Interaction of Live Sapwood and Fungi Commonly Found in Discolored and Decayed Wood

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ABSTRACT

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Interactions between fungi found in discolored and decayed wood of living sweetgum and yellow-poplar trees and living sapwood were studied in vitro. The results indicated that early colonizers (*Ceratocystis coerulescens, Fusarium oxysporum,* and *F. moniliforme*) of discolored wood found within 30 days of wounding: (i) usually grow on live sapwood without inducing wood discoloration, (ii) grow at concentrations of gallic acid inhibitory to decay fungi, (iii) do not persist on dead wood, and (iv) initially outcompete other fungi that colonize wood, but are readily replaced by decay fungi on dead wood. More persistent colonizers (*Phialophora bubakii* and *P. melinii*) of discolored wood: (i) neither grow on live sapwood nor induce its discoloration, (ii) grow at levels of gallic acid inhibitory to decay fungi, (iii) persist in wood by utilizing some cell wall substances, and (iv) cannot compete with either early colonizers or decay fungi on living or dead sapwood. Decay fungi (such as *Pleurotus ostreatus*) which commonly are found on living trees: (i) grow on live sapwood only if they induce discoloration and grow well only after dark pigments have been removed, (ii) are inhibited by levels of gallic acid that have no effect on the growth of the former two groups of fungi, and (iii) replace early colonizers in dead wood. It was concluded that some decay fungi may cause wood discoloration following wounding independent of pioneer fungi and induce a host response which suppresses their growth and temporarily allows pioneer fungi and bacteria to flourish. This view is contrary to the current concept of succession of microorganisms leading to decay in living trees.

Bacteria and nonhymenomycetous fungi commonly are isolated from discolored wood associated with wounds and decayed wood in living trees. It is thought that these microorganisms intensify wood discoloration by interacting with live wood, and may be important in decay caused by wood destroying Basidiomycetes (10). Evidence of interactions is based primarily on the constant association of bacteria and nonhymenomycetous fungi with the discoloration of wood (12).

The purposes of this study were to test: (i) the capacity of fungi isolated from stemwood of sweetgum (*Liquidambar styraciflua* L.) and yellow-poplar (*Liriodendron tulipifera* L.) during the development of discoloration and decay (13), to grow on live wood and to induce discoloration, (ii) the capacity of living sapwood to inhibit growth of fungi, and (iii) the capacity of fungi to grow on media containing gallic acid and to utilize cell wall substances. Competition among such fungi in living and nonliving sapwood was also studied in vitro.

MATERIALS AND METHODS

Substrates.—Wood blocks (0.5 × 4 × 5 cm) used to test growth and discoloration were aseptically split tangentially from freshly cut, 5-cm-thick disks and placed in sterile moist chambers. The wood was from 5 to 25 yr old. Disks containing sapwood, discolored wood, or heartwood, were taken from sweetgum, yellow-poplar, red maple (*Acer rubrum* L.), and white oak (*Quercus alba* L.). All trees were 40 to 50 yr old and were cut in August through October, 1973. One 36-yr-old sound, healthy sweetgum was cut in April, 1972 for decay tests. Blocks were placed in moist chambers consisting of 100 × 15-mm glass petri dishes containing two pieces of filter paper and 8.5 ml of distilled water, and initially sterilized by autoclaving for 20 min at 121 C. Larger blocks initially were surface-disinfested, and rinsed before being split into test blocks, but later this was found to be unnecessary to prevent contamination. Blocks of apple tissue (cultivar Golden Delicious) were used to compare results obtained with sapwood.

After the blocks were placed in chambers, half were heat-treated to kill living parenchyma. Initially, heat treatments consisted of autoclaving for 20 min at 121 C. Later, it was found that steaming blocks at 100 C for 2 to 4 min gave equivalent results.

To determine if the wood parenchyma cells were alive, small samples (0.5 × 1 × 1 cm) were incubated in freshly prepared 1% aqueous triphenyltetrazolium chloride for 24 hr in darkness at 23-28 C. They were considered to be living if the test sample became distinctly reddish-pink in color. Parenchyma of freshly-excised sapwood blocks that had been heat-treated failed to stain reddish-pink. Freshly excised discolored wood and heartwood did not react to vital staining.

Inoculum.—The following fungi were isolated from sweetgum and yellow-poplar (13): *Ganoderma lucidum* (Leys.) Karst., *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm.,

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Polyporus spraguei B. and C., *P. versicolor* L. ex Fr., *Ceratocystis coerulescens* (Munch) Bak., *Fusarium moniliforme* Sheldon, *F. oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Phialophora bubakii* (Laxa) Schol-Schwarz, *P. melinii* (Nannf.) Conant, *Phialophora* sp., species of *Cephalosporium, Cladosporium, Cytosporina, Epicoccum, Gliomastix, Nodulisporium, Pestalotia,* and four unidentified fungi. *Polyporus versicolor,* Madison isolate 697, and *Lenzites trabea* Pers. ex Fr., Madison isolate 617, also were used for comparative purposes. *Hypoxylon deustum* (Hoffm. ex Fr.) Grev. and *Stereum murrayi* (B. and C.) Burt were isolated from sugar maple. *Polyporus dryophilus* Berk., oak isolate P18, was provided by F. H. Berry (Forest Insect and Disease Lab., Delaware, OH 43015), and *P. melinii,* isolate P5, was provided by A. L. Shigo (Northeastern Forest Experiment Station, Durham, NH 03824).

Fungal growth and wood discoloration.—Live sapwood, heat-killed sapwood, discolored sapwood, and heartwood blocks were inoculated in triplicate by placing onto the center of each block a 3 mm agar plug cut from 1- to 2-wk-old cultures of fungi grown on malt-yeast agar. Blocks were incubated in moist chambers in darkness at 23-28 C. Maximum spread of mycelium over the long (5 cm) and short (4 cm) axes of the test block, when observed under × 10 magnification, was used as an index of growth. The presence or absence of discoloration similar in color and intensity to that which occurs following wounding in living trees, also was recorded.

Fungal growth on gallic acid media.—Growth on gallic acid agar medium was determined in triplicate as for the Bavendamm reaction (1). Growth in liquid culture was determined in triplicate as oven-dry weight of mycelium in a basal medium (14), which contained 4 g carbon (10 g glucose), in which gallic acid is substituted for D-glucose at rates of 0, 1/100, and 1/10.

Decay tests.—Test blocks (0.5 × 1.5 × 4 cm) of sweetgum sapwood were split from 4 cm disks. The blocks were oven-dried to constant weight at 104 C, aspirated in distilled water, autoclaved 15 min at 121 C in moist chambers, and placed on inoculated filter paper in mineral-vitamin agar (2). One each of triplicate blocks were harvested at 6, 9, and 12 wk, the oven-dry weight was determined, and weight loss due to decay was expressed as a percentage of original oven-dry weight. Means over all three harvests were used for statistical comparison of weight loss due to decay.

Interspecific competition.—Test blocks of live and heat-killed sapwood were inoculated in the center with *Pleurotus ostreatus* as previously described. Agar plugs with four different fungi were placed between the center plug and the corners of the block. Five different combinations of fungi were used. Four wood chips were taken between the center and outer plugs after 7 and 14 days of incubation from duplicate blocks inoculated with each of the five combinations of fungi on live and heat-killed sapwood.

RESULTS

Fungal growth and discoloration of living and nonliving tissue.—None of the nonhymenomycetous fungi tested induced discoloration of live sapwood similar in color and intensity to that found in living trees (Tables 1 and 2). *Ceratocystis coerulescens* caused a typical blue stain wherever it grew. Some of the decay-causing Hymenomycetes tested induced discoloration of live sapwood, but not of heat-killed sapwood (Tables 1 and 2; Fig. 1). *Hericium erinaceous* and *Ganoderma lucidum* were tested only on sweetgum sapwood, and induced discoloration of live sapwood; *Polyporus sulphureus* and *Lenzites trabea* neither grew on living sweetgum sapwood

TABLE 1. Growth of fungi and wood discoloration resulting from the inoculation of living sapwood, heat-killed sapwood, and discolored wood (sweetgum, red maple) or heartwood (white oak)

Type and identity of fungal inoculum	Index of growth and discoloration after 10 days[a]								
	Live sapwood			Killed sapwood[b]			Discolored wood		Heartwood
	Gum	Maple	Oak	Gum	Maple	Oak	Gum	Maple	Oak
Nonhymenomycetes:[c]									
Ceratocystis coerulescens	20–	20–		20–	20–		0–	0–	
Fusarium spp.	t–		0–	20–		20–	20–		20–
Phialophora spp.	0–	0–	0–	20–	20–	8–	4–	5–	0–
Hymenomycetes:									
Pleurotus ostreatus	5+	4+	4+	20–	20–	20–	20–	3–	20–
Polyporus versicolor	1+	0–	0–	20–	20–	20–	20–	0–	20–
Polyporus dryophilus	0–	0–	0–	20–	20–	20–		2–	12–

[a]Growth index = mean spread of mycelium in long axis (5 cm) × spread in short axis (4 cm) of triplicate wood blocks. Maximum index = 20 cm^2, t = trace of growth on block, + = dark discoloration induced in wood beyond margins of mycelium, – = no discoloration induced, and t = trace.

[b]Freshly excised sapwood blocks autoclaved 20 min at 121 C and one atmosphere of gauge pressure (15 psi) in early tests and later steamed 2 min at 100 C with equivalent results.

[c]*Fusarium oxysporum, F. moniliforme, Phialophora bubakii, P. melinii.*

nor induced its discoloration.

Growth of all fungi was greatest on rapidly killed sapwood and least on live sapwood, except for *C. coerulescens* which grew equally well on live or heat-killed sapwood of sweetgum and red maple (Tables 1 and 2). *Ceratocystis coerulescens* (isolate from sweetgum) failed to grow on live sapwood of yellow-poplar (Table 2). *Fusarium moniliforme* (isolate from yellow-poplar) grew poorly on sweetgum.

Growth of fungi on discolored wood and heartwood, both dead products of sapwood in living trees, was highly variable (Tables 1 and 2). *Ceratocystis coerulescens* grew poorly or not at all on discolored wood and heartwood, but all other fungi tested exhibited a capacity to grow on these types of wood.

Growth of the decay fungus *Pleurotus ostreatus* increased with time of prior exposure of the wood to steam heat and reached a maximum when wood parenchyma cells no longer reacted to vital staining (Table 3). Initial exposure to steam heat enhanced the discoloration reaction, which no longer occurred once vitality was lost (Table 3).

The incubation of sapwood in moist chambers 12 days prior to inoculation resulted in an increase in bacterial populations from $<10^3$ to $>10^6$ cells/g wood, loss of starch, slight discoloration, and some loss in vital staining reactions. Growth of *F. moniliforme* increased greatly. Growth of *P. ostreatus* and *Polyporus versicolor* increased slightly and discoloration enhanced by the partial loss of cell vitality. *Phialophora melinii* and *Polyporus dryophilus* neither grew nor induced discoloration before or after incubation.

Fungi (*C. coerulescens, F. oxysporum, P. ostreatus,* and *P. versicolor*) that grew on some species of living sapwood also grew on living parenchymatous apple tissue. Fungi (*Phialophora bubakii* and *P. dryophilus*) that failed to grow on living sapwood did not grow on living parenchymatous tissue.

Fungal growth on gallic acid media.—Growth of decay fungi (*Fomes igniarius, F. connatus, Polyporus glomeratus, Stereum murrayi, Pholiota adiposa, Stecharinum septentrionale, Ganoderma applanatum,* and *Daedalea unicolor*), which are found most commonly decaying trees lacking heartwood (beech and maple), was inhibited by gallic acid under conditions of the Bavendamm reaction —one part gallic acid to three parts

TABLE 2. Growth of fungi and wood discoloration resulting from the inoculation of living sapwood, heat-killed sapwood, and heartwood of yellow-poplar

Type and identity of fungal inoculum	Index of growth and discoloration after 10 days[a]		
	Live sapwood	Killed sapwood[b]	Heartwood
Nonhymenomycetes:			
Ceratocystis coerulescens	0–	20–	0–
Fusarium moniliforme	7–	20–	6–
Phialophora bubakii	0–	2–	0–
Hymenomycetes:			
Pleurotus ostreatus	0+	20–	20–
Polyporus versicolor	1+	20–	7–
Polyporus spraguei	7–	20–	10–

[a]Growth index = mean spread of mycelium in long axis (5 cm) × spread in short axis (4 cm) of triplicate wood blocks. Maximum index = 20 cm^2, + = dark wood discoloration induced in wood beyond margins of mycelium, – = no discoloration induced in wood.
[b]Freshly excised sapwood blocks autoclaved 20 min at 121 C and one atmosphere of gauge pressure (15 psi) in early tests and later steamed 2 min at 100 C with equivalent results.

TABLE 3. Effect of steam heating upon vitality and discoloration of living sapwood and growth by *Pleurotus ostreatus*

Source of sapwood	Exposure (min)[a]	Vitality[b]	Growth[c]	Discoloration[d]
Sweetgum	0	+	5	13
	1	+	10	16
	4, 6, 10, 15, 20	–	20	0
Yellow-poplar	0	+	0	0
	0.5	+	0	1
	1.0	+	8	12
	1.5	+	20	0
	2.0, 2.5, 3.0	–	20	0

[a]Time exposure to steam at 100 C.
[b]Symbols: + = positive reaction to vital staining with 1% aqueous triphenyltetrazoliumchloride; and – = no reaction.
[c]Growth index at 9 days for sweetgum, 12 days for yellow-poplar. Growth index = mean spread of mycelium in long axis (5 cm) × spread in short axis (4 cm) of triplicate wood blocks. Maximum index = 20 cm^2, t = trace of growth on block, + = dark discoloration induced in wood beyond margins of mycelium, – = no discoloration induced, and t = trace.
[d]Size of discolored area expressed in same manner as growth index.

malt extract (1). Although these fungi initially cannot use nutrients in the medium for growth, they produce a strong oxidase reaction in the substrate surrounding the inoculum. *Pleurotus ostreatus* and *H. erinaceous* were two fungi representative of this group that consistently induced a discoloration of live sapwood (Tables 1 and 2). Both fungi cause decay of maple and oak.

Gallic acid did not inhibit the growth of decay fungi (*Lenzites trabea, L. saepiaria, Poria oleraceae, P. incrassata, P. vallanti, Daedalea quercina, Polyporus adustus, P. palustris, Stereum frustulatum,* and *Schizophyllum commune*) commonly found on wood products of beech, maple, and oaks (1)–except *P. versicolor* which acted like the fungi that decay living trees. None of the saprobic fungi, except *P. versicolor,* produced an oxidase reaction. This was true for fungi of this group that caused whiterot as well as for those that caused brownrot. *Polyporus versicolor* sometimes induced discoloration of live sapwood; *L. trabea* neither grew on live sapwood, nor induced its discoloration.

Growth of some fungi (*Polyporus fissilis, P. zonalis, P. hispidus, Fomes everhartii,* and *Poria andersonii*) which decay living trees with nonliving heartwood as well as sapwood (oaks), was inhibited by gallic acid, whereas growth of others (*Polyporus dryophilus, P. curtisii, Stereum gausapatum, S. subpiliatum, S. frustulatum, Ganoderma lucidum, Fomes geotropus,* and *Lentinus tigrinus*) was not (1). The fungi that were inhibited and produced a strong oxidase reaction included the canker-rot fungi of oak (*Polyporus hispidus, Poria andersonii,* and *Fomes everhartii*) and the canker-rot fungi of maple (*Polyporus glomeratus, Stereum murrayi, Daedalea unicolor,* and *Fomes igniarius* f. sp. *laevigatus*). *Polyporus dryophilus* which only decays the heartwood of mature oak trees (4), was not inhibited by gallic acid and failed to induce a discoloration of living sapwood in all tests (Tables 1 and 2). It also failed to grow on live apple tissue.

Growth of most fungi (*Pleurotus ostreatus, Hericium erinaceous, Armillaria mellea, Polyporus beikeley,* and *P.*

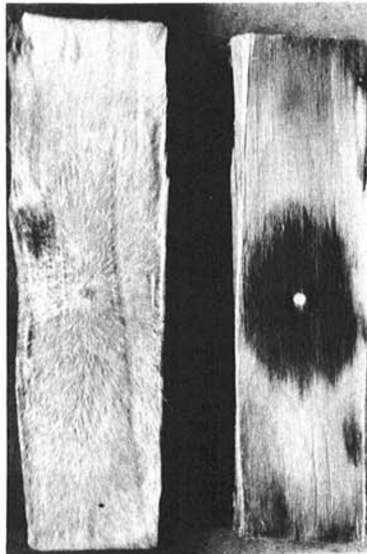

Fig. 1. *Pleurotus versicolor* mycelium covers heat-killed sapwood of yellow-poplar (2 × 5 × 20 cm block, steamed 4 min) after 10 days (left), but only traces of mycelium are found on live sapwood where discoloration was induced (right).

TABLE 4. Effect of increasing level of gallic acid in basal medium upon the mycelium weight of three fungi isolated from discolored wood of sweetgum

Fungal species	Mycelial growth after 21 days[a]		
	Gallic acid/glucose ratios of		
	0/100	1/100	10/100
Fusarium oxysporum	43	46	44
Phialophora melinii	41	41	42
Pleurotus ostreatus	54	61*	19*

[a]Mean of triplicate 250-ml flasks containing 25 ml of medium from which the mycelium was filtered, washed, and oven-dried 24 hr at 105 C. Asterisk (*) indicates mean was significantly different from glucose control ($P \leqslant 0.05$).

TABLE 5. Mycelial growth at 12 wk and weight loss due to decay caused by wood-destroying fungi (Hymenomycetes) and other wood-inhabiting fungi (non hymenomycetes) on heat-killed sweetgum sapwood

Inoculum	Wt. loss (%)[a]	Growth index[b]
Hypoxylon deustum	31*	+++
Phialophora sp.	7*	+++
P. bubakii	6*	+++
P. melinii 2)[c]	6*	+++
Cytosporina sp.	6*	+++
Pestalotia sp.	6*	+++
Nodulisporium sp.	6*	+++
Cladosporium sp.	3	+
Gliomastix sp.	2	+
Epicoccum sp.	2	+
Cephalosporium sp.	1	+
Ceratocystis coerulescens	1	+
Fusarium oxysporum 2)	1	−
F. moniliforme	1	−
F. solani	1	−
Nonidentified fungi 4)	1	−
Hymenomycetes:		
Polyporus spraguei	14*	+++
Ganoderma lucidum	6*	+++
Pleurotus ostreatus	5*	+++
Polyporus versicolor	1	+++
Stereum murrayi	1	+++

[a]Weight loss is mean of determinations after 6, 9, and 12 wk of incubation in agar block decay chambers. Asterisk (*) indicates mean was significantly different from noninoculated control ($P < 0.05$).

[b]Symbols representing growth index: +++ = entire block covered with mycelium; ++ = portion of block covered with mycelium; + = trace of mycelial growth; and − = no mycelial growth.

[c]Number of isolates in preceding right parenthesis.

obtusus) which commonly are found decaying both living maples and oaks, was inhibited by gallic acid, except for the two brownrot fungi which are of importance on living hardwood trees (*Polyporus spraguei* and *P. sulphureus*). These two fungi grew on gallic acid medium without inducing a strong oxidase reaction. *Polyporus spraguei* grew on living yellow poplar sapwood (Table 2) at a reduced rate compared to that on nonliving sapwood, but without inducing a discoloration. *Polyporus sulphureus* failed to grow on living sweetgum sapwood.

Most nonhymenomycetous fungi (23 of 28 species from maple, and 21 of 29 species from sweetgum and yellow-poplar; one to nine isolates per species, tested in triplicate) isolated from discolored sapwood of maple, sweetgum, and yellow-poplar grew on gallic acid medium and produced a wide variety of color reactions under conditions specific for the Bavendamm reaction. Growth of two common fungi found in discolored wood (*Fusarium oxysporum* and *Phialophora melinii*) was unaffected by substitution of gallic acid for carbohydrate (Table 4). Growth of *Pleurotus ostreatus* was strongly inhibited at a gallic acid/glucose ratio of 1/10 but was slightly stimulated at a ratio of 1/100 (Table 4). Inhibition of growth was accompanied by dark discoloration of the culture medium.

Decay tests.—Seven species of nonhymenomycetous fungi grew well on wood blocks and caused significant weight loss due to decay (Table 5). Twelve species of nonhymenomycetous fungi grew poorly or not at all and caused no decay. All five hymenomycetous fungi grew well, but only three caused decay. A wild-type isolate of *P. versicolor* from yellow-poplar grew more slowly on nonliving sweetgum sapwood than the Madison isolate 697 that was used in other tests.

Interspecific competition.—*Ceratocystis coerulescens* and *F. moniliforme*, rapid-growing, early colonizers of sapwood exposed by wounding in trees, were recovered most frequently from both living and nonliving sapwood after 7 days (Table 6). These fungi were quickly replaced by decay fungi at 14 days on nonliving sapwood. *Polyporus ostreatus* was recovered only where none of the more strongly saprobic decay fungi (*P. versicolor, P. dryophilus*, or *P. spraguei*) was introduced. *Ceratocystis coerulescens* and *F. moniliforme* persisted for 14 days on living sapwood; however, *P. ostreatus* overgrew these fungi in 30 days on two blocks where incubation was continued after isolations were made. The slow-growing *Phialophora bubakii* and *P. melinii* could not compete with either early colonizers or decay fungi under the indicated test conditions.

DISCUSSION

Species of *Ceratocystis* and *Fusarium* which were

TABLE 6. Postinoculation recovery of inoculum from live sapwood and heat-killed sapwood blocks inoculated with different combinations of five different fungi

Recovery of fungi from sapwood blocks or chips									
Live sapwood					Heat-killed sapwood[x]				
Recovery after 7 days[y]:									
Cc	Fo	Po	Pb	E	*Cc*	*Fo*	Po	Pb	*E*
Fm	Pv	Po	Pm	Lt	*Fm*	Pv	Po	Pm	Lt
Cc	Pv	Po	Pd	P	*Cc*	Pv	Po	Pd	P
Fm	He	*Po*	Pd	Pb	*Fm*	He	*Po*	Pd	*Pb*
Cc	He	Po	Pd	Ps	*Cc*	He	Po	Pd	*Ps*
Recovery after 14 days[y]:									
Cc	*Fo*	Po	Pb	E	*Cc*	Fo	*Po*	Pb	E
Fm	Pv	Po	Pm	Lt	Fm	*Pv*	Po	Pm	Lt
Cc	Pv	Po	Pd	P	Cc	*Pv*	Po	Pd	*P*
Fm	He	Po	Pd	Pb	Fm	He	Po	*Pd*	Pb
Cc	He	Po	Pd	Ps	Cc	He	Po	Pd	*Ps*

[x]Fresh sapwood steamed 3 min.

[y]Symbols that are underlined indicate fungi that were isolated from wood chips (four chips/block) taken from the surface of duplicate wood blocks by the same method used to isolate fungi from discolored wood of living trees (13).

[z]Abbreviations: Hymenomycetes—Po = *Pleurotus ostreatus*, Pv = *Polyporus versicolor*, Pd = *Polyporus dryophilus*, Ps = *Polyporus spraguei*, He = *Hericium erinaceous*, and Lt = *Lenzites trabea*. Nonhymenomycetes—Cc = *Ceratocystis coerulescens*, Fm = *Fusarium moniliforme*, Fo = *Fusarium oxysporum*, Pm = *Phialophora melinii*, Pb = *Phialophora bubakii*, P = *Pestalotia*, and E = *Epicoccum* sp.

found most often during early stages of discoloration in vivo (13), had the capacity to colonize live sapwood in vitro. Neither fungus induced a dark discoloration of sapwood. *Ceratocystis coerulescens* caused a typical bluestain such as observed along with darker wood discolorations during the first month after summer wounds (13). *Fusarium* sometimes caused a pink stain. Initial mycelial growth was strong on sapwood and these fungi outgrew all competing fungi. These pioneer invaders, which do not use cell wall substances for food, were replaced by decay fungi, which do.

Species of *Phialophora* and other genera of softrot fungi, which were found most often in older columns of discolored wood in vivo (13), lacked the capacity to colonize live sapwood and to cause its discoloration in vitro. *Phialophora* could not compete with either the early pioneers or decay fungi on sapwood. However, *Phialophora* differs from the early pioneers by its capacity to use some cell wall substances for food and to maintain strong mycelial growth on wood much longer than the pioneer fungi. It differs from the decay fungi in possessing the capacity to flourish on substrates in which polyphenols such as gallic acid strongly inhibit growth of decay fungi. *Phialophora melinii* uses gallic acid as a carbon source (14). Thus, accumulation of phenols in tissues in response to injury and infection, a phenomenon known in living trees (7, 8) and in many other plant tissues (5, 6), would hinder the growth of decay fungi and favor that phenol-tolerant fungi. Death of sapwood accompanying phenol production may promote development of propagules of fungi such as *Phialophora* observed in low populations in healthy sapwood (13). Replacement of *Phialophora* and its associates by decay fungi is inevitable once levels of phenols no longer are limiting and cell wall substances become the major source of food. The principle of a nondecay fungus removing an inhibitory substance from wood and rendering it susceptible to decay is well documented (3), and has been demonstrated in vitro for *Phialophora* and for a fungus able to cause decay of live maple trees (14).

The fungi that decayed live trees induced discoloration of live sapwood in vitro; the pioneer fungi did not. If discoloration did not occur, the decay fungi did not grow on live sapwood. If discoloration did occur, growth of decay fungi was retarded compared to growth on heat-killed sapwood, where parenchyma cells no longer function, and where discoloration did not occur. Mycelial growth on discolored wood was sparse until the wood was "decolorized" (Fig. 2-B). This loss of pigmentation occurred in trees (Fig. 2-A) at the same point where decay fungi are readily isolated (13) and phenols reach a low concentration (15).

Fungi which primarily decay trees lacking heartwood (e.g., beech and maple) which they strongly discolored were inhibited by gallic acid, whereas most decayers of dead wood products and more than half the fungi that usually decay trees with heartwood (oaks) were neither inhibited by gallic acid nor caused its discoloration. The

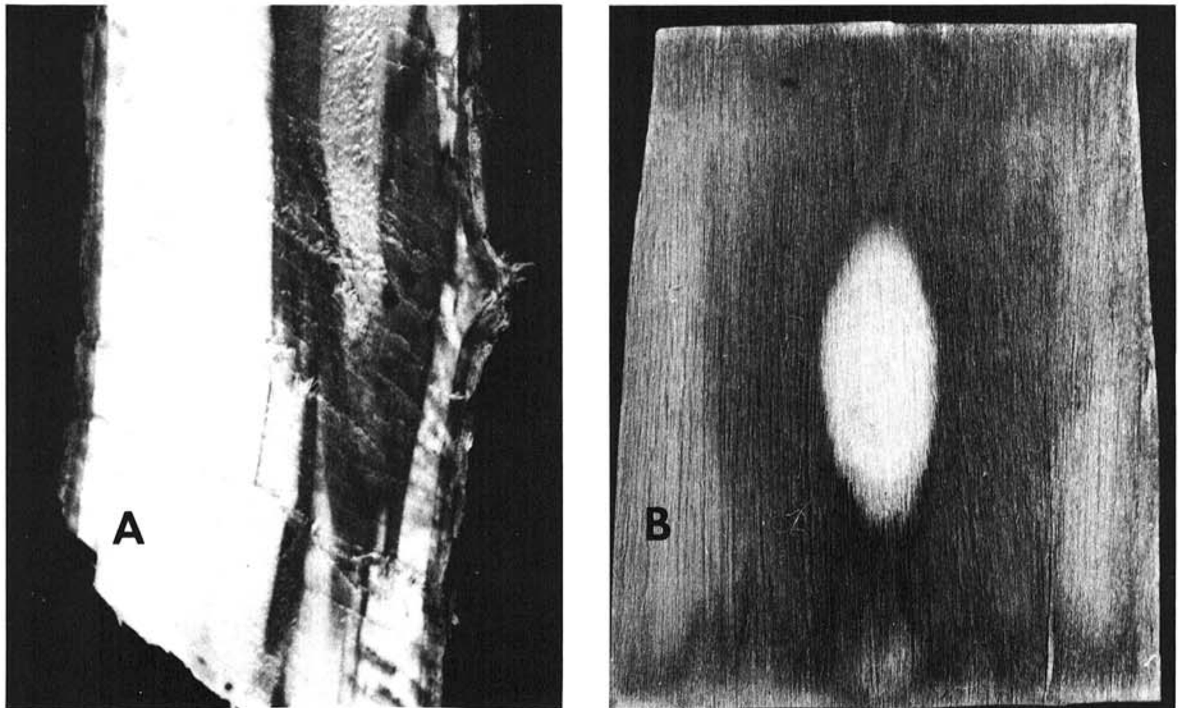

Fig. 2-(A, B). A) Column of discolored and decayed wood of sweetgum from which *Pleurotus ostreatus* was isolated. **B)** Discoloration reaction of *P. ostreatus* (same isolate) on live sweetgum sapwood followed by decolorization of wood in center. Mycelial growth of *P. ostreatus* on discolored wood was very sparse; growth on decolorized wood was moderately dense.

majority of species called pioneer fungi grew on gallic acid medium, as did saprobic decay fungi, and caused a wide variety of color reactions. Thus, common saprobes of wood, both decay and nondecay fungi, would be favored by phenol-enriched environments, but pathogenic decay fungi that attack living trees by becoming established in live sapwood are not so favored. Although temporarily overgrown by fast-growing pioneers like *Ceratocystis* and the more persistent, phenol-tolerant pioneers like *Phialophora,* these decay fungi would eventually succeed. These pathogenic decay fungi cannot compete with saprobic decay fungi on dead wood, but are given a selective advantage by early colonization of sapwood exposed by wounding.

The capacity of pathogenic decay fungi to gain a foothold in live sapwood may account for the fact that decay of living trees generally begins in discolored sapwood, even in species with heartwood (11, 13). It would also explain why trees lacking heartwood are nearly exclusively decayed by pathogenic decay fungi as described herein. Furthermore, all canker-rot fungi had the same gallic acid reaction as fungi that caused decay in trees lacking heartwood.

The current explanation for the phenomenon of succession of microorganisms during discoloration and decay of living trees is that tree parenchyma cells respond to injury and infection, pioneer fungi and bacteria interact with these cells as a part of the discoloration process, and decay fungi invade following pioneer fungi (9, 10). There is another possible explanation which is consistent with the patterns of isolations from living trees and with the experiments presented in this study. Early colonizers of living sapwood consist of: (i) decay fungi that induce wood discolorations and which are inhibited by this reaction, and (ii) nondecay fungi which are able to grow on living sapwood and do not induce discoloration. These early colonizers act independently and only the decay fungi persist, although their growth is strongly suppressed. Phenol-tolerant, small-spored, saprobic fungi, which may be present in sapwood prior to wounding (13), or which may invade after initiation of wood discoloration, persist in discolored wood until it becomes "decolorized". At this point, the nonhymenomycetous fungi would be isolated more often from discolored wood than the suppressed, phenol-intolerant decay fungi (11, 12, 13). Finally, decay fungi, which have survived the decolorization process, actively decay the decolorized wood because they are no longer inhibited by the products of dying sapwood parenchyma cells.

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