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Title: Use of Fluorescent-Labeled Lectins for Studying Progressive Stages of Fungal Decay in Douglas-fir and Ponderosa Pine

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The ability of the fluorescent-coupled lectins wheat germ agglutinin (WGA) and concanavalin A (Con A) to react with selected Basidiomycetes, Ascomycetes, and Fungi Imperfecti was evaluated using pure cultures of thirty-five fungi grown on malt extract agar. WGA, which is specific for residues of N-acetylglucosamine present in fungal chitin, reacted with nearly all hyaline fungal structures, but did not react with dematiaceous (dark) structures. Con A, which is specific for α-D-mannosyl and α-D-glucosyl residues, reacted with about one half of the fungi that reacted with WGA. This lectin was less useful for detecting fungal decay. The results indicate that WGA is a highly specific probe for detecting fungal chitin and fungal colonization by non-dematiaceous fungi.

The sequence of changes that occurred in wood over progressive stages of fungal decay was also studied using Douglas-fir heartwood [Pseudotsuga menziesii (Mirb.) Franco] and ponderosa pine sapwood (Pinus ponderosa Dougl. ex Laws.). The fungi used were Poria carbonica Overh., a brown-rot fungus; Coriolus versicolor (L. ex Fr.) Quel., a white-rot fungus; and Chaetomium globosum Kunze ex Fr., a soft-rot fungus. Incident light and fluorescence microscopy were used to

observe wood sections. *Poria carbonica* and *Coriolus versicolor* caused high weight losses at the early stages of exposure; while *Chaetomium globosum* caused lower weight losses. As expected, weight losses were generally greater in pine blocks. *Poria carbonica* displayed a greater tendency to attack ponderosa pine than Douglas-fir. The cellulolytic enzymes of the brown-rot fungus in ponderosa pine were capable of penetrating and acting within the cell walls at early stages of decay, while attack of Douglas-fir was concentrated on the more readily accessible ray parenchyma. The attack patterns found with *Coriolus versicolor* were similar to those found with *P. carbonica* on Douglas-fir, but bore holes were less common in the advanced stages of white rot attack. The hyphae of *C. versicolor* were widespread in ponderosa pine in early stages of decay. Passive penetration via the pits was prevalent in the early stages of decay ($\leq$4.35%), while active penetration through the cell walls was prevalent in the advanced stages of decay. *Chaetomium globosum* lacked the ability to cause soft rot damage (Type 1 or 2) in Douglas-fir blocks and failed to produce soft rot cavities (Type 1) in ponderosa pine blocks. The soft rot fungus preferentially invaded the ray cells of both wood species, then grew into the lumens of the adjacent tracheids.

FITC-WGA improved hyphal visualization when compared with results obtained using a conventional safranin-O/picro-aniline blue method. Double-staining, combining FITC-WGA and safranin-O stains, improved lectin contrast, increased the visibility of hyaline hyphae, and permitted more detailed examination of fungal colonization.

Use of Fluorescent-Labeled Lectins for Studying Progressive Stages of Fungal Decay in Douglas-fir and Ponderosa Pine

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My Parents

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USE OF FLUORESCENT-LABELED LECTINS FOR STUDYING PROGRESSIVE STAGES OF FUNGAL DECAY IN DOUGLAS-FIR AND PONDEROSA PINE

INTRODUCTION

Wood products undergo substantial changes when attacked by decay fungi, resulting in decreased service life and economic loss. While it is important to detect decay as early as possible, decay detection in the incipient stages poses considerable difficulty. The hyphae that colonize wood vary in their reactivity to biological stains, making it difficult to perform detailed observations of fungi during early stages of colonization (Wilcox, 1978). Most methods used for staining or detecting decay fungi in wood are only applicable to a few wood or fungal species (Eslyn, 1979; Gibson, et al., 1985). However, recent studies have shown that reacting hyphae or wood sections with fluorescent-coupled lectins enhances hyphal visibility (Morrell, et al., 1985; Krahmer, et al., 1986).

Lectins are plant or animal derived proteins that bind selectively to specific carbohydrates or glycoproteins (Liener, 1976). Each lectin is highly specific, reacting with only one or two carbohydrate groups. Several plant-derived lectins have been proven useful as selective probes for detecting fungal chitin (Morrell, et al., 1985).

This study evaluates the process of colonization and decomposition of wood blocks by brown, white, and soft rot fungi at the light microscopic level with the use of fluorescent-labeled lectins.

The objectives of this study were:

a) To determine the reactivity of hyphae from selected Basidiomycetes, Ascomycetes, and Fungi Imperfecti to fluorescent-labeled wheat germ agglutinin and concanavalin A.

b) To observe the progress of decay in Douglas-fir and ponderosa pine by *Poria carbonica*, *Coriolus versicolor*, and *Chaetomium globosum* using fluorescein isothiocyanate-labeled wheat germ agglutinin (FITC-WGA).

LITERATURE REVIEW

Effects of Decay on Wood Microstructure, Chemistry, and Attack Patterns

Wood in service or in storage may undergo degradation due to the action of wood destroying fungi. Fungal degradation depends on hyphae which enable the fungus to penetrate and move from cell to cell in the wood. On the basis of the physical and chemical changes they produce in wood, fungi are classified as brown rots, white rots, or soft rots.

A. Brown Rot Fungi

In general, softwoods are more susceptible to brown-rot attack due to high accessibility of cellulose (Cowling, 1957). Brown rot fungi primarily attack the cell wall carbohydrates, leaving behind a network of lignin and small amounts of more resistant, crystalline cellulose. The residual lignin is also modified to some extent by demethylation (Kirk, 1975). In the first stages of brown rot, the hyphae degrade the cell from the lumen, where the hyphae lie against the S-3 layer of the secondary wall. As decay proceeds, the S-2 layer is heavily degraded, while the S-3 layer remains relatively unattacked (Highley and Murmanis, 1985). Brown rot fungi have been reported to produce enzymes which are capable of diffusing considerable distances from the hyphae, causing higher strength losses at given weight losses than other types of decay (Cowling, 1961). Cowling (1961) reported that the rapid depolymerization of the hollocellulose led to the strength loss, which was followed by a gradual weight loss as the breakdown products were utilized. The presence of hemicellulose was also found to be required before these fungi could degrade cellulose (Highley, 1977).

Koenigs (1974) proposed that brown-rot Basidiomycetes attacked cellulose and partly decayed wood via an H_2O_2-Fe++ system. Low concentrations of H_2O_2 and Fe++ were found to stimulate rapid weight loss of sweetgum and loblolly pine sapwood. The degree of cellulose polymerization decreased rapidly at low weight losses in woods with added peroxide and iron, then diminished more gradually as weight losses increased. These results suggested that brown-rot was oxidative rather than being strictly hydrolytic. The H_2O_2-Fe++ system oxidizes cotton cellulose, and essentially reproduced characteristic decay patterns of brown-rot Basidiomycetes in wood and wood cellulose. The brown rot fungi appear to produce H_2O_2 from native substrates in wood. The formation of oxalic acid by the brown-rot fungi was found to bring the cellulolytic enzymes in contact with the cellulose in the tracheid walls by hydrolyzing the hemicellulose to produce water-soluble sugars that were subsequently used by the fungus as carbon sources (Bech-Andersen, 1987).

B. White Rot Fungi

White rot fungi are capable of decomposing all wood components and produce extracellular enzymes which oxidize phenolic compounds related to lignin. In some species, lignin is preferentially removed and this fact has been exploited for bio-pulping. White rot fungi generally degrade cell wall material at the same rate as they metabolize it and the activity of the enzyme is limited to the area adjoining the hyphae. Murmanis et al. (1984) reported that fungal hyphae colonized the lumens of tracheids and parenchyma cells, but ultrastructural evidence of cell wall degradation was not readily apparent. Small, electron-dense

particles were found to be associated with the degraded lignin. Evidence has shown that lignin isolated from the white rotted wood has been heavily modified in a process that was largely oxidative (Kirk and Chang, 1974). These results suggested that these organisms are important in the bioconversion of wood and several studies have demonstrated that enough lignin decomposition occurs in wood chips pretreated with a cellulase-less mutant of a white rot fungus to decrease the energy demand for production of thermo-mechanical pulp. In general, both softwoods and hardwoods are susceptible to white rot fungi (Cowling, 1957; Duncan and Lombard, 1965).

C. Soft Rot Fungi

Soft rot fungi are mostly Ascomycetes and Fungi Imperfecti which attack the S-2 layer of the secondary cell wall by the formation of diamond-shaped cavities parallel to the microfibrils or erode the cell wall much in the same manner as the brown rot fungi (Corbett, 1965). Soft rot cavities are produced by the secretion of enzymes from the hyphae that penetrate the walls in a direction parallel to the cellulose microfibrils or by secretion from hyphae lying within the cell lumen (Preston, 1979). The cavities form in chains and each develops as a smooth tunnel with pointed conical ends. Soft rot fungi appear to degrade wood components in a manner similar to the white rot fungi (Eslyn et al., 1975). Their attack pattern is usually confined to the wet, outer portions of the wood. Courtois (1963) studied the decay patterns caused by microfungi and found some were capable of causing erosion of the S-2 cell wall layer from within the lumen. This erosion was later termed "Type 2" and the formation of cavities "Type

1" (Corbett, 1965). Both types of attack were found to be produced by some soft rot fungi (Corbett, 1965). In general, hardwoods are more susceptible than softwoods to soft-rot attack, possibly due to the lower lignin content of the hardwood cell wall (Savory, 1954). Although non-wood fibers not containing lignin are also attacked by Type 1 soft-rot organisms (Nilsson, 1974), the presence of lignin has been reported to be required for cavity formation (Zainal, 1978; Preston, 1979; Morrell and Zabel, 1988).

Weight and Strength Losses Associated with Wood Decay

In the process of degradation, wood strength properties are affected to varying degrees. The strength properties most sensitive to the onset of decay are toughness and impact bending (Wilcox, 1978). Softwood colonized by both brown-rot and white-rot fungi was found to have toughness losses of more than 50 percent with a 1 percent weight loss (Richards, 1954). Both brown and white-rot fungi were reported to cause impact bending strength losses of at least 50 to 80 percent in hardwoods when weight losses reached 5-10 percent (Henningsson, 1967).

The properties that appear to be the next most sensitive to decay are the measurements of work associated with bending (Wilcox, 1978). Brown rot of softwood was found to produce losses of 50-70 percent at weight losses of 5-10 percent. Modulus of rupture (MOR) and modulus of elasticity (MOE) were also reported to be reduced by 60-70 percent (Kennedy, 1958).

Most other strength properties decrease less dramatically in the early stages of decay, even though essentially no strength remains at the late stages of decay. At 10 percent weight loss, brown rot in the

softwood produced losses of about 60 percent in compression perpendicular (Toole, 1971), 40 percent in compression parallel (Mizumoto, 1966), 50-60 percent in tension parallel (Kennedy and Ifju, 1962), and 20 percent in shear and hardness (Mizumoto, 1966).

There is little information concerning strength losses induced by soft rot fungi, but these fungi also produced severe strength losses at relatively low wood weight losses (Armstrong and Savory, 1959; Baechler et al., 1961; Henningsson, 1967; Morrell and Zabel, 1985). Soft rot attack was reported to reduce strength of the attacked region to zero (Henningsson and Nilsson, 1976) and was estimated to reduce the bending strength of a pole by 4 percent per year (Friis-Hansen, 1976).

From these studies, it would appear that strength loss is a more sensitive and realistic measure of fungal attack than weight loss. Because major effects on wood strength occur very early in decay, decay detection must occur as early as possible.

Methods for Detecting Decay: Laboratory Tests

The laboratory assessment of decay generally involves microbiological culturing, chemical indicators, or microscopic examination of wood sections. Culturing is a relatively simple method for detecting fungi in wood and assessing the risk of decay. This is particularly useful in the early stages of decay, when fruiting bodies are lacking and the wood is not visibly damaged. Conversely, culturing is time-consuming, and while it permits identification of the fungi, it does not indicate the degree of decay or wood strength reduction. In addition, the services of a trained mycologist are required to insure accurate identification of the fungal species. This is particularly

important since not all fungal species cause wood decay.

While culturing can detect incipient decay, it is sometimes difficult to culture fungi from some wood species (Scheffer et al., 1984; Zabel et al., 1985). In these cases, the use of indicators to detect subtle changes in wood chemistry may also be useful.

Chemical indicators which change color in the presence of decayed wood have been used in a number investigations (Curtin, 1928; Eslyn, 1979; Smith, 1982). Curtin (1928) found that seventeen major wood-decay fungi evolved metabolic acids ranging in concentration from pH 5 to 3 or less. The production of such acids preceeding fungal growth lowered the pH of the infected wood and the increased acidity was used to detect the presence of decay. Eslyn (1979) also used pH-indicators to detect decay in southern pine and found nine chemicals or chemical combinations that produced obvious color differences when applied to sound and decayed wood. Of these, three indicators: butter yellow plus methylene blue followed by bromcresol green plus methyl orange; chrome azurol-S; and methyl orange plus indigo carmine were suitable for detecting internal decay in pine poles. Smith (1982) evaluated color indicators for detecting early decay in Douglas-fir plugs decayed to varying weight losses. Chrome azurol-S plus sodium acetate was found to be the most effective indicator of decay by <u>Poria placenta</u> but not by other decay fungi commonly isolated from Douglas-fir. Methyl orange plus indigo carmine, a good indicator of decay in Douglas-fir sapwood, often reacted with sound Douglas-fir heartwood.

The use of chemical tests to detect decay has taken on added importance as more sophisticated devices have become available. The

infrared (IR) spectrophotometer, a powerful tool for the studying the chemical structures of solid materials, enables analysis of gross chemical structures of wood in the native state and provides an effective means for examining chemical variations of wood in any portion of a tree. Chow (1972) found different IR spectra from earlywood to latewood and from sapwood to juvenile wood for Douglas-fir, white spruce, western hemlock and grand-fir. Current inspection methods emphasize detecting decay using warm water extracts of the affected wood before major strength reductions occur. Gibson, et al. (1985) used infrared spectroscopy of warm water extracts to detect the presence of incipient brown rot decay in Douglas-fir and southern yellow pine by the appearance of an absorption peak between 1,500 cm^{-1} and 1,800 cm^{-1} in infrared spectrum. Holt (1985) subsequently isolated the compounds found in extracts of incipiently decayed Douglas-fir and southern yellow pine that gave rise to an absorbance peak of 1720 cm^{-1} in the IR range and identified the compounds. The IR spectra of these compounds exhibited both acid and aromatic characteristics, indicating that the decay compounds probably arose from enzymatic oxidative degradation of lignin.

Krahmer et al. (1982) detected incipient brown rot in southern yellow pine by using fluorescence microscopy. Non-decayed wood stained with acridine orange fluoresced green, wood with 1 to 3 percent weight loss fluoresced greenish orange, and wood with greater than 3 percent weight loss fluoresced orange. This technique could not distinguish the extent of decay at weight losses between 3 and 10 percent, nor was it applicable to Douglas-fir heartwood. In addition, operator variables were also found to influence test results.

While culturing and chemical indicators are useful for detecting fungi and the changes they induce in wood, microscopic examination of the affected wood provides a direct comparison between wood damage and the degree of fungal attack.

Microscopic examination was considered the most reliable means of determining the presence of decay in wood (Wilcox, 1964a, b). The major application of microscopic examination in the study of wood deterioration is the qualitative assessment of microbiological damage. The woods examined are rated on the basis of the size and quantity of bore holes, the occurrence of cell wall separation, the degree of cell wall thinning as observed in cross section, and the presence of hyphae. Good reviews of histological stains and staining are given by Sass (1958), Conn et al. (1960), and Johansen (1940). Certain methods were found to be suitable for the study of decayed wood. Sequential staining with safranin and picro-aniline blue provided the best results for differentiating hyphae from wood. The procedure in use is a modification of that originally developed by Cartwright (1929), in which the staining solutions have been diluted as suggested by Proctor (1941). By this method, wood cell walls appear pink, and hyphae appear blue, but hyphae are usually difficult to detect at early stages of decay, especially in wood with spiral thickenings on the tracheid walls (e.g. Douglas-fir). In addition, hyphal staining is not always uniform.

Lectins, which are proteins that selectively bind with various carbohydrates and glycoproteins (Liener, 1976) can also be used as fungal "stains" (Morrell, et al., 1985). Each lectin is highly specific, reacting with only one or two carbohydrates, and can be

visualized when coupled with the appropriate fluorochrome and observed using a fluorescence microscope (Liener, 1976). Morrell et al. (1985) evaluated the reactivity of plant-derived lectins and found only wheat germ agglutinin (WGA) reactive with hyphal fragments or hyphae present in wood cells. A comparison of hyphae stained with picro-aniline blue alone and with FITC-labeled WGA alone indicated that the latter reaction improved visualization of fungal hyphae with little interference from normal wood structure. Hyphae stained with lectins lose their fluorescence with time, especially when exposed to fluorescent light. However, hyphal fluorescence can be recovered, even after drying during storage, by restaining with the lectin. Although WGA is only useful for detecting chitin, which is present in nearly all higher fungi, it improves hyphal visualization in wood sections at the initial stages of deterioration, when conventional stains are more variable. This lectin, which is specific for N-acetylglucosamine and sialic acid, has high specificity for fungal chitin; however, lignin also fluoresces and interferes with lectin visualization. Double-staining with fluorescent-labeled WGA followed by conventional histological stains can eliminate some of this wood autofluorescence to improve hyphal visualization (Krahmer, et al., 1986).

At present, there are no detailed studies of lectin reactivity with fungal hyphae through progressive stages of decay.

MATERIAL AND METHODS

I. Reactivity of hyphae from selected Basidiomycetes, Ascomycetes, and Fungi Imperfecti to fluorescent-labeled wheat germ agglutinin and concanavalin A.

Thirty-five fungi (listed in Appendix A) were grown on 1 percent malt extract agar until they had formed normal anatomical structures. Small pieces of agar containing the fungal hyphae were asceptically removed, placed on clear glass slides, and allowed to air dry. A small drop of fluorescein isothiocyanate coupled wheat germ agglutinin (FITC-WGA) (Vector Laboratories Inc. Burlingame, CA) diluted 1:50 in phosphate buffered saline (pH 7.0)(PBS) was added to the hyphae and thoroughly mixed with a micropipettor. The lectin-hyphal mix was incubated for 10-15 minutes in darkness to minimize quenching by fluorescent light. Excess lectin was pipetted off and the slide was rinsed thoroughly with PBS to remove unreacted lectin. A second lectin, tetra-methylrhodamine isothiocyanate coupled concanavalin A (TRITC-CON A), was then reacted using the same procedure. After rinsing, the slide was blotted dry, and a drop of water was added to the hyphae. A glass coverslip was placed on the hyphae, which were observed with a Leitz fluorescence microscope for hyphal morphology under normal bright field illumination and for fluorescent-labeled lectins under incident illumination from a xenon light source through a Ploemopak Module M-2 filter for TRITC or H-2 filter for FITC. Structures observed for lectin reactivity included hyphae, conidiophores, and conidia. Photomicrographs were taken using Ektachrome 400 color slide film.

II. Application of fluorescein isothiocyanate-labeled wheat germ agglutinin (FITC) to observe the progress of decay in Douglas-fir and ponderosa pine by Poria carbonica, Coriolus versicolor, and Chaetomium globosum.

Wood Specimens - Douglas-fir heartwood [Pseudotsuga menziesii (Mirb.) Franco] and ponderosa pine sapwood (Pinus ponderosa Dougl. ex Laws.) were used for the fungal decay tests. Samples selected for use were defect free.

Test Fungi - Three wood decay fungi were tested.

Poria carbonica Overh., (Isolate number 1978) a Basidiomycete causing a brown rot, obtained from the Forest Research Laboratory of Oregon State University.

Coriolus versicolor (L. ex Fr.) Quel., (Isolate number R-105) a Basidiomycete causing a white rot, obtained from the Forest Products Laboratory, Madison, Wisconsin.

Chaetomium globosum Kunze ex Fr., (Isolate number WFPL-172A) an Ascomycete causing Types 1 and 2 soft rot damage, obtained from Forintek Canada Corp, Vancouver, B.C.

Blocks of Douglas-fir and ponderosa pine (1.9 cm cubed) were dried for at least 24 hours at 54°C, and their weights recorded. The blocks were then vacuum impregnated in distilled water, placed in petri plates and autoclaved for 20 minutes at 121°C.

The decay chambers were 454 g French squares, filled with 40 ml of 2.5 percent malt extract agar. The chambers were autoclaved for 20 minutes at 121°C and laid horizontally to cool, prior to inoculation by transferring a small section of agar removed from a plate containing an actively growing culture of the test fungus. Each chamber was incubated for one week at 28°C, then a sterile U-shaped glass rod was

placed on the agar surface in each chamber, and two wood blocks with cross-sections facing upwards were placed on the glass rod. Twelve chambers of each wood species per test fungus were incubated for up to 3 months at 28°C. One chamber of each wood species per test fungus was harvested weekly. The blocks were removed and gently scraped clean of adhering mycelium, and weighed to determine the moisture content of the block. One block from each fungal species was dried for at least 24 hours at 54°C and reweighed to determine weight loss due to fungal exposure. The other block was immediately aspirated in formalin acetic acid (FAA) and stored for later sectioning.

The FAA fixed blocks were sectioned on a sliding microtome. Transverse, tangential, and radial sections first were stained with 1 percent safranin O in distilled water, rinsed thoroughly in distilled water, and blotted dry on glass slides. The FITC-WGA (diluted 1:50) in phosphate-buffered saline (PBS) was dropped on to the sections, which were then incubated in darkness for 10-15 minutes. The sections were rinsed thoroughly with PBS and then mounted in water on glass slides. Cover glasses were placed over the stained sections and the sections were observed immediately using a Leitz fluorescence microscope. Incident illumination was provided by a xenon light source through a Ploemopak Module H-2 filter. Photomicrographs were taken using 35mm color slide film (Ektachrome 400 ASA).

RESULTS AND DISCUSSION

I. Reactivity of hyphae from selected Basidiomycetes, Ascomycetes, and Fungi Imperfecti to fluorescent-labeled wheat germ agglutinin and concanavalin A.

Lectin reactivity of the fungi varied widely, but the variation consistently occurred in dark pigmented (dematiaceous) fungal structures (Tables I-1; I-2). WGA, which is specific for residues of N-acetylglucosamine present in fungal chitin, reacted with nearly all hyaline or slightly colored hyphae (Figure I-1), but did not react with darker hyphae. Hyaline conidia were also reactive, even when they were produced by dark, non-reactive conidiophores (Figure I-2). This lack of reactivity suggests that the dark pigments are externally deposited in a manner that blocks the reactive sites on the chitin or that the darker pigments somehow quench the fluorescence. The latter explanation seems unlikely, and it appears that hyphal accessibility to the lectin is an important factor in lectin reactivity. Although dark pigmented hyphae were non-reactive, these hyphae were easily seen with conventional, bright field microscopy. FITC-WGA made the extremely fine Basidiomycete hyphae that are typically difficult to detect by conventional means, readily visible.

Con A, which is specific for α-D-mannosyl and α-D-glucosyl residues, reacted with about one half of the fungi that reacted with WGA. This reactivity suggests that many fungal structures are composed of compounds containing these subunits. The relatively fine diameter and thick walls of the hyphae produced by most fungi prevented precise determination of the point of localization in the cell. Thus, Con A may actually be fluorescing from within the fungal structure. The

variable reactivity with Con A indicates that this lectin will be less useful for detecting fungal decay, although this variation may be useful for studying fungal structures.

Chitin, which constitutes interwoven microfibrils in fungal cell wall, is present in the cell walls of most fungi (Alexopoulos and Mims, 1979); however, a few fungi contain cellulose but lack chitin in their walls (Moore-Landecker, 1982). These fungi should not react with WGA. In Table I-1, Fomitopsis cajanderi was the only Basidiomycete which did not react with WGA, suggesting that cell wall composition varied, or the deposition of other materials masked the presence of chitin (Alexopoulos and Mims, 1979). Fomitopsis cajanderi, Haematostereum sanguinolentum, Phlebia subserialis and Schizophyllum commune were the only Basidiomycetes which did not react with Con A (Table I-2), suggesting that α-D-mannosyl and α-D-glucosyl are not the common constituents in these Basidiomycetes.

Previous studies indicate that each lectin is highly specific and can be used to determine the identity of specific compounds present within various cell types (Goldstein and Hayes, 1978; Liener, 1976; Lis and Sharon, 1973), thus, WGA and Con A can be used to accurately detect the presence of the chemicals for which they have specificity.

The results indicate that fluorescent-coupled wheat germ agglutinin is a useful probe for detecting fungal chitin, although one Basidiomycete was not reactive, and is a highly specific probe for detecting fungal colonization by non-dematiaceous fungi, especially at the early stages of decay.

Table I-1. Reactivity of selected fungi to fluorescein coupled wheat germ agglutinin.

Fungal species	LECTIN REACTIVITY[1] FITC-WGA Hyphae	Conidiophores	Conidia
Alternaria alternata	-2	-2	-2
Aspergillus niger	-	-2	-2
Aureobasidium pullulans	-	-2	-2
Ceratocystis albida	+	-	-
Chaetomium globosum	++	-2	-2
Cladosporium elatum	++	+2	++
Coriolus versicolor	+	+	+
Crustoderma dryinum	++	++	++
Epicoccum nigrum	-2	+	-2
Fomitopsis cajanderi	-		-
Gloeophyllum saepiarum	++	-	-
Gloeophyllum trabeum	++		
Haematostereum sanguinolentum	++		
Heterobasidion annosum	++		
Hyalodendron lignicola	+	-2	+
Irpex lacteus	++		
Lentinus lepideus	++		
Leptodontium elatius	++	-2	+
Oidiodendron griseum	++	-2	++
Paecilomyces variotti	+	-	-
Penicillum italicum	+	-	-
Peniophora gigantea	+		
Phellinus weirii	+		
Phialocephala dimorphospora	+	-2	+
Phialophora fastigiata	++/-	-2	+
Phialophora sp. 3	++	-2	+
Phanerochaete sordida	+	-	-
Phlebia radiata	++		
Phlebia subserialis	++		
Poria carbonica	++		
Poria placenta	++		
Poria xantha	++		
Schizophyllum commune	++		
Scytalidium sp.	++	-2	-2
Sistotrema brinkmanii	++		

1. Lectin reactivity based on: (++) strongly reactive, (+) moderately reactive, (+/-) variably reactive, and (-) non-reactive.

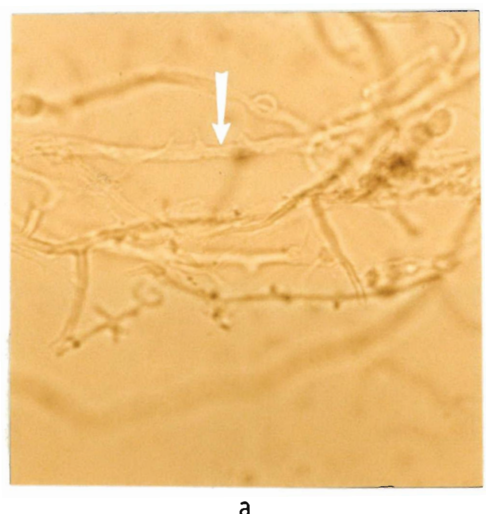
2. Denotes the formation of fungal structures containing dark pigments.

Table I-2. Reactivity of selected fungi to rhodamine coupled concanavalin A.

	LECTIN REACTIVITY[1] TRITC-CON A		
Fungal species	Hyphae	Conidiophores	Conidia
Alternaria alternata	-	-	-
Aspergillus niger	-	-	-
Aureobasidium pullulans	-	-	-
Ceratocystis albida	-	-	-
Chaetomium globosum	-	-	-
Cladosporium elatum	-	-	-
Coriolus versicolor	+	+	+
Crustoderma dryinum	++	++	++
Epicoccum nigrum	+	-	-
Fomitopsis cajanderi	-		
Gloeophyllum saepiarum	++		
Gloeophyllum trabeum	++		
Haematostereum sanguinolentum	-		
Heterobasidion annosum	++		
Hyalodendron lignicola	+	-	#
Irpex lacteus	++		
Lentinus lepideus	++		
Leptodontium elatius	++	-	+
Oidiodendron griseum	+	-	-
Paecilomyces variotti	-	-	-
Penicillum italicum	+	-	-
Peniophora gigantea	+		
Phellinus weirii	+		
Phialocephala dimorphospora	+	-	+
Phialophora fastigiata	+/-	-	-
Phialophora sp. 3	-	-	-
Phanerochaete sordida	+	-	-
Phlebia radiata	++		
Phlebia subserialis	-		
Poria carbonica	++		
Poria placenta	++		
Poria xantha	++		
Schizophyllum commune	-	-	-
Scytalidium sp.	-	-	-
Sistotrema brinkmanii	++		

1. Lectin reactivity based on: (++) strongly reactive, (+) moderately reactive, (+/-) variably reactive, and (-) non-reactive.

2. Denotes the formation of fungal structures containing dark pigments.



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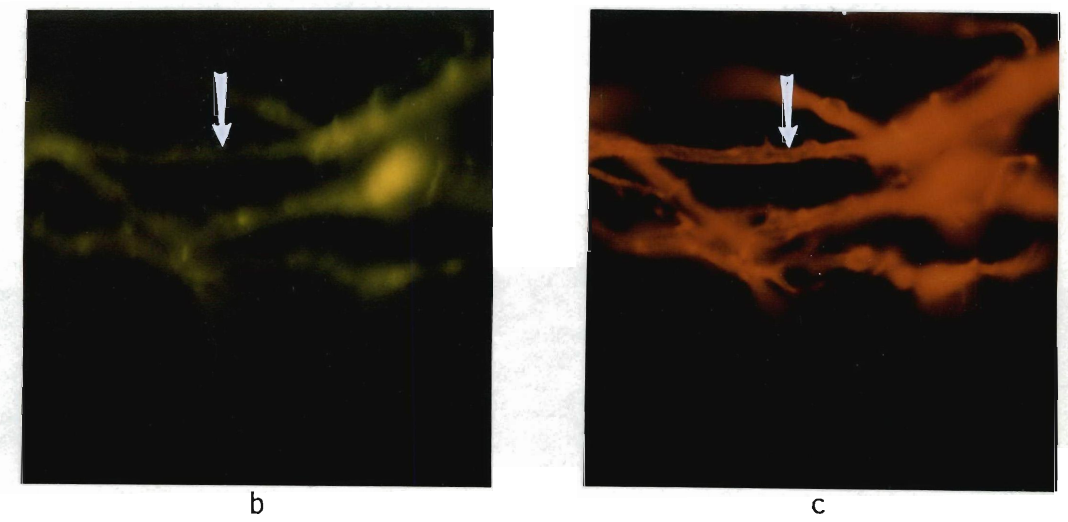
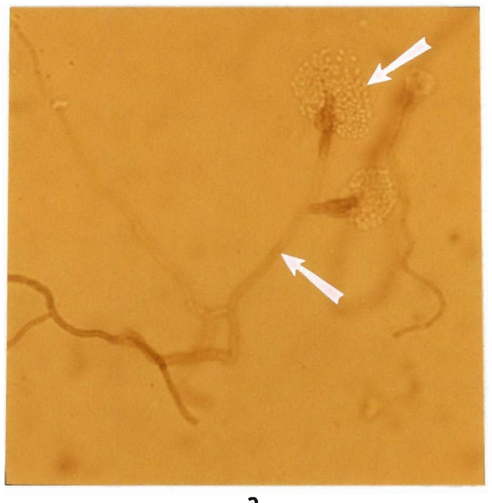


b c

Figure I-1. Micrographs of fungal structure produced by Poria carbonica (400X) under normal bright field illumination (a); under incident illumination from a xenon light source following reaction with FITC-WGA, and observed with a Leitz fluorescent microscope equipped with Ploemopak module H-2 for fluorescein (b) and under the same illumination following reaction with TRITC-Con A using a Ploemopak module M-2 for rhodamine (c). Note the improved appearance of hyaline hyphae (arrows).



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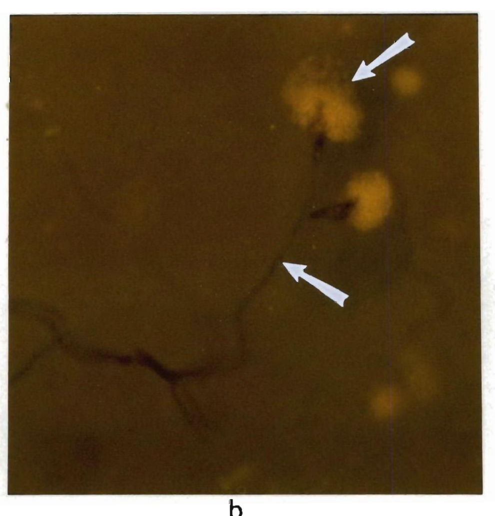

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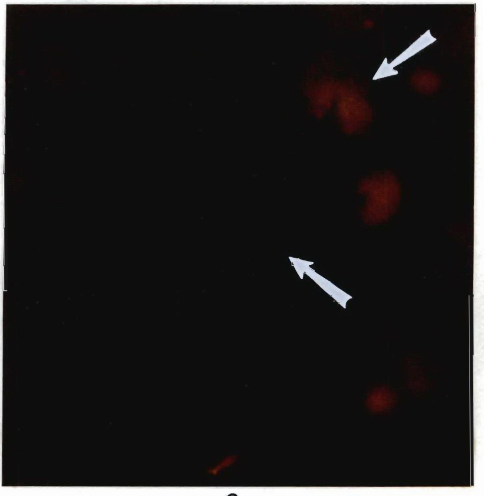


c

Figure I-2. Micrographs of fungal structure produced by Phialocephala dimorphospora (400X) under normal bright field illumination (a); under incident illumination from a xenon light source following reaction with FITC-WGA, and observed with a Leitz fluorescent microscope equipped with Ploemopak module H-2 for fluorescein (b) and under the same illumination following reaction with TRITC-Con A using a Ploemopak module M-2 for rhodamine (c). Note non-reactive, dark conidiophore producing reactive, hyaline conidia (arrows).

II. Application of fluorescein isothiocyanate-labeled wheat germ agglutinin (FITC-WGA) to observe the progress of decay in Douglas-fir and ponderosa pine by Poria carbonica, Coriolus versicolor, and Chaetomium globosum.

All the test fungi caused measurable weight losses in Douglas-fir and ponderosa pine, indicating substantial destruction of cell wall material (Table II-1). In general, weight losses were greater in pine blocks. This result was not unexpected, since sapwood is more susceptible to decay (Scheffer and Cowling, 1966).

Poria carbonica exposed to Douglas-fir and ponderosa pine blocks produced weight losses which far exceeded those of C. versicolor and C. globosum (Figure II-1, 2). Poria carbonica caused weight losses on ponderosa pine blocks of 14.41 percent over the 12 week incubation period. Exposure of Douglas-fir blocks to the same fungus resulted in a weight loss of 9.46 percent for the same incubation period. Chaetomium globosum produced low weight losses on both wood species, with weight losses of 2.29 percent and 2.46 percent for ponderosa pine and Douglas-fir, respectively, following a 12 week exposure.

The weight losses indicate that decay rates were not uniform. These differences may reflect the variations of wood moisture content. Poria carbonica and C. versicolor caused high weight losses at the early stages of exposure, while C. globosum caused lower weight losses.

Table II-1. Weight losses caused by *Poria carbonica*, *Coriolus versicolor*, and *Chaetomium globosum* on Douglas-fir and ponderosa pine blocks in decay chambers incubated from 1 week to 12 weeks at 28°C.

Fungus	Decay Type	Decay Period (weeks)	Wood Weight Loss (%)	
			Douglas-fir	Ponderosa pine
P. carbonica	Brown Rot	1	1.10	0.66
		2	1.60	1.20
		3	2.69	2.71
		4	2.75	5.94
		5	2.84	6.90
		6	4.35	8.30
		7	5.85	10.09
		8	6.00	11.21
		9	8.26	12.53
		10	8.54	13.30
		11	9.23	14.04
		12	9.46	14.41
C. versicolor	White rot	1	0.90	0.71
		2	1.04	1.82
		3	2.58	3.55
		4	3.49	4.31
		5	3.52	4.35
		6	3.96	7.48
		7	3.97	10.91
		8	4.01	10.93
		9	4.25	12.76
		10	4.33	12.78
		11	4.97	14.23
		12	4.98	14.38
C. globosum	Soft Rot	1	0.50	0.33
		2	0.62	0.41
		3	0.83	0.64
		4	0.83	1.20
		5	1.01	1.43
		6	1.01	1.60
		7	2.03	1.65
		8	2.14	1.82
		9	2.15	1.84
		10	2.15	1.98
		11	2.46	2.28
		12	2.46	2.29

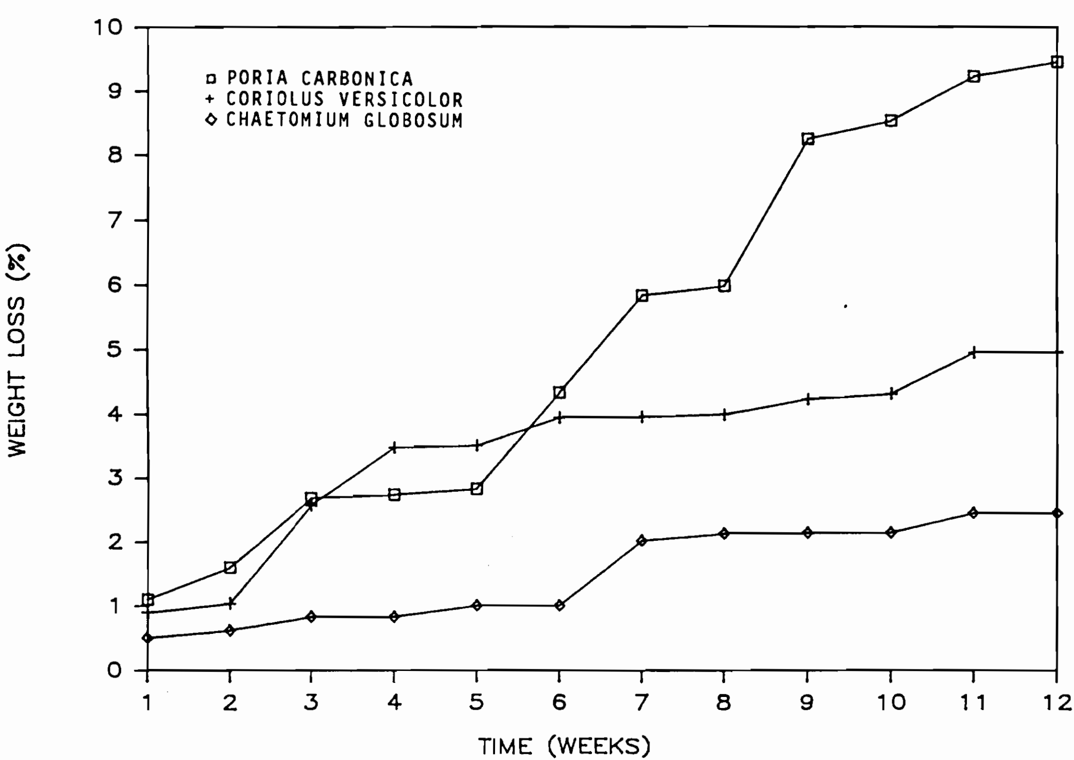


Figure II-1. Weight losses caused by <u>Poria carbonica</u>, <u>Coriolus versicolor</u>, and <u>Chaetomium globosum</u> on Douglas-fir blocks in decay chambers incubated from 1 week to 12 weeks at 28°C.

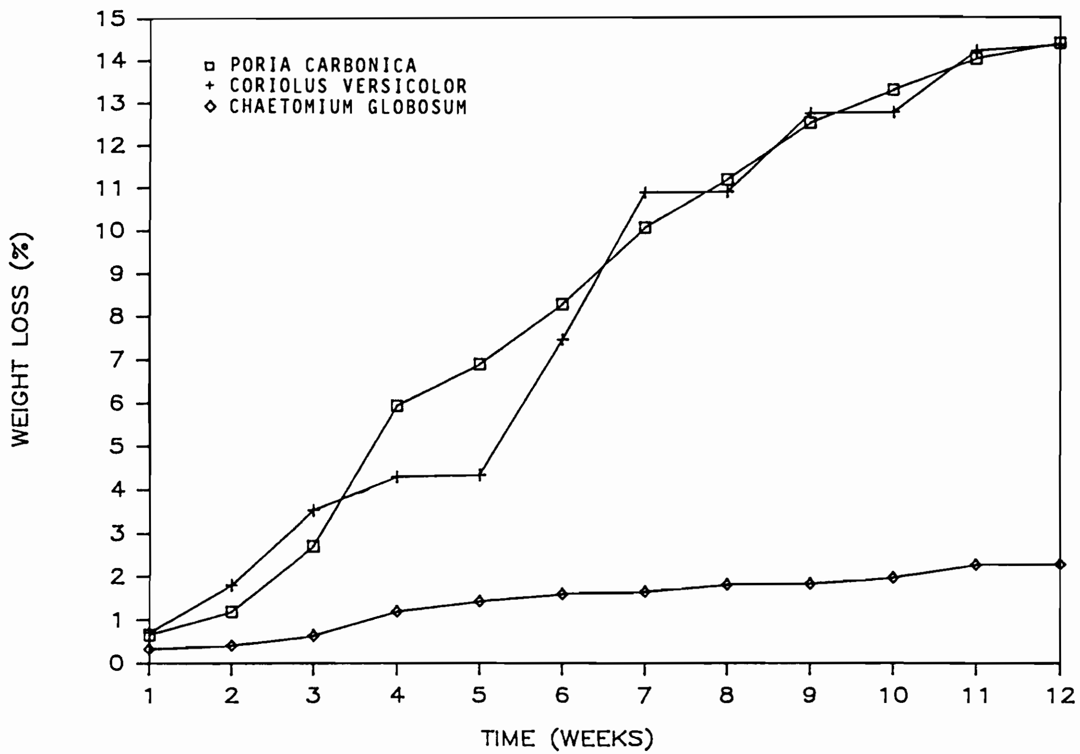


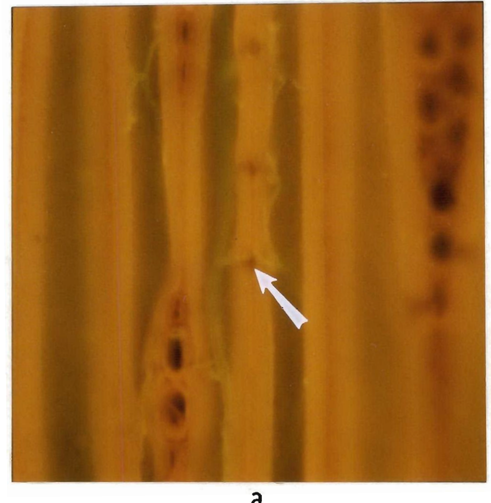
Figure II-2. Weight losses caused by Poria carbonica, Coriolus versicolor, and Chaetomium globosum on ponderosa pine blocks in decay chambers incubated from 1 week to 12 weeks at 28°C.

Douglas-fir exposed to Poria carbonica

After one week of exposure (wt. loss: 1.10%), the bases of the blocks were colonized with a sparse mycelium. Microscopically, hyphae were present in the rays, and some bordered and cross-field pit penetration was evident (Figure II-3-a). The attack pattern remained similar after three weeks (wt. loss: 2.69%), but the surface of the blocks became more heavily colonized. After five weeks (wt. loss: 2.84%), some bore holes through the cell wall were noted (Figure II-3-b). As colonization proceeded, blocks exposed for seven weeks (wt. loss: 5.85%) contained more abundant hyphae in the ray cells and more numerous bore holes (Figure II-3-c). This trend continued through the nine (wt. loss: 8.26%) and eleven week (wt. loss: 9.23%) exposures. At the end of the exposure period, the ray parenchyma cells were heavily attacked, while the ray tracheids appeared relatively unaffected. The results suggest that the fungus concentrated its efforts on the more readily accessible ray parenchyma; however, previous studies indicate significant strength effects occur at low weight losses ($\leq$5.00%) due to extensive migration of cellulase enzymes produced by brown rot fungi (Wilcox, 1978). As a result, microscopic evaluation does not fully delineate the potential effects of the brown rot fungi.

Ponderosa pine exposed to Poria carbonica

After one week of exposure (wt. loss: 0.66%), the bases of the blocks were colonized with a sparse mycelium. Microscopically, hyphae were primarily present in the earlywood zone and some bordered, cross-field pit and ray cell penetration was evident (Figure II-4-a,



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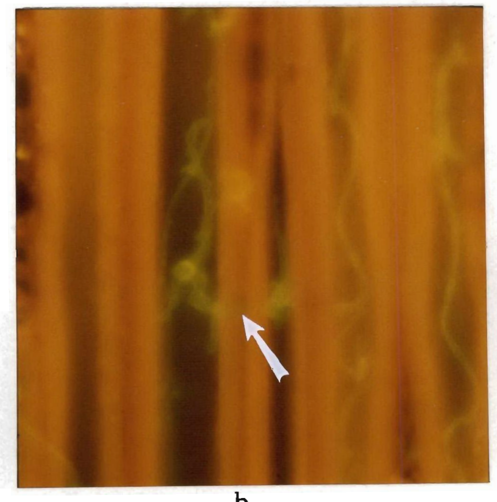

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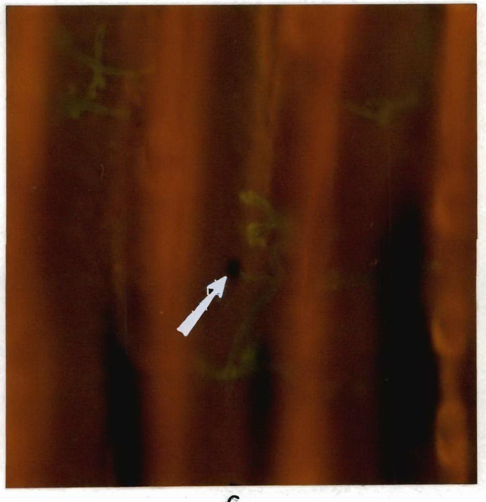


c

Figure II-3. Tangential sections of Douglas-fir exposed to Poria carbonica for one (a), five (b), or seven weeks (c) with arrows showing a) bordered pit penetration (400X), b) fungal bore hole penetrating directly through the cell wall (400X), and c) the presence of fungal bore holes in the wood cell wall (400X).

b). After three weeks (wt. loss: 2.71%), the blocks were colonized with a denser mycelium, hyphae were present in the ray cells, cell wall penetration was noted (Figure II-4-c). After five weeks (wt. loss: 6.90%), more abundant hyphae were present in the tracheids and ray cells (Figure II-4-d). The attack pattern remained similar after seven weeks (wt. loss: 10.09%), but the surface of the blocks became more heavily colonized. After nine weeks (wt. loss: 12.53%), hyphae passed into the ray tracheid lumens via the pits (Figure II-5-a). No further attack was observed over the eleven week (wt. loss: 14.04%) exposure. At the end of the exposure period, the thin-walled ray parenchyma were heavily attacked and degraded (Figure II-5-b). The results indicate that the rays in ponderosa pine provide the initial penetration path for the fungus. Cell wall penetration noted in the block exposed for three weeks (wt. loss: 2.71%) suggests the cellulolytic enzymes of the brown-rot fungus were capable of penetrating and acting within the cell walls at early stages of decay.

Poria carbonica displayed a greater tendency to attack ponderosa pine than Douglas-fir. The hyphae in ponderosa pine were abundant in both rays and tracheids, whereas the hyphae in Douglas-fir were abundant in rays but sparse in the tracheids.

Douglas-fir exposed to Coriolus versicolor

After one week of exposure (wt. loss: 0.90%), the bases of the blocks were colonized with a sparse mycelium. Microscopically, few hyphae were present in the ray cells and some bordered pit penetration was found. After three weeks (wt. loss: 2.58%), more hyphae had colonized the tracheids. After five weeks (wt. loss: 3.52%),

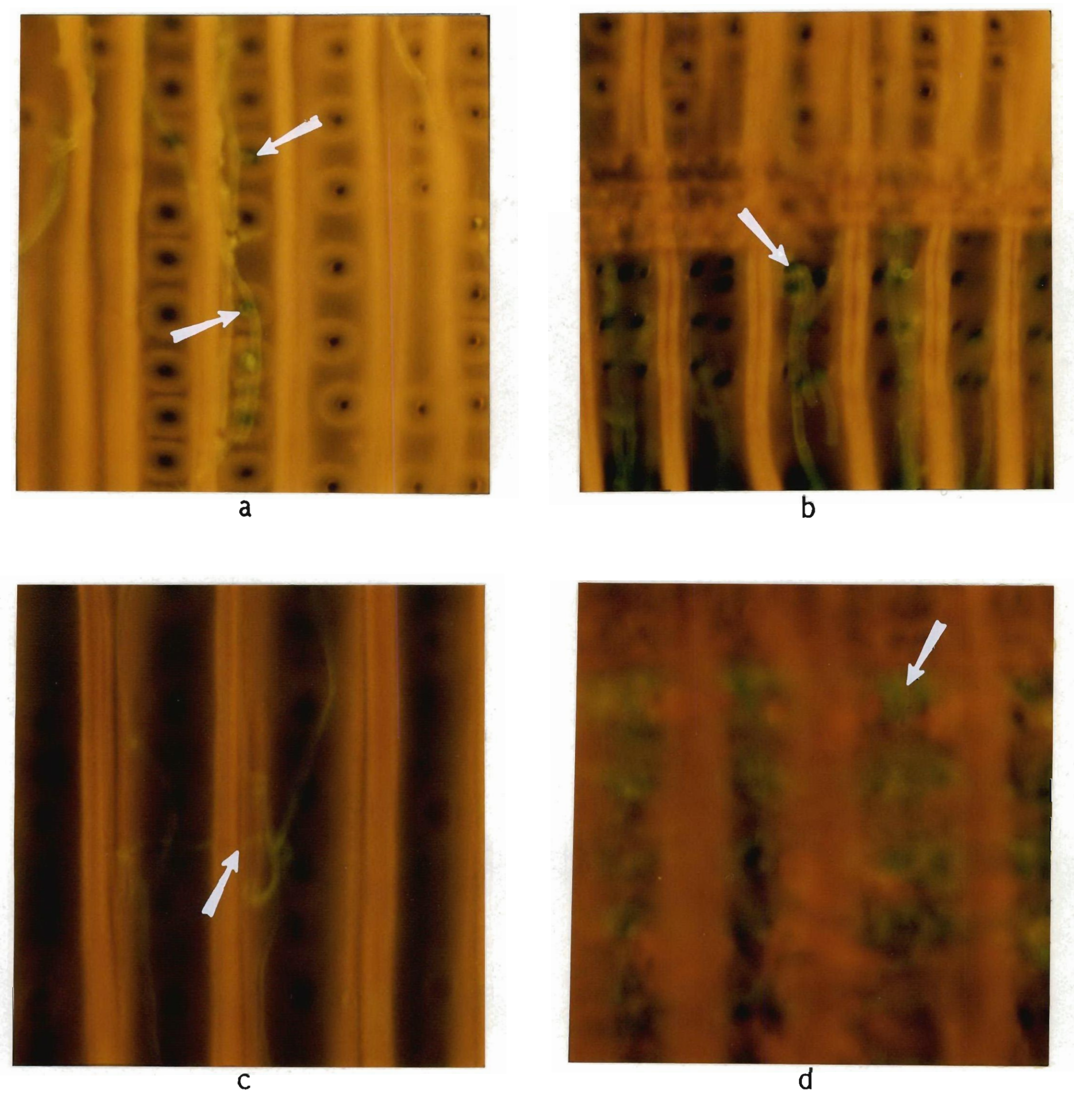


Figure II-4. Radial sections of ponderosa pine exposed to <u>Poria carbonica</u> for one (a, b), three (c), or five weeks (d) with arrows showing a) bordered pit penetration (275X), b) cross-field pit penetration (275X), c) direct cell wall penetration (400X), and d) fungal colonization of the ray parenchyma (400X).

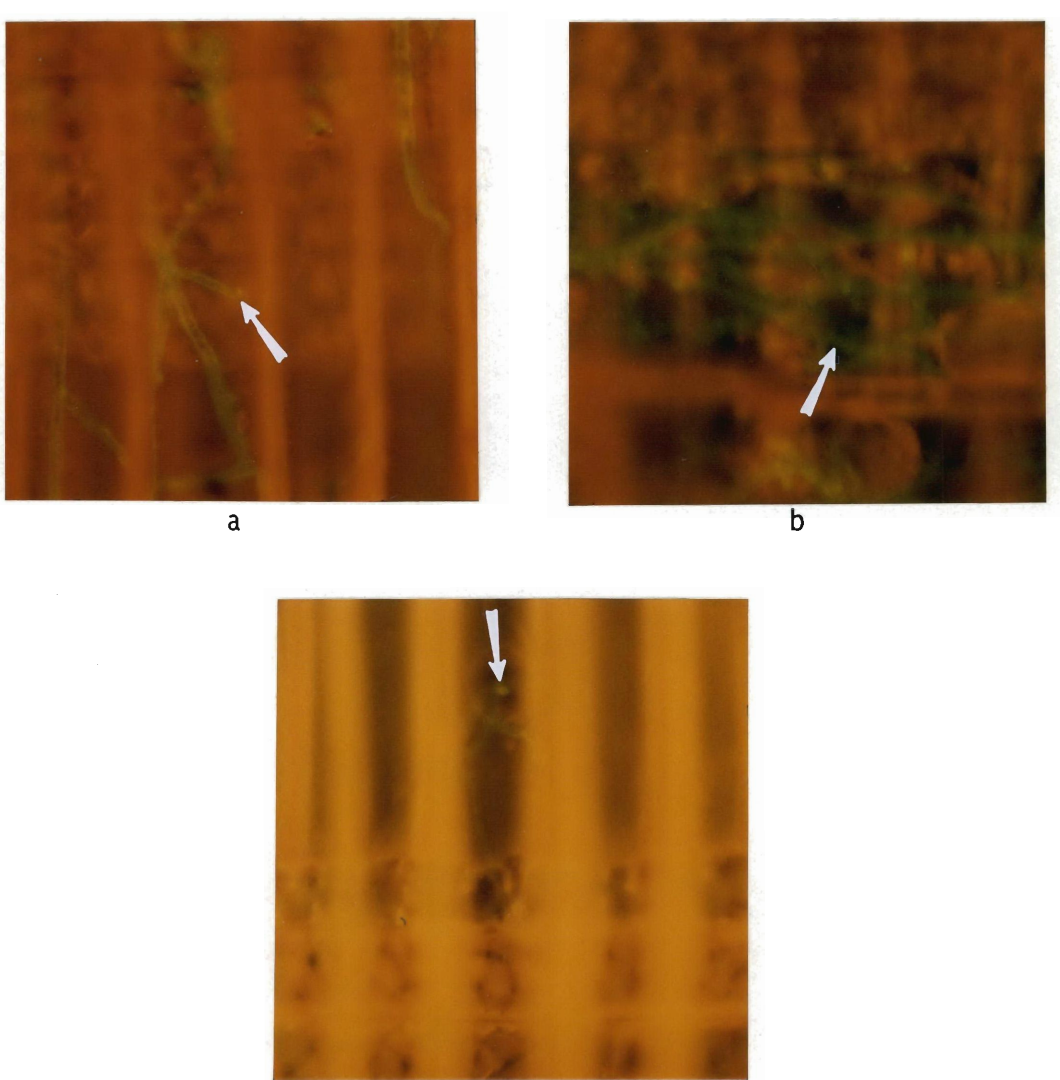


Figure II-5. Radial sections of ponderosa pine exposed to _Poria carbonica_ (a, b), or _Coriolus versicolor_ (c) for three (c), nine (a), or eleven weeks (b) with arrows showing a) hyphae passing into the ray tracheids via the pits (400X), b) severe degradation of the ray parenchyma (400X), and c) bordered pit penetration (400X).

cross-field pit and cell wall penetration was evident. After seven weeks (wt. loss: 3.97%), more hyphae colonized ray parenchyma cells. The attack pattern remained similar after nine weeks (wt. loss: 4.25%), but the block surface became more heavily colonized. The blocks exposed for eleven weeks (wt. loss: 4.97%) contained more abundant hyphae in the ray parenchyma cells, but these cells were not completely deteriorated. The results suggest that the fungus attacked the ray cells which were more accessible than the rest of the wood components. In addition, passive hyphal penetration via the pits was more prevalent than direct cell wall penetration.

The attack patterns found with C. versicolor were very much like those found with P. carbonica on this wood species. Both decay types preferentially penetrated pits in early stages of decay, but bore holes were more numerous in advanced stages of brown rot than of white rot.

Ponderosa pine exposed to Coriolus versicolor

After one week of exposure (wt. loss: 0.71%), the bases of the blocks were colonized with a sparse mycelium. Microscopically, the wood appeared sound and only a few hyphae were present in the tracheids. After three weeks (wt. loss: 3.55%), the blocks were covered with thin mycelium, more abundant hyphae were present in the tracheids, hyphae had penetrated bordered pits, and ray cells were slightly colonized (Figure II-5-c). The attack pattern remained similar after five weeks (wt. loss: 4.35%). After seven weeks of exposure (wt. loss: 10.91%), the blocks were covered with thick mycelium, and cross-field pit penetration with some bore holes through the cell wall were noted. In addition, ray parenchyma cells were

heavily attacked and degraded. This trend continued through the nine (wt. loss: 12.76%) and eleven (wt. loss: 14.23%) week exposures. At the end of the exposure period, cell wall penetration was prevalent. The results indicate that passive penetration via the pits was prevalent in the early stages of decay (wt. loss below 4.35%), while active penetration through the cell walls was prevalent in the advanced stages of decay; however, white rot fungi have been reported to affect wood strength properties significantly at low weight losses (≤5.00%) (Wilcox, 1978). Consequently microscopic evaluation did not fully delineate the potential damage caused by white rot fungi.

Douglas-fir exposed to Chaetomium globosum

After one week of exposure (wt. loss: 0.50%), the blocks remained sound, and no mycelium or perithecia were found. Microscopically, only a few hyphae were present in the tracheids. The attack pattern was similar after three weeks (wt. loss: 0.83%). After five weeks (wt. loss: 1.01%), the bases of blocks were colonized by a sparse mycelium and the ray cells were lightly colonized. As colonization proceeded, blocks exposed for seven weeks (wt. loss: 2.03%) contained more hyphae in the ray cells. This trend continued through the nine (wt. loss: 2.15%) and eleven (wt. loss: 2.46%) week exposures. At the end of the exposure period, abundant hyphae colonized the tracheids, while the ray cells were attacked but not completely deteriorated. The results suggest that not all stored materials in the cell lumens were utilized by the fungus over the twelve week exposure. Previous studies suggest that soft rot fungi only begin to penetrate and attack cell-wall material after these substances are no longer available (Greaves and

Levy, 1965; Krapivina, 1960). This fungus apparently lacked the ability to cause soft rot damage in Douglas-fir blocks; however, the use of more severe decay tests such as a vermiculite burial method might alter these results (Nilsson, 1973).

Ponderosa pine exposed to Chaetomium globosum

After one week of exposure (wt. loss: 0.33%), the blocks were covered with a thin mycelium. Microscopically, bordered pit penetration was evident (Figure II-6-a). After three weeks (wt. loss: 0.64%), ray parenchyma cells were colonized (Figure II-6-b). After five weeks (wt. loss: 1.43%), cross-field pit penetration was noted. After seven weeks (wt. loss: 1.65%), the blocks were covered with mycelium and a few perithecia. Microscopically, hyphae were abundant within the tracheids and cell wall penetration was noted (Figure II-6-c). As colonization proceeded, blocks exposed for nine weeks (wt. loss: 1.84%) contained more abundant hyphae in the ray cells, and erosion (Type 2) of the cell wall. This trend continued through the eleven week (wt. loss: 2.28%) exposure. At the end of the exposure period, the ray parenchyma cells were heavily attacked, while the ray tracheids remained unaffected. Cell wall penetration was also prevalent in these sections. The results suggest that penetration in early stages of attack was primarily through pits, while cell wall penetration began when the storage materials present in the ray cells were utilized. The failure to obtain soft rot cavities in this wood species might be due to the short exposure period or the test conditions (Nilsson, 1973).

In both of Douglas-fir and ponderosa pine blocks, the soft rot

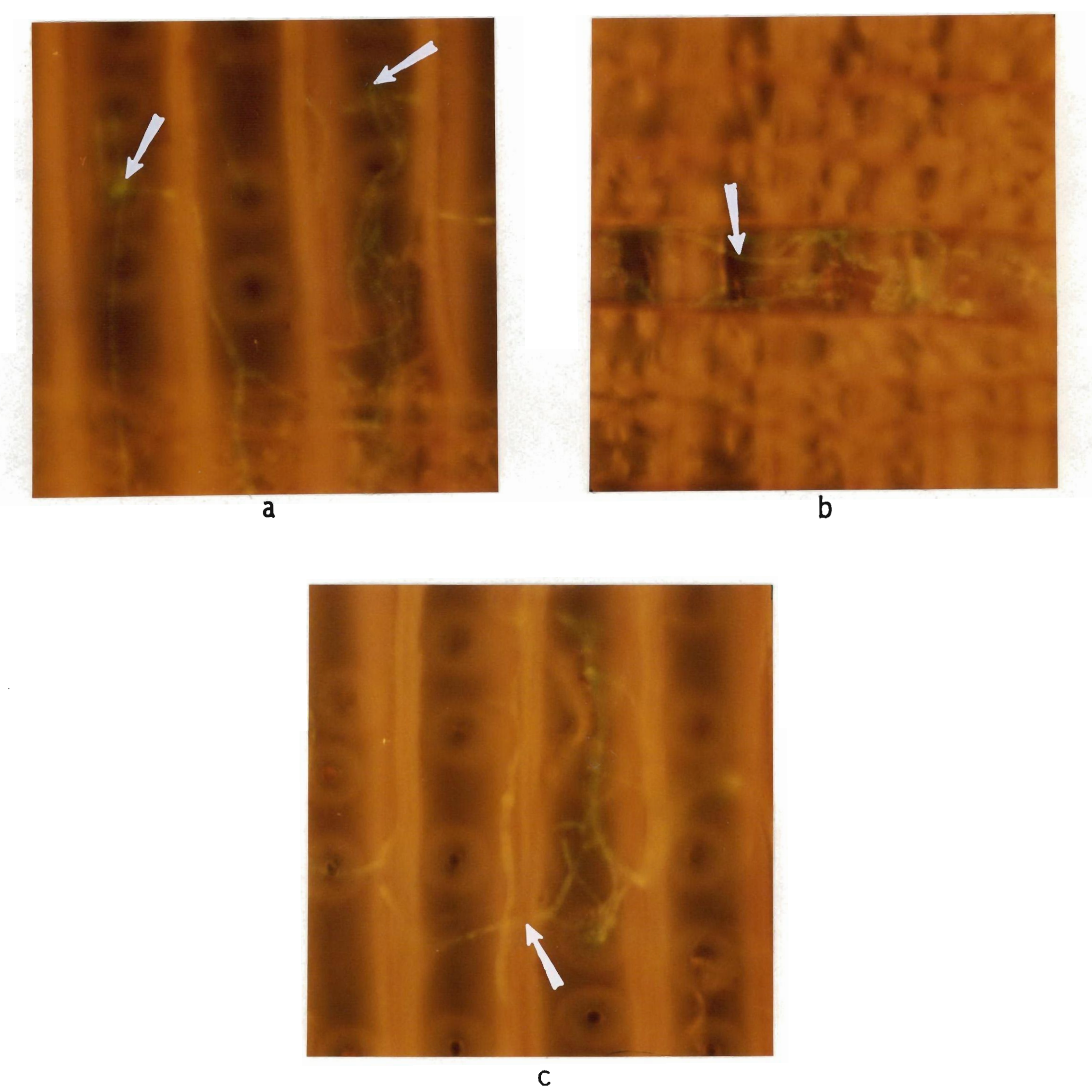


Figure II-6. Radial sections of ponderosa pine exposed to Chaetomium globosum for one, three, or seven weeks (a-c, respectively) with arrows showing a) bordered pit penetration (400X), b) colonization of the ray parenchyma (400X), and c) direct cell wall penetration (400X).

fungus preferentially invaded the ray cells, then grew into the lumens of the adjacent tracheids.

Effectiveness of FITC-WGA for fungal visualization

Wood sections stained with safranin-O retained a reddish color under the filter specific for the green-fluorescing FITC-WGA, making the hyaline hyphae more visible than those under bright field illumination (Figure II-7). Combining fluorescent-labeled wheat germ agglutinin with safranin-O stains improved the lectin contrast and increased the visibility of scattered hyphae, especially in Douglas-fir which had spiral thickenings on the tracheid walls (Figure II-8). In addition, double-staining permitted more detailed examination of ray cell colonization and made cross-field pit penetration more visible.

Although FITC-WGA was useful for observing fungal hyphae, there were some difficulties in lectin application. Fungal bore holes were not readily apparent in lectin stained sections (Figure II-9); however, previous studies suggest that double-staining with brilliant vital red and FITC-WGA enhances the visibility of fungal bore holes (Krahmer, et al., 1986). In general, cell wall damage or erosion (Type 2 soft rot attack) was less visible in the lectin stained sections; however, examination of safranin-O stained sections with bright field illumination clearly indicated the presence of this damage. Nevertheless, the lectin did improve hyphal visibility, and in combination with safranin-O, permitted detailed study of fungal colonization. Although no Type 1 soft rot cavities were detected in this study, previous studies suggest that most cavity detail is obscured by intense fluorescence due to the presence of a slime matrix

around the cavity hyphae that inhibits movement of the lectin from the cavity (Morrell, et al., 1985). Thus, FITC-WGA does not appear to be useful for detecting soft rot damage.

The results indicate that FITC-WGA improves hyphal visualization when compared with results obtained using a conventional safranin-O/picro-aniline blue method (Morrell et al., 1985; Krahmer, et al., 1986). Although dark-pigmented hyphae can not be visualized using a FITC-WGA, they can be readily visualized using conventional bright field microscopy and their lack of lectin reactivity should not diminish the usefulness of this technique.

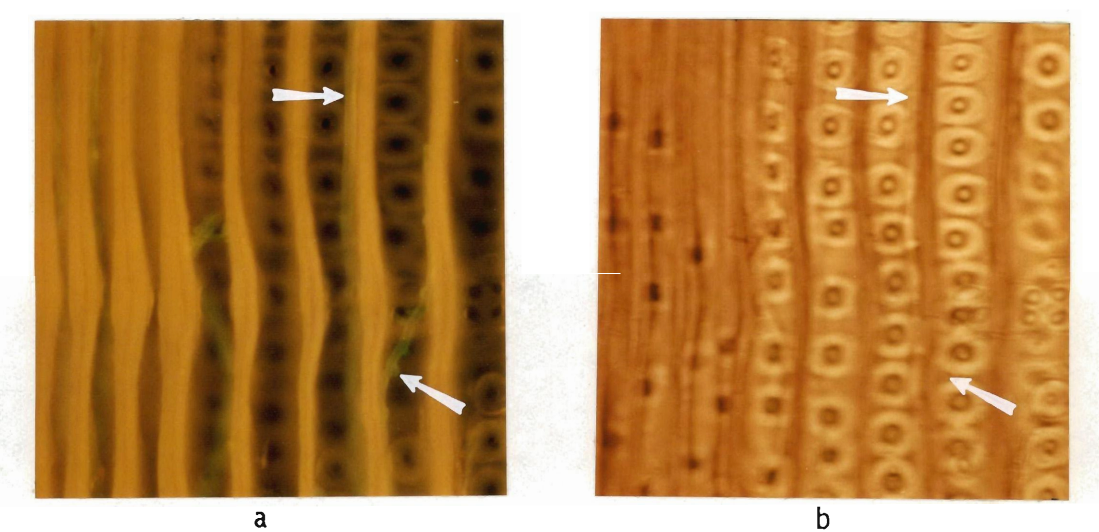
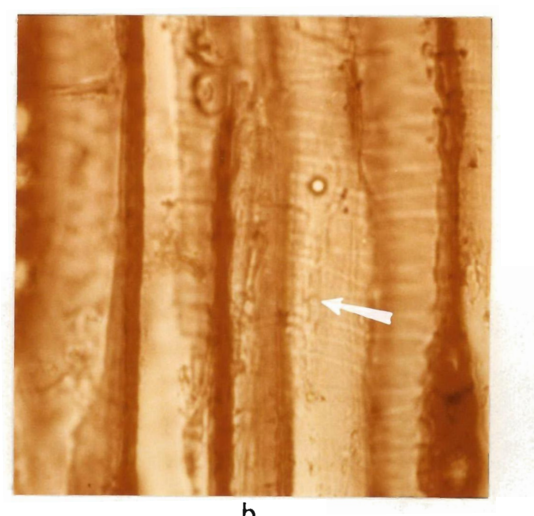


Figure II-7. Radial sections of ponderosa pine exposed to _Poria carbonica_ for one week (275X), then stained with a) safranin-O followed by FITC-WGA, or b) safranin-O. Note the improved hyphal visibility in the WGA stained sections (arrows).

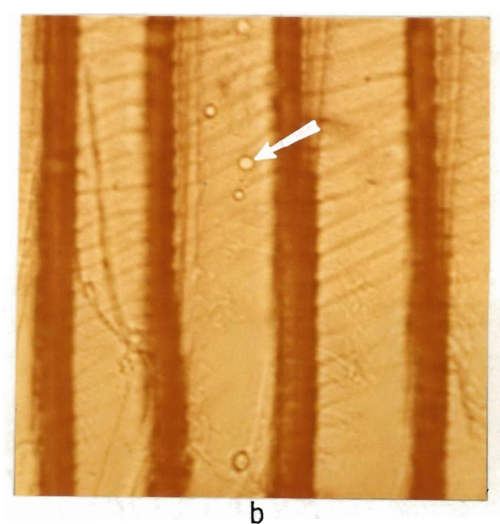
a



b

Figure II-8. Tangential sections of Douglas-fir exposed to Poria carbonica for nine weeks (400X), then stained with a) safranin-O followed by FITC-WGA, or b) safranin-O. Note the ease of locating WGA stained hyphae and the lack of interference from spiral thickenings (arrows).

a



b

Figure II-9. Radial sections of Douglas-fir exposed to Poria carbonica for nine weeks (400X), then stained with a) safranin-O followed by FITC-WGA, or b) safranin-O. Note the difficulty of detecting fungal bore holes in the FITC-WGA stained sections (arrows).

CONCLUSIONS

The results of the lectin screening indicate that FITC-WGA is useful for detecting fungal chitin. This lectin improved hypha visualization when compared with results obtained using a conventional safranin-O/picro-aniline blue method (Morrell, et al., 1985; Krahmer, et al., 1986). Double-staining, with safranin-O followed by FITC-WGA improved the lectin contrast, increased the visibility of hyaline hyphae, and permitted more detailed examination of fungal colonization; however, the lectin did not appear to be useful for detecting soft rot damage or fungal bore holes. Although dark pigmented hyphae did not react with FITC-WGA, they could be readily visualized using conventional bright field microscopy.

Poria carbonica and Coriolus versicolor caused high weight losses at the early stages of exposure; while Chaetomium globosum caused lower weight losses. Weight losses were generally greater in pine blocks. Poria carbonica displayed a greater tendency to attack ponderosa pine than Douglas-fir. The cellulolytic enzymes of the brown-rot fungus in ponderosa pine were capable of penetrating and acting within the cell walls at early stages of decay, while attack was concentrated on the more readily accessible ray parenchyma in Douglas-fir. Coriolus versicolor attacked the more accessible ray cells in Douglas-fir, and passive hyphal penetration via the pits was more prevalent than direct cell wall penetration. The attack patterns found with C. versicolor were very similar to those produced by P. carbonica on Douglas-fir. Both decay types preferentially penetrated pits in early stages of decay, but bore holes were more numerous in advanced stages of brown rot. The hyphae of C. versicolor were widespread in ponderosa pine in

early stages of decay. Passive penetration via the pits was prevalent in the early stages of decay (wt. loss below 4.35%), while active penetration through the cell walls was prevalent in the advanced stages of decay. White rot fungi have been reported to affect wood strength properties significantly at low weight losses ($\leq$5%) (Wilcox, 1978). Thus, the microscopic evaluation did not fully delineate the potential damage caused by white rot fungi. Chaetomium globosum lacked the ability to cause soft rot damage in Douglas-fir blocks; however, the use of more severe decay tests such as a vermiculite burial method might alter these results (Nilsson, 1973). Penetration of C. globosum through ponderosa pine in early stages of attack was primarily through pits, while cell wall penetration began once the fungus had thoroughly penetrated the ray cells. The failure to obtain soft rot cavities in both wood species might be due to the short exposure period or the test conditions (Nilsson, 1973). In both Douglas-fir and ponderosa pine blocks, the fungus preferentially invaded the ray cells, then grew into the lumens of the adjacent tracheids.

Although WGA was only useful for detecting chitin, which is present in nearly all higher fungi, it did improve hyphal visibility in wood sections where conventional stains were more variable.

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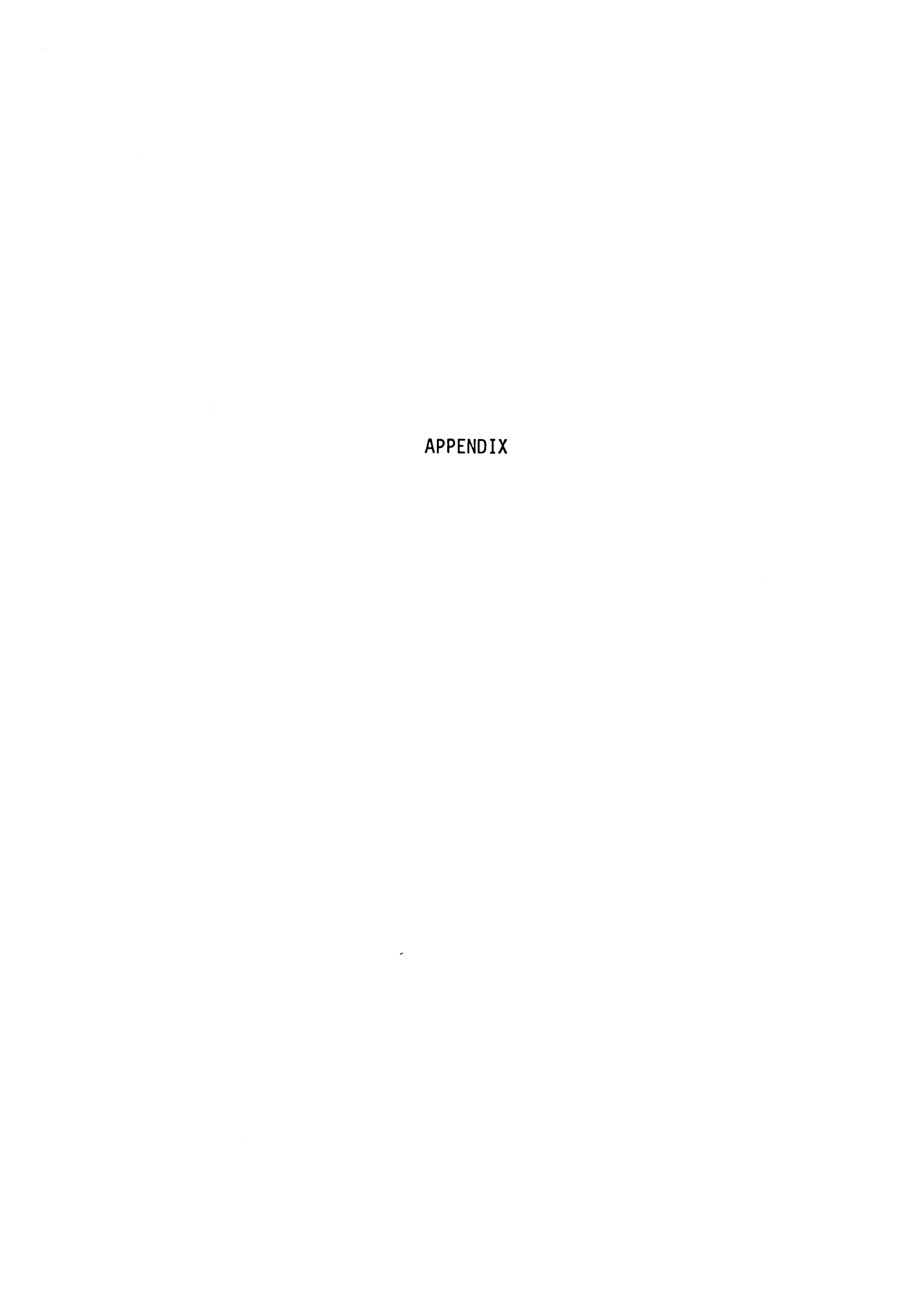

APPENDIX

APPENDIX A

Fungi Tested and Their Origins

Alternaria alternata (Fr.) Kiessler, isolate number ED 113, obtained from the SUNY College of Environmental Science and Forestry, Syracuse, N.Y.

Aspergillus niger Van Tiegh, isolate number ATCC 64045, obtained from the American Type Culture Collection, Rockville, Md.

Aureobasidium pullulans (de Bary) Arn., isolate number IMI 17,468 WY 4162, obtained from Forintek Canada Corp, Vancouver, B.C.

Ceratocystis albida (Mathiesen-K.) Hunt, isolate number WFPL-297A, obtained from Forintek Canada Corp, Vancouver, B.C.

Chaetomium globosum Kunze ex. Fr., isolate number WFPL-172A, obtained from Forintek Canada Corp, Vancouver, B.C.

Cladosporium elatum (Harz) Nannf., isolate number 563-0-I1 F.S.T., obtained from the Forest Research Laboratory, Oregon State University.

Coriolus versicolor (L. ex. Fries) Quel., isolate number R-105, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Crustoderma dryinum (B. & C.) Parm., isolate number 62C 1 Eug. Inf. St. 6 mo., obtained from the Forest Research Laboratory, Oregon State University.

Epicoccum nigrum Link, isolate number 7E F4 Orov. D.D. 1 yr., obtained from the Forest Research Laboratory, Oregon State University.

Fomitopsis cajanderi (Karst.) Kot. & Pouzar, isolate number FP-10796, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Gloeophyllum saepiarum (Wulf.:Fr.) Karst., isolate number FP-100242-S, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Gloeophyllum trabeum (Pers.:Fr.) Murr., isolate number Madison 617-R, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Haematostereum sanguinolentum (Alb. & Schw.:Fr.) Pouzar, isolate number 42M Scappoose, obtained from the Forest Research Laboratory, Oregon State University.

Heterobasidion annosum (Fr.) Bref., isolate number 10MB Grants Pass, Or. (18 mo.), obtained from the Forest Research Laboratory, Oregon State University.

Hyalodendron lignicola Diddens, isolate number D 5354 #1, obtained from the Forest Research Laboratory, Oregon State University.

Irpex lacteus (Fr.:Fr.) Fr., isolate number FP 105915-sp, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Lentinus lepideus Fr.:Fr., isolate number Madison 534-R, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Leptodontium elatius (Mang.) de Hoog, obtained from the SUNY College of Environmental Science and Forestry, Syracuse, N.Y.

Oidiodendron griseum Robak, isolate number OSU 504-1-0, obtained from the Forest Research Laboratory, Oregon State University.

Paecilomyces varioti Bainier, isolate number OSU 541-1, obtained from the Forest Research Laboratory, Oregon State University.

Penicillum italicum Wehmer, isolate number OSU 54, obtained from the Forest Research Laboratory, Oregon State University.

Peniophora gigantea (Fr.) Massee, isolate number 5WE5 Oroville DD, obtained from the Forest Research Laboratory, Oregon State University.

Phellinus weirii (Murr.) Gilbn., isolate number FP 91601, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Phialocephala dimorphospora Kendrick, obtained from the SUNY College of Environmental Science and Forestry, Syracuse, N.Y.

Phialophora fastigiata (Lagerb., Lundb. & Melin) Conant, isolate number #14, obtained from the Forest Research Laboratory, Oregon State University.

Phialophora sp. 3, isolate number Carranza 334-E, obtained from the SUNY College of Environmental Science and Forestry, Syracuse, N.Y.

Phanerochaete sordida (Karst.) Eriksson & Ryv., isolate number 36 T2 Taylor-Sheridan A.S., obtained from the Forest Research Laboratory, Oregon State University.

Phlebia radiata Fr., isolate number L-15608-sp, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Phlebia subserialis (B. & G.) Donk, isolate number 8CK5 Peavy Arb. Chem. Test, obtained from the Forest Research Laboratory, Oregon State University.

Poria carbonica Overh., isolate number 1978, obtained from the Forest Research Laboratory, Oregon State University.

Poria placenta (Fr.) Cooke, isolate number FP-94267A, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Poria xantha (Fr.) Cooke sensu Lind, isolate number FP 105494-sp, obtained from the U.S. Forest Products Laboratory, Madison, Wisconsin.

Schizophyllum commune Vaill.:Fr., isolate number 107 T4 Rochester, obtained from the Forest Research Laboratory, Oregon State University.

Scytalidium sp., isolate number Fy-FRL, obtained from the Forest Research Laboratory, Oregon State University.

Sistotrema brinkmanii (Bres.) Eriksson, isolate number 3UD9 Scappoose DD 2Yr. Untreated, obtained from the Forest Research Laboratory, Oregon State University.