Development of Discoloration, Decay, and Microorganisms Following Wounding of Sweetgum and Yellow-Poplar Trees

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ABSTRACT

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Patterns of development of discoloration, decay, and microorganisms were studied in 122 naturally or experimentally wounded sweetgum (*Liquidambar styraciflua*) and yellow-poplar (*Liriodendron tulipifera*) trees. Heartwood and wound-initiated discolorations were found in yellow-poplar, but sweetgum contained only wound-initiated discoloration. Barrier zones of abnormal cells were found in both species and appeared to account, at least in part, for compartmentalization of the stem. Xylem formed after wounding was free of discoloration and decay, and of the large populations of microorganisms that developed in wood formed prior to wounding. Sparse populations of bacteria (200-4,000 cells/g wood) and small-spored fungi (5-150 propagules/g wood) were detected by dilution plating of homogenates of normal sapwood from wounded and nonwounded trees. Some genera of the fungi found in normal sapwood, (*Phialophora*, *Fusarium*, *Cephalosporium*, and *Streptomyces*) colonized discolored sapwood. Abundant populations of bacteria ($>10^5$ cells/g) and these same fungi (up to 10^3 propagules/g) were found in discolored wood from which most bits of tissue plated in agar media also yielded microorganisms. The tissue plating method seldom yielded bacteria when populations were $<10^3$ cells/g, but bacteria were isolated routinely when populations were $>10^3$ cells/g. These results indicate that some of the bacteria and fungi that colonize discolored wood exist as sparse populations in normal sapwood.

Decay of living trees is the most destructive disease of hardwood timber in the southern United States. In 1952, annual losses in this region were estimated to be about $196 \times 10^6 m^3 (7 \times 10^9 ft^3)$ of timber (14). At 1975 market prices, this volume of timber would be worth about $160 million on the stump and about $4 billion as finished lumber, pulp, or other wood products.

Decay in living trees once was thought to occur mainly in the heartwood. But decay also occurs in living trees such as beech, birch, and maple, which do not form heartwood (12, 32, 34).

Wounding of xylem in trees usually leads to discoloration and later to decay of the discolored tissue. Successions of microorganisms have been associated with these processes: bacteria and certain nonhymenomycetous fungi develop rapidly in discolored sapwood, and decay-causing hymenomycetes become abundant in later stages of the succession.

The purposes of this investigation were to determine and compare the spatial and temporal patterns of development of discoloration, decay, and populations of bacteria and fungi in stemwood of living sweetgum (*Liquidambar styraciflua* L.) and yellow-poplar (*Liriodendron tulipfera* L.) trees and to determine, quantitatively as well as qualitatively, the changes in the microbial populations associated with discoloration and decay.

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MATERIALS AND METHODS

Ten trees of each species with major wounds, and ten with minor wounds only, were selected for observation. Major wounds consisted of broken tops, branch stubs, and basal injuries caused by fire, logging, or parent stumps of sprout stems. All trees had minor wounds such as small branch stubs, superficial scrapes, and insect bore holes. The trees ranged from 30 to 60 yr of age and 8 to 35 cm diameter at breast height (dbh). The trees were located in two natural, mixed-hardwood stands on the Schenck Forest near Raleigh and the Hill Forest near Rougemont, North Carolina. Three additional sweetgum and two yellow-poplar trees wounded by fire were cut in a 30-year plantation at Bolton, N.C.

All trees were cut into 1-m bolts and dissected so that the patterns of discoloration and decay associated with each wound could be observed and photographed in transverse and radial view. Representative samples of healthy, discolored, and decayed wood were used for isolation of microorganisms and microscopic examination of the xylem.

Experimentally wounded trees.—In May-August 1971, superficial and deep wounds were inflicted in 16 trees each of sweetgum and yellow-poplar in the same stands as naturally wounded trees. Superficial wounds were made by removing bark from a diamond-shaped area equal in width and height to 40% and 80% of stem circumference, respectively. Deep wounds were made by making two parallel transverse cuts 2.5 cm apart to a depth of 0.4 × diameter and removing the wood between them with a chisel. Two to six trees were harvested 15, 30, 90, 360, and 720 days following wounding between June and September. The distance to which discoloration and decay had progressed from the wound surface was measured, patterns of discoloration and decay were observed and photographed in transverse and radial view, and representative wood samples were taken for isolation of bacteria and fungi and microscopic examination of the tissues.

In a second experiment, deep wounds were inflicted in 40 sweetgum trees in August, 1973. At 1-wk intervals after wounding, three to five trees per week were harvested for 8 wk after wounding. The average volume of discolored wood induced by wounding was calculated from the discolored wood that was visible on cross-sectional disks cut at 5-cm intervals above and below the wound. Populations of microorganisms were estimated quantitatively by dilution plating of homogenates of discolored and nondiscolored tissues in each disk. Populations of microorganisms in nonwounded trees were determined from 10 disks cut in duplicate from five trees before the experiment began.

Isolation of microorganisms.—Fungi and bacteria were isolated from wood by tissue plating on malt-yeast agar (36) or by dilution plating by methods modified (37) from those of Levy (23) and Greaves (10). Results of tissue plating are reported as relative occurrence as defined by Butcher (3):

$$\frac{\text{no. of isolations of a taxon}}{\text{total no. of isolations attempted}} \times 100$$

The media used for dilution plating included enriched nutrient agar (8 g nutrient broth + 5 g malt extract + 1 g yeast extract + 15 g agar/liter) with and without two drops of 50% lactic acid per plate or 50 mg/liter rose bengal + 30 mg/liter streptomycin. The results are reported as number of viable cells per gram of moisture-free wood.

Samples used for tissue plating were blocks 5 × 5 × 5 cm, split from the 5-cm disks and used immediately or stored up to 2 wk at 2 C. Sterilized control blocks were wrapped in aluminum foil and autoclaved for 1 hr at 121 C. Sample blocks were split aseptically and six to ten small wood chips were plated in agar media, three to five chips per plate. Fungi that grew from chips were subcultured and identified to genus and species if possible.

Samples for dilution plating were blocks that either were split or drilled (16mm diam × 5 mm deep) aseptically to expose a fresh tangential or transverse surface, respectively. Sterilized controls consisted of blocks treated as described above or blocks taken from 5-cm disks of 50-cm bolts, which were wrapped in kraft paper, autoclaved for 1 hr at 121 C, and cooled 4-6 hr. Wood shavings were obtained with an electrically-driven sterile 9.5-mm diameter flathead drill bit and collected in 100 ml of sterile distilled water in a Waring Blendor cup held beneath the block being drilled. Shavings from four holes 2 cm deep yielded 1-2 g of moisture-free wood. Shavings were blended for 60 sec, larger particles were allowed to settle, and the resulting suspension was plated with a spreader-bar at dilutions of 1/200, 1/5,000, 1/50,000, and 1/500,000 in duplicate or triplicate. To detect low populations, 10 ml of suspension was dispersed in 25 ml of melted medium at 42 C in 150 × 20 mm petri dishes. Wood fragments were recovered quantitatively from the Blendor cup by filtering on a fritted-glass crucible (porosity C), before drying for 24 hr at 104 C to determine moisture-free weight. Dry weights and counts of microbial colonies were used to estimate numbers of cells per gram of wood.

Microscopic examination of wood.—Radial and transverse freehand sections from representative wood samples were mounted in water or lactophenol-cotton blue. Sections were examined by light- and phase-contrast microscopy to locate cells of fungi and bacteria and deposits of extraneous materials. Starch, the predominant storage product of sweetgum and yellow-poplar (16), was detected with 2% IKI in 70% ethanol (43). Living cells were detected by plasmolysis and deplasmolysis of cells that accumulated neutral red overnight (9) or by incubation in freshly prepared 1% aqueous triphenyl tetrazolium chloride in darkness for 24 hr (43). Blocks steamed for 20 min at 100 C were included as controls for vital staining.

RESULTS

The sapwood of sweetgum trees without major aboveground wounds was not colored throughout the length of the stem except for small localized zones associated with insect bore holes or other minor wounds. Yellow-poplar trees without major wounds invariably contained a central core of yellowish-green tissue (heartwood) which was continuous, uniform in color, circular in cross-section, conical in longitudinal form, and surrounded by 20-30 annual rings of nondiscolored sapwood. Starch and living parenchyma cells were found in nondiscolored sapwood, but not in colored tissues of any type in either tree species. Deposits of colored substances were found in many parenchyma cells and fiber tracheids within darkly colored tissues, but were only scattered in lightly colored and nondiscolored tissues. Since sweetgum trees without major wounds contained only nondiscolored sapwood with living parenchyma cells from cambium to pith, this species is considered to contain sapwood only.

Yellow-poplar differed from sweetgum not only with respect to heartwood, but also in natural pruning. Branches of yellow-poplar separate from the stem by decay or abscission inside the bark, thus usually leaving no branch stub exterior to the stem. Discolored branchwood was surrounded by heartwood and sapwood.

Branches of sweetgum often decayed or broke off leaving a stub external to the stem. If the stub was smaller than 3 cm in diameter, discolored branchwood usually was surrounded by sapwood. If the stub was larger than 3 cm in diameter, the stemwood which surrounded the branch and which had been formed before the branch died, was often discolored either by narrow streaks or columns. The larger the branch stub, the more discolored and decayed wood was found associated with it (41).

Patterns of discoloration and decay in wounded trees.—Sweetgum and yellow-poplar trees had discolored wood associated invariably with major wounds. Discolored wood in the center of sweetgum stems was surrounded by a few to many annual rings of nondiscolored sapwood. The discolored wood varied from continuous columns of uniform color to discontinuous irregular columns. Continuous columns usually were associated with broken tops, which, except for branch stubs, constituted the most common type of wound observed in the sweetgum trees that were studied. Discolored wood in yellow-poplar trees was most commonly associated with basal wounds caused by fire, logging, and cutting or breakage of one or more stems in a sprout cluster. Both sapwood and heartwood were discolored in wounded yellow-poplar trees.

The discolored wood of sweetgum usually was some shade of reddish brown, whereas discolored wood of yellow-poplar ranged from light to dark yellow-green or green to red, blue, purple, brown, and black (24). Concentric patterns of variable color usually could be traced to multiple wounds. Circular separations (ringshake) sometimes developed between discolored zones or between discolored wood and sapwood formed after wounding when the wood was dried.

In both species, discolored wood was associated with all wounds regardless of size or type of wound; decay was found in discolored wood, never in nondiscolored sapwood. Tissues formed after wounding were free of discoloration and decay. Heartwood of yellow-poplar decayed after undergoing color changes to shades of brown (15), but decay usually began in discolored sapwood rather than heartwood.

Development of discoloration and decay in experimentally wounded trees.—Superficial wounds inflicted on stems of sweetgum and yellow-poplar during the summer months resulted in the formation of a thin sheet of intensely discolored wood on the exposed wound surface within 1 mo after wounding (Table 1). This sheet of discolored tissue extended vertically beyond the margins of the wound to a maximum distance of 11 cm in yellow-poplar and 1 cm in sweetgum at 720 days after wounding. Increases in radial depth of discolored tissues beyond 2-4 mm consistently were associated with insect bore holes or cracks in the wood (Fig. 1-A). Wood within 2-4 mm of the exposed surface became dark brown to black in sweetgum and dark purple to black in yellow-poplar. Discolored wood beneath this zone was similar in color to that associated with deep wounds of natural or experimental origin.

Deep wounds resulted in the formation of columns of discolored wood which began as a series of vertical streaks in exposed sapwood (Fig. 1, B-E, H; 2). The streaks coalesced into columns which increased in volume from the exposed surface axially during the first several weeks after wounding (Fig. 3). The rate of column development varied widely among trees (Table 1, Fig. 1-C). Decay became apparent in columns of discolored wood 360-720 days after wounding (Table 1, Fig. 1-F, I).

Xylem formed soon after either superficial or deep wounding differed anatomically from that present at the time of wounding. A "barrier zone" (28) could be seen to extend up to 1 m or more above and below most wounds and sometimes completely around the stem if wounds were severe (Fig. 1-G). Microscopic observation of zones visible to the naked eye revealed rows of traumatic resin canals in sweetgum (7) and incompletely differentiated cells filled with extraneous materials in yellow-poplar. No attempt was made to describe either the total extent of

TABLE 1. Depth of discolored wood and decayed wood in experimentally wounded sweetgum and yellow-poplar trees

Symptom development and time after wounding (days)	Depth (range in mm)[a] of discolored and decayed wood following:							
	Superficial wounding[b]				Deep wounding[b]			
	Sweetgum		Poplar		Sweetgum		Poplar	
Discolored wood								
15	0-1	2)	2-3	2)	1-2	2)	0	2)
30	1-3	4)	3-5	4)	10-100	6)	0-550	4)
90	1	2)	5-8	2)	10-270	2)	0-780	2)
360	2-6	4)	5-32	4)	50-250	4)	520-950	3)
720	2-5	2)	5-10	2)	500-550	2)	900	1)
Decayed wood								
360							250-350	3)
720					200-250	2)	300	1)

[a]Depth is measured perpendicular to the cambium in superficially wounded trees and to the transverse surface exposed in deeply injured trees. Numbers in parentheses are trees observed.

[b]Superficial wounds were made by removing bark from a diamond-shaped area equal in width and height to 40 and 80%, respectively, of the stem circumference. Deep wounds were made by making two parallel transverse cuts 2.5 cm apart to a depth of 0.4 × the stem diameter.

each zone or the exact nature of anatomical changes within it.

Relative occurrence of microorganisms.—More than 4,000 isolations were made by tissue plating from sapwood, heartwood (yellow-poplar only), discolored wood, and decayed wood. Bacteria were found in all of these tissues. The relative occurrence of bacteria in sapwood varied from 1-2% in young, outer sapwood, to 17-24% in older, inner sapwood. Populations of bacteria in sapwood from nonwounded and from wounded sweetgum trees prior to discoloration averaged 10 cells/g of wood with a maximum of 10^3 cells/g of wood (Table 2). Populations increased to an average of 10^4 cells/g of wood with a maximum $>10^5$ cells/g of wood during early discoloration in deeply wounded sweetgum trees. This increase occurred during 4-8 wk in the field. The same increase in numbers was obtained in only 3 days in excised wood blocks incubated in a moist chamber, and no dark wood discoloration occurred. Six wood samples from a badly decayed yellow-poplar tree yielded bacterial populations of $>10^7$ cells/g from discolored and decayed wood and $<10^3$ cells/g from sapwood.

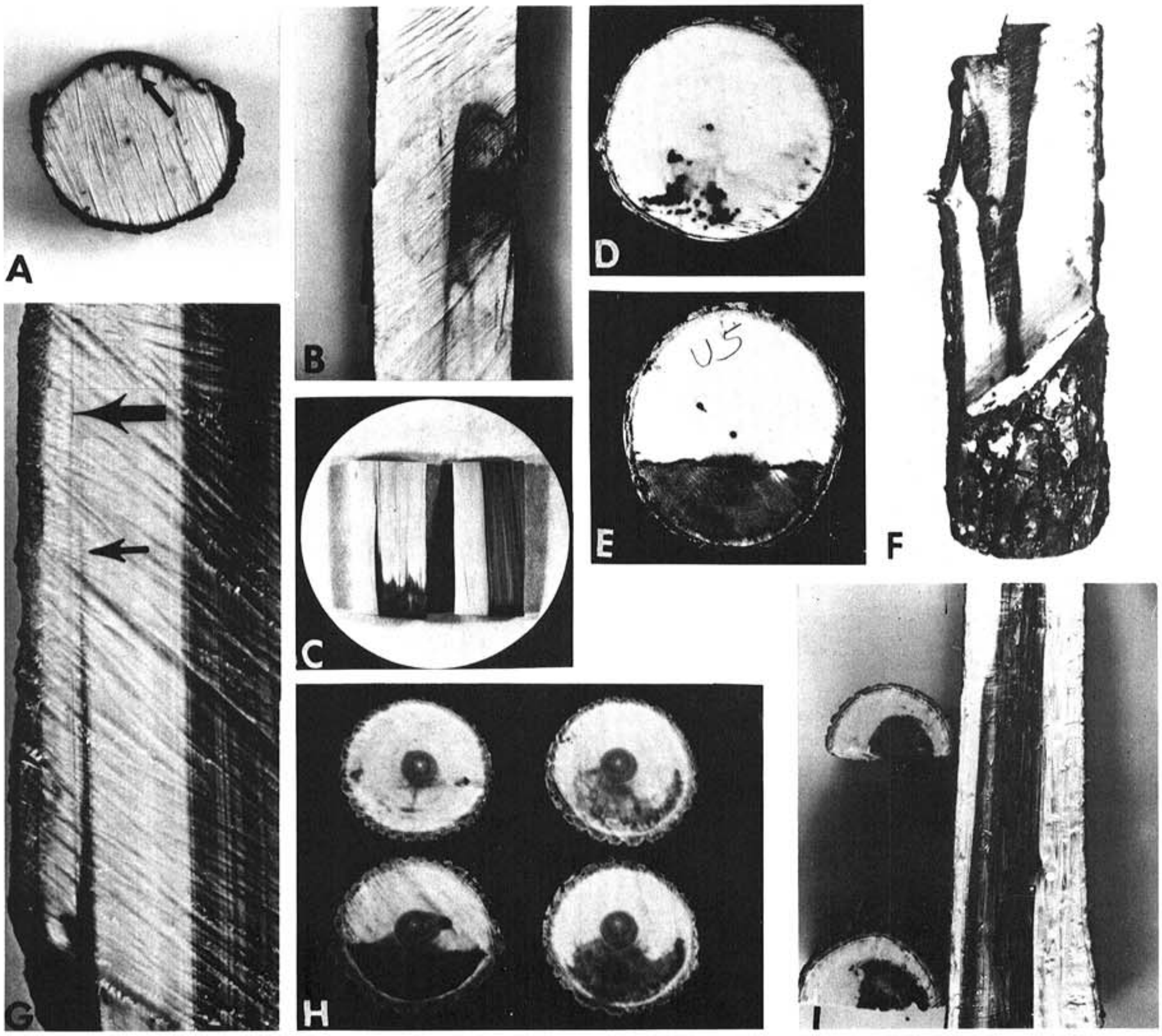

Fig. 1-(A to I). A) Discoloration of superficially wounded sweetgum occurred within 1 cm of the exposed surface except where the surface was penetrated by insects (arrow) and by cracks during drying. **B)** Discoloration of deeply wounded sweetgum occurred as columns, which varied in rate of development; e.g. **C)** left column extended 3 cm above wound 250 days after wounding, right column extended 20 cm beyond the 10 cm shown 150 days after wounding. **D)** Columns began as multiple longitudinal streaks seen as spots on the transverse surface (5 cm above the wound, 30 days after wounding), **E)** coalesced into solid zones 5 cm above wound 60 days after wounding, and **F)** decayed after 360-720 days. A zone of abnormal cells was observed in wood formed after superficial or deep wounding of yellow-poplar (G, large arrow) or sweetgum. **H, I)** Discoloration and decay of deeply wounded yellow-poplar trees occurred as in sweetgum. Superficial wounds were made by removing bark from a diamond-shaped area equal in width and height to 40 and 80%, respectively, of the stem circumference. Deep wounds were made by making two parallel transverse cuts 2.5 cm apart to a depth of 0.4 × the stem diameter.

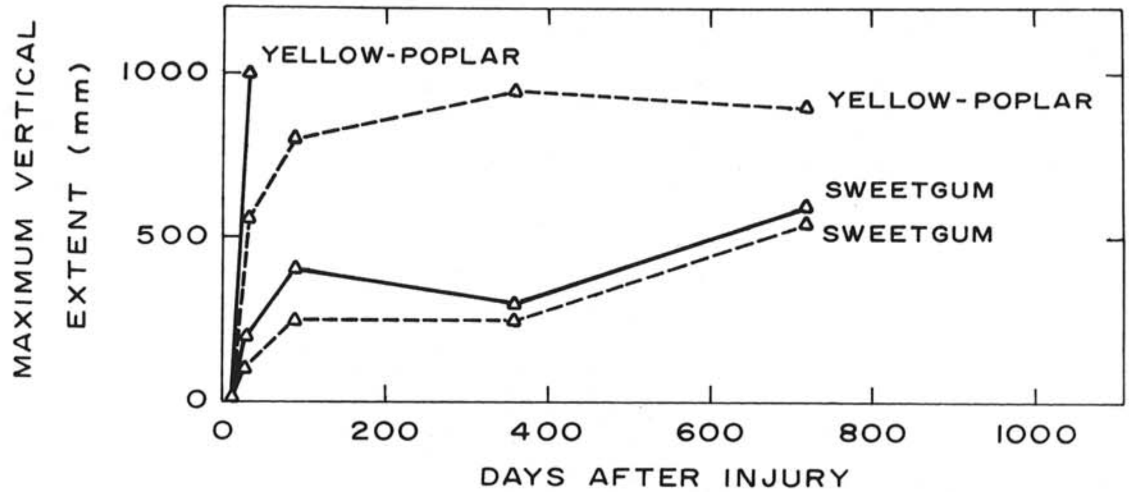

Fig. 2. Development of streaks (——) and columns (- - -) of discolored wood following deep wounding of sweetgum and yellow-poplar trees. Deep wounding was achieved by making two transverse cuts 2.5 cm apart and to a depth of 0.4 × the stem diameter.

Direct comparisons were made between relative occurrence data derived from plating wood chips and numbers of cells per gram of wood derived from dilution plating of wood homogenates. The relative occurrence of bacteria was as follows: 2% of 120 chips from 20 samples yielded <10 cells/g; 3% of 456 chips from 76 samples yielded no more than 10^3 cells/g; and 90% of 90 chips in 15 samples yielded 10^4 or more cells per gram of wood.

Bacteria isolated from sapwood and from discolored wood were similar in appearance. The most common bacteria were small (0.2 × 0.8 nm to 1.5 × 2.5 nm), Gram-negative, motile, asporogenous rods.

Fungi were found in all tissues where bacteria were found. The relative occurrence of fungi in sapwood was 1-3%. The taxa found in sapwood were mostly *Phialophora*, *Fusarium* (with microspores), *Cephalosporium*, *Streptomyces*, and *Trichocladium canadense* Hughes. The latter produced small phialospores in addition to the dark aleuriospores on which its classification is based. This was observed earlier in red maple isolates (29). *Trichocladium canadense* was the most common fungus isolated from the heartwood of yellow-poplar (5%) and from long columns of discolored wood associated with broken tops in sweetgum (11%), but the fungus was not found in discolored wood formed after experimental wounding or in discolored wood surrounding decayed wood within 1 m of an open wound.

The fungi most commonly found in discolored and decayed wood were the wood-destroying hymenomycetes, *Ceratocystis*, *Fusarium*, and *Phialophora*. *Cephalosporium* (5%) was found in discolored wood associated with some wounds of yellow-poplar.

Other fungi (listed in order of decreasing occurrence) having relative occurrence of less than 2% were of the form genera *Gliomastix*, *Cytospora*, *Pestalotia*, *Cladosporium*, *Penicillium*, *Streptomyces*, *Paecilomyces*, *Nodulisporium*, *Pyrenochaeta*, *Verticillium*, *Candida*, *Epiccocum*, *Phoma*, *Tubercularia*, and *Gliocladium*.

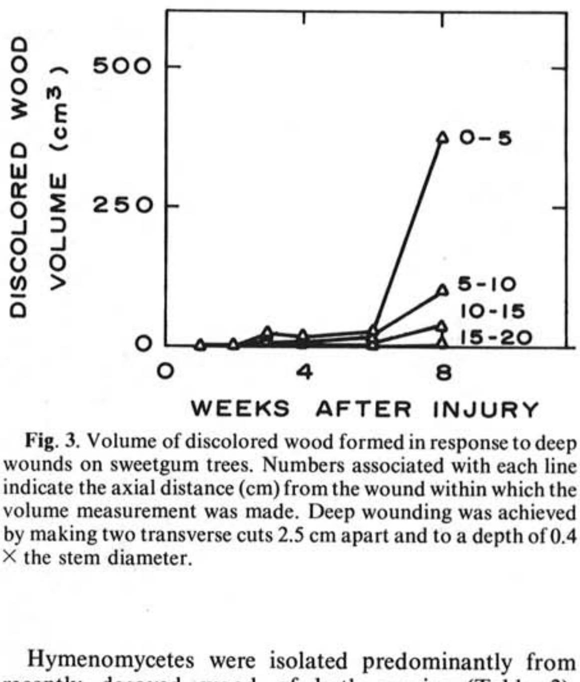

Fig. 3. Volume of discolored wood formed in response to deep wounds on sweetgum trees. Numbers associated with each line indicate the axial distance (cm) from the wound within which the volume measurement was made. Deep wounding was achieved by making two transverse cuts 2.5 cm apart and to a depth of 0.4 × the stem diameter.

Hymenomycetes were isolated predominantly from recently decayed wood of both species (Table 3). Hymenomycetes were found in all types of discolored and decayed wood, but not in nondiscolored sapwood or heartwood.

Ceratocystis spp. were isolated predominantly from the discolored wood formed first, but not during later stages of discoloration and decay (Table 3). *Fusarium* spp. were isolated predominantly from yellow-poplar during both early and later stages of wood discoloration. *Phialophora* spp. were isolated predominantly during later stages of wood discoloration of sweetgum.

Few fungi were isolated from the small amount of discolored wood formed in superficially wounded trees. *Cytospora* and *Cytosporina* were most commonly found in sweetgum and *Fusarium oxysporum* in yellow-poplar.

Populations of fungi in sapwood from nonwounded and from wounded sweetgum trees prior to discoloration averaged 10 propagules/g wood with a maximum of 10^2 propagules (Table 2). Populations increased to an average of 10^2 propagules/g with a maximum of 10^3 propagules during early discoloration, which took 4-8 wk in deeply wounded sweetgum trees.

DISCUSSION

Although sweetgum is reported to have heartwood (2, 25), all central cores of colored wood in sweetgum were associated with major wounds as in northern hardwoods (35). Previous observations indicating the irregular occurrence of such cores of colored wood are as follows: (i) 10% of sampled sweetgum trees over 35 years of age

TABLE 2. Average populations of bacterial and fungal propagules found in stemwood of sweetgum

Type of tissue	Propagules per gram (dry wt) of wood			
	Bacteria		Fungi	
	Average	Maximum	Average	Maximum
Autoclaved sapwood (control)	0	0	0	0
Nondiscolored sapwood	10	10^3	10	10^2
Discolored sapwood	10^4	$> 10^5$	10^2	10^3

TABLE 3. Fungal taxa most frequently isolated 15-720 days after deep experimental wounding[a], and more than 720 days after a variety of natural wounds

Type of tissue and time after wounding (days)	Relative occurrence[b] of:			
	HYM[c]	CER[d]	FUS[e]	PHI[f]
Sweetgum				
Discolored				
15-30	2	38	5	8
360-720	5	0	0	41
> 720	9	0	8	10
Decayed				
360-720	92	0	0	0
> 720	32	0	7	5
Yellow-poplar				
Discolored				
15-30	1	23	21	0
360-720	18	0	29	0
> 720	3	0	11[g]	6
Decayed				
360-720	100	0	0	0
> 720	75	0	0	4

[a]Deep experimental wounding: two transverse cuts 2.5 cm apart and to a depth of 0.4 × the stem diameter.

[b]Relative occurrence

$$= \frac{\text{number of isolations of a taxon}}{\text{number of isolations attempted}} \times 100.$$

[c]Abbreviation HYM = Hymenomycetes. Isolates included *Armillaria mellea* Vahl., *Daedalea quercina* ex Fr., *Ganoderma applanatum* (Pers. ex Wallr.) Pat., *G. lucidum* (Leys.) Karst., *Hericium erinaceous* Fr., *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm., *Polyporus spraguei* B and C., *P. sulphureus* Bull. ex Fr., and *P. versicolor* L. ex Fr.

[d]Abbreviation CER = *Ceratocystis*. Isolates included *C. coerulescens* (Munch) Bak. (most common), *C. allantospora* Griffin. *Ceratocystis* sp., and *Chalara* sp. (possible imperfect state of *Ceratocystis*).

[e]Abbreviation FUS = *Fusarium*. Isolates included *F. oxysporum* Schlecht. (most common), *F. moniliforme* Sheldon (yellow-poplar only), and *F. solani* (Mart.) Sacc. (sweetgum only).

[f]Abbreviation PHI = *Phialophora*. Isolates included *P. bubakii* (Laxa) Schol-Schwarz (most common), *P. melinii* (Nannf.) Conant (common), *P. alba* Beyma, and several unidentified *Phialophora* spp.

[g]Relative occurrence of *Fusarium* includes 5% *Cephalosporium* spp.

had no discolored heartwood (17), (ii) it was not uncommon to find second-growth sweetgum trees 46 cm dbh without colored heartwood (19), and (iii) in the lumber trade, "sapgum" trees which do not have colored heartwood are distinguished from "redgum" trees which do (2). In yellow-poplar trees more than 30 yr old a colored core of heartwood is universally observed.

Development of discoloration and decay in both species was similar, although they differed with respect to heartwood formation. Superficial wounding resulted in the formation of a "protective zone" on the exposed surface of wood present at the time of wounding, as described for fire-scarred sweetgum by Hepting and Blaisdell (13). This sheet of heavily infiltrated discolored wood was more effective in sweetgum than in yellow-poplar, which may account in part for the greater amount of basal defect observed in yellow-poplar than in sweetgum. Failure of the protective zone to prevent internal discoloration was observed when the zone was physically disrupted by boring insects or cracks that developed during drying.

Deep wounding resulted in the formation of columns of discolored sapwood in sweetgum and yellow-poplar and discolored heartwood in yellow-poplar. Columns of discolored wood began to decay within 2 yr after wounding. These columns began as multiple vertical streaks which coalesced into columns similar in shape to those described by Shain (26, 27) in pine and spruce infected with *Fomes annosus*. Such columns in pine and in spruce were surrounded by a "reaction zone" (26), in which phenolic substances accumulated. Preliminary studies indicated that a similar phenomenon was taking place in sweetgum (Shortle, *unpublished*). Studies of a reaction zone in yellow-poplar are in progress (4).

Observation of xylem formed after wounding indicated that a "barrier zone" is formed in sweetgum and yellow-poplar, as in maple (28). Traumatic resin canals (7) were found in this zone in sweetgum, unlike yellow-poplar or maple. Storax, the natural product of these resin canals in sweetgum, contains 5-15% free cinnamic acid (40), which has been shown to inhibit both a decay fungus and a nondecay fungus in vitro (39) and is a precursor of other natural fungitoxic substances in plants (22). A thin film of storax spread on wood blocks completely inhibited the growth of the decay fungus, *Pleurotus ostreatus* (38). This suggests the production of a chemical barrier in wood formed after wounding.

The extent to which anatomical and chemical barriers are formed by the cambium after wounding is not known. It has been recognized for over four decades that sapwood formed after wounding does not decay in sweetgum and some other southern hardwood tree species (12). Hepting (12) postulated that the new sapwood was different in character from that present at time of wounding. A chemical and/or anatomical "barrier wall" could be the character postulated to account for "compartmentalization" of tree stems following wounding (34).

Propagules of bacteria and some of the same taxa of fungi found to colonize discolored sapwood appeared to be present in sapwood prior to its discoloration. Propagules may be deposited in sapwood through the many tiny wounds found on all tree stems (30) or they may be deposited by the transpiration stream following root injury. Low populations of bacterial cells ($<10^3$ propagules/g of wood) are not readily detected by tissue plating of wood chips and thus may be easily overlooked by assays utilizing that method. The common hyphomycetes with small spores found in discolored wood (8, 31, 33, 42) also are commonly found in soil (1) and bark (6). Small, Gram-negative, motile bacteria are common in soil and have been reported by others in apparently healthy stemwood (11, 18, 20, 21). Direct evidence of systemic movement of bacteria in Persian walnut has been demonstrated by use of double-marker mutants (5).

Bacterial populations did not increase in sapwood until after discoloration began. Increases in populations of fungi lagged behind those of bacteria. The predominant early colonizer, *Ceratocystis*, did not persist in discolored wood, but small-spored fungi such as *Phialophora* and *Fusarium* did. These more persistant colonizers of discolored wood were replaced by decay-causing hymenomycetes as wood began to decay, thus completing a succession (32, 34). Two points regarding the concept of succession require clarification: (i) "pioneer invaders" of sapwood may develop, at least in part, from internal sparse populations of microorganisms as the result of discoloration and (ii) if pioneer colonizers of sapwood do not cause discoloration, how important are they to the decay process relative to the role of the tree and decay-causing hymenomycetes?

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