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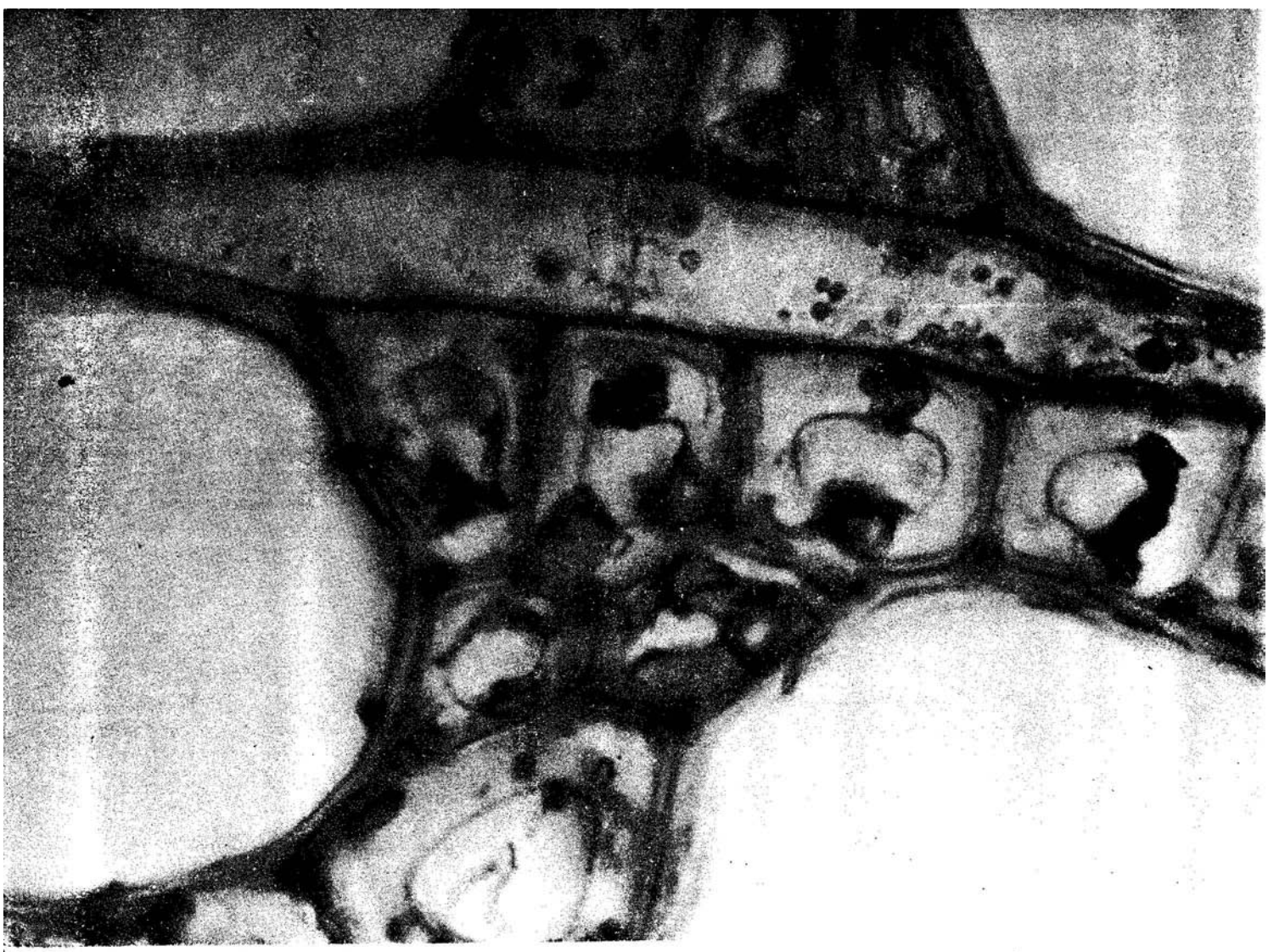

SUMMARY

A laboratory procedure is outlined that incorporates techniques found to promote soft rot by several fungi. This procedure employs either an agar or a soil substrate. Also presented are the principal findings of experiments underlying the procedure.

Results of tests conducted according to the suggested procedure are illustrated. The overall decay resistance of the softwoods typically was greater than that of hardwoods. The levels of decay in softwoods, however, were substantially higher than previously obtained. There was a tendency for decay in soil to be somewhat greater than on agar; however, a particular preference for either substrate was indicated only in a few instances.

DETERMINING RESISTANCE TO SOFT-ROT FUNGI

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INTRODUCTION

Fungi Imperfecti and Ascomycetes may cause a soft-rot type of wood decay by their utilization of cellulose in the secondary walls of tracheary cells (3,7,12).[2] Soft rot tends to be the dominant form of deterioration under extreme wetness or frequent dryness--conditions that retard or inhibit development of the more aggressive Basidiomycete wood-destroying fungi. It may be desirable to know what woods or preservative treatments can be used to avoid suchdeterioration under these environments.

To obtain at least preliminary information of this kind, there is need for an accelerated procedure that can be used satisfactorily with several of the principal soft-rot species. A soft-rot test procedure would, of course, also be valuable for purposes other than testing; it could serve advantageously for a variety of studies of the fungi developing in wood.

Methods of determining resistance to soft rot or of studying the causal fungi, in which soil or agar was used as the substrate, have been tried (5,6,9,11,12,14,15,17). These methods, generally, have not been adaptable to a sufficient variety of soft-rot fungi, and have the questionable feature of being designed to test preservatives without incorporating them in wood.

In particular, the standard soil-block testing technique, adopted by the American Society for Testing and Materials (ASTM D1413-61) (2) and used SO successfully with the Basidiomycete fungi, has been an unsatisfactory procedure for use with soft-rot isolates (7). Its inadequacy appears to be largely an inability to maintain sufficient moisture in the wood throughout the test period.

When definitely ascertained that the standard ASTM soil-block procedure could not be used satisfactorily with soft-rot fungi, an attempt was made to devise a procedure that could satisfac-

[1] Maintained at Madison, Wis., in cooperation with the University of Wisconsin.
[2] Underlined numbers in parentheses refer to Literature Cited at the end of this report.

torily be used with a number of fungi.

This report outlines a procedure, employing either an agar or a soil substrate, that incorporates techniques found to favor soft rot in the laboratory by several fungi. The experiments underlying the procedure are not described here in detail, but the principal findings, used as guidelines, are presented.

FACTORS THAT PROMOTE SOFT ROT

Results of miscellaneous previous studies and of experiments made specifically in search of an improved procedure indicated certain factors that seem to favor soft rot. Therefore, these factors should be given particular consideration in attempting to improve techniques of culturing the causal fungi. In all of the miscellaneous studies and experiments, unless otherwise indicated, the agar or soil substrate contained four basic salts and a complement of microelements and vitamins. The salts are ammonium nitrate, mono- and dibasic- potassium phosphate, and magnesium sulphate. The microelements and vitamins are boron, calcium, cobalt, copper, iodide, iron, manganese, molybdenum, zinc, glycine, nicotinic acid, thiamine, and pyrodoxine.

Research has shown a definite need for these microelements and vitamins in the nutrition of specific fungi; however, whether these requirements are the same for all fungi under all cultural conditions has not been established (10). A source of carbon, other than that in the test wood, was provided by a filter paper strip on the surface of the agar substrate or soil.

The factors that seem to have most influence in promoting soft rot under laboratory conditions and some of the observations pointing to them are discussed in the following paragraphs.

Minerals.--A solution containing ammonium nitrate, mono- and dibasic- potassium phosphate, and magnesium sulphate with a complement of microelements and vitamins applied either to the wood, in an agar substrate (covered by a strip of filter paper), or to the soil generally

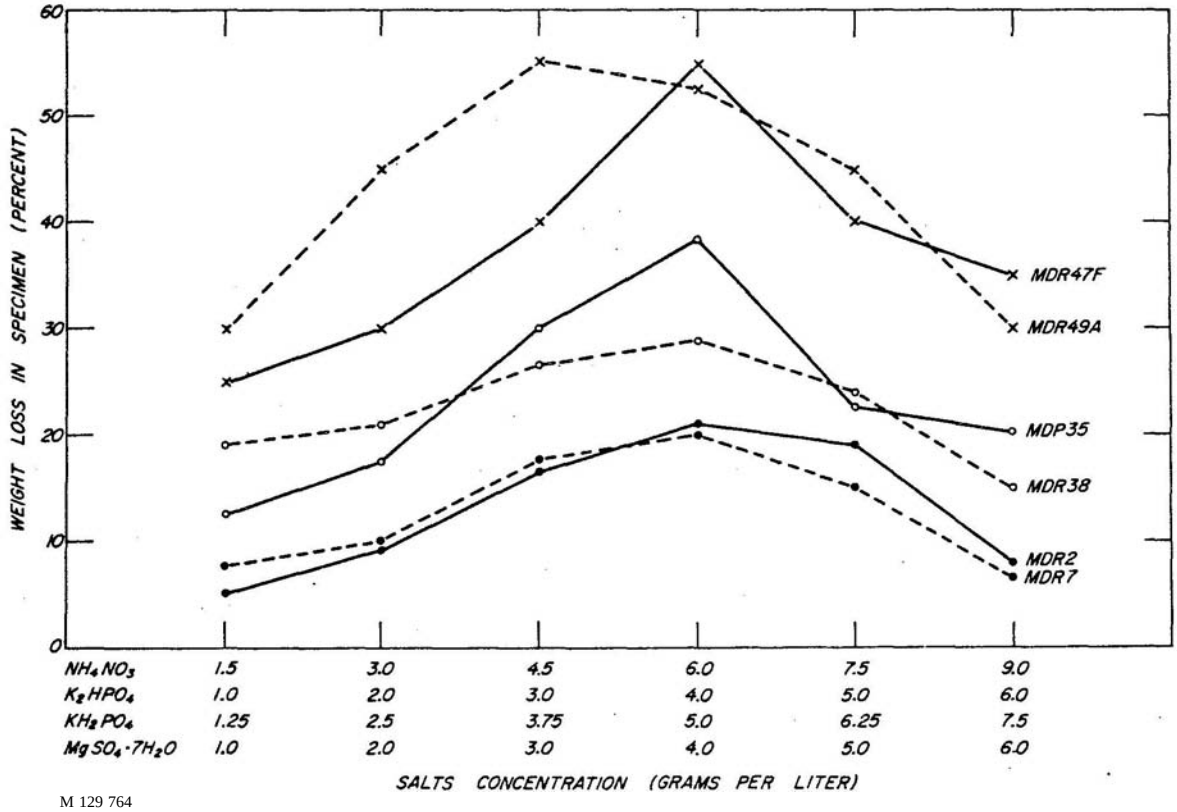

Figure I.--Effect of a salt mixture (in an agar substrate, containing microelements and vitamins, with a filter paper strip on its hardened surface) on soft rotting of sweetgum sapwood specimens during an 8-week incubation period. Legend: MDR47F, Graphium; MDR49A, Chaetomium; MDP35, Orbicula; MDR38, Monosporium; MDRZ, Phoma; MDR7, Phialophora.

increased soft-rot attack. An example of the advantage of using the nitrogen, phosphate, and magnesium medium is shown in figure 1. In the experiment on which the figure is based, specimens of sweetgum sapwood were laid on a square of filter paper resting on the agar. The rate of soft rot was maximum when the concentrations of salts, in grams per liter, were 6 of NH_4NO_3, 4 of K_2HPO_4, 5 of KH_2PO_4, and 4 of $MgSO_4 \cdot 7H_2O$.

Nitrogen.--In addition, it was found that although some of the fungi may prefer one form of nitrogen over another, all the fungi tested apparently utilize ammonium nitrate relatively well (table 1). At equal concentrations of nitrogen, this salt contributed as much to soft rot on an agar substrate as did the organic compounds, asparagin and urea. Also, ammonium nitrate was generally more effective than the other tested inorganic sources--sodium nitrate or ammonium sulphate.

Similarly, Savory (13) found that additional amounts of Abrams' salts mixture[3]--up to about half the normal amount--increased decay, by Chaetomium globosum, proportional to the salt concentration. Concentrations of the salts greater than two times the normal amount did not further increase the decay.

Glucose.--The addition of glucose to the agar medium, containing basic mineral salts, microelements, and vitamins and covered by a filter paper strip in amounts up to 0.25 percent, Caused

Table I.--Effect of nitrogen source in an agar substrate[1] on soft rotting of sweetgum sapwood specimens

Fungus	Nitrogen source and corresponding weight loss in test specimens[2]				
	NH_4NO_3	$NaNO_3$	$(NH_4)_2SO_4$	Asparagin	Urea
	Pct.	Pct.	Pct.	Pct.	Pct.
Orbicula sp. (MDP35)	48	35	40	50	46
Chaetomium globosum (MDR66)	56	40	42	56	53
Coniothyrium sp. (MDP36)	45	40	30	42	29
Phoma sp. (MDR2)	25	20	15	30	29
Pestalozzia sp. (MDP40)	30	25	28	35	32
Cephalosporium sp. (MDR35)	16	20	22	20	25
Monosporium sp. (MDR38)	30	21	25	29	28
Bisporomyces sp. (MDP6B)	19	15	18	22	21
Phialophora sp. (MDR7)	17	12	20	25	14
Graphium sp. (MDR47F)	58	50	48	45	40

[1] One liter of the agar medium contained 2.0 grams nitrogen as NH_4NO_3, $NaNO_3$, $(NH_4)SO_4$, asparagin, or urea; 4.0 grams K_2HPO_4; 5.0 grams KH_2PO_4; 4 grams $MgSO_4 \cdot 7H_2O$, microelements and vitamins, and 2.5 grams glucose. A filter paper strip was laid on the agar substrate.

[2] Each weight loss is an average of 10 test pieces.

[3] Abrams' salt solution contained, in grams per liter, 3 of NH_4NO_3, 2 of K_2HPO_4, 2.5 of KH_2PO_4, and 2 of $MgSO_4 \cdot 7H_2$.

a progressive increase in soft rot. The amount at 0.25 percent was nearly 15 percent greater than that produced without any glucose supplement. With 0.5 percent glucose, decay lessened slightly, and with 1.0 percent, it was about the same as at zero (0.0) concentration (table 2). Siu (16) referred to a partial inhibition of cellulytic activity by high concentrations of sugars. He suggested that this was probably due to the preferential utilization of the simple carbon compound.

High wood-moisture.--A comparatively high moisture content of the wood was found to be a necessary condition for rapid soft rotting (7). Very wet wood was best obtained by impregnating the test block with water before testing. Also, a thin test block placed directly on the wet soil or agar was more likely to retain a favorable moisture content.

Hardwoods vs. softwoods.--Hardwoods are recognized as being more susceptible to soft rotting than softwoods, at least in situations not involving ground contact. Provided with sufficient moisture, sweetgum or beech showed little resistance to a variety of fungi; whereas, only about half of the fungi tested appeared capable of seriously attacking a softwood such as pine sapwood, and none of them could attack the pine as readily as sweetgum or beech (7). One of the main objectives of the present study was to obtain a substantially more rapid attack on pine or other nondurable softwoods.

Temperature.--The temperature optimum of approximately one-half of the 40 species of soft-rot fungi studied earlier was found to be considerably higher than that among wood-destroying Basidiomycetes (7). Table 3 indicates that incubation at 32° C. generally promoted more rapid

Table 2.--Effect of amount of glucose in an agar substrate[1] on soft rotting of sweetgum sapwood specimens.

Fungus	Percentage of sugar and corresponding weight loss in test specimens[2]				
	0.0	0.1	0.25	0.5	1.0
	Pct.	Pct.	Pct.	Pct.	Pct.
Orbicula sp. (MDP35)	38	40	48	45	40
Chaetomium sp. (MDR66)	50	52	56	50	42
Coniothyrium sp. (MDP36)	40	43	45	44	41
Phoma sp. (MDR2)	20	20	25	21	19
Pestalozzia sp. (MDP40)	21	25	30	30	28
Cephalosporium sp. (MDR35)	12	14	16	15	10
Monosporium sp. (MDR38)	19	25	30	24	15
Bisporomyces sp. (MDP6B)	15	15	19	18	15
Phialophora sp. (MDR7)	12	15	17	15	14
Graphium sp. (MDR47F)	52	56	58	55	50

[1] One liter of agar substrate contained, in addition to different percentages of sugar, 6 gram NH_4NO_3, 4 grams K_2HPO_4, 5 grams KH_2PO_4, and 4 grams $MgSO_4 \cdot 7H_2O$, and microelements and vitamins.

[2] A filter paper strip was laid on the agar substrate.

[3] Each weight loss is an average of 10 test pieces.

Table 3.--Effect of temperature on decay of sweetgum sapwood by soft-rot fungi[1]

Fungus	Weight loss[2] caused by decay under indicated incubation temperatures		
	20° C.	26.7° C.	32° C.
	Pct.	Pct.	Pct.
Orbicula sp. (MDP35)	30	38	55
Chaetomium sp. (MDR66)	42	50	65
Coniothyrium sp. (MDP36)	34	40	48
Phoma sp. (MDR2)	5	21	28
Pestalozzia sp. (MDP40)	21	22	35
Cephalosporium sp. (MDR35)	10	18	20
Monosporium sp. (MDR38)	19	29	30
Bisporomyces sp. (MDP6B)	18	20	22
Phialophora sp. (MDR7)	9	19	30
Graphium sp. (MDR47F)	35	53	64

[1]The soil-burial culture technique described in the suggested test method was used for this experiment.
[2]Each weight loss is an average of 10 test pieces.

(about 12 percent) decay than 26.7° C., the temperature used. The latter temperature is considered near optimal for the Basidiomycetes in the standard test of resistance to decay by ASTM D1413-61.

pH.--More than half of 32 species of soft-rot fungi studied for pH relations grew at maximum rate (between pH 6 and 7) in a broad pH range (7).

Aeration.--The soft-rot fungi were found to have a greater tolerance of poor aeration than the Basidiomycetes (7, 8). This does not necessarily indicate that oxygen may not accelerate the decay process; nevertheless, there have been indications that increased carbon dioxide may stimulate soft rot, at least initially. This phase of the physiological studies has not yet been completed.

SUGGESTED PROCEDURE

Observed physiological characteristics of the soft-rot fungi and earlier findings of the general requirements of Basidiomycetous fungi in tests of decay resistance were used as guidelines. Numerous procedures that offered promise for evaluating--in the laboratory--resistance to a variety of soft-rot fungi were tried. From these trials, materials and methods that appeared to be broadly suitable for the purpose were selected. These are described in the following sections in the form of suggestions. For simplicity and clarity, the suggestions are generally quite specific but do not intend or imply a lack of room for additional refinements.

Specimens

Selection of wood.--Wood used for specimens should be free of excessive resins or unusual accumulation of extractives, and should not show any visible signs of previous infection by molds, stain, or wood-destroying fungi.

The sapwood or heartwood of any species may be tested for its natural resistance to a soft-rot

fungus. However, it should be all sapwood or all heartwood in any given comparative series. To determine only whether a particular fungus isolated from soft-rotted wood is the causative organism, a nondurable hardwood like the sapwood of beech or sweetgum is especially suitable--regardless of the species of the original wood.

The sapwood of sweetgum (Liquidambar styraciflua) is well suited for determining the relative fungicidal value of a given preservative. If desirable to include a softwood as well as hardwood, the sapwood of ponderosa pine (Pinus ponderosa) may be used.

Size and number of specimens.--A good specimen size is 0.4 by 1.5 by 3.0 centimeters. For the most rapid decay, the short axis of the piece should be parallel to the grain of the wood. This type of cut, however, provides a proportionately large cross sectional face which may accentuate the loss of preservative. Therefore, when testing involves a preservative, the piece should be cut so that the long dimension is parallel to the grain of the wood.

The number of replicate specimens needed for exposure to a particular fungus may vary, but a minimum of five should be used. For each replicate group designated for exposure to a fungus, there should be an equal number of specimens given identical treatment and handling--except that they are not exposed. The purpose of these is to reveal any losses in weight, during the exposure period, not caused by fungal activity.

Fungi

Fungi selected should be commonly associated with soft-rotted wood, grow well under laboratory conditions that favor substantial attack on both sweetgum and ponderosa pine sapwood, and be relatively tolerant of a number of chemicals (7). Fungi in the following genera are suggested for use in appraising soft-rot resistance:

Graphium
Phoma sp.
Orbicula
Pestalozzia
Chaetomium

Culture Container and Substrate

Container.--A 237-milliliter (8-ounce) French square bottle such as used in ASTM D1413-61(2) is satisfactory as a culture chamber. Either an agar or soil substrate may be used. Results obtained with the two tend to be comparable, as

Table 4.--Natural decay resistance of 12 woods against soft rot fungi.[3] Results were obtained by the soil-burial and mineral-agar testing procedures

Wood species[1]	Incubation substrate[2]	Weight loss in test pieces caused by indicated fungi[3]									
		Orbicula sp. (MDP35)	Chaetomium globosum (MDR66)	Coniothyrium sp. (MDP36)	Phoma sp. (MDR2)	Pestalozzia sp. (MDP40)	Cephalosporium sp. (MDR35)	Monosporium sp. (MDR38)	Bisporomyces sp. (MDP68)	Phialophora sp. (MDR7)	Graphium sp. (MDR47F)
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
					HARDWOODS						
Sweetgum (Liquidambar styraciflua)	S	45	60	43	24	30	16	30	15	20	60
	A	44	65	40	22	29	18	27	17	18	58
Sugar maple (Acer saccharum)	S	40	48	35	19	31	20	22	12	11	58
	A	38	50	35	18	30	19	19	12	12	59
Cherry birch (Betula lenta)	S	51	52	32	30	38	25	45	33	24	55
	A	50	55	30	32	36	21	44	30	24	52
Chestnut (Castanea dentata)	S	20	29	18	8	25	7	13	8	12	30
	A	22	33	20	10	22	5	12	9	13	28
White oak (Quercus alba)	S	38	45	34	21	32	20	27	20	15	40
	A	35	52	35	19	29	19	22	17	12	38
Black locust (Robina pseudoacacia)	S	15	12	20	12	10	4	11	7	7	19
	A	9	14	12	10	10	6	9	6	7	20
					SOFTWOODS						
Ponderosa pine (Pinus ponderosa)	S	12	15	10	5	7	17	9	8	6	25
	A	7	5	9	5	6	10	9	4	4	20
White spruce (Picea canadensis)	S	18	19	15	10	12	17	10	8	9	38
	A	10	6	12	8	10	12	9	3	7	32
Douglas-fir (Pseudotsuga menziesii)	S	10	7	9	7	8	11	6	6	4	19
	A	8	3	7	5	5	9	5	4	2	10
Redwood (Sequoia sempervirens)	S	5	9	6	5	4	6	3	2	4	12
	A	2	4	4	3	3	5	3	2	4	5
Western redcedar (Thuja plicata)	S	6	8	5	8	7	6	4	2	5	10
	A	3	2	5	7	6	5	6	2	3	8
Western hemlock (Tsuga heterophylla)	S	17	16	16	12	10	15	9	7	10	22
	A	12	6	10	10	7	14	7	6	8	20

[1] Sweetgum and pine are sapwood; all other species are heartwood.
[2] Incubation substrate: S = soil-burial; A = mineral agar. Both techniques are described in text.
[3] The weight losses are net losses, representing those over and above the losses that occurred in control blocks similarly exposed, except for the presence of a fungus. Each is an average result for 10 test pieces. Soft rot was microscopically found in all test pieces.

illustrated in table 4.

Agar substrate. --The following method, employing an agar substrate, is based on the mycelial-mat method described by Abrams (1) for testing the resistance of rot-proofed fabrics against Chaetomium globosum. It is also similar to that described in the British standard (4) for testing wood or preservatives against the same fungus. The medium should contain the following ingredients, each added to water in the order listed:

Ammonium nitrate (NH_4NO_3)	6 grams
Potassium hydrogen phosphate (K_2HPO_4)	4 grams
Potassium dihydrogen phosphate (KH_2PO_4)	5 grams
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	4 grams
Glucose	2.5 grams
Microelement and vitamin concentrate-[4]	10 milliliters
Agar	15 grams
Distilled water	1,000 milliliters

Adjust the pH of the liquefied mixture to 6, place 50 milliliters of the medium in each test bottle, and autoclave at 121° C. for 15 minutes. After removal from the autoclave, allow the bottles to cool in an upright position. After the agar substrate hardens, a sterile piece of ashless filter paper, approximately 1.7 by 3.2 centimeters, should be placed aseptically on the surface.

Soil substrate.--The selected soil should have a water-holding capacity of at least 40 percent and a pH between 5 and 7 (note 4, 7b in ASTM D1413-61) (2). Soil for tests, from which close comparisons are to be made, should be mixed after breaking up all clumps, sifted through a No. 6 sieve, and air dried. The soil is air dried so that nutrients, dissolved in the water to be added, will be equally distributed.

Fill the 237-milliliter culture bottle about half full with the sifted air-dry soil. Lightly compact the soil by tapping. Place a 1.7- by 3.2-centimeter piece of ashless filter paper on the leveled soil surface. Wet the soil in the completed test bottle until the percentage of water is at least 100 percent of the water-holding capacity. The added water should contain the same nutrient ingredients as listed for the agar substrate. If the water is introduced directly onto the filter paper, it will not disturb the leveled condition of the soil surface.

Autoclave the prepared bottles, with the caps slightly loosened, at 121° C. for 30 minutes.

Preparation of Cultures

After the culture bottles and contents have been sterilized and cooled, they may be inoculated. The soft-rot fungi do not spread rapidly, but complete and early coverage of the filter paper and growth into soil is desired. This can be obtained by inoculating the substrate at a large number of places so that growth can start from many areas as follows:

A 2-week-old agar-slant culture is covered with approximately 10 cubic centimeters of sterile distilled water and the mycelium aseptically scraped loose. The water and mycelium is then transferred to a sterile tissue grinder (or a blender), where the mycelium is broken up into small fragments. The minced mycelium is then suspended in 50 milliliters of sterile distilled water. Finally, approximately 1 milliliter of the water and the mycelial fragments (together with the spores, if present) is pipetted onto the surface of the filter paper resting on the agar or soil substrate.

At approximately 32" C. and 70 percent relative humidity, incubate the inoculated bottles, with the lids released one-half turn from a tightened position, until the filter paper is heavily covered by the mycelium. The bottles are then ready to receive the specimens.

Preparation of Specimens

The specimens to be tested should be dried at approximately 105" C. for 12 hours, or conditioned

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[4] A liter of the concentrate contains, in grams, the following: 0.075 of KI, 0.44 of $MnSO_4$, 0.05 of $CuSO_4$, 0.15 of $ZnSO_4$, 0.16 of H_3BO_3, 0.05 of $CoCl_2 \cdot 6H_2O$, 0.10 of $CaCl_3$, 0.10 of $Na_2MoO_4 \cdot 2H_2O$, 0.2 of glycine, 0.05 of nicotinic acid, 0.01 of thiamine hydrochloride, 0.05 of pyridoxine hydrochloride 0.341 of $FeCl_3 \cdot 6H_2O$, and 0.465 of $Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$. The latter two compounds must be dissolved and mixed together before adding them to the mixture. Unused concentrate should be frozen.

to constant weight at a uniform relative humidity and temperature. Then weigh them to the nearest 0.001 gram. This weight is considered the initial weight.

Testing natural decay resistance.--To test the natural resistance of a wood sample, first impregnate the weighed specimen5 with distilled water. To do this, weight the specimens in a beaker and cover them with the water. Place the beaker in a desiccator or bell jar attached to a vacuum pump and to a manometer. Reduce the pressure to 10 centimeters or less of mercury and hold for 20 to 30 minutes or until all the specimens sink in the water when the pressure is released. To complete preparation, quickly surface dry them between towels, place them in closed containers, and steam for 20 minutes at atmospheric pressure. They are then ready for introduction into the test chamber.

Testing a preservative.--To test the effectiveness of a preservative against soft rot, make up treating solutions in gradient concentrations. The specimens should contain retentions running from below to above an anticipated threshold (the .minimum retention needed to reduce decay to a level where it is practically uncorrelated with retention). The lowest retentions should permit fungus attack and consequent decay sufficient to produce definite weight loss. The required solution concentrations should be made up with distilled water if the preservative is to be carried in water, and with toluene if it is to be carried in oil.

If the approximate threshold is not known, it is helpful to run a reconnaissance test employing a relatively wide range of retentions. Then, in the final test, narrow the range of retentions to the general level of the anticipated threshold.

The specimens should be treated according to the procedure outlined in ASTM D1413-61, 9(d) (2). The retentions, using the volume of a specimen (0.4 by 1.5 by 3.0 centimeters) as 1.8 cubic centimeters, can be calculated as in 9(e) (2). It is not considered necessary, however, to calculate retentions for all specimens individually. If the specimens are accurately cut and have been selected with similar weights (within + 0.01 gram), all will absorb essentially the same amount of preservative from a solution of a given concentration.

Impregnated specimens should be dried for 48 to 72 hours in the laboratory and then placed in a constant temperature-humidity room (conditioning room) for 21 days. Then, they are ready for the weathering procedure.

Wood that is prone to considerable leaching is most likely to be attacked by soft-rot fungi. Consequently, it seems logical that a weathering phase should routinely be included when permanence of the preservative under wet conditions is needed as an integral part of its appraisal. The ASTM D1413-61 (2) weathering phase does not provide for the leaching expected of preservatives under very wet conditions. Therefore, the following procedure is at present suggested. The actual extent of leaching should be determined by chemical assay.

To leach, place dry specimens of the same kind or treatment in a beaker, weight them down, and cover with distilled water. The water covering the specimens should be approximately 10 times the volume of the specimens. Place the beaker in a desiccator or bell jar attached to a vacuum pump or to a manometer. Reduce the pressure to 10 centimeters or less of mercury and hold for 20 to 30 minutes, or until all the pieces sink when the pressure is reduced. Change the water in the beaker every 2 hours between 8 and 17 hours, and leave the specimens in distilled water overnight. Repeat this cycle for 4 days. At the end of the fourth day, quickly remove surface water from the specimens by blotting them between towels. Place the specimens of the same kind or treatment in tightly covered containers, and steam them for 20 minutes at atmospheric pressure. The weathered specimens are then ready for test chambers.

Exposure of Specimens

When the specimens have been prepared for exposure to the fungi by impregnation with distilled water, steamed aseptically for 20 minutes at atmospheric pressure, and cooled, they are ready for placement aseptically in the test chamber. If the agar substrate is being used, each piece should be placed with a tangential or cross sectional face on the fungus-covered filter paper. If the soil substrate is being used, the piece should be pressed edgewise (penetrating the filter paper) into the soil until its upper edge is just below the soil surface.

Incubation and Duration of Test

Keep the test bottles for 12 weeks in an incubation room maintained at approximately 32° C. and 70 percent relative humidity.

Handling Specimens after Exposure

At the end of the incubation period, remove the specimens and carefully free them of surface mycelium. Superficial growth of soft-rot fungi forms a slime which is difficult to eliminate without removing some of the softened wood. Careful brushing under a stream of warm water may prove helpful. Preliminarily air dry the pieces in the laboratory. Then, condition or ovendry them in exactly the same way as was done preceding the weighings, and obtain their final weights.

Appraisal of Attack

Attack by a test fungus can be appraised in one or more of three ways: visual inspection, microscopical examination, and loss in weight.

Visual inspection, --Examine the blocks after they have been dried and weighed at the completion of the test. Distortion, shrinkage, and softening of a specimen, especially the surface wood, are indications of rot. Longitudinal checks along the grain are often additional evidence of attack.

Microscopical examination. -- Microscopical examination cannot be used to estimate closely the amount of decay. However, it will usually disclose whether the cell walls have been attacked in the typical manner of soft rot (3, 7, 12). In soft rot, the fungus hyphae ramify within the cell wall and make cavities with pointed ends. The cavities run spirally lengthwise in the cell wall, following the direction of the cellulose fibrils. In cross sections, the cavities appear as holes that equal or exceed the diameter of the hyphae. Soft rot is generally confined to the less lignified secondary walls of the tracheary cells and fibers. It is more conspicuous in summerwood than in springwood, especially in a softwood.

Loss in weight.--Weight losses of sufficient magnitude to be measured reliably furnish the simplest means of observing the activity of the fungus. If the weight loss is less than 5 percent, rot should be verified microscopically since the fungus may utilize sugars, extractives, or resins, causing a small weight loss without any attack of wood cellulose. This loss in weight will not be shown by the control specimens in test chambers without the fungus.

Calculate the percentage weight loss of the pieces from their initial or initial plus preservative weight and their final weights. Both sets of weights should be taken on blocks similarly ovendried or conditioned at a constant moisture and humidity. Consider the loss due to fungus activity to be the difference between the weight loss of blocks subjected to the fungus and that of similar blocks not exposed to the fungus.

Examples of Test Data

The data presented in table 4 show typical results of tests conducted according to the suggested procedures to determine the natural soft-rot resistance of a waterborne preservative of a number of hardwoods and softwoods. All 10 species of fungi were able to attack most of the hardwoods severely. Thus, apparently any of them would serve adequately for testing hardwoods. Chaetomium globosum was expected, based on earlier experiments and reports of others, to be one of the most destructive species. This proved to be the case. However, the Graphium exhibited at least an equal capacity to attack the hardwoods. Two additional species, Orbicula and Coniothyrum, were only moderately less destructive--and then only on the most susceptible woods.

Correlations between weight losses produced by the 10 fungi in the different hardwoods are shown in table 4. There is, nevertheless, enough disagreement to suggest that probably none of the fungi would, by itself, depict relative rot resistance in hardwoods sufficiently to warrant a restriction in any testing procedure.

The overall rot resistance of the softwoods typically was materially greater than that of the hardwoods. The most susceptible softwoods incurred weight losses little or no larger than corresponding losses in the most resistant hardwood--black locust. Nevertheless, the culturing technique and appropriate selection of fungi resulted in levels of rot in the softwoods that were substantially higher than previously obtained reliably. But most important, the weight losses produced by five of the fungi were sizable enough to easily qualify the combination of procedure and fungi for testing softwoods.

In particular, Orbicula, Chaetomium, Coniothyrium, and Cephalosporium, in each case, caused losses ranging between 15 and 20 percent. The Graphium was outstanding in causing losses to 38 percent. Results with four of these five fungi were in agreement--relating the highest rot resistance to the redwood and western redcedar, and intermediate resistance to the Douglas-fir,

On the whole, the weight losses developed in testing natural resistance were much. alike on the soil and agar substrates. There was a small tendency for the losses in soil to be somewhat larger than those on agar. However, only in a few instances could the differences be considered large, Thus, no particular superiority of performance was indicated for either substrate,

Table 5 shows results typical of those obtained when using the procedure for appraising preservatives. It also shows results typical of those obtained in analyzing the procedure for suitability as a means of appraising preservatives for protection against soft rot, The wood in which the preservative was incorporated in this instance was sweetgum sapwood. When there is need to use a softwood, this can be done successfully by combining a relatively susceptible species like ponderosa pine sapwood with fungi most able to attack softwoods.

In testing preservatives, one of the criteria of suitable procedure is its sensitivity to small differences in amount of the same preservative. By this standard, the procedure used here has performed adequately, with either soil or agar substrate, and with any of the four fungi represented in table 5. The threshold retention of preservative may be somewhat higher whenusing

Table 5.--Resistance to soft rot provided by a copper-chrome-arsenate salt mixture. The results on fungus tolerance to preservatives were obtained by the soil-burial and mineral agar procedures

Copper-chrome-arsenate salt mixture in water		Weight loss in test pieces[1] caused by indicated fungi			
Concentration	Retention	Phoma sp. (MDR2)	Coniothyrium sp. (MDP36)	Graphium sp. (MDR47F)	Chaetomium globosum (MDR66)
Pct.	Lb. per cu. ft.	Pct.	Pct.	Pct.	Pct.
		SOIL-BURIAL SUBSTRATE			
0.0	0.00	20	44	60	60
.2	.075	18	40	51	50
.4	.17	14	31	38	53
.6	.26	12	20	32	41
.8	.35	9	5	26	30
1.0	.43	8	2	14	13
1.5	.64	7	0	10	10
2.0	.85	0	0	8	4
		MINERAL-AGAR SUBSTRATE			
0.0	0.00	22	41	46	65
.2	.075	16	31	36	51
.4	.17	13	22	26	52
.6	.26	6	17	17	45
.8	.35	4	4	12	34
1.0	.43	3	0	6	16
1.5	.64	0	0	4	5
2.0	.85	0	0	4	0

[1] The weight losses are net losses, representing those over and above the losses that occurred in control blocks similarly exposed, except for the presence of a fungus. Each is an average for 10 test pieces. Soft rot was microscopically found in all test pieces where a weight loss is indicated.

soil rather than agar for the substrate. However, without specific evidence to indicate otherwise, this should not necessarily be regarded as an advantage.

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