial wall or original wall formed in the meristem; and secondary wall, consisting of an outer layer (S1), central layer (S2), and inner layer (S3). The term compound middle lamella denotes the unit composed of the true middle lamella and the two adjacent primary walls. References to direction within the cell wall also correspond to the concept of Kerr & Bailey (1934) of "inner" and "outer" layers. Therefore, "outward" denotes the direction from lumen toward middle lamella and "inward" the opposite direction.

DECAY

The term decay is here applied to the effects produced in wood by the action of fungi belonging to the class Basidiomycetes. Two major types of decay are recognized, based on action within the wood and on reaction of the causal fungus to tests for extracellular oxidase (Nobles, 1965). In brown rot only the carbohydrate fraction of the wood is removed to a significant degree, while in white rot both the lignin and carbohydrate fractions of the wood eventually are removed. More detailed classifications of rot types have been proposed but will not be employed here (Björkman et al., 1949; Meier, 1955). Earlier reviews of literature on the microscopical characteristics of decay were presented by Cartwright & Findlay (1943; 1958) and Wilhelmsen (1965).

Hyphal Distribution. *White Rot.* White-rot fungi have been reported to first extensively colonize the vessels or rays during attack of hardwoods, entering the fibers only as decay progressed (Bayliss, 1908; Cowling, 1961; Greaves &

Levy, 1965; Scheffer, 1936). In other work, hyphae were observed in almost all cells early in decay of a hardwood (Wilcox, 1968), although they were more numerous in vessels and rays. In coniferous wood, hyphae from a white-rot fungus were more numerous in rays and resin canals but were present in all cells in early stages of decay (Wilcox, 1968).

Brown Rot. In some of the brown rots investigated, the mycelium had permeated small study blocks in early stages of decay—in one study, using sweetgum cubes 0.75 inch on a side, prior to 5% weight loss (Cowling, 1961). In most reports hyphal distribution was quite uniform, with nearly every cell containing at least one hypha (Cowling, 1961; Pechmann & Schaile, 1950). However, one study of brown rot in a coniferous wood indicated that hyphae were abundant in rays but sparse in the tracheids (Wilcox, 1968). It has been observed that certain brown-rot fungi advance longitudinally through the lumina of wood cells as individual hyphae (Falck, 1919; Meier, 1955; Waterman & Hansbrough, 1957; Wilcox, 1968). Greaves & Levy (1965) found that in brown-rotted pine and birch the rays were heavily colonized with hyphae in early stages of decay, while in beech it was the vessels rather than the rays that had heavy colonization. In a study of microscopical characteristics of decay and toughness of decayed wood, no correlation was found between the quantity of hyphae visible in spruce tracheids and the amount of wood deterioration indicated by loss in toughness (Waterman & Hansbrough, 1957).

Hyphal Penetration. Fungi of the two major decay types also appear to differ with respect to the means of penetration from cell to cell. Hubert suggested that production of bore holes is a characteristic that can be used to distinguish most decay fungi from other wood-inhabiting fungi (Hubert, 1924). However, some fungi of white-rot and brown-rot decay types apparently penetrated through pits instead of producing bore holes in early stages (Bayliss, 1908; Cartwright, 1930; Cowling, 1961; Greaves & Levy, 1965; Pechmann & Schaile, 1950; Scheffer, 1936; Schmid & Liese, 1964; Wilcox, 1968). In most of these cases both brown- and white-rot fungi eventually produced bore holes in later stages of decay. It has been suggested that the number of bore holes may be an indicator of the stage of decay (Waterman & Hansbrough, 1957; Zycha & Brand, 1959). Other work, however, indicates only a rough correlation between degree of decay and the amount of mycelium or the condition of the cell walls (Cartwright et al., 1931), and Wilcox (Wilcox & Garcia, 1968) found bore holes to occur in about the same number both inside and outside the advanced decay pockets caused by the brown-pocket-rot fungus *Polyporus amarus* Hedgcock. Bore holes have variously been reported to be more numerous in advanced stages of brown rot than of white rot (Cowling, 1961; Waterman & Hansbrough, 1957), and vice versa (Wilcox, 1968). It has also been pointed out that pit cavities were enlarged in white rot (Jurášek, 1964; Long, 1930; Wilcox, 1968) and that enlarged pits and bore holes became indistinguishable in advanced stages of decay (Wilcox, 1968). In addition to forming bore holes perpendicularly to the axis of the cell, hyphae of brown-rot fungi have been observed to grow longitudinally within the S2 layer (Meier, 1955), and cavities of axial orientation have been observed in both white rot and brown rot

(Courtois, 1963b; Liese, 1956; Liese & Schmid, 1966; Sen, 1948; Tamblyn, 1937; Wilcox, 1968).

The mechanism of bore-hole formation by decay fungi has been investigated by several workers. It was reported that the hyphae either swelled slightly before penetration (Bayliss, 1908) and constricted into a fine thread to perform the first actual penetration (Meier, 1955; Nutman, 1929; Sen, 1948), or that there was no change in diameter upon penetration (Bailey, 1934; Cartwright, 1930). Liese & Schmid (Schmid & Liese, 1965; Liese & Schmid, 1966) observed specialized structures, microhyphae and transpressoria, which formed at the tip of *Trametes* (*Fomes*) *pini* (Thore ex Fr.) Fr. hyphae and appeared to function in bore-hole production, possibly in a mechanical role. Several workers (Bailey, 1934; Cartwright, 1930; Nutman, 1929; Proctor, 1941; Sen, 1948) concluded that white-rot and brown-rot fungi produced bore holes by a process of enzymatic digestion in advance of the hyphal tip. The enlargement of bore holes to several times the diameter of the penetrating hyphae, even after penetration has been completed, was thought to indicate that the wall-deteriorating enzymes are not liberated exclusively at the hyphal tips but are also exuded from the lateral surfaces of the hyphae (Bailey, 1934; Cartwright, 1930; Cartwright & Findlay, 1943; Cowling, 1961; Nutman, 1929; Waterman & Hansbrough, 1957). Furthermore, Liese (1963b, 1965; Liese & Schmid, 1962b, 1966) reported evidence of local wall dissolution around hyphae of white-rot fungi lying on the wall surface, indicating ability of these fungi to secrete enzymes along the length of their hyphae. Hyphae that originally had been sharply constricted during the process of wall penetration sometimes filled out to their normal size following enlargement of the bore holes (Nutman, 1929).

Microstructural Changes. A major difference between white rot and brown rot appears to be the microscopical appearance of the residual cell walls following removal of wall substance. White rot often involved a progressive thinning of the secondary wall, beginning at the lumen and progressing outward toward the middle lamella (Cartwright & Findlay, 1943; Cowling, 1961; Hubert, 1924; Liese & Schmid, 1962a; Scheffer, 1936; Schmid & Liese, 1964; Waterman & Hansbrough, 1957; Wilcox, 1968), while brown rot reportedly involved no such thinning of the secondary wall, or if thinning occurred it did so only in very late stages of decay (Cartwright & Findlay, 1943; Liese & Schmid, 1962a; Pechmann & Schaile, 1950). Pechmann & Schaile (1950) observed that strength loss in residual wood far exceeded the visible effects upon the walls in brown-rotted wood but closely paralleled these effects in white-rotted wood. This could be explained by results of chemical and microscopical analyses which indicated that in white rot decomposition was restricted to surfaces, while in brown rot the attack of carbohydrate was spread through the wall and hence did not become microscopically visible until advanced stages of decay (Cowling, 1961; Wilcox, 1968). Many such differences have been reported in the details of the removal of cell-wall substance resulting from the action of various decay fungi.

White Rot. White-rot fungi appear to follow one of two different modes of

action in their attack on wood cell walls (Björkman et al., 1949; Meier, 1955). The effects upon microstructure reportedly differ depending upon whether lignin is preferentially decomposed early in decay, or lignin and cellulose are decomposed simultaneously throughout decay.

Jurášek (1955) reported that the white rotter, *Panus stipticus* (Bull.) Fr., began its deterioration of wood by delignifying the cells progressively from the S3 to the middle lamella and finally caused a loosening of the bond between cells. This same organism also was reported to destroy the entire S3 layer early in decay (Nečesaný, 1957; Nečesaný & Jurášek, 1956). Nečesaný & Cetlová (1963) found a correlation between amount of cell separation along the middle lamella and loss in lignin content. *Polyporus versicolor* L. ex Fr., a white-rot fungus that appeared to attack the lignin and cellulose simultaneously (Cowling, 1961; Scheffer, 1936) caused a progressive thinning of the cell walls by decomposing each wall layer successively, beginning with the S3 and progressing toward the outside of the cell (Cowling, 1961; Liese & Schmid, 1962a; Meier, 1955; Scheffer, 1936; Schmid & Liese, 1964; Wilcox, 1968). However, evidence was found that the lignin-destroying enzymes of this fungus may precede the action of the cellulolytic enzymes in the lignin-poor regions of the secondary wall (Wilcox, 1968). A similar progressive thinning was reported for *Trametes gibbosa* Fr. (Jurášek, 1964). Long (1930) reported delignification of ray cells and tracheids and destruction of the middle lamella of vessels and rays in early stages of white rot caused by *Ganoderma* (*Polyporus*) *Curtisii* (Berk.) Murr. in oak. Lutz (1931, 1943) reported the first stage of the white rots studied, including that caused by *Coriolus* (*Polyporus*) *versicolor* (L. ex Fr.) Quél., to be delignification. The cell-wall lignin was reportedly completely destroyed before cellulose was attacked, but attack on the middle lamella was not observed until cellulose decomposition was nearly complete (Lutz, 1931, 1943). In light of current knowledge of lignin content in the middle lamella, the observations of Lutz can be interpreted as similar to those of Wilcox (1968) in which lignin decomposition appeared to precede cellulose decomposition in lignin-poor regions of the secondary wall, although the two processes became more equalized when delignification reached the lignin-rich compound middle lamella. This may explain the remarkable proportionality between the rates of lignin and cellulose decomposition throughout all stages of decay by *Polyporus versicolor* L. ex Fr. as reported by Scheffer (1936) and Cowling (1961). White-rot fungi progressively decomposed the ray cells (Bayliss, 1908; Buro, 1954; Greaves & Levy, 1965; Waterman & Hansbrough, 1957) and greatly enlarged pit canals (Cowling, 1961; Scheffer, 1936; Wilcox, 1968). Wilcox (1968) found attack of ray cell walls in white rot of a coniferous wood but not in a hardwood. It has been reported that even in advanced stages of white rot little or no shrinkage or cell collapse occurred, and the original shape and outward appearance of the wood were maintained (Cartwright & Findlay, 1943; Scheffer, 1936; Wilcox, 1968). However, Greaves & Levy (1965) found a loss of structure in advanced stages of white rot in beech.

Radial cracks or checks in the secondary wall occurred during early stages

of white rot (Jurášek, 1964; Wilcox, 1968); these were interpreted as evidence of penetration of enzymes into the wall in advance of complete destruction (Jurášek, 1964). A separation between cells, within or adjacent to the compound middle lamella, also was observed in early stages of white rot (Wilcox, 1968). No changes other than pit cavity enlargement, wall checking, and cell separation were observed by Jurášek (1964) in a white-rotted coniferous wood, even at moderate stages of decay. Liese & Schmid (1966) observed cell separation along the middle lamella in late stages of white rot caused by *Trametes* (*Fomes*) *pini* (Thore ex Fr.) Fr.

Electron microscope studies of decayed wood have added considerable knowledge of microstructural changes and have contradicted some conclusions based upon light microscopy. Cell-wall thinning in white-rotted wood, as observed with the light microscope, appeared very uniform within each cell and from cell to cell (Cowling, 1961; Scheffer, 1936); however, upon observation with the electron microscope the thinning of individual cell walls appeared less uniform (Cowling, 1961; Meier, 1955). Minute pockets, indicating the removal of cell wall material, were found in both the secondary wall and the compound middle lamella. The occurrence of these pockets preceded over-all thinning of the walls (Cowling, 1961). A similar increase in cell-wall porosity in white-rotted wood has been reported by others (Buro, 1954; Liese & Schmid, 1962a; Schmid & Liese, 1964). Rhomboid cavities similar to but smaller than those typically produced by soft-rot fungi have been reported in the secondary walls of wood attacked by basidiomycetous, wood-destroying fungi (Courtois, 1965; Liese, 1963b, 1964; Liese & Schmid, 1962b, 1966; Schmid & Liese, 1964). Liese & Schmid (1962b; Liese, 1963b) concluded that such cavities may be caused by all types of wood-destroying fungi that hydrolyze cellulose. The cavities were produced in the S2 layer of the secondary wall, often where no hyphae were apparent, and it was presumed that the fungal cellulase had diffused through the S3 layer to produce this effect (Liese & Schmid, 1962b, 1966). Ability of the enzymes of a white-rot fungus to diffuse some distance from the producing hyphae also was postulated by Cowling (1961), based upon the observation that thinning of cell walls occurred at approximately the same rate in cells lacking hyphae as it did in cells containing hyphae.

Although reaction to guaiacol is used as a diagnostic test for identifying fungi that will produce white rot, Nečesaný (1965) found no quantitative relationship between the intensity of color change and the extent of wood decomposition visible in the electron microscope.

Brown Rot. The extensive, progressive cell-wall thinning typical of white rot has not been observed in brown-rotted wood (Liese & Schmid, 1962a; Jurášek, 1964; Pechmann & Schaile, 1950; Wilcox, 1968), the form of the cells apparently being maintained by the residual lignin framework (Cartwright & Findlay, 1943; Jurášek, 1964; Meier, 1955; Pechmann & Schaile, 1950). Changes in the cell wall in advanced stages of brown rot of a coniferous wood have been described by Wilcox (1968) as collapse rather than thinning; the wall at first retained its appearance while cellulose removal occurred throughout. In advanced stages of decay, presumably when residual material no longer

possessed the strength to maintain the original form, the wall collapsed and caused decreased cell size and wall thickness. Cellulose decomposition occurred in the secondary wall of the hardwood studied without producing noticeable change in the size or shape of the residual middle lamella as was true of the wall collapse noted in the coniferous wood (Wilcox, 1968). However, secondary wall decomposition in the hardwood was unlike the progressive thinning of white rot, as it began with the formation of large cavities in the S2 layer (Wilcox, 1968). Similar observations of brown rot in a coniferous wood were made by Jurášek (1964).

A number of other microscopical characteristics that distinguish effects of certain brown-rot fungi from those of white-rot fungi have been reported. Schulze & Theden (1938) found that cell-wall decomposition by brown-rot fungi was very irregular. Deterioration of cellulose was well advanced in some cells, while in adjacent cells the cellulose seemed to be little affected. They concluded that this irregular destruction offered an explanation for the cracked appearance of brown-rotted wood. Cartwright (1930) reported attack on tracheid walls by the brown-rot fungus *Trametes serialis* Fr. similar to that by white-rot fungi in that the attack began at the lumen. Jurášek (1955) reported that removal of cell-wall substance in brown rot began in the S2 layer of the secondary wall by the formation of oblong cavities parallel to the cellulose fibrils. The S1 layer and the compound middle lamella were destroyed after decomposition of the S2 layer. Liese (1963b, 1965; Liese & Schmid, 1962b) also found that the S3 layer resisted degradation while the S2 layer was being destroyed, even when the hyphae were in the lumen lying against the S3. Rhomboid cavities similar to those formed by soft-rot fungi were observed in the S2 layer, (Liese & Schmid, 1962b, Liese, 1963b). Extensive cracking within the cell wall has been associated with moderate degrees of brown rot; in advanced stages of decay the summerwood tracheids were found to be penetrated throughout with spiral cracks (Pechman & Schaile, 1950). In one study, ray parenchyma cells were attacked in early stages of brown rot and walls were completely destroyed by advanced stages (Pechmann & Schaile, 1950). However, Wilcox (1968) found thinning of ray cell walls in brown rot of a coniferous wood, but noted little or no effect on ray cells in a hardwood. Greaves & Levy (1965) also observed destruction of ray cell walls in advanced stages of brown rot in a coniferous wood. In brown-rotted beech the cellulose of pit borders was attacked in

TABLE I

SUMMARY OF MICROSTRUCTURAL CHANGES CAUSED BY DECAY

	White Rot	Brown Rot
General effect on cell wall	progressive thinning	collapse in late stages
Uniformity of decomposition from cell to cell	uniform	irregular
Order of attack on secondary wall layers	progressive from lumen outward	S2 first or simultaneous
Effect on middle lamella	destruction; sometimes attacked in early stages	little detectable degradation

early or intermediate stages of decay; in advanced stages the vessel walls had disintegrated completely while cells other than the earlywood fibers showed no signs of attack (Greaves & Levy, 1965). In advanced stages of brown rot in birch, cellulose decomposition was detected in all cells except the latewood fibers (Greaves & Levy, 1965).

The results of Szuleta (1947) appear to be contradicted by current knowledge. Although reporting on the the effects of brown-rot fungi, *Poria vaporaria* Fr., *P. vaillantii* (DC.) Fr. and *Merulius lacrymans* Fr., the author indicated that decomposition began in the region of the compound middle lamella and moved to the secondary wall only in late stages of decay; early decomposition reportedly consisted of the removal of lignin. Such activity presumably could only occur with lignin-decomposing white-rot fungi.

In a detailed electron-microscopical study, Meier (1955) observed that the effects of two different brown-rot fungi were similar whether the attack was on a softwood (spruce) or a hardwood (birch). In both cases the S2 layer of the secondary wall was the first to be attacked by the fungi; it was progressively decomposed toward the middle lamella, while the S3 layer remained essentially intact. The removal of the S2 layer from the birch was not uniform—in some places it appeared essentially intact, while in others cavities existed. Such cavities were not observed in the spruce, as the form of the deteriorated S2 layer was maintained by a loose lignin framework that still appeared homogeneous in the electron microscope. This was assumed by Meier (1955) to be an indication that the S2 layer of birch was much less lignified than that of spruce. Following complete removal of the cellulose from the S2 layer, and in birch the removal of the entire layer, the S3 was found to be decomposed; however, even in this advanced stage the S1 layer, the primary wall, and the middle lamella were still intact. Similar observations were made by Wilcox (1968) using light-microscope techniques, except that in sweetgum the S1 layer was attacked before the S3. Courtois (1965) found that the S3 layer often was attacked prior to S2 when decomposition started from the lumen. An increase in cell-wall porosity at the submicroscopical level similar to that observed in white-rotted wood was detected in cells attacked by a brown-rot fungus (Cowling, 1961).

Relative Resistance of Wood Elements and Cell-wall Layers to Fungal Degradation. Variations among the different cell-wall layers in their ability to resist degradation have been detected. It has been reported that the S3 layer of the secondary wall of some woods is highly resistant to degradation by brown-rot fungi (Jurášek, 1958, 1964; Liese, 1963a; Meier, 1955; Nečesaný, 1963; Wilcox, 1968); it appears resistant also to the action of acids and alkalis (Lange, 1958; Liese, 1963a; Wardrop & Dadswell, 1957). Its resistance may be due to a greater degree of order in the cellulose microfibrils (Lange, 1954; Meier, 1955), to a greater degree of lignification (Jurášek, 1955, 1958, 1964; Liese, 1963a; Wilcox, 1968), or to actual differences in the chemical composition of the microfibrils (Meier, 1955). The S3 layer appears to retain its resistance to the action of brown-rot fungi, even when it has first been delignified chemically (Meier, 1955). Meier (1955) reported that the S3 layer showed

resistance even to the action of certain white-rot fungi. Nečesaný (1963) found that the microfibrils of the S3 layer were resistant to degradation by white-rot fungi, particularly in early stages of decay, but even in advanced stages of white rot the microfibrils appeared more intact than those of brown-rotted wood. The S2 appeared to be the layer least resistant to the action of brown-rot fungi (Meier, 1955); Nečesaný (1957) suggested that it had greater resistance to lignin-destroying enzymes than to cellulose-destroying enzymes. However, Wilcox (1968) found evidence of action of lignin-destroying enzymes of a white-rot fungus in advance of the action of the cellulolytic enzymes in the secondary walls of sweetgum fibers. The S1 layer has been reported resistant to attack by both white- and brown-rot fungi (Meier, 1955); this was attributed to a greater density in the S1 layer than in the S2, or to differences in chemical composition (Meier, 1955, 1957). Yazawa (1943) reported that brown-rot fungi destroyed the birefringence of the secondary wall, while a white-rot fungus did not. However, I could not determine if Yazawa observed that all secondary wall layers retained their birefringence or if only the S1 was involved. Similarly, Schulze, Theden, & Vaupel (1937) found the X-ray interference due to cellulose disappeared gradually in wood decayed by several brown-rot fungi but remained in white-rotted wood. These results could be explained by the progressive action of white rot on wall surfaces observed by Cowling (1961) and Wilcox (1968), which would allow residual cellulose to retain its crystallinity.

Meier (1955) found that some of the differences between the effects upon wood of white-rot and brown-rot fungi disappeared if the wood to be decayed was first macerated. He subjected macerated spruce wood, which he considered to be essentially pure cellulose, to attack by several brown-rot and white-rot fungi and observed that both types of fungi decomposed this material at approximately the same rate and produced similar morphological effects.

The compound middle lamella appears to be the region most resistant to attack of both white-rot and brown-rot fungi (Cowling, 1961; Meier, 1955; Wilcox, 1968). Nevertheless, this region was eventually attacked in advanced stages of white rot (Meier, 1955; Wilcox, 1968). The thickened areas of the middle lamella at the cell corners resisted degradation the longest (Meier, 1955; Wilcox, 1968). However, attack on the pit membranes was the only decomposition of the middle lamella by a white-rot fungus observed by Cowling (1961) in sweetgum at weight losses up to 79%. In birch, Meier (1955) found that the vessels also were resistant to the attack of brown-rot fungi, while in sweetgum both vessels and rays were resistant to attack of a white-rot and a brown-rot fungus (Wilcox, 1968).

On the basis of a differential swelling rate, Kisser & Lohwag (1937) concluded that the walls of earlywood tracheids and radial walls of latewood tracheids had a denser structure than did tangential walls of latewood tracheids. This was offered as the explanation for the fact that the white-rot fungus *Fomes Hartigii* (Allesch.) Sacc. and Trav. attacked principally the latewood and dissolved the tangential walls first. Liese & Schmid (1966) found bore holes of the white-rot fungus *Trametes* (*Fomes*) *pini* (Thore ex Fr.) Fr. more prevalent on tangential than on radial tracheid walls, and attributed this to

differences in lignification. They observed the first signs of deterioration to be the shrinkage of the tangential walls of the first few rows of latewood tracheids (Liese & Schmid, 1966).

Differences in decay resistance of various regions within annual rings also have been reported. Schulze & Theden (1938) observed that earlywood of pine and spruce was more resistant to brown rot than was latewood. Conversely, Meier (1955) reported that latewood of spruce was more resistant to the action of brown-rot fungi than was earlywood, but that earlywood was more resistant to white-rot fungi than was latewood.

Summary. In general, it appears that hyphal distribution is not a function of the type of decay produced by the particular fungus, since both uniform and irregular distributions have been reported for fungi which cause white rot as well as for those causing brown rot. The anatomical effects of decay on the wood, however, appear to be more uniform with white rot than brown rot. Some fungi of both decay types preferentially penetrate pits in early stages of decay, but most decay fungi do produce bore holes at some stage of decay. Both types of fungi are capable of producing cavities more or less parallel to the microfibrils of the secondary wall or to the cell axis. The action of white-rot fungi on hardwood fibers and softwood tracheids most often involves a progressive decomposition of both lignin and cellulose from the lumen outwards, resulting in a progressive thinning of the wall throughout decay, although the rate of decomposition of the two components may differ. Action of brown-rot fungi on cellulose occurs in a diffuse manner, through the entire wall and with residual lignin maintaining the cell shape, so that little damage to the wall is apparent until late stages of decay when residual wall materials collapse. Differences in susceptibility to decay of various tissues, cell types, and wall layers appear to be correlated with differences in chemical composition, primarily with lignin content.

SOFT ROT

The type of cell-wall decomposition typical of soft rot was observed microscopically and attributed to fungus action as early as 1863 (Duncan, 1960; Cartwright & Findlay, 1958; Schacht, 1863). For some time it was considered simply as incipient decay (Bailey, 1913). The relationship of this type of wood deterioration to non-Basidiomycete fungi was surmised by Bailey & Vestal (1937) and confirmed culturally by Barghoorn & Linder (1944). In 1954, Savory clearly described the occurrence of this form of deterioration and its symptoms and coined the term "soft rot" (Savory, 1954). Various aspects of the type of wood decomposition known as soft rot have been reviewed by Bellmann (1961), Cartwright & Findlay (1958), Levy (1965a), and Wilhelmsen (1965).

Although fungi causing soft rot have been clearly differentiated from Basidiomyetes which cause decay, the distinction between them and other non-Basidiomycete wood-inhabiting fungi has not been clear. Soft-rot fungi appear to belong to both the Ascomycetes and the Fungi Imperfecti, but so do those fungi which cause stain and mold. Differentiation has been based upon the anatomical aspects of the deterioration: the distinctive, often diamond-shaped, spiraling cavities in the S2 wall layer, and the extent to which the wood is de-

graded. This type of distinction has led to some confusion, and there is increasing evidence that it will lead to more if the definition of soft rot is not made more selective.

Thus, Krapivina (1960) reported studies on fungi that because of their pigmentation produced stain in wood, and described anatomical changes that they produced in wood in terms appropriate to classical definitions of soft rot. Merrill (1965) indicated that some species of mold fungi, such as *Trichoderma viride* Pers., were capable of producing a few diamond-shaped cavities in the secondary walls of some wood species and not of others. Duncan (1960) found fine, spiraling cavities in the secondary wall in the case of the white rot produced by the Basidiomycete *Poria nigrescens* Bres., and diamond- or rhomboid-shaped cavities have been found in the walls of both white-rotted and brown-rotted wood by Liese (Liese, 1963b; Liese & Schmid, 1962b, 1966). Corbett (1965) has evaluated the soft-rot fungi as intermediate in properties and action between the stain and decay fungi, because they can sometimes display properties of both. Levi & Preston (1965) concluded that if differences between soft-rot fungal action and that of other wood-inhabiting fungi are due to quantitative differences in enzymes, the soft rotters are most like the brown-rot fungi, while if due to qualitative differences they constitute a distinct group of wood destroyers. Soft rot differs from both decay and stain because it is primarily a surface form of deterioration starting in outer layers of exposed wood and moving inward as outer surfaces deteriorate (Corbett & Levy, 1963; Courtois, 1963). The decomposition extends somewhat more deeply into the wood in tracheids adjacent to rays (Corbett & Levy, 1963). However, under certain conditions (Liese, 1961; Schulz, 1964) penetration and destruction of the wood may occur even more rapidly than would be expected with Basidiomycetes.

Hyphal Distribution. In early stages of deterioration, the action of soft-rot fungi and decay fungi was similar (Greaves & Levy, 1965). Hyphae travelled through cell lumina, becoming most numerous first in the rays in several species studied (Corbett, 1965; Greaves & Levy, 1965; Levy, 1965b; Levy & Stevens, 1966). Vessels were the primary entrance path in beech (Greaves & Levy, 1965), while Courtois (1963) found that tracheids and fibers were attacked first, followed by parenchyma cells and finally vessels. It was not until bore-hole formation (the "active penetration" noted by Greaves & Levy, 1965) that differences between soft-rot and decay fungi appeared (Greaves & Levy, 1965).

Corbett (1965) found that decomposition of test blocks occurred at a different rate, depending on which block face was placed in contact with the fungal culture. The order of the effect of the contacting face upon rate of decomposition from fastest to slowest, was, transverse, tangential, and radial. A higher lignin content of the radial walls was considered a possible explanation for their slow destruction (Corbett, 1965).

Hyphal Penetration. Penetration in early stages of soft rot was primarily through pits (Greaves & Levy, 1965; Levy, 1965b; Levy & Stevens, 1966). After destruction of all storage materials in the cell lumina, bore-hole formation began

(Greaves & Levy, 1965; Krapivina, 1960). Pits continued to be the primary passageways for tangential spread (Corbett, 1965), although bore hyphae can grow tangentially as well as radially (Liese, 1964). The hyphae running longitudinally through cell lumina were relatively large and thick-walled, and formed fine, hyaline branches at right angles to the hyphal axis which penetrated the cell wall through bore holes the same size as the fine hyphae (Corbett, 1965; Corbett & Levy, 1963; Krapiniva, 1960; Levy, 1965b; Levy & Stevens, 1966). These tiny bore holes appeared not to increase in size after penetration (Corbett, 1965). The penetrating branches often passed through the double cell wall and branched again in the adjacent lumen to form another thickened longitudinal hypha (Corbett, 1965; Corbett & Levy, 1963; Krapivina, 1960; Levy, 1965b). The cavities in the secondary wall may arise in several ways: branching of a penetrating hypha in the S2 layer, or change in direction of a hypha passing through a pit (Levi, 1965; Levi & Preston, 1965). The penetrating hypha may branch in the S2 layer forming a T, with the two branches growing at the same rate in opposite directions and approximately parallel to the microfibrils (Corbett, 1965; Corbett & Levy, 1963; Krapivina, 1960; Levy, 1965b). In some studies the penetrating hyphae usually passed through the first S2 layer encountered and formed the T branch in the second contiguous S2 (Corbett, 1965; Corbett & Levy, 1963; Levy & Stevens, 1966; Liese, 1964), although Levy & Stevens (1966) found T-branching also in the first S2 layer encountered by the penetrating hypha, and Liese (1964) observed that several cells might be penetrated before branching occurred. Penetration of soft-rot fungi was considered identical with that of stain fungi up to the point of T-branch formation (Corbett & Levy, 1963).

Several theories have been suggested to explain the abrupt change in direction involved in the formation of T branches selectively in the S2 layer (Levi & Preston, 1965). First, certain properties of the S3 layer, such as degree of crystallinity, high degree of polymerization, the presence of an inhibitory substance, etc., may cause it to act as a barrier. Second, bore hyphae may be passing through plasmadesmata or other capilliaries that may be blind. Third, there may be localized regions in the S2 layer that readily support hyphal growth.

T branching was rarely found in hardwoods, and the predominant form of decomposition consisted of V-shaped notches in the secondary wall with fine hyphae passing through the wall connecting notches in adjacent fibers (Corbett, 1965).

Microstructural Changes. Corbett (1965) recognized two types of degradation produced by soft-rot fungi. Type 2, which occurred predominantly in hardwoods, consisted of erosion of the wall along hyphae lying in the lumen and formation of V-shaped notches at branches from the longitudinal hyphae (Corbett, 1965; Levy, 1965b; Levy & Stevens, 1966). These notches penetrated as far as the S1 layer or to the compound middle lamella (Corbett, 1965; Levy & Stevens, 1966), and notches in adjacent fibers often were connected by a fine hyphal branch passing through the cell wall at right angles to the fiber axis (Corbett, 1965). The fine, side branches reportedly became bulbous

before forming a fine penetration hypha (Levy & Stevens, 1966) in a manner similar to the formation of a transpressorium as reported by Liese & Schmid (1964) for blue-stain fungi. This suggests that the initial penetration of soft-rot fungi may be mechanical (Levy & Stevens, 1966). It also suggested to Levy & Stevens (1966) that the hyphae of soft-rot fungi obtain their nutrition by direct wall erosion when the boundary layer can be decomposed enzymatically; where it cannot, the hyphae penetrate the barrier mechanically until they reach an area of the wall that can be attacked enzymatically. This theory could provide an explanation for the difference between the Type 1 and 2 forms of attack.

Corbett's Type-1 deterioration, the classical form of deterioration attributed to soft-rot fungi, is the primary form of attack in softwoods (Corbett, 1965). This type occurs after formation of T-shaped vertical branches in the S2 wall layer, or the entrance into the S2 layer of a hypha in a pit chamber. The two vertical branches of the T grow in length at about the same rate (Corbett, 1965). As the vertical hyphae extend through the S2 layer, cavities form around them at the tip (Corbett, 1965; Krapivina, 1960). The rate of hyphal extension may be reduced while the cavities enlarge laterally (Corbett, 1965), or the cavities may enlarge as the hypha grows (Krapivina, 1960). The tapered or conical tips of cavities arise as the cavities enlarge (Corbett & Levy, 1963; Courtois, 1963). The vertical hyphae may branch and give rise to branching chains of cavities (Corbett & Levy, 1963; Krapivina, 1960). Length of cavities appears to be inversely related to the diameter, with the longest cavities having the smallest diameter (Corbett, 1965). Formation of cavities appears closely related to hyphal growth for, unlike the deterioration caused by Basidiomycetes, there appears to be limited diffusion of enzymes away from hyphal surfaces (Levi, 1965; Liese, 1964; Meier, 1955), and hyphae must actually be present in the cell before deterioration takes place (Levy, 1965a). Fracture lines at the cavity ends suggested at least limited diffusion and action of the enzymes outside the cavities (Liese, 1964), and Levi & Preston (1965) concluded, from evidence of loss of microfibrillar structure at a distance from hyphae, that diffusion of enzymes to significant distances from the hyphae must occur in late stages of decomposition.

Courtois (1963) recognized 14 types of soft-rot attack, basing his differentiations upon shape, orientation, and location of cavities in the wall. The characteristics of these types were influenced more by properties of the cell wall, such as fine structure, thickness, cell type, and chemical composition, than by the species of soft-rot fungus involved (Courtois, 1963).

The classical location for soft-rot cavities is the S2 wall layer, although both the S2 and S3 are involved in hardwood decomposition (Corbett, 1965; Courtois, 1963). The S3 layer of softwoods is more resistant to attack than the S2 (Courtois, 1963; Liese, 1965), but it may also be attacked in later stages of decomposition (Courtois, 1963). Corbett (1967) found that soft-rot attack of tension wood fibers and cotton fibers, which contained essentially no lignin, produced a general attack (sculpturing or thinning) along the exposed wall surfaces, suggesting that S3 layer resistance and the formation of discrete cavities may be controlled at least in part by lignin content or distribution. However, there is evidence that at least some soft-rot fungi can decompose

lignin to a limited degree (Krapivina, 1960; Levi, 1965; Levi & Preston, 1965).

Most workers have reported that in cases where cavities in the S2 layer are formed by soft-rot fungi, the cavities spiral along the cell axis with their sides approximately parallel to the microfibrils (Duncan, 1960; Meier, 1955). Liese (1964) found that cavities paralleled microfibrils only in the more aggressive species of fungi; however, Courtois (1963) concluded that cavity orientation was controlled more by the properties of the cell wall than by the species of fungus, and Willeitner (1965) observed different cavity shapes when the same species of fungus attacked three different species of wood. The conical tips of the cavities form a fairly constant angle to the microfibril axis, 23^0 as measured by Meier (1955). Such a constant angle has been attributed to enzymatic decomposition along given hydrolysis planes in the cellulose molecule (Frey-Wyssling 1938; 1956), or to a faster rate of diffusion of a limited quantity of enzyme along the dissolving microfibrils than across them (Roelofsen, 1956). Liese (1964) reported that the cavities enlarged more tangentially with regard to each cell than they did radially, leading to an oval shape. The regularity of this shape suggested that it was controlled by enzyme diffusion (Liese, 1964).

There are several possible explanations for the narrowing between each cavity in a chain (the formation of conical cavity tips) even though the hypha that formed them runs continuously through each one. One explanation could be based on Corbett's observation (Corbett, 1965) that hyphal elongation slows down while the cavity behind the tip enlarges. Possibly, two different locations of enzyme secretions might be alternately involved in the processes of elongation and cavity enlargement (Levi & Preston, 1965). Another theory is that the shape of the cavities is due to morphological or chemical properties of the cell wall (Liese, 1964). This explanation would not, however, seem applicable to situations where cavities enlarge to the point of coalescence and general destruction of the S2 layer (Levi, 1965; Levy & Stevens, 1966). Perhaps the most plausible theory assumes restricted release of cellulolytic enzymes along certain portions of the hyphae (Levi, 1965; Levi & Preston, 1965; Liese, 1964), perhaps at septa (Levi, 1965; Levi & Preston, 1965). Levi (1965) postulated that the rate of deterioration, and presumably the cavity shape, might be controlled by the rate of lignin modification rather than the rate of cellulose decomposition. Therefore, longitudinal enzyme diffusion would be between microfibrils, and transverse diffusion would be through the lignin between crystalline regions (Levi, 1965).

Cavities have been reported to be more conspicuous in latewood than in earlywood, especially in softwoods (Duncan, 1960; Savory, 1954), while Levy & Stevens (1966) reported them more prevalent in earlywood than in latewood in hardwoods.

Summary. In the degree of wood decomposition and the aggressiveness of the causal fungi, soft rot appears to be intermediate between stain and decay. Fungi that cause soft rot penetrate through pits and live on storage material during early stages of their development in wood. Only after these substances are no longer available do they begin to penetrate and dissolve cell-wall material, and even then there is evidence that early wall penetration may be mechanical rather then enzymatic. Decomposition consists of localized cavities in the vicinity

of the hyphae, primarily on the surface exposed to the lumen in hardwoods and within the S2 wall layer in softwoods. Wall decomposition is often limited to the exposed surfaces of the deteriorating wood rather than being distributed throughout, as in the case of decay. A characteristic of soft rot, particularly in softwoods, is the abundance of chains of conically tipped or diamond-shaped cavities in the S2 wall layer running helically around the cell axis, often approximately parallel to the microfibrils. Similar cavities have been observed in wood decomposed by other types of cellulose-destroying fungi, but not with the frequency with which these features are found in soft rot.

MOLD AND STAIN

Mold and stain are considered together because of the similarity of action of the causal fungi on wood microstructure. Mold and stain (along with soft rot) are caused by members of the Ascomycetes and Fungi Imperfecti. Duncan (1960) and Krapivina (1960) showed that some fungi previously known as stain or mold producers can also cause soft rot under proper conditions. Merrill (1965) reported that some mold fungi that produced little effect on the cell walls of poplar wood were known to produce typical soft rot in oak. It is probable that given the right conditions other mold and stain fungi also may be able to produce soft rot. The difference in action is one of the level of aggressiveness upon the wood substance. In soft rot a significant portion of the wood substance is decomposed by the fungus. With mold and stain, the fungus uses the wood substance primarily as a habitat and draws most of its food from stored materials in the wood. The major portion of the deterioration attributable to mold and stain is not decomposition but discoloration caused by pigment within the penetrating hyphae (in the case of stains) or by pigments only in the surface-formed conidia (in the case of molds). In either case, the attack on the wood is similar to the early stages of soft rot except that if deterioration is considered mold or stain, it does not proceed beyond the stage of fine bore hole formation the penetrating hyphae (in the case of stains) or by pigments only in the surface-formed conidia (in the case of molds.). In either case, the attack on the wood is similar to the early stages of soft rot except that if deterioration is considered mold or stain, it does not proceed beyond the stage of fine bore hole formation into a phase of cell-wall decomposition.

Hyphal Distribution. The hyphae of blue-stain fungi primarily occupied the rays (Konstantnaja, 1964; Liese & Schmid, 1961; Scheffer & Lindgren, 1940). The hyphae tended to follow the rays into the wood (Liese & Schmid, 1961; Scheffer & Lindgren, 1940), passing primarily through the ray parenchyma cells and occurring only rarely in ray tracheids (Liese & Schmid, 1961). Hyphae also were present in fibers and tracheids and, although not as abundant, were able to advance through the wood more rapidly in these elements (Liese & Hartmann-Fahnenbrock, 1953; Liese & Schmid, 1961; Scheffer & Lindgren, 1940). Krapivina (1962) reported differences in distribution of the hyphae of mold fungi within the annual ring, with species of *Fusarium* fairly uniformly distributed within both earlywood and latewood while species of *Penicillium* and *Verticillium* were limited primarily to the earlywood. A stain fungus that

attacks the heartwood of living balsam fir was found to develop mainly in the tracheids (Pomerleau & Etheridge, 1961).

The fungi causing stain in hardwoods, on the other hand, were found to colonize primarily the vessels during early stages of infection and to be present only to a limited extent in the rays (Campbell, 1959). As stain progressed, hyphae became more abundant in the vessels and fibers but not in the rays (Campbell, 1959). The ray cells generally were occupied by finer, lighter-colored hyphae than were present in the vessels and fibers (Campbell, 1959).

Hyphal Penetration. The hyphae of mold and stain fungi penetrate mainly through pits (Campbell, 1959; Hubert, 1929; Konstantnaja, 1964; Krapivina, 1962; Liese & Schmid, 1961, 1962b; Scheffer & Lindgren, 1940), but most have been shown to produce bore holes as well (Campbell, 1959; Cartwright & Findlay, 1943; Hubert, 1929; Krapivina, 1962; Liese & Schmid, 1961). Pit penetration appears to involve penetration through the torus rather than the margo of the pit membrane (Krapivina, 1962; Liese & Hartmann-Fahnenbrock, 1953; Liese & Schmid, 1961; 1962b). Liese & Schmid (1961, 1962b) observed no appressorium, no hyphal constriction in passing through the torus, and no other evidence of enzyme action. These considerations, along with detection of crushing of the pit membrane (Liese & Schmid, 1961), indicated that penetration must be mechanical. However, Krapivina (1962) observed extensive torus decomposition resulting from the penetration of mold hyphae, suggesting enzymatic action. In the genus *Pestalotia,* which has been shown also to produce soft rot (Duncan, 1960; Savory, 1954), directional branching toward pits by means of specialized, naked-protoplasmic outgrowths was observed (Ritchie, 1967). Liese & Schmid (1961) observed branch formation opposite pits, but once branching had occurred there was no apparent directional effect of pits on hyphal growth.

The formation of bore holes through tracheid walls appears to be a more complex process than pit penetration. Prior to bore hole formation, the hyphal tip swells and forms an appressorium (Cartwright & Findlay, 1943; Lagerberg et al., 1927; Liese, 1963b; 1965; Liese & Schmid, 1961, 1962b; Schmid & Liese, 1965). This structure is sufficiently distinctive that Liese & Schmid (1964) coined the term "transpressorium" to describe it. From the appressorium a fine bore hypha is formed that penetrates the wall (Lagerberg et al., 1927; Liese, 1963b; Liese & Schmid, 1961, 1962b). Although Krapivina (1962) found histochemical evidence of enzymatic action during bore hole formation by several mold fungi, and Liese (1965) considered penetration by stain fungi to involve both mechanical and enzymatic processes, most workers have observed little or no evidence of enzymatic action and consider at least the initial penetration to be by mechanical means (Cartwright & Findlay, 1943; Lagerberg et al., 1927; Liese & Schmid, 1961, 1962b, 1964; Scheffer & Lindgren, 1940). Liese & Schmid (1964) reported mechanical penetration of metal foils by the bore hyphae of stain fungi. The bore hyphae produced by appressoria, which were about 1/5 the size of normal hyphae, did not grow from the tip but were pushed into the cell wall by intercalary growth at the appressorium

(Liese & Schmid, 1961). Other workers have reported extreme constriction of hyphae forming bore holes through tracheids (Hubert, 1929; Roff, 1964; Scheffer & Lindgren, 1940; Siepmann & Johnson, 1960), which may be another interpretation of the same phenomenon. More bore holes were observed in sapwood near the cambium than in older sapwood (Liese & Schmid, 1961).

Microstructural Changes. Although there is evidence that most mold and stain fungi do not attack the walls of tracheids and fibers other than by bore holes, they apparently can attack parenchyma cells. Hyphae have been observed growing within the walls of ray parenchyma cells (Liese & Schmid, 1961) and in some cases have partly or completely destroyed ray parenchyma cell walls (Lindgren, 1952: Scheffer & Lindgren, 1940) and the parenchyma cells in longitudinal resin ducts (Scheffer & Lindgren, 1940).

Liese (1964) reported formation of lysis zones in tracheid walls around the hyphae of *Alternaria* sp., which involved both the S3 and S2 of conifers in a manner similar to that of some soft-rot fungi, and Krapivina (1962) reported an alteration of the secondary wall indicated by differences in staining reaction. Merrill (1965; Merrill & French, 1965) observed a few diamond-shaped cavities in the cell walls of wood attacked by the common mold *Trichoderma veride*. Pers. Merrill & French (1965) reported that occasional attack on lumen surfaces was the only evidence of attack by mold fungi that was visible even up to 20% weight loss in particleboard. The gelatinous layer in tension wood fibers was partially decomposed by mold fungi (Merrill & French, 1965; Zenker, 1963). Many workers, however, have reported no effects upon tracheid cell walls by hyphae of stain and mold fungi other than the formation of bore holes (Liese, 1965; Liese & Hartmann-Fahnenbrock, 1953; Liese & Schmid, 1961, 1962b). Liese & Hartmann-Fahnenbrock (1953) reported the possibility of hyphae in the middle lamella, but the observation was not confirmed (Liese & Schmid, 1961), and Krapivina (1962) reported a weakening of the middle lamella. The fungus *Ceratocystis fagacearum* (Bretz) Hunt, which invades living oaks, was found to degrade both the secondary wall and the compound middle lamella during penetration (Sachs et al., 1967). The large openings in the pit tori (formed by passage of hyphae) and the extensive damage to ray parenchyma cell walls could account for increased permeability to liquids in stained or molded wood (Liese & Hartmann-Fahnenbrock, 1953; Lindgren, 1952).

Summary. Mold and stain fungi apparently cause little damage to the structure of wood they inhabit, provided their action does not reach a more aggressive stage where it would be considered soft rot. Hyphae may be present in most wood elements but are often more numerous in ray parenchyma cells. Penetration is primarily through pits, with hyphae passing directly through the torus, but bore holes are formed through tracheids and fibers. The bore hypha is considerably smaller than the rest of the hypha and may actually arise from an appressorium. The bore holes apparently do not enlarge after formation. There is evidence that penetration through pit tori and cell walls may be primarily mechanical.

BACTERIA

Although the deterioration produced by bacteria in wood does not seriously affect most wood properties, it does represent another step in the continuum of aggressiveness of wood decomposition by wood inhabiting microorganisms.

Distribution. Bacterial cells in wood appear to be associated primarily with parenchymatous tissues, regardless of the type of wood. Bacterial cells have been found to accumulate in rays (Courtois, 1966; Greaves, 1965; Greaves & Levy, 1965) and in resin ducts and other parenchyma cells (Boutelje & Kiessling, 1964; Courtois, 1966; Knuth, 1964). They eventually move from these parenchyma cells to surrounding prosenchyma (Courtois, 1966; Greaves, 1965; Greaves & Levy, 1965). They have been observed to accumulate in pit chambers and show an affinity for the S3 layer in softwood tracheids and hardwood fibers and vessels (Knuth, 1964). Greaves (1969), in a review of numerous observations, reported that all wood elements may be colonized by bacteria and are equally susceptible to attack, despite differences in lignification.

Microstructural Changes. The first change produced by the presence of bacteria appears to be decomposition of storage material in the rays (Boutelje & Kiessling, 1964; Ellwood & Ecklund, 1959; Greaves, 1969; Greaves & Levy, 1965; Knuth, 1964). Following this the walls of the ray parenchyma cells may be attacked and destroyed (Ellwood & Ecklund, 1959; Greaves, 1965, 1969; Greaves & Levy, 1965; Knuth, 1964; Lutz et al., 1966). Knuth (1964) reported that although the walls of ray parenchyma cells in pine were decomposed, in sweetgum they were not; however, Greaves & Levy (1965) reported similar ray decomposition in pine, beech, and birch. The attack on ray parenchyma cell walls was found to involve a progressive attack on crystalline cellulose, beginning at the lumen and working outward (Greaves, 1965; Greaves & Levy, 1965). Many workers reported no effects upon the walls of tracheids, ray tracheids, and fibers (Ellwood & Ecklund, 1959; Liese & Karnop, 1968; Lutz et al., 1966; Knuth, 1964). Except for their pits, vessels were rarely attacked (Greaves, 1969). However, attack by bacteria on tracheid cell walls was observed by Courtois (1966), and attack on both tracheids and fibers was reported by Greaves (1969).

Courtois (1966) found depressions in the walls of tracheids underneath places where bacterial colonies had adhered to the wall. As the colonies enlarged, both S3 and S2 wall layers were attacked, and the cavities became irregular etchings. The affinity of colonies for the S3 layer of longitudinal elements observed by Knuth (1964) could be similar to the observations of Courtois but representative of an earlier stage because of the wide difference in incubation times in the two studies. Courtois (1966) observed that decomposition sometimes began in the rays and moved outward through the compound middle lamella to attack the secondary walls of tracheids. The middle lamella was resistant at first but was eventually attacked so that all wall layers were subject to decomposition (Courtois, 1966). Because decomposition was limited to portions of the wall in contact with bacterial colonies, and because the stage of decomposition varied widely even in adjacent cells, Courtois (1966) concluded that enzyme diffusion

from the bacterial cells was restricted and that actual contact between the cells and the walls was necessary for decomposition. Courtois (1966) observed that latewood was more resistant to bacterial attack than was earlywood.

Greaves (1969) reported attack on both tracheids and fibers, the most common effect being erosion zones that extended into the wall as far as the middle lamella. Attack on the middle lamella occurred primarily as an extension of attack on pit membranes (Greaves, 1969). Under long-term exposure complete thinning of the secondary wall was observed, involving both the S2 and S3 cell-wall layers; such decomposition often occurred in patches associated with rays and vessels (Greaves, 1969). Unpublished data were cited that provided evidence that some bacterial enzymes are able to diffuse freely from the site of production in the lumen, through the S3, into the S2 (Greaves, 1969).

Harmsen & Nissen (1965a, 1965b) observed conical depressions in tracheids extending from the lumen into the secondary wall but not affecting the middle lamella. Although they isolated a bacterium and an Actinomycete from the material, they were unable to reproduce the observed effects in culture even though the organisms were cellulolytic, and therefore could not positively ascribe the damage to bacterial attack (Harmsen & Nissen 1965a, 1965b).

The most common effect of bacterial infection upon wood properties appears to be a striking increase in porosity or liquid permeability (Ellwood & Ecklund, 1959; Greaves, 1965; Greaves & Levy, 1965; Knuth, 1964; Knuth & McCoy, 1962; Liese & Karnop, 1968). Several effects of bacterial infection could account for the increased permeability. Knuth (1964) and Liese & Karnop (1968) reported attack on pit membranes. The membranes of sweetgum were thinned significantly, and the tori of pine were destroyed and the margo disrupted (Knuth, 1964). Using light microscopes, Greaves (Greaves, 1965; Greaves & Levy, 1965) observed no effect on pits and concluded that increased permeability resulted from alteration of cellulose structure in the walls of ray parenchyma cells. However, in a later paper, Greaves (1969) reported that pit degradation was common, including destruction of the border and even the margo and torus.

Summary. Bacteria appear to focus their major effects upon the parenchyma cells of the rays. Only when action on the rays is well advanced do effects on other elements usually appear, if then. The major effect upon wood properties produced by bacteria appears to be an increase in permeability to liquids. This may be accomplished by destruction of storage material in the rays, by destruction or alteration of pit membranes, or by decomposition of ray parenchyma cell walls.

ALTERATION OF WOOD STRUCTURE BY CELL-FREE ENZYME PREPARATIONS

Until recently it has been thought impossible to degrade wood significantly with cell-free enzyme systems unless the wood was first reduced to a very small particle size—by ball-milling, for example. Several recent results, however, suggest that such processes are possible. Experiments based upon cell-free, or even isolated or purified, enzyme systems may provide the information necessary

to define more clearly the similarities and differences between the action of organisms in various categories of wood deterioration.

Stárka & Schâněl (1962; Schâněl & Stárka, 1963) found that they could induce dissolution of the middle lamella and cell separation, bulging of the primary and secondary cell walls, and destruction of the cell wall through the application of cell-free culture fluid from the cultures of a number of Basidiomycetes and one Ascomycete. Nicholas & Thomas (1968) found that application of pectinase to pine specimens degraded the pit membranes and ray parenchyma cell walls, while cellulase removed the margo fibrils and partially degraded the torus, and hemicellulase had little detectable effect. King (1968) achieved rapid loss of birefringence by applying a concentrated enzyme preparation from the brown-rot fungus *Coniophora cerebella* (Pers.) Duby to spruce holocellulose, and total dissolution in four hours of specimen material that was only one cell thick. Several other workers also have been successful in degrading wood with cell-free enzyme preparations, but their work has involved chemical rather than microscopical analysis of the effects.

LANDMARK DISCOVERIES

Every field of knowledge has its significant advances—its landmark discoveries. Even though the ranking of such advances involves personal value judgment, it is useful to do so in order to put research results into proper perspective and to aid in decisions as to where future research effort may best be directed. I recognize five categories into which contributions to a scientific field may be placed and will consider the advances in this field in the context of these categories.

Prerequisite Discoveries. Contributions in this first category are fundamental to the development of the field itself and therefore have not been dealt with in the limited scope of this review. They would include the invention of both the light and electron microscopes and subsequent evolution of specialized optical systems, and the establishment of pure culture techniques, particularly with respect to ligno-cellulose destroying microorganisms.

Independent Additions to Basic Knowledge. In this category fall the basic discoveries, the landmark observations in a virgin subject area. This field has had its share of such discoveries, beginning perhaps with Hartig's diagnosis of the causal relationship between Basidiomycetous fungi and wood decay (Cartwright & Findlay, 1958; Duncan, 1960; Hartig, 1878). Another such discovery was the establishment of the role of non-Basidiomycete fungi in wood deterioration, soft rot, by the observations of Bailey & Vestal (1937) and cultural confirmation of Barghoorn & Linder (1944). The distinctive cavities in wood cell walls constituting this type of deterioration were observed microscopically by a number of workers as early as 1863 (Cartwright & Findlay, 1958; Duncan, 1960; Schacht, 1863). With the work of Ellwood & Ecklund (1959), Knuth (1964), and Greaves & Levy (Greaves, 1965; Greaves & Levy, 1965), it was microscopically established that bacteria can cause a degree of structural damage to wood. Finally, the results of a number of investigators are providing

evidence that microscopically detectable effects on whole wood may be induced by the application of culture filtrates or purified enzyme preparations (Nicholas & Thomas, 1968; Schánĕl & Stárka, 1963; Stárka & Schánĕl, 1962).

A further conclusion, based on results of microscopical examination of biologically deteriorated wood, is that there are differences in the effects of microorganisms in wood that correspond closely with the taxonomic differentiations of these organisms.

Evidence for Established Hypotheses. The example chosen for this category is microscopical confirmation of hypotheses on enzyme action developed by Cowling (1961) to explain results of chemical analyses of decaying wood. Cowling hypothesized that the lignin-destroying enzymes of a white-rot fungus penetrated the wall structure in advance of the cellulolytic enzymes, and that action of the cellulolytic enzymes of a white-rot fungus was restricted to exposed wall surfaces while that of a brown-rot fungus extended generally throughout the wall. Confirming evidence of these hypotheses has been developed by Jurášek (1964) and Wilcox (1968).

As evidence of microscopically detectable attack of wood by culture filtrates and enzyme preparations is substantiated, it will confirm the widely held hypothesis that enzymes of wood-inhabiting fungi can act at some distance from the hyphae.

Development of New Hypotheses. Contributions in this category are of great long-term significance because they may open the way for whole fields of new research approaches. The field of microscopical study of the deterioration of wood has had its share of contributions of this nature. The examples cited concern information made available by the microscopical study of deteriorating wood which may serve as valuable keys or tools for further chemical and physical study of the cell wall.

Decay fungi may show a preference between hardwood and softwood as a substrate (Cowling, 1957; Duncan & Lombard, 1965; Scheffer, 1964). Microscopical workers have shown that wood-inhabiting fungi may produce different forms of deterioration in the two types of wood, suggesting chemical or physical differences in the wood cell walls (Levy, 1965b; Meier, 1955; Wilcox, 1968). It has been observed that various layers of the cell wall within a given species of wood may differ in their resistance to the action of a decay fungus. Jurášek (1958, 1964) and Wilcox (1968) observed that wall layers showing a high degree of decay resistance also appeared to have a higher lignin content than did more susceptible layers, and then suggested that the relative resistance might be due to lignin content. It has also been suggested that lignin content may play a role in the differences between hardwood and softwood with respect to the action of various fungi. These hypotheses offer new challenges to the study of the chemistry of the cell wall.

Since the discovery of the significance of soft-rot cavities, the peculiarity of their shape and orientation has interested many observers. These cavities may prove to be valuable tools in the investigation of cell-wall and cellulose ultrastructure and chemistry—as for example, in the theories of Frey-Wyssling (1938, 1956), Levi (1965), and Roelofsen (1956).

Preliminary Discoveries Establishing the Direction for Further Study. There are probably many observations that could be placed in this category. I have picked three that are particularly intriguing. First, by pooling the results of a number of observers it can be concluded that members of all the groups of wood-inhabiting fungi—white rot, brown rot, soft rot, mold and stain—are known to produce bore holes in ligno-cellulose cell walls. However, many of these fungi are known not to utilize lignin to any significant degree. What are the properties of lignin in the cell-wall complex or, conversely, of the non-lignin-destroying organisms, that allow such organisms to dissolve holes through substrates containing significant proportions of a substance they are incapable of destroying? Or does mechanical penetration play a greater role in the movement of some of these organisms than has been suspected?

Of interest in this category as well are the observations of differences in decay resistance between springwood and summerwood of the same annual rings (Meier, 1955; Schulze & Theden, 1938) and even between radial and tangential walls of the same cells (Meier, 1955). These observations may indicate fundamental differences in the chemical composition or physical structure of the cell walls and might provide necessary tools for the investigation of these properties.

Finally, the curious uniformity of shape and orientation of the cavities produced in cell walls by soft-rot fungi and their possible importance to other fields of investigation have already been noted. For some time these have been considered diagnostic features of soft rot. However, there is increasing evidence (Courtois, 1965; Jurášek, 1955; Liese, 1963b; Liese, 1964; Liese & Schmid, 1962b, 1966; Schmid & Liese, 1964; Tamblyn, 1937; Wilcox, 1968) that cavities within the S2 layer, approximately parallel to the microfibrils, are sometimes produced by Basidiomycetes as well. Liese (1963b; Liese & Schmid, 1962b) has proposed that such cavities are not characteristic of soft-rot fungi in particular, but of fungi which produce cellulases. This again poses the question of the role played by lignin present in the wall structure in influencing the form of attack upon the cell wall. In addition, it opens up an entirely new facet of, and provides increased impetus to, research on the nature and causes of the shapes and orientations of nearly longitudinal cavities within ligno-cellulose cell walls.

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