

AN ABSTRACT OF THE THESIS OF

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Title: ABILITY OF SELECTED FUNGI FROM DOUGLAS-FIR POLES
TO DEGRADE WOOD AND THEIR TOLERANCE TO WOOD-
PRESERVING CHEMICALS

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Wood degrading ability and tolerance to wood-preserving chemicals of several fungi isolated from Douglas-fir utility poles were investigated by the agar-stick and soil-block methods. Birch (Betula sp.) wood sticks and western hemlock (Tsuga heterophylla) wood blocks were used.

The soil-block and agar-stick tests provided identical weight loss rankings of the eight fungi from Douglas-fir poles. Breaking radius appeared to be a more sensitive and rapid indicator of decay than was weight loss and provided a reasonable basis for detecting decay fungi. Decay caused by the brown-rot fungi, Poria carbonica and Poria monticola was more severe with increase in incubation time expressed either as strength reduction or weight reduction. The white-rot fungus, Schizophyllum commune caused relatively little

weight loss and gave erratic results. Except for Phialophora fastigiata, the fungi-imperfecti caused relatively little weight loss but a relatively large change in breaking radius after twelve weeks.

In the preservative tolerance test, the brown-rot fungi were highly resistant to ammoniacal copper arsenite and moderately tolerant to creosote and pentachlorophenol. The white-rot fungus was susceptible to all preservatives except creosote. The fungi-imperfecti, Hyalodendron lignicola and Phialophora fastigiata were highly tolerant to all preservatives.

In a study of classifying hymenomycetes isolated from Douglas-fir utility poles into brown-and white-rot fungi by the oxidase test method, brown-rots were more prevalent than white-rot fungi. The intensity of the oxidative reaction and the time required showed considerable variation among the white-rot fungi.

Ability of Selected Fungi from Douglas-Fir
Poles to Degrade Wood and Their
Tolerance to Wood-Preserving
Chemicals

by

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ABILITY OF SELECTED FUNGI FROM DOUGLAS-FIR POLES TO DEGRADE WOOD AND THEIR TOLERANCE TO WOOD-PRESERVING CHEMICALS

INTRODUCTION

Wood poles have been used by utilities to support conductors from the beginning of transmission of electrical energy. In the United States, the trend in transmission and distribution of electrical energy is toward high voltages that require tall structures. Wood poles as long as 40 meters are being used and the remaining source for such material in this country is the forests of the Pacific Northwest. With western red cedar (Thuja plicata D. Don), the long-preferred species, in short supply, Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) is the principal timber available to meet the demand. However an important limitation to the usefulness of wood as utility poles is its susceptibility to decay in particular conditions of use.

Large Douglas-fir poles normally are dried to meet treating requirements and not to moisture content that they will attain in service, thus the heartwood moisture content of about 30 to 40 percent frequently remains unchanged until the treated pole is installed. In service, the interior of the pole above the ground line drops below the fiber saturation point and seasoning checks may extend beyond the treated shell. Wood destroying organisms, mostly fungi, enter the

poles through the exposed untreated wood and may cause extensive internal deterioration in this relatively thin sapwood species.

Research has shown that certain agricultural fumigants placed in holes in Douglas-fir poles diffuse for considerable distances as gases to eliminate decay fungi and to provide residual protection for at least five years. However emphasis has been placed on the principal decay fungi, Poria carbonica and Poria monticola while little attention has been given to other fungi found in the preservative treated shell of poles, in the untreated heartwood, or in the re-invasion of fumigant treated poles. This research will therefore explore the role of both decay and non-decay fungi in the initial invasion of wood as determined by their tolerance to preservatives used to protect wood and their ability to degrade wood.

OBJECTIVES

The objectives of this study were:

1. To determine the ability of selected decay and "non-decay" fungi from Douglas-fir poles to degrade wood.
2. To determine the tolerance of the more prevalent fungi isolated from Douglas fir poles to ammoniacal copper arsenite, creosote and pentachlorophenol.
3. To classify wood-inhabiting hymenomycetes isolated from Douglas-fir poles, into brown- and white-rot fungi.

APPLICATION OF FINDINGS

Knowledge of the tolerance of the principal fungi in Douglas-fir poles to wood preserving chemicals will aid in:

1. Predicting the need for supplemental treatment for the outer treated shell of poles.
2. Determining the amount of chemical needed to protect wood.
3. Selecting fungi for further studies of their role in the breakdown of preservatives or the development of biological buffers to decay fungi.

LITERATURE REVIEW

Wood degrading Fungi

Decay in wood is a form of decomposition, produced by enzymes of fungi (6). According to Alexopoulos (1) fungi include nucleated, spore-bearing, achlorophyllous organisms which generally reproduce sexually and asexually, and whose usually filamentous, branched somatic structures are typically surrounded by cell walls containing cellulose or chitin or both.

Wood decay is generally caused by fungi belonging to the large class called Basidiomycetes which includes such familiar plants as mushrooms or toadstools (33). Most of the wood decay species occur in the subclass "Homobasidiomycetes" in one order known as the "Agaricales" (20). They are mainly in four families: Agaricaceae, Hydnaceae, Polyporaceae and Thelephoraceae.

Another group of fungi belongs to Ascomycetes and fungi-Imperfecti which cause so called soft-rot in wood. They occur in association with decay fungi and could be important as inhibitors of decay fungi (28). As reported by Unligil (64) some can detoxify a preservative and thus allow decay fungi to become established.

Wood Decay Requirements

The conditions necessary for development of decay organisms

in wood are (31, 57):

1. A suitable source of energy and nutrients.
2. Moisture near or in excess of the fiber-saturation point of the wood.
3. Oxygen in adequate supply.
4. A favorable temperature about 50° to 90° F.

A deficiency in any of these requirements will inhibit the growth of a fungus even though it already may be well established.

Fungal Infection and Spread

Davidson (21) reports that most decay that takes place in trees, logs or lumber products starts from spores. Spores are disseminated by being forcibly ejected from the sporophore into the air. Their small size and weight permits their being carried for considerable distances by slight or strong wind currents (53). They finally may land on another tree, log, or pole and settle in a favorable spot for germination and growth. On germination the delicate mycelial filaments penetrate into and through wood cells by dissolving the cell walls and using the substances thus obtained for further growth and expansion (21). The penetrating hyphae use certain parts of the cell walls and continue to multiply and concentrate action of growth until much of the content of the cell walls are broken down constituting what is termed decay.

Decay may be incipient or advanced depending on the extent of damage. Cartwright and Findlay (12) report that in the earliest or incipient stage of decay the hyphae may spread through the wood in all directions from the point of infection, usually passing from cell to cell through bore holes which they form at the points of contact between the hypha and cell wall, or through the natural openings. During this invasion stage, there is no apparent change in appearance of the wood, other than a slight discoloration of the infected piece. Scheffer (56, 58) pointed out that the signs of incipient decay varied and often more than one symptom needs to be considered for a convincing diagnosis. He further stated that the existence of the decay frequently can be confirmed by examining the wood microscopically for the presence of hyphae or by incubating bits of wood on nutrient agar to see if a decay fungus will appear.

Advanced decay is characterized by three features (56): the deterioration extends deep into the wood, strength is much lower and the wood has an abnormal color. In the late or advanced stage of decay the wood may become punky, soft or spongy depending upon the nature of the attacking fungus and the extent of its effect on the wood.

Types of Wood Decay and Extent of Damage

There has been much discussion about the classification of fungi according to the type of degradation they cause in wood (44, 37).

Falck and Haag (32) divided wood destroying fungi into Korrosionsfäule and Destruktionsfäule. These correspond to Hartigs white and brown rots; cellulose and lignin being removed by the former and only cellulose by the latter (35). Later Campbell (10, 9) further divided the white rots according to the following methods of attack:

Group 1: Lignin and pentosans attacked in the early stages, cellulose later.

Group 2: Cellulose and its associated pentosans attacked in early stages, lignin and the non-associated pentosans later.

Group 3: Both lignin and cellulose attacked at all times.

These methods of classification have been combined by Meier (44) and Kawase (37) who recognized four types of decay:

Brown rots: The cellulose and its associated pentosans are attacked the lignin remaining largely unchanged, any changes being of a secondary nature. The enzymes produced dissolve the carbohydrates, and the cell wall is uniformly changed.

White rots: The lignin decreases steadily, the cellulose being attacked only in the later stages of decay.

Simultaneous rots: The lignin and cellulose are attacked together, the cell wall becoming thinner.

Soft rots: The cellulose is removed, cavities being formed in the secondary walls. This type of rot is caused by Ascomycetes and Fungi Imperfecti.

Among the above four types of fungi, brown-rot and white-rot are the two most common types prevalent in literature. In both types of decay hyphae penetrate the cell walls transversely through pits or by formation of boreholes and then ramify through the cell cavities. However, Wilcox (68) reports that while the hyphae of the white-rot fungus (Polyporus versicolor L.) penetrated the wood of sweetgum and Southern pine through the pits as well as by means of numerous boreholes through the cell wall, the hyphae of the brown-rot fungus (Poria monticola Murr.) penetrated almost exclusively through the pits. In the later stages of decay, both fungi enlarged pit canals to such an extent that they could no longer be distinguished from true boreholes.

Cowling (17), Wilcox (69) and others have shown that considerable differences exist in the manner of degradation of the cell walls by brown- and white-rot fungi. The hyphae of the brown-rot fungi liberate enzymes which diffuse from the cell lining, where the hyphae are located, through the secondary cell wall. In the hardwoods they appear to attack carbohydrates in the S_2 layer of the secondary wall first, followed by the S_1 and finally the S_3 layers, leaving the middle lamella more or less untouched. In softwoods there is considerable variation in the order in which the three layers of the secondary wall

are attacked. Decay may start in the S_2 layer and then spread to the S_1 and S_3 layers. In contrast enzymes secreted by the white-rot fungi, both in conifers and hardwoods, produce a gradual erosion of all the cell wall constituents from the lumen outward. The S_3 layer is attacked first, followed progressively by the other layers as each becomes eroded.

More recently it has been recognized that fungi other than Basidiomycetes are capable of causing decay (15, 26, 39). Since the surface of the affected wood is typically softened, the term soft rot has been applied to this type of decay caused by the Ascomycetes and Fungi-Imperfecti groups of fungi. Soft rot is characterized by hyphae which, unlike those of the Basidiomycetes fungi, produce tunnels in the cell walls that run along the grain and are generally confined to the less-lignified S_2 layers of the secondary walls (41). The hyphae are in the form of spirals contained within the eroded areas in the secondary walls. The eroded areas are in the form of cavities with pointed ends, aligned in the same direction as the microfibrils. In cross section the cavities produced by hyphae appear as holes equal to or exceeding the diameter of the hyphae (26). In some wood, especially hardwoods, soft-rot may break down the S_3 and S_2 cell wall layers from the cell lumen side. This type of deterioration is generally found on surfaces exposed persistently to damp conditions

resulting in moisture content considerably above that tolerated by the Basidiomycetes.

Effect of Decay on Wood Properties

Cartwright and Findlay (12) report that toughness or resistance to impact is the strength property that is affected first by fungal infection; it is followed in the approximate order of susceptibility by reduction in bending strength, compression strength, hardness, and bending elasticity.

At equivalent losses of weight, white rots generally cause less loss in strength than do brown rots (17). Kennedy (38) reports that modulus of rupture and work-to-maximum load in static bending often were reduced drastically with little or no loss of weight; decay, caused by brown rot, Poria monticola, generally induced higher losses of strength than that caused by the white rot, Polyporus versicolor.

Chemical action of fungi has been studied by noting changes in solubility of wood in selected solvents, changes in chemical composition and degree of polymerization of cellulose (17, 8). Cowling (17) found that a white rot fungus caused a gradual change in solubility, chemical composition and degree of cellulose polymerization as decay progressed. On the other hand a brown rot fungus caused solubility

to increase rapidly during early stages and decrease during later stages of decay, rapid decrease in cellulose content with an increase in proportion of beta-cellulose and rapid lowering of the degree of cellulose polymerization, but little decrease in lignin content based on moisture-free weight of original wood. Differences in effect of these fungi on wood were attributed by Cowling (17) to the enzyme system present in each fungus.

Soft-rot fungi have been reported to degrade wood chemically. Duncan (23) found that most of them could cause substantial degradation of sweetgum (*liquidambar styraciflua*) sapwood. Exemplified by Chaetomium globosum, they differ from decay fungi in the way they modify the wood chemically, resembling white-rot fungi in causing a comparatively small increase in alkali solubility yet behaving like brown-rot species in being unable to utilize the lignin extensively (55, 40). Duncan (23) reports that soft-rot fungi are more prevalent in hardwoods than in softwoods.

Enzymatic Degradation of Wood

Enzymes are protein molecules produced by living cells; they act as organic catalysts, making it possible for the biochemical reactions necessary for physiological processes to take place (15). According to Boswell and Campbell (8, 9), the mechanisms of fungal degradation of wood, now generally accepted, provides that the

fungi to utilize cellulose associated with lignin. The linear anhydro-glucose chains produced by the action of "X" are hydrolyzed by a series of enzymes, designated C_X , yielding cellobiose, which is then hydrolysed to glucose and further metabolised.

The production of enzymes by various species of wood-destroying fungi has been studied by Lyr (42) who arrived at the following conclusions. Enzyme production in general is greatest in brown rots and least in soft rots where the cellulase production is very low. Xylanase actively is always present wherever the fungal substrate, though cellulase is not, and xylanase activity reaches a maximum before the cellulase activity. The last two points have been confirmed for Chaetomium globosum by Lyr. Activity of the cellulolytic enzymes of white-rot fungi is restricted to the cell wall surface (69). These fungi consume both the crystalline and amorphous cellulose of the cell walls within a limited area. The cellulolytic enzymes of the brown-rot fungi and the lignin-destroying enzymes of white-rot fungi are able to penetrate and act within the cell walls (69). Therefore, brown-rot fungi consume polysaccharides within the crystalline and amorphous regions of the cell walls. This occurs over an expanded area and causes a rapid depolymerization of carbohydrates (16).

Preservatives and Decay Evaluation Methods

Various methods have been employed for evaluating wood preservatives (12). The Petri-dish, flask, or toximetric test (60) in which the preservative material is dispersed in a malt-agar medium, should not be underrated in its own field, namely, in the determination of relative toxicities. However, a more realistic laboratory test is to expose treated wood blocks to the action of wood-destroying fungi. The agar-block or soil-block culture techniques can be used to screen out the less effective and to test, under laboratory conditions, the relative permanence of the more effective preservative materials.

Permitting better control of moisture content, the soil-block technique approaches the ideal test more closely than does the agar-block procedure. It is also the more severe of the two, demanding in general higher preservative retentions for inhibition of decay.

Soil Block Method

Starting in 1950 and ending in 1957, Duncan (25) was able to standardize the soil-block method. She simplified the method, improved its reproducibility, established the validity of various phases of the procedure and developed a reasonably suitable weathering schedule. Much of the data obtained served as a background for

"Tentative Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures," of the American Society for Testing and Materials Designation: D1413-61.

In this standard soil-block test simple jars are employed and soil is used as the moisture regulating medium. In addition, special wood feeder strips of southern pine sapwood are used as nutrient substrate. The jar, filled with moist soil and furnished with a wood feeder strip, is sterilized and inoculated with a pure culture of specified fungus and left after inoculation for at least four weeks. Thereafter, treated and untreated 3/4" cubes of southern pine sapwood are placed on the feeder strips and the lids are screwed on loosely. The blocks are thus exposed to the fungus at a temperature of 80° F for twelve to twenty-four weeks. The effectiveness of the preservative is then evaluated by the extent of weight-loss of the wood cubes. The retention of the test preservative that will just cause no measurable weight-loss is defined as the threshold value.

Although the absolute variation in weight-loss among replicates is not great when it is under well-controlled conditions, this method is extremely time-consuming and not sufficiently sensitive because strength properties of wood might be seriously affected before any apparent weight-loss occurs (46).

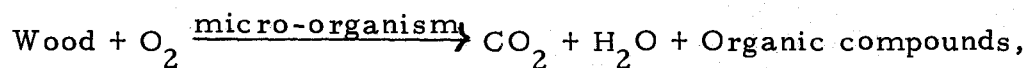
Agar-Stick Method

Because weight-loss is a slow indicator of incipient fungal degradation, investigators have been seeking a substitute using other approaches.

Wang and Graham (66) used a simple agar-stick bioassay procedure devised at the U. S. Forest Products Laboratory during World War I for testing aircraft veneer. In the procedure, inoculum from the margin of an isolate selected for testing was transferred to each of three potato dextrose agar slants containing two sterile dry birch sticks (3 by 6 by 100 mm long) inserted into the agar about 32 mm. The sticks had been selected previously for straightness of grain and freedom from defects. After an incubation period of six weeks, the sticks were removed, wiped free of agar and then one stick from each of the three tubes was soaked in water while the other three sticks were dried at room conditions for at least 24 hours. Each stick was tested on a series of 17 successively smaller mandrels from 3.50 to 0.125 inches in radius to determine its breaking radius. The lower third (in agar), middle third (just above agar) and top third of each stick was tested at two locations. The portion of each stick having advanced decay was assigned a bending radius of 4 inches and for incipient decay, these breaking radii appear to be close to 1-3 inches when tested dry and 1-2 inches when tested wet.

Oxygen and Carbon Dioxide Measuring Methods

The simple reaction:



in fungal metabolic mechanism was used by Smith (62). He determined CO_2 production by fungi and used it as a measure of microbial activity. His method consisted of slowly passing oxygen through a system in which a decaying piece of wood was located. The gas was picked up at the end of the system and analyzed for carbon dioxide. The amount of carbon dioxide present in the spent gas could then be used to assess fungal activity in the system. Smith's method was a rather fundamental approach to wood decay requiring relatively high-skilled personnel and expensive instrumentation. Halabisky and Ifju (34) did some pioneering work using respirometry to measure the O_2 consumption by fungi while working on wood. The procedure they used was quite similar to the soil-block test except that the test block was moved into a Warburg reaction vessel after a three or four weeks incubation period. The Warburg vessel contained a ten percent solution of KOH. In a twenty-four hour duration, the O_2 consumption was measured by the movement of Brodie's solution in a manometer attached to the vessel. Behr (5) recently extended the study of Halabisky and Ifju by using different fungi and preservatives for the test.

Although the O_2 measuring method is sensitive enough to determine the threshold values of preservatives, it uses a relatively indirect interpretation of decay. The O_2 uptake by fungi after certain periods of incubation cannot indicate the degree of wood decay. Conversely, weight-loss is a description of the actual condition of the decayed wood. Another drawback is that the O_2 consumption measured after different incubation times can be affected by the declining supply of food. Thus for preservative-treated wood, a decrease in O_2 consumption can either be the effect of the preservative or of the declining supply of food.

Zone of Inhibition Method

More recently Scheffer and Graham (59) devised a standardized procedure for appraising the protection afforded by residual pentachlorophenol in the sapwood of sprayed western red cedar poles. The assay is made by placing samples of the sapwood at a prescribed distance from a decay fungus, Poria monticola, growing on malt agar, and later measuring the distance (zone of inhibition) by which the fungus is prevented from reaching the wood. The method proved distinctly more practical and informative than chemical analysis for the purpose. It clearly showed practically significant differences in existing levels of protection between pole lines sprayed five years earlier.

Fungal Resistance to Wood Preservatives

Many cases of wood inhabiting fungi demonstrating considerable resistance to specific toxicants are reported in the phytopathological literature. Richards (50, 49) reports that 18 common wood-destroying fungi differ in their relative resistance to sodium fluoride and zinc chloride. Lenzites trabea was shown to be resistant to both sodium fluoride and zinc chloride. Poria incrassata and Lentinus lepideus were found to be least resistant respectively to sodium fluoride and zinc chloride. In studies on treatment of wood by a double diffusion method, Baechler (4) indicated Lenzites trabea to be arsenic tolerant and susceptible to copper and Coniophora cerebella to be rather tolerant to copper and susceptible to arsenic. Cartwright and Findlay (12) report Lenzites trabea highly resistant to arsenic, Lentinus lepideus resistant to creosote and tar oils, Coniophora cerebella resistant to zinc and cadmium compounds, and Poria vaporaria resistant to copper. In preservative evaluation studies, Duncan and Richards (27) selected test fungi chiefly on the basis of observed resistance to the particular preservatives under test. Lentinus lepideus was more resistant to creosote. Lenzites trabea was less inhibited by pentachlorophenol than other test fungi. Poria monticola was resistant to creosote, pentachlorophenol and copper. Hirt (36) isolated Poria xantha frequently from wood products which

failed in service. Laboratory tests revealed this organism to have resistance to copper.

Christensen (14) reports resistance to high concentrations of creosote by a non-wood destroying fungus Hormodendrum resinae. Recent studies by Marsden (43) on this unique fungus indicates that H. resinae actually metabolizes portions of creosote and may decrease its toxicity since creosote may serve as its sole source of nitrogen and carbon. Duncan and Deverall (28) showed that wood-inhabiting fungi, not necessarily responsible for decay, were capable of degrading a toxic compound into a less potent form, thus rendering it less effective in protecting wood from decay by less-tolerant basidiomycetous wood-destroyers. In their experiment pine sapwood blocks treated with pentachlorophenol and sodium arsenate were exposed progressively to a soft-rot fungus, Chaetomium globosum and a decay fungus, Lenzites trabea. Chemical analyses of treated blocks indicated that in the first exposure the fungus had substantially depleted pentachlorophenol and sodium arsenate. Recently Unligil (64) tested 27 strains of fungi isolated partly from wood treated with fungicides for tolerance to pentachlorophenol (PCP) on malt agar. Trichoderma viride, Gliocladium viride, Cephaloascus fragrans and Pullularia pullulans proved to be the most tolerant among the tested fungi. In the soil-block tests pine sapwood blocks impregnated with sodium pentachlorophenate were exposed for 52 days to a common

mold Trichoderma viride and a basidiomycete, Coniophora puteana, a fungus with relatively high sensitivity to pentachlorophenol on malt agar. Analyses of wood blocks revealed that a substantial part of the loss of pentachlorophenol from wood blocks were due to fungal activity. Trichoderma viride, while penetrating into the wood without causing any appreciable weight loss, depleted 62 percent of pentachlorophenol from sample containing 5.8 kg PCP/m³. The PCP depleting activity of Coniophora puteana equalled that of Trichoderma viride and, in addition, it caused heavy weight loss of the wood.

MATERIALS

The major experimental variables were: eight fungi, three preservatives and 12 preservative retention levels, Birch (*Betula* sp.) sticks, Western Hemlock [*Tsuga heterophylla* (Raf.) Sarg.] wood blocks and increment cores from Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] utility poles.

Wood Specimens

Birch (sp. gr. 0.53¹) sticks 0.05 inch thick by 0.25 inch wide by 2.25 inch along the grain and western hemlock (sp. gr. 0.47¹) wood blocks 0.5 inch thick by 1 inch by 0.5 inch along the grain were used as test specimens for the agar-stick and soil-block tests respectively. The specific gravity and dimensions of the birch sticks were the average values of ten sticks selected randomly from a box of 1,000 sticks. The wood blocks were sawed from Western hemlock sapwood. These wood specimens were selected because of their susceptibility to attack by wood-degrading fungi.

Test Fungi

The eight fungi used in this study were cultured from increment cores taken from Douglas fir poles in Western Oregon (Table 1).

¹Oven dry weight and volume basis.

Table 1. Fungi Selected for Tests of Decay and Preservative Tolerance.*

Name of Fungus	Isolated Number
<u>BASIDIOMYCETES</u>	
Polyporaceae:	
<u>Poria carbonica</u> Overh.	SS 1
<u>Poria monticola</u> Murr.	SS 2
Schizophyllaceae:	
<u>Schizophyllum commune</u> Fr.	SS 3
<u>FUNGI IMPERFECTI</u>	
Dematiaceae:	
<u>Periconiella</u> sp. A.	SS 4
<u>Phialophora fastigiata</u> Conant.	SS 5
<u>Rhinoctadiella mansonii</u> Schol.	SS 6
<u>Scytalidium lignicola</u> Pesante	SS 7
Moniliaceae:	
<u>Hyalodendron lignicola</u> Diddens	SS 8

* Isolated and identified by Dr. Elwin Stewart, Department of Botany, now with the Department of Plant Pathology, University of Minnesota.

These species include economically important brown- and white-rot fungi from two families of Hymenomycetes and more prevalent fungi from two families of fungi-imperfecti. Cultures were isolated and identified by Dr. Elwin Stewart, Department of Botany and Plant Pathology. The test fungi, Poria monticola, Murr. and Poria carbonica Overh. were selected because they are the primary cause of internal decay of Douglas-fir. Schizophyllum commune Fr. was selected because it is known to attack wood slowly. The others were selected because of their higher frequency of occurrence in Douglas-fir poles.

Preservatives

The preservatives used in this investigation, ammoniacal copper arsenite, creosote and pentachlorophenol (Table 2) were selected because they are widely used commercially to protect wood.

Table 2. Description of Preservatives Used. *

Name and type of preservative	Active ingredients per-cent weight basis	Solvent
Water-Soluble Preservative:		
1. Ammoniacal Copper Arsenite (ACA)	AS_2O_5 -- 48.6 CuO -- 51.4	water
Oil-Type Preservatives:		
2. Creosote	Creosote -- 100.00	Toluene
3. Pentachlorophenol (PCP)	Chlorinated phenols - 5.0 Heavy oil - 95.0	Toluene

* The preservatives were obtained from the following firms:

No. 1: J. H. Baxter & Co., Eugene, Oregon

No. 2: L. D. McFarland & Company, Eugene, Oregon

No. 3: McCormick & Baxter Creosoting Co., Portland, Oregon

PROCEDURES

Ability of Fungi From Douglas-Fir
Poles to Degrade WoodAgar-Stick Test (Wang)

Preliminary Test:

Preliminary studies were made to determine optimum conditions for decay in the shortest time. I investigated the effect of initial moisture content of the sticks and their location in or on the agar on the rate of decay. All sticks were selected for straightness of grain, freedom from defects, and uniform thickness.

Dry birch sticks were weighed and wrapped in a wet cheese cloth and autoclaved at a pressure of 20 psi for 15 minutes. Autoclaved sticks were weighed to the nearest 0.01 gm, oven-dried for 24 hours at 214⁰ F, reweighed and moisture contents were determined. The procedure gave moisture contents of 40 to 50 percent that are favorable for decay. Dry birch sticks at average moisture content of 6 percent also were wrapped in aluminum foil and autoclaved at a pressure of 20 psi for 15 minutes. The dry and wet sticks were placed in or laid flat on agar slants with the agar surface completely covered by a decay fungus, Poria monticola. One-half of the dry and wet sticks were inserted into the agar and the remaining sticks were laid

flat on the fungus mycelium (Figure 1). Sticks were removed after incubation periods of 2, 3, 4, 5, and 6 weeks. Adhering mycelium and agar were cleaned off and the sticks were weighed.

Each stick was tested wet to failure on a series of 17 successively smaller mandrels from 3.50 to 0.125 inches in radius by pressing the sticks tightly against the mandrels with the thumbs touching to determine its breaking radius (Figure 2). Sticks placed flat on agar were tested for breaking radius at the mid-point. Sticks inserted into agar were tested for breaking radius just above the agar surface and below the agar surface as controls. Broken sticks were oven dried for 24 hours and weighed to determine weight change and moisture content.

The procedure of placing the sticks flat on agar gave favorable results and was therefore employed for subsequent tests (Tables 4 and 5).

Testing the Ability of Fungi to Degrade Wood

Birch sticks selected for straightness of grain, freedom from defects, and uniform thickness were oven dried for 24 hours weighed to the nearest 0.01 gm and sterilized by autoclaving at 20 psi for 15 minutes. Malt agar slants were inoculated by aseptically planting 3-millimeter discs of actively growing mycelium of the test fungi (Table 1), about 1.5 inches from opposite ends of the agar slants.

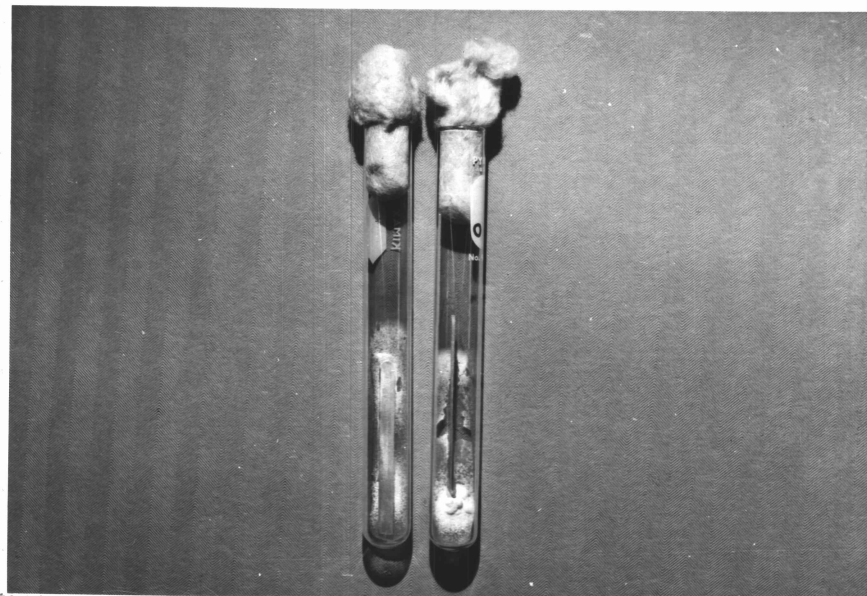


Figure 1. Birch sticks placed on (left) and inserted into (right) malt-agar slants inoculated with a brown-rot fungus, Poria monticola Murr.



Figure 2. Testing sticks for breaking radius on a series of 17 successively smaller mandrels from 3.5 to 0.125 inches in radius. Pressure is exerted on the stick with the thumbs touching.

After incubation for seven days at 80° F, the fungi formed mats of mycelium over the surface of the agar in the tubes. The sterile sticks were laid flat on the malt-agar slants covered with the test fungus (Figure 3). Three replicate slants were prepared for each combination of the eight test fungi and incubated at a temperature of 80° F. Sticks were removed after incubation periods of 2, 4, 6, 8 and 12 weeks. Adhering mycelium and agar were cleaned off and each stick was tested wet at the mid-point on a series of 17 successively smaller mandrels from 3.50 to 0.125 inches in radius by pressing the sticks tightly against the mandrels with the thumbs touching to determine its breaking radius. Broken sticks were oven-dried for 24 hours and weighed to determine weight change.

Soil-Block Method (Modified)

Preparatory:

Sixteen-ounce French bottles with loose-fitting screw caps were used as decay chambers. The decay chambers were filled about half full of oven dried screened top soil. A circular-shaped filter paper strip was placed on top of the soil. Sufficient water containing five percent malt extract by weight of soil was poured evenly onto the filter paper in each chamber to obtain a soil moisture content of 60 percent based on oven-dry weight of soil. The oven-dry weight of



Figure 3. Birch sticks bioassayed on malt-agar slants inoculated with a brown-rot fungus, Poria monticola (left) and an Imperfect fungus, Rhinocladiella mansonii (right).

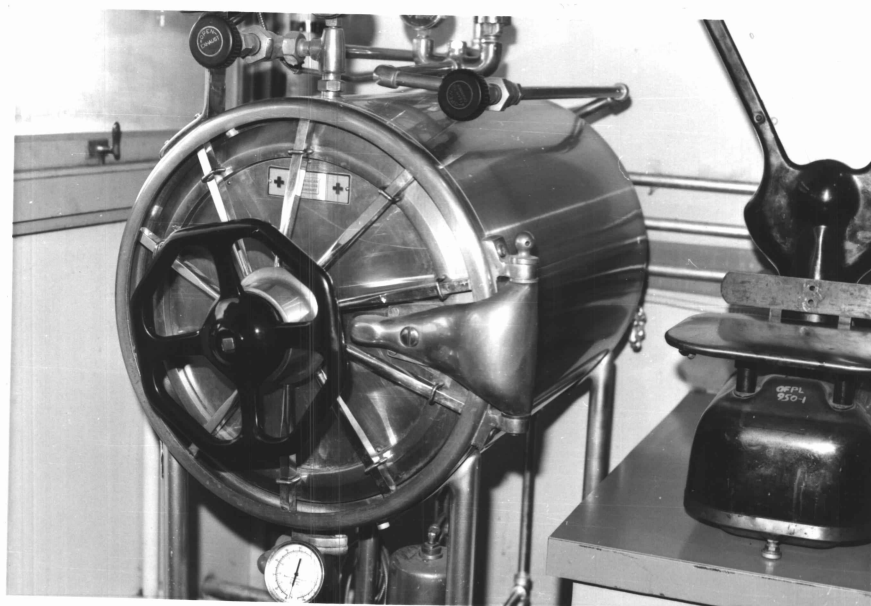


Figure 4. Autoclave used for sterilizing.

soil was the average determination for three extra bottles. It was assumed that the amount of soil in a decay chamber was essentially the same as those in the extra bottles.

The chambers were sterilized by autoclaving at a pressure of 20 psi for 15 minutes and allowed to cool thoroughly. They were then inoculated by aseptically planting two 6-millimeter discs of actively growing mycelium of the test fungi (Table 1), about 1-5 inches from opposite ends of the chambers. After incubation for seven days at 80° F, the fungi formed mats of mycelium over the surface of the filter paper to reach the soil in the decay chambers.

Testing the Ability of Fungi to Degrade Wood

Sterile Western hemlock [*Tsuga heterophylla* (Raf.) Sarg] wood blocks 0.5 x 1 x 0.5 inch along the grain which had been oven dried and weighed were then placed on the mycelial mats. The decay chambers with the lids loosely turned down were incubated at a temperature of 80° F for 12 weeks. Sample chambers of assembly are shown in Figure 5. Five replicate chambers were prepared for each of the eight fungi. Each chamber was examined fortnightly and visible evidence of fungal penetration of the test blocks was noted. At the end of the incubation period, the chambers were opened and the block removed. Adhering mycelium was removed and the blocks were then

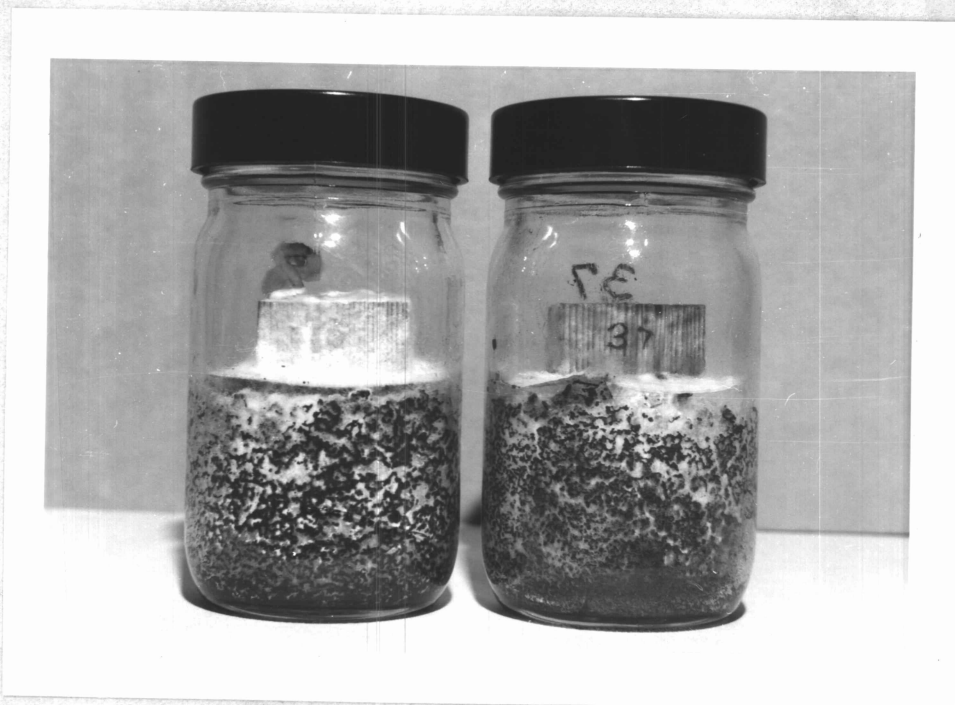


Figure 5. Decay chambers assembly with Western hemlock wood-blocks inoculated (from left) with a brown-rot fungus, Poria monticola and Imperfect-fungus, Rhinoctadiella mansonii, respectively.

oven dried for 24 hours and weighed to determine weight losses caused by each fungus.

The following criteria were used to identify decayed blocks by visual observation:

1. Blocks not contacted by the mycelium were considered free of decay.
2. Blocks exhibiting obvious breakdown of the wood were considered decayed.
3. Blocks subjected to test fungi were oven dried, those deformed by drying were considered decayed (Figure 12).

Control blocks were not deformed.

Tolerance of Test Fungi to Wood Preservatives

Agar Stick Method (Wang)

Preparation of Test Sticks:

Birch sticks selected for straightness of grain, freedom from defects and uniform thickness were cut to standard length of 2 1/4 inches, oven dried for 24 hours and weighed to the nearest 0.01 gm. The width and thickness of the sticks were measured to the nearest 0.001 inch using a screw-gauge micrometer. Average weight, volume and specific gravity were determined for ten sticks which

were randomly selected from a box of 1,000 sticks.

Preparation of Preservative Solutions:

To estimate the expected preservative retentions, preliminary tests of absorption capacity of the sticks of pure toluene solvent (diluent for penta and creosote) and distilled water (diluent for chemonite) were run according to the ASTM standard treatment procedures (2). The balance used to weigh sticks before and after preservative treatment was accurate to 0.001 gm. Information obtained from the preliminary test was used to prepare four solutions forming a progression of increasing concentrations of each preservative (Table 3). Treating solutions of each preservative were prepared by diluting aliquots of master solutions using toluene and distilled water for the oil-soluble and water-soluble preservatives respectively.

Preservative Treatment:

The apparatus for preservative treatment and the procedures of treatment were organized according to ASTM standard (D 1413) - Test for Wood Preservatives by Soil-block Cultures. The treating apparatus comprised of a vacuum desiccator as an impregnation chamber, a separatory funnel above for filling with preservative before impregnation, and a vacuum pump connected to the desiccator for reducing the pressure before impregnation (Figure 6).

Table 3. Preservative retentions of Ammoniacal Copper Arsenite, Creosote and Pentachlorophenol Obtained After Treatment of Test Sticks.

Preservative retention * in pounds per cu. ft.	Concentration of treating solution in percent
AMMONIACAL COPPER ARSENITE	
0.05	0.20
0.10	0.30
0.20	0.60
0.50	0.30
CREOSOTE	
1.0	5.30
2.0	10.60
5.0	26.50
10.0	53.00
PENTACHLOROPHENOL	
0.05	0.30
0.10	0.60
0.20	1.00
0.50	2.70

* Each retention is computed from the average of five sticks.

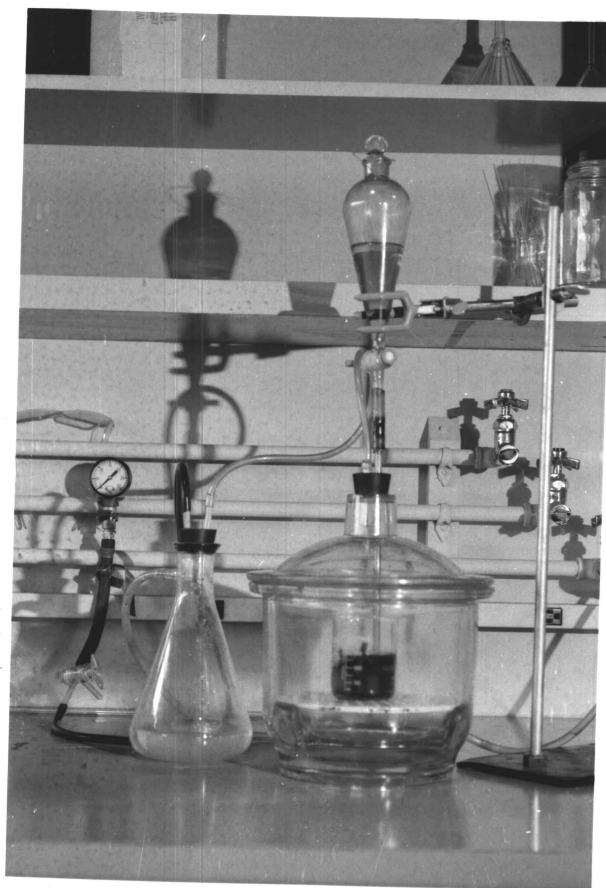


Figure 6. Schematic apparatus for preservative treatment of test sticks.

The sticks were placed in a beaker contained in the desiccator attached to the suction and to the manometer. The pressure in the desiccator was reduced to a differential value of 6 cm. of mercury and held approximately 15 minutes. Each of the preservatives was diluted to obtain a gradient series of retention in the sticks above and below that at which decay might be expected to occur. The dilution of a preservative made just prior to its use, was poured into the globe separatory funnel with the stem extending through the top of the desiccator and into the beaker containing the sticks. The suction pump then was turned off and the separatory funnel stopcock opened to let the diluted preservative material run directly into the beaker in the evacuated desiccator. Enough preservative was used to cover the sticks completely. After the vacuum was released, the beaker containing the sticks was removed and covered. The sticks were taken out individually, the excess liquid was wiped off, and the sticks weighed. They were then dried in oven at 130° F for 24 hours to eliminate toluene.

Calculation of Retention:

The retention (R) in the sticks in terms of pounds of the preservative per cubic foot of wood (Table 3) was calculated by the following formula (2);

$$R = \frac{G C (62.4)}{100 V} \text{ lb/cu. ft. where,}$$

G - Grams of treating solution absorbed by the sticks.

C - Grams of material in 100 grams of treating solution.

V - Volume of test stick.

62.4 - Factor for converting grams per cubic centimeter to pounds per cubic feet.

Testing:

Malt-agar slants were inoculated by aseptically planting a 3-millimeter disc of actively growing mycelium of the test fungi on the agar slants about 1.5 inches from each end. After incubation for seven days at 80° F, the fungi formed mats of mycelium over the surface of the agar in the tubes. The treated sticks were sterilized by rapidly rotating them individually over an alcohol flame and then laid flat on the fungus mat. Three replicate slants were prepared for each combination of the eight test fungi and 12 preservative retentions and incubated at a temperature of 80° F. One untreated control was prepared for each combination. Sticks were removed after an incubation period of six weeks. Adhering mycelium and agar were cleaned off the sticks and weighed. Their breaking radii at mid-point was determined on smaller mandrels. Broken sticks were oven-dried for 24 hours and weighed.

Detecting and Classifying Wood-Inhabiting
Hymenomycetes in Douglas-Fir Poles

Cultures

The cultures of wood-inhabiting hymenomycetes classified were obtained from cores taken with an increment borer from Douglas-fir utility poles on Murder Creek, Stayton-Lyons, and Stayton-Jefferson lines in Western Oregon. Since no fruiting bodies were available at the time of collection, all the fungi classified were in culture. With few exceptions, the cultures remained in good condition and early isolates showed little deterioration during the long period they were in culture.

Method of Isolating Fungi

The cores were surfaced sterilized by flaming and incubated on malt agar prepared according to the following formula:

Malt Agar

Difco Bacto Malt Extract	12.5 gm
Difco Bacto Agar	20.0 gm
Distilled Water	1000 ml

The cultures were grown on malt agar in petri dishes from center inoculum for one week. Two to 3 mm. cubes of inocula were transferred from actively growing mycelium to the edge of 9 cm. petri

dishes, each containing about 30 ml. malt agar. Five plates were inoculated for descriptive studies and two for purposes of classification. These were incubated for six weeks at room temperatures in the dark, being brought into the light at weekly intervals for examination and description. The following data were recorded:

1. The rate of growth expressed as the number of weeks required by the fungus to cover the surface of the agar in the petri dish.
2. The form and character of the advancing zone.
3. The color, topography and texture of mat.
4. The presence and absence of fruiting bodies.
5. The color changes in the agar induced by the growth of the fungus referred to as "Reverse."
6. The odor.

In addition, permanent slides were prepared of mycelium from the advancing zone, from the older parts of the mat, both aerial and submerged and from fruiting areas by mounting in five percent aqueous KOH and staining with an aqueous solution of phloxine for examination and description at suitable intervals.

Oxidase Test

The presence or absence of extracellular oxidase was determined by the Bavendamn test, following the procedure described by

Davidson, Campbell and Blaisdell (22). Cultures were grown from center inocula in petri dishes on agar made according to the following formula:

Tannic Acid Agar

Difco Bacto malt extract	15.0 gm
Difco Bacto agar	20.0 gm
Distilled water	1000 ml
Tannic acid	5.0 gm

The agar and malt were added to 850 ml. of water, the remaining 150 ml. of water being placed in a separate flask. The contents of both flasks were sterilized for 20 minutes at 15 lb. pressure. While the sterilized water was still hot, the tannic acid was dissolved in it, and this solution was added to and thoroughly mixed with the slightly cooled agar which was poured directly into sterile petri dishes. After incubation for one week, the presence and intensity of diffusion zones, indicating the formation of extracellular oxidase, or their absence, was recorded (Figure 7).

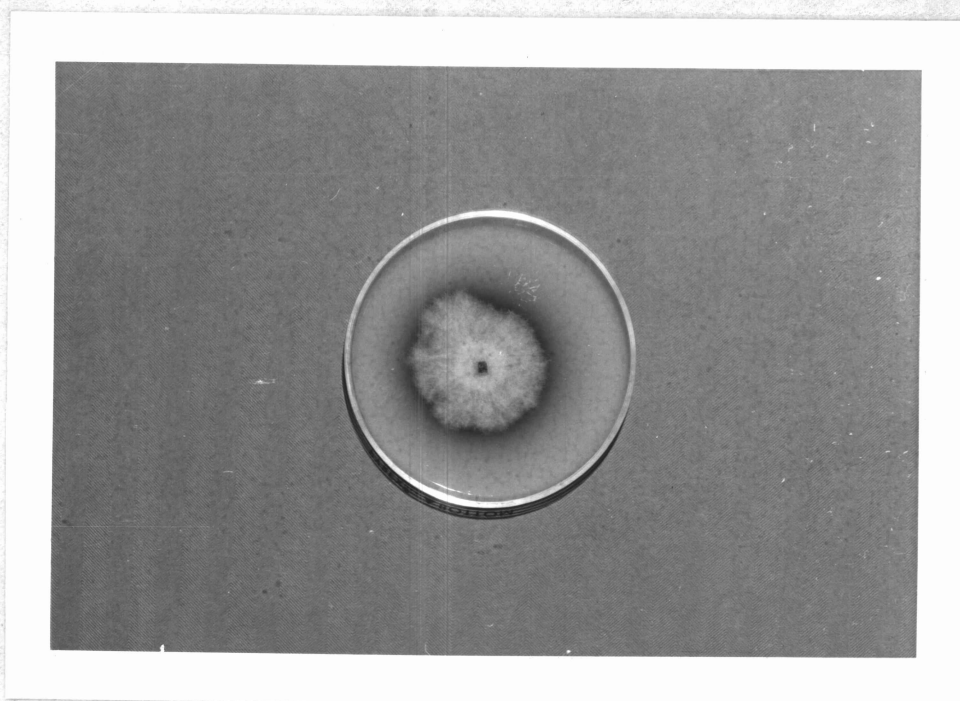


Figure 7. Oxidase test of fungus culture, showing dark diffusion zone on tannic acid agar indicating presence of extracellular oxidase of a white-rot fungus.

RESULTS AND DISCUSSION

Ability of Fungi From Douglas-fir Poles to Degrade Wood

Agar-Stick Test (Wang)

Preliminary Test:

Increase in weight was regularly observed in the test sticks during initial stages of decay (Tables 4 and 5). Similar results have been reported elsewhere (46). The observed gain in weight may be attributable largely to the method of testing for decay. In growing from the malt agar onto the wood specimens, the fungal mycelium may increase the weight of the sample due to the diffusion of sugars from the malt agar medium to the wood. Mulholland (46) attributed weight gain in incipiently decayed sitka spruce to an increase in the equilibrium moisture content of the samples.

Weight loss occurred at the fourth week of bioassay, which is in accordance with the findings of Smith (63). Since a brown-rot fungus was used in this study, the weight loss was also an indication of increased hydrolysis of the cellulose chains with a consequent increase in the number of short-chained carbohydrates. For both dry and wet sticks, weight loss was greater during the sixth week. However, sticks placed flat on agar showed greater weight loss than those

Table 4. Preliminary Test of the Effect of Initial Moisture Content of Sticks on their Rate of Decay When Inserted One-Half Their Length in Agar Slants Inoculated with Poria monticola.¹

Incubation Time (wks)	Moisture ² Content (%)	Weight Change (%)	Breaking Radius ³	
			At agar Surface (ins.)	In agar (ins.)
<u>Sticks inserted dry:</u> ⁴				
2	--	+0.4	---	---
3	57	+0.8	0.63	0.29
4	78	-1.8	1.50	0.19
5	71	-2.4	1.75	0.25
6	90	-3.4	3.00	0.25
<u>Sticks inserted wet:</u> ⁵				
2	--	+2.7	---	---
3	76	+3.0	0.75	0.25
4	74	+1.4	1.33	0.33
5	85	-0.8	2.00	0.46
6	79	-1.3	3.00	0.33

¹ Each value is the average of three sticks, one in each of three agar slants.

² After incubation.

³ Average breaking radius of ten sound, wet sticks was 0.145 inch.

⁴ Inserted at a moisture content of 6 percent.

⁵ Inserted at a moisture content of 40 to 50 percent.

Table 5. Preliminary Test of the Effect of Initial Moisture Content of Sticks on Their Rate of Decay when Laid Flat on Mycelium of *Poria monticola* on agar slants.¹

Incubation Time (wks.)	Moisture Content (%) ²	Weight Change (%)	Breaking Radius ³ (ins.)
Dry sticks: ⁴			
2	---	+3.4	---
3	104	+1.4	0.58
4	117	-4.9	2.25
5	106	-6.9	2.41
6	128	-22.1	3.10
Wet sticks: ⁵			
2	---	+1.5	---
3	112	+0.8	0.66
4	114	-5.3	1.50
5	133	-13.3	3.50
6	155	-23.3	3.50

¹ Each value is the average of three sticks, one in each of three agar slants.

² After incubation.

³ Average breaking radius of ten sound wet sticks was 0.145 inch.

⁴ Placed at a moisture content of 6 percent.

⁵ Placed at a moisture content of 40 to 50 percent.

inserted into agar. This difference is attributed to much larger surface area exposed to the fungus by the flat sticks.

Successful use of this bioassay procedure for determining the ability of fungi to decay wood will depend on the selection of dry and wet breaking radii that separate decay from non-decay fungi. According to Wang and Graham (66) these breaking radii appear to be close to 1.3 inch when dry and 1.2 inch when tested wet. These values were attained by the end of the fourth week. The breaking radii of dry and wet sticks inserted or placed flat on agar were within these limits.

Moisture content of the bioassayed sticks increased with time of incubation due to absorption of water from agar and the action of the fungus and was highest in the sticks placed flat on the agar. As previously mentioned, the fungus used in this study was a brown-rot organism which brings about the breakdown of wood substance through a reaction similar to that of dilute acid hydrolysis. This hydrolysis occurs in the cellulose chains which presumably then afford a greater opportunity for the absorption of water molecules. Another factor contributing to increased moisture content during decay is an increase in chemically combined water of constitution as a result of hydrolysis.

Testing the Ability of Fungi to Degrade Wood

Table 6 shows the effect of each fungus on weight loss and breaking radius of birch sticks during incubation periods of 2, 4, 6, 8, and 12 weeks. Loss in strength as measured by an increase in breaking radius due to decay often occurred before appreciable weight losses were noted. These findings agreed with results of other investigators (13, 46, 52). Cartwright and others (13), using a brown-rot fungus, Trametes serialis to decay sitka spruce reported a reduction of over 25 percent in bending strength before weight losses became apparent. They concluded that loss in strength would appear to be due to a chemical action on the cell wall substance rather than to a physical breaking down of the walls by hyphal penetration. In a similar investigation, Mulholland (46), using Poria monticola, a brown-rot organism, to decay sitka spruce, noted reductions in modulus of rupture and work values in static bending before weight was significantly reduced. Richards (52) found that toughness of loblolly pine and red gum was reduced at least 50 percent at a weight loss of only two percent.

Decay caused by the brown-rot fungi, Poria monticola and Poria carbonica was more severe than that caused by the white-rot fungus, Schizophyllum commune and the fungi-imperfecti (Table 6). The data in Table 6 have been averaged for weight loss and breaking radius

Table 6. Average Weight Losses and Breaking Radii of Birch Sticks Inoculated with Eight Test Fungi and Incubated for a Maximum Period of 12 Weeks.¹

Test fungus	Incubation time (wks)	Weight loss (%)	Breaking Radius ²	
			inches	change (%)
<u>Typical Decay Fungi:</u>				
<u>Poria</u> <u>monticola</u>	2	2.6	1.0	700
	4	5.8	2.3	1740
	6	15.2	3.0	2300
<u>Poria</u> <u>carbonica</u>	2	2.0	1.0	700
	4	4.9	2.1	1580
	6	14.5	2.8	2140
<u>Schizophyllum</u> <u>commune</u>	2	0	0.3	140
	4	0.8	0.8	540
	6	1.2	1.1	780
	8	1.8	1.4	1020
	12	2.5	1.9	1420
<u>Other Fungi:</u>				
<u>Phialophora</u> <u>fastigiata</u>	2	0	0.1	0
	4	0.6	0.2	60
	6	1.0	0.5	300
	8	1.5	1.0	700
	12	2.2	1.2	860
<u>Periconiella</u> <u>sp. A.</u>	2	0	0.1	0
	4	0.1	0.3	140
	6	0.3	0.5	300
	8	0.5	0.5	300
	12	0.7	0.9	620

Table 6. Continued.

Test fungus	Incubation time (wks)	Weight loss (%)	Breaking Radius ²	
			inches	change (%)
Other fungi:				
<u>Scytalidium</u> <u>lignicola</u>	2	0	0.2	60
	4	0	0.3	140
	6	0.1	0.3	140
	8	0.3	0.4	220
	12	0.6	0.5	300
<u>Hyalodendron</u> <u>lignicola</u>	2	0	0.1	0
	4	0	0.1	0
	6	0.2	0.3	140
	8	0.3	0.3	140
	12	0.5	0.8	540
<u>Rhinocladiella</u> <u>mansonii</u>	2	0	0.1	0
	4	0	0.1	0
	6	0.05	0.1	0
	8	0.1	0.3	140
	12	0.1	0.5	300

¹ Each value is the average of five sticks, one in each of five agar slants, except when contamination occasionally reduced number of sticks to not less than three.

² Average breaking radius of sound, wet birch sticks was 0.125 inch.

values for these fungi and plotted against incubation time in Figures 8 and 9. Both weight loss and breaking radius changed most rapidly with incubation time for birch sticks decayed by brown-rot fungi. However, the white-rot fungus, Schizophyllum commune, caused more rapid progression than the imperfect fungi.

Decay by brown-rot fungi, Poria monticola and Poria carbonica averaged only about two percent loss in weight after two weeks incubation, however strength losses as a measure of breaking radius were higher, which were in conformity with previous work (38, 46, 13).

White-rot fungus, Schizophyllum commune caused relatively little weight loss even after six weeks incubation which agreed with results of earlier experiments (51). The breaking radii of sticks attacked by this fungus are not far above the minimum criteria for decay and well below those of other decay fungi. However, these results confirm the suspicions of Richards (51) that this fungus may not be as harmless as reported. The different degrees of strength reductions observed among the bioassayed sticks may be due to chemical nature of decay which have been explained by Booth and Campbell (11, 12).

Except for Phialophora fastigiata, the fungi-imperfecti caused relatively little weight loss but a relatively large change in breaking radius after twelve weeks. From previous findings it is possible to

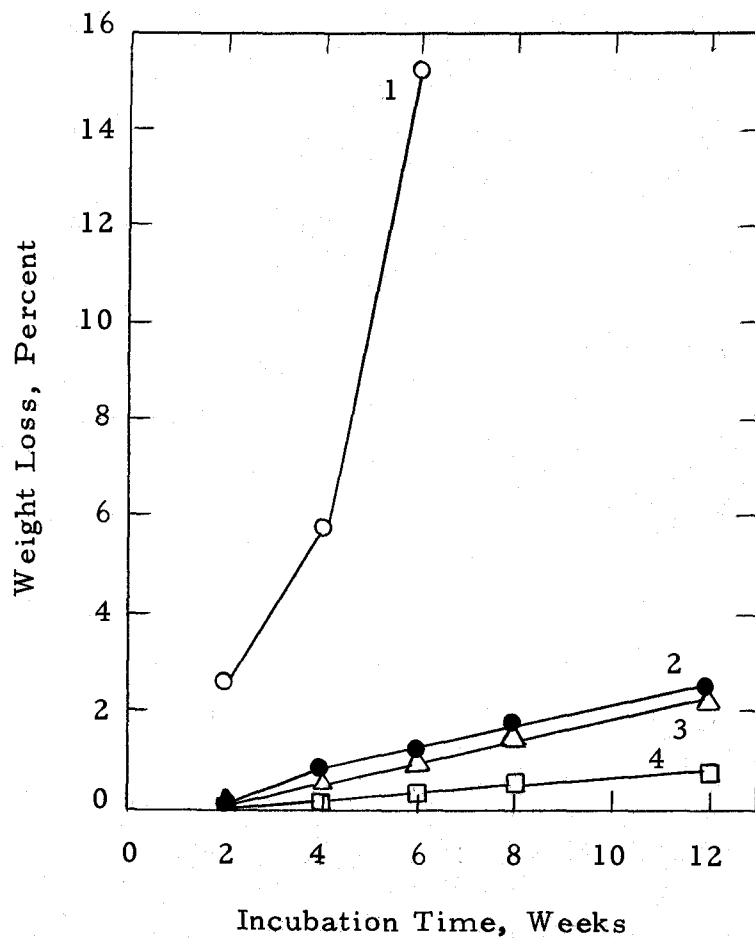


Figure 8. Relationship between incubation time and weight losses of birch sticks caused by 1) a brown-rot fungus, Poria monticola, 2) a white-rot fungus, Schizophyllum commune, 3) a soft-rot fungus, Phialophora fastigiata and 4) an imperfect-fungus, Periconiella sp. A.

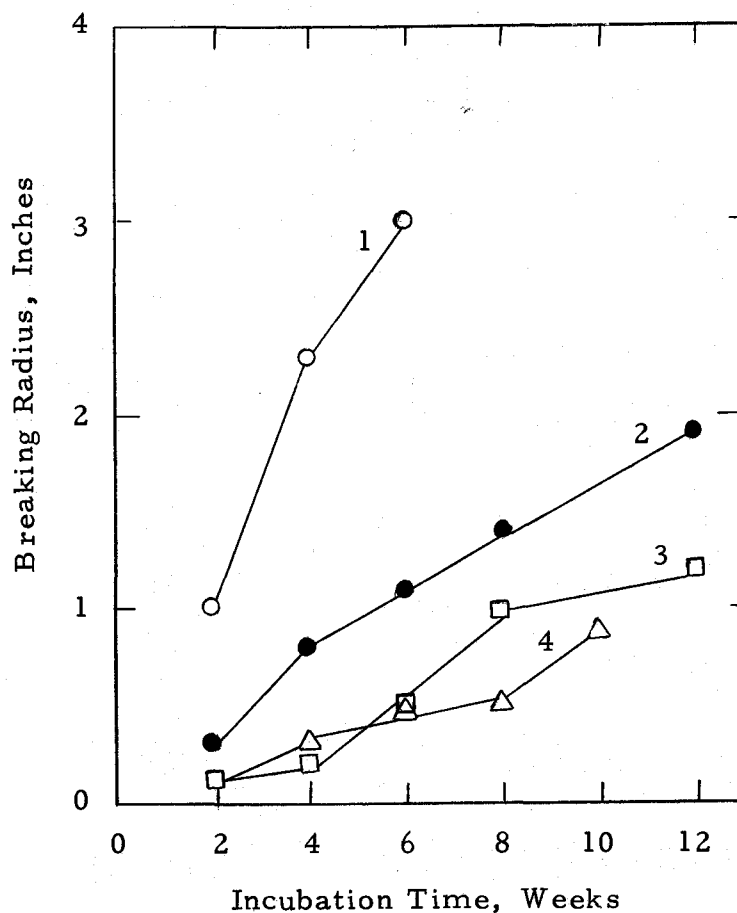


Figure 9. Relationship between incubation time and breaking radius caused by 1) a brown-rot fungus, *Poria monticola*, 2) a white-rot fungus, *Schizophyllum commune*, 3) a soft-rot fungus, *Phialophora fastigiata* and 4) an imperfect-fungus, *Periconiella* sp. A.

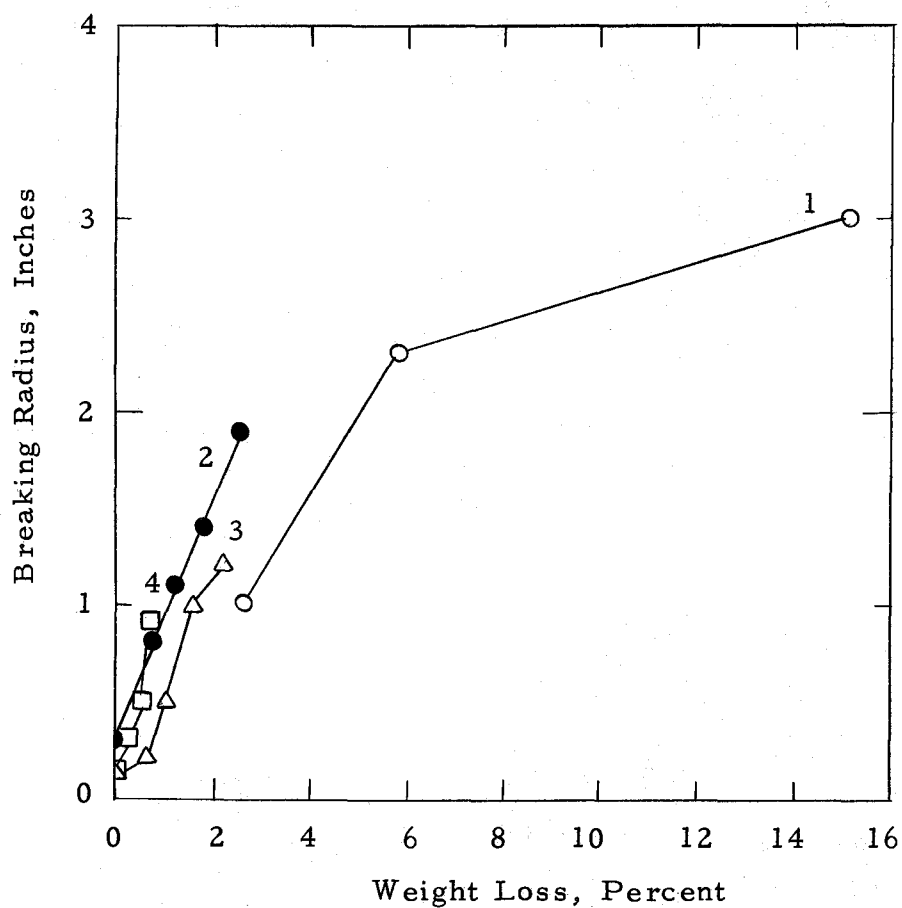


Figure 10. Relationship between weight loss and breaking radius caused by 1) a brown-rot fungus, Poria monticola, 2) a white-rot fungus, Schizophyllum commune, 3) a soft-rot fungus, Phialophora fastigiata and 4) an imperfect-fungus, Periconiella sp. A.

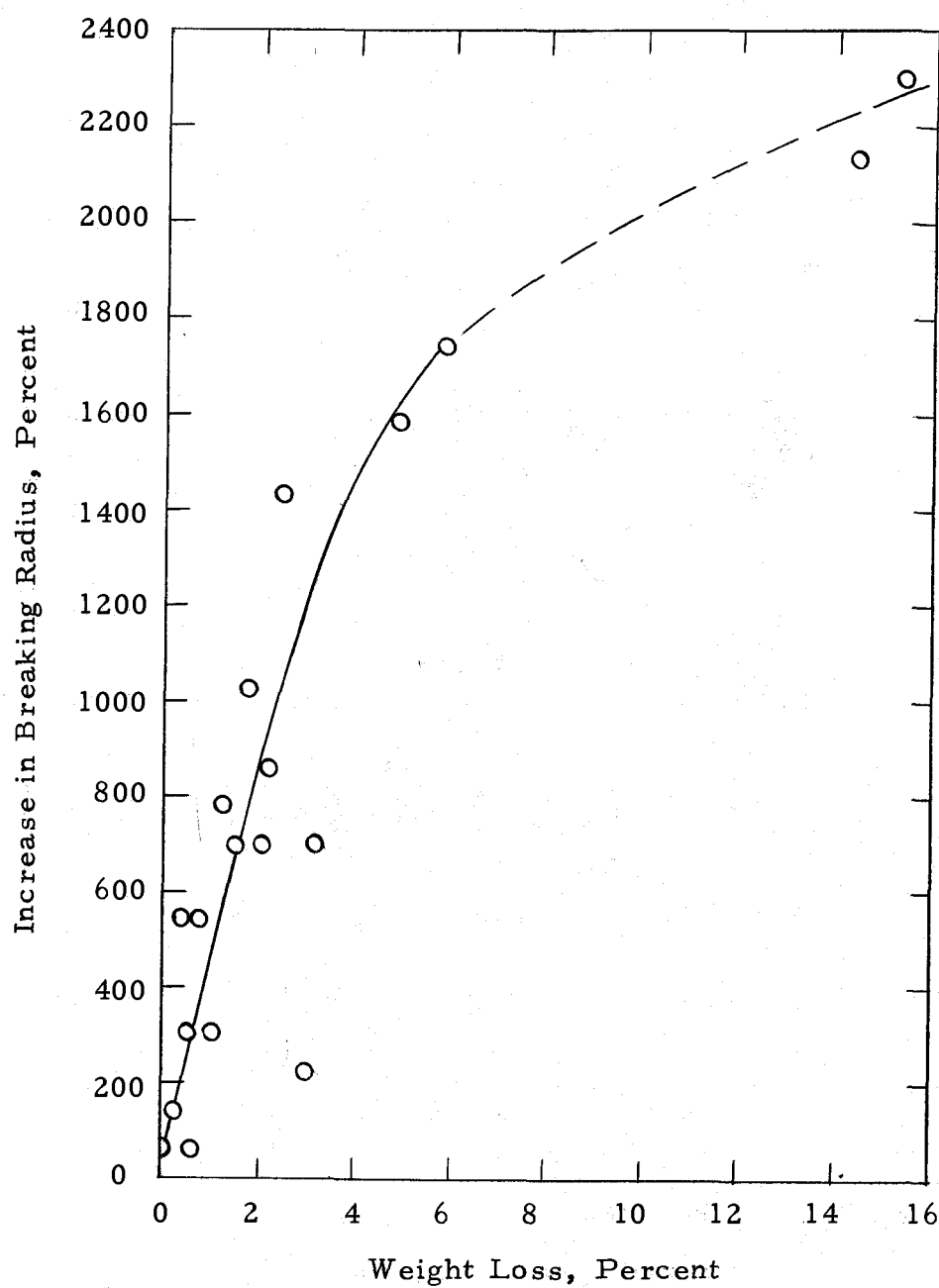


Figure 11. Change in properties of birch sticks exposed on malt-agar to eight fungi isolated from Douglas-fir poles.

expect strength reductions from small weight losses caused by these fungi (38, 51, 13).

Phialophora fastigiata, which has been classified as a soft-rot fungus (29) caused substantial weight and strength reductions approaching that of Schizophyllum commune. Both fungi sustained relatively little weight loss (2.2 - 2.5%) even after 12 weeks incubation, but breaking radius of sticks attacked by these fungi was increased by 960 to 1520 percent. The breaking radius increased with increasing weight loss of the sticks which was in agreement with the findings of Duncan (26). She found that there was a decrease in the bending tolerance (as determined on mandrels) of sweetgum veneer strips infected with 60 different soft-rot isolates. Moreover, this bending tolerance was closely correlated with weight losses, suggesting that it could be used, if particular care was exercised in selection of test material, as a suitable measure of the relative amounts of attack by soft-rot fungi. It is possible to expect strength reductions from the small weight losses caused by Phialophora fastigiata because working with beech soft-rotted by Chaetomium globosum, Armstrong and Savory (3) found there was a sharp decrease in resistance to impact loading before significant weight losses occurred in the wood similar to decay by Basidiomycetes.

The other imperfect-fungi caused much greater changes in breaking radius than in weight loss (Table 6) indicating that their

presence in wood should not be ignored. Duncan and Deverall (28) suggest that they also may be important for decay fungi to become well established.

Soil-Block Test

The average percentage weight loss for each of the test fungi decaying western hemlock wood blocks is given in Table 7. In 12 weeks, the brown-rot fungi, Poria monticola and Poria carbonica caused extensive weight losses and produced brown crumbly rot in localized areas. The wood blocks decayed so badly that it was difficult to clean off the adhering mycelium, some blocks disintegrated on cleaning and others shrunk extensively when oven dried (Figure 12). None of the other fungi caused visible decay of the blocks. The white-rot fungus, Schizophyllum commune caused little weight loss and no visible signs of decay.

The soft-rot fungus, Phialophora fastigiata was the most degradative among the imperfect-fungi. The fungus caused weight reduction of three percent in 12 weeks, whereas none of the other imperfect-fungi caused weight reduction of even one percent at the same incubation time.

As with birch sticks, the other imperfect-fungi caused little weight loss of the test blocks, however Periconiella sp. A. was more important than H. lignicola and S. lignicola in terms of weight losses

Table 7. Effect of Fungi Isolated from Douglas-Fir Poles on Properties of Hemlock Blocks and Birch Sticks.¹

Fungus	Incubation time (wks)	Weight Loss		Breaking radius of birch sticks ²	
		Hemlock blocks	Birch sticks	Radius (in)	Change (%)
<u>Typical decay fungi:</u>					
<u>Poria</u>	6	--	15	3.0	2400
<u>monticola</u>	12	49	45 ³	4.2 ³	3260
<u>Poria</u>	6	--	14	2.8	2240
<u>carbonica</u>	12	43	42 ³	3.8 ³	2940
<u>Schizophyllum</u>					
<u>commune</u>	12	3.2	2.5	1.9	1520
<u>Other fungi:</u>					
<u>Phialophora</u>					
<u>fastigiata</u>	12	3.0	2.2	1.2	960
<u>Periconiella</u>					
<u>sp. A</u>	12	0.8	0.7	0.9	720
<u>Scytalidium</u>					
<u>lignicola</u>	12	0.7	0.6	0.5	400
<u>Hyalodendron</u>					
<u>lignicola</u>	12	0.6	0.5	0.8	640
<u>Rhinocladiella</u>					
<u>mansonii</u>	12	0.5	0.1	0.5	400

¹ Hemlock blocks were exposed to fungi in a soil-block test. Birch sticks were exposed to fungi in test tubes and tested to failure on mandrels of successively smaller diameter.

² Breaking radius of sound, wet birch sticks was 0.125 inch.

³ Data extrapolated from curves.

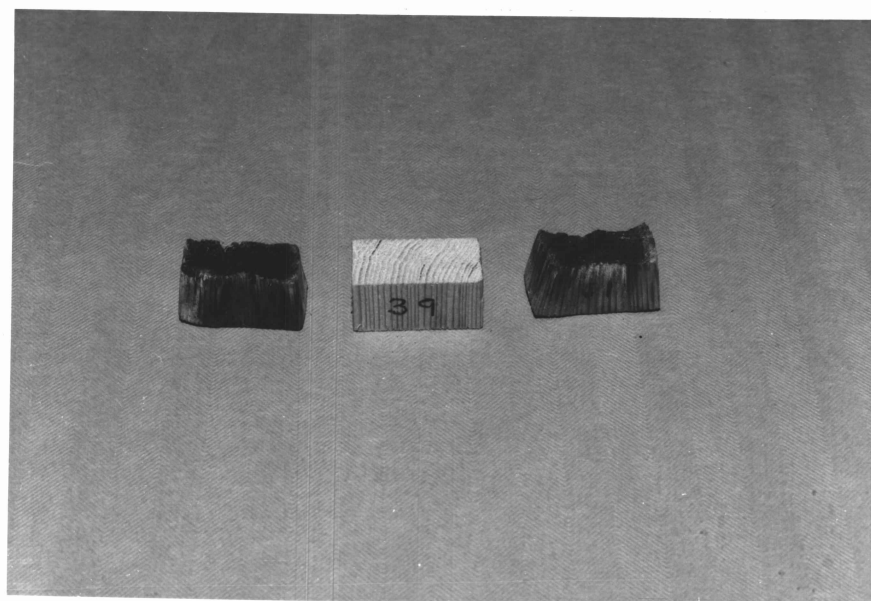


Figure 12. Results of the soil-block test. Wood blocks decayed by the brown-rot fungus, Poria monticola deformed on drying (extreme right and left). The "sound" block (middle) was attacked by the Imperfect-fungus, Rhinocladiella mansonii.

under this test method. Rhinocladiella mansonii showed the least effect on weight reductions of the test blocks. Effect of fungi on properties of hemlock blocks and birch sticks are compared in Table 7. Weight losses by both methods were very similar but breaking radius was a much more sensitive and rapid indicator of decay.

Weight loss, which is the most commonly used measure of decay, has some disadvantages. The chief difficulty is that there is commonly a great lag in commencement of weight loss than most of the other effects that have been studied. This lag is to be expected since release of CO_2 and metabolic H_2O , which causes most of the loss of weight is the last stage of the chemical changes induced by the fungi in the cell wall material. As a striking example, Cartwright and others (13) found no weight loss caused by Poria monticola in spruce at three weeks in an experiment in which moduli of rupture and elasticity showed 20 percent reduction in one week.

Tolerance of Test Fungi to Wood Preservatives

The relationships between retention of preservatives and average weight loss and breaking radius of the test sticks are classified by test fungi and presented in Table 8. Weight losses and breaking radius of individual sticks within a series showed considerable variation which leads to an understanding that weight loss alone does not necessarily mean that decay has occurred. Part or all of the weight loss

Table 8. Average Weight Losses and Breaking Radius of Test Sticks Treated to Various Retentions of Pentachlorophenol, Ammoniacal Copper Arsenite and Creosote by Five Test Fungi Using the Agar-Stick Method.¹

Preservative Retention (lb/ft ³)	<u>P. carbonica</u> weight breaking		<u>P. monticola</u> weight breaking		<u>S. commune</u> weight breaking		<u>H. lignicola</u> weight breaking		<u>P. fastigiata</u> weight breaking	
	loss %	radius % change	loss %	radius % change	loss %	radius % change	loss %	radius % change	loss %	radius % change
<u>Pentachlorophenol</u>										
0.50	0	0	0	60	0	0	0	0	0	0
0.20	0	155	0	220	0	0	0	0	0.3	155
0.10	1.2	460	2.9	540	0	155	0.1	60	0.4	220
0.05	3.5	620	4.2	700	0	300	0.1	155	0.5	220
Control	14.0	2140	15.0	2300	1.5	700	0.2	155	1.0	380
<u>Ammoniacal Copper Arsenite</u>										
0.40	4.0	300	4.5	460	0	0	0	0	0	0
0.20	5.8	460	6.6	700	0	0	0.2	0	0.1	60
0.10	6.3	700	7.2	1100	0	220	0.1	155	0.3	0
0.05	7.2	1100	8.0	1340	0	380	0.3	60	0.6	155
Control	14.7	1900	14.8	2220	1.2	700	0.2	155	0.8	460
<u>Creosote</u>										
10.0	0	60	0	0	0	0	0	0	0	60
5.0	0	155	0	0	0	0	0	0	0.3	155
2.0	3.8	380	3.2	155	0.6	300	0.2	155	0.6	155
1.0	5.1	540	4.5	460	1.0	460	0.1	60	0.8	300
Control	15.0	2300	16.0	2300	1.3	620	0.4	155	1.2	460

¹ Each value is the average of five sticks, one in each of five agar slants, except when contamination occasionally reduced number of sticks to not less than three.

² Average breaking radius of sound wet treated sticks was 0.125 inch.

may be due to evaporation of the preservative material during the test period. In evaluation of oil-type wood preservatives by soil-block and agar-block techniques, Duncan (24) found that it was quite possible, when testing blocks containing the higher retentions of some preservatives, to have a weight loss of ten percent with no visible attack by the fungus. On the other hand, decay was present when testing other preservatives, when there was a weight loss of only two percent. Therefore, to say there is no decay below or above a set percentage weight loss would lead to error.

In this discussion emphasis is placed on the lowest preservative retentions (threshold) which controlled decay in the test sticks. The threshold value determined are approximate in that they measure only the least retention of the preservative, in ranges studied, that protected the test sticks.

The test fungi in many instances demonstrated important differences in resistance to the preservatives under test. The soft-rot fungus, Phialophora fastigiata proved to be the most resistant to pentachlorophenol although weight losses and breaking radius of test sticks decayed by this fungus were very much below those of the decay fungi at the corresponding preservative retentions. The approximate threshold for this organism was 0.50 lbs. per cu. ft. of pentachlorophenol. Poria carbonica, P. monticola, and H. lignicola did not cause

decay at retention of 0.20 lbs. per cu. ft. Schizophyllum commune was very susceptible to pentachlorophenol.

Except for Schizophyllum commune, striking tolerance of the test fungi occurred with ammoniacal copper arsenite. Poria carbonica and P. monticola demonstrated strong tolerance of ammoniacal copper arsenite. In the retention ranges employed in this test only slight weight loss was observed for H. lignicola and P. fastigiata. Breaking radius values obtained for the test fungi were the highest in this test which was enough evidence for the resistance of the fungi to ammoniacal copper arsenite. Hyladendron lignicola did not cause decay at retention of 0.40 lbs. per cu. ft. of ammoniacal copper arsenite.

The soft-rot fungus, Phialophora fastigiata and the imperfect-fungus, H. lignicola exhibited considerable resistance to creosote. An approximate retention of 10 lbs. per cu. ft. prevented decay. The other fungi Poria monticola, P. carbonica and S. commune caused no decay at approximate retention of 5.0 lbs. per cu. ft. of creosote.

Comparison of these approximate threshold retentions emphasizes the resistance certain fungi show to specific preservatives and that these resistance patterns vary with the fungi and with the preservatives (Table 8). Vapors from the various treated test sticks appeared to differentially affect the growth rate and cultural characteristics of the test fungi. Creosote and pentachlorophenol treated sticks

caused mycelium in their vicinity to turn brown and appeared to initially repel the mycelium. These effects were evident the first few weeks of incubation for only sticks with lower retentions of preservative. Vapors from ammoniacal copper arsenite sticks did not cause any change of color of the mycelium in their vicinity.

The results confirmed the reported tolerance of the brown-rot fungi P. carbonica and P. monticola, to copper containing compounds (70), soft-rot fungus, Phialophora fastigiata and imperfect-fungus, H. lignicola to wood-preserving chemicals (23) and also the reported susceptibility of the white-rot fungus S. commune to copper containing compounds (70) and pentachlorophenol (19).

Young (70) in his work suggested that the high tolerance of the brown rots to copper-containing compounds is due to their habit of producing oxalic acid which it was supposed precipitates the copper into the insoluble form of the oxalate thus rendering the copper metabolite inert. The tolerance of soft-rot fungi to wood-preserving chemicals has been confirmed by several investigators (23, 54, 61, 47). In toxicity studies of soft rot in wood, Duncan (23) concluded that the various fungi capable of producing soft-rot show wide variations in tolerance to different wood preservative chemicals which in general represents as much or greater tolerance to preservatives than a number of common basidiomycetes wood-destroyers. In laboratory tests by Savory (54), the amount of pentachlorophenol

needed to reach the toxic limit for Chaetomium globosum was considerably higher than for Polyporus versicolor - one of the most phenol-tolerant Basidiomycetes in hardwoods. Scholles (61) found that the toxic limits for Chaetomium globosum might possibly represent the group, since five other soft-rot fungi had similar tolerances.

The smaller weight losses caused by the imperfect-fungi in the preservative evaluation method, indicate that in an intricate group of micro-organisms living in close association in preservative-treated shell of poles, the imperfect-fungi may not be necessarily responsible for major decay but may be capable of degrading the toxic constituents of the preservative into a less potent form, thus rendering it less effective in protecting wood from decay by less-tolerant basidiomycetes wood destroyers.

The results therefore indicate that considerable variation in tolerance to preservative materials exists within and among species of fungi that destroy wood. This variation shows that the isolates and species of fungi used in preservative evaluation tests should be selected on the basis of their tolerance to preservatives as well as for their prevalence in causing decay. Tests with susceptible fungi tend to overestimate the effectiveness of a toxicant. It is, therefore, desirable to use as test organisms the more tolerant fungi that frequently cause decay. Complete understanding of the phenomenon of resistance to toxicants in fungi requires more than merely determining

its incidence and magnitude. Variation in responses to toxic materials reflects physiologic differences among the fungi. Thorough study of these fundamental differences is necessary to explain this important and perplexing aspect of the variability found in biologic populations.

Characteristics of Hymenomycetes Isolated from Douglas-Fir Poles

Descriptions of the ten different hymenomycetes isolated from Douglas-fir poles are summarized in Table 9. All but one of the ten cultures were isolated from cores with visible decay. Most of the decay in the poles sampled was internal, usually several inches from the surface. Probably external rot was not encountered for one reason that most of the older lines sampled had been given groundline treatment within the last five years, which involved removal of external decay before treatment. The decay in the poles sampled was often associated with large cracks which may have developed after treatment and allowed fungi access to the poorly protected inner sapwood.

Six of the cultures in Table 9 showed the presence of moderate to strong brown diffusion zones indicating the formation of extracellular oxidase (Figure 7) whereas the remaining four of the cultures produced no diffusion zones, indicating lack of extracellular oxidase.

Table 9. Cultural Characteristics of Wood-Inhabiting Hymenomycetes Isolated from Douglas-Fir Poles.

Culture No. ¹	Rate of growth ²	Advancing Zone	Color of Mat	Fruiting Body in Culture	Reverse ³	Odor	Oxidase test
MJ 1/2	3	Even	white	similar to those produced in nature	unchanged	honey	+
MJ 4/2	4	appressed	white w/tinge of pale	none	unchanged	none	-
MJ 7/2	6	hyaline & appressed	pale yellow	none	unchanged	odor of apples	-
SJ 2/20	2	Even	white	none	bleached	none	+
SJ 11/19	6	Even	barium yellow	none	unchanged	strong odor of carbide	-
SJ 13/16	4	Even	cream	pored fruiting surface	unchanged	very strong suggesting turpentine	-
SJ 14/11	7	Bayed	honey yellow	none	dark	slightly spicy	-
SJ 15/21	4	even & submerged	white	immature fruit bodies	unchanged	very strong odor of pepper	-
SL 14/24	3	even & appressed	white w/tinge of cream color	none	bleached	strong "fish"	+
SL 15/4	6	even	straw yellow	compact raised areas	brown	none	+

¹ Includes Pole Line M - Murder Creek, J - Jefferson, S - Stayton, L - Lyons and pole number.

² Rate of growth is expressed as the number of weeks required by the fungus to cover the surface of the agar in the petri dish.

³ Reverse is the color changes in the agar induced by the growth of the fungus.

As stated by Davidson, Campbell and Blaisdell (22), species whose cultures lack extracellular oxidase are usually associated with brown rots and those species whose cultures produce extracellular oxidase are associated with white rots. The presence of the diffusion zones is the result of the oxidation of the tannic acid agar by the extracellular enzyme (oxidase) produced by the fungus hyphal body. This information about the type of rot that the species produce based on the oxidase test aid in classification by separating the species into two major groups as brown- and white-rot fungi.

Microscopic examination revealed that most of the cultures classified have simple and nodose septations at different locations on the fungus hyphal body which are typical of basidiomycetous fungi. Asexual spores, conidia, chlamydospores and oidia were present in some of the isolates which are rather unusual with hymenomycetes.

A large number of the Douglas-fir poles, from which the fungal isolates included in Table 9 were obtained, had been treated with pentachlorophenol prior to installation. This raised the question of the possible screening effect this treatment might have had on the fungal flora of these poles. It has been concluded from observations made over the years at the Forest Products Laboratory in Madison, Wisconsin that there is no evidence that the preservative itself has significant influence in determining the species of fungi infecting the

central, untreated interior of poles (30). These fungi apparently gained entry largely through seasoning checks or, perhaps, to a lesser extent, through inadequately treated sapwood.

CONCLUSIONS

On the basis of the experimental results, the following conclusions may be drawn:

1. When placed on malt agar, dry sticks at an average moisture content of about six percent decay about as rapidly as sticks at an initial moisture content of 40 percent.
2. Location of sticks, in or laid flat on agar, influences weight loss but not breaking radius; sticks on agar lost the most weight.
3. Moisture content of sticks on malt agar increased with incubation time.
4. Breaking radius appears to be a more sensitive and rapid indicator of decay than is weight loss and provides a reasonable basis for detecting decay fungi.
5. High breaking radius, an index of strength loss, often were attained with little weight loss.
6. Decay caused by the brown-rot fungi, Poria carbonica and Poria monticola was more severe and induced higher strength losses than that caused by the white-rot fungus, Schizophyllum commune and the fungi-imperfecti.
7. Schizophyllum commune caused relatively little weight loss and gave erratic results, however this organisms should not be regarded as harmless.

8. Except for Phialophora fastigiata, a soft-rot fungus, the non-basidiomycetes were not capable of causing appreciable weight losses of birch and western hemlock. They did, however, cause marked changes in breaking radius of birch sticks.
9. The soil-hemlock block and agar-birch stick tests provided identical weight loss rankings of the eight fungi.
10. The fungi-imperfecti, Hyalodendron lignicola and Phialophora fastigiata were more tolerant to wood preservatives than the brown- and white-rot fungi.
11. The brown-rot fungi, Poria carbonica and Poria monticola were highly tolerant to ammoniacal copper arsenite.
12. The white-rot fungus, Schizophyllum commune was susceptible to all preservatives except creosote.
13. Breaking radius merits consideration as a rapid and simple means of evaluating decay resistance of wood and preservatives for protecting wood.
14. The probable role of fungi-imperfecti in the performance of treated as well as untreated wood warrants more study because of their close association with wood-decaying basidiomycetous fungi in the interior of Douglas-fir utility poles.
15. The oxidase test method proved to be effective for classifying fungal isolates of wood-inhabiting hymenomycetes from Douglas-fir utility poles into brown- and white-rot fungi.

16. With white-rot fungi, the intensity of reaction and the time required for the reaction to take place showed considerable variation.

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APPENDIX

Sample of Data Recorded for Test Sticks Treated with Ammoniacal Copper Arsenite and Inoculated with a Brown-rot Fungus, Poria monticola.

Stick No.	Oven-Dry Weight gm	Treated Weight gm	Increase in weight gm	Concentration of solution % ¹	Chemical Retained		Oven-Dry Weight after incubation gm	Weight Loss percent
					gm	lb/cu. ft. ²		
101	0.225	0.505	0.280	1.30	0.0036	0.43	0.218	4.7
102	0.229	0.518	0.289	1.30	0.0038	0.43	0.222	4.7
103	0.233	0.528	0.295	1.30	0.0033	0.43	0.226	4.4
106	0.227	0.501	0.274	0.70	0.0019	0.29	0.214	6.6
107	0.224	0.520	0.296	0.70	0.0021	0.29	0.212	6.3
108	0.229	0.512	0.283	0.70	0.0020	0.14	0.214	7.4
112	0.224	0.500	0.276	0.30	0.0008	0.14	0.207	7.9
113	0.213	0.501	0.288	0.30	0.0009	0.14	0.198	7.5
117	0.231	0.504	0.273	0.20	0.00055	0.07	0.212	8.5
118	0.230	0.534	0.304	0.20	0.00060	0.08	0.212	8.1

¹ Grams of material in 100 grams of treating solution

² Pounds of material per cubic foot of wood in the stick after impregnation