A Laboratory Evaluation of Wood Preservatives

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Evolution of a simple laboratory technique for the assay of materials proposed for use in the preservation of wood is reported in this paper. This test involves a measurement of the actual decay resistance of the treated wood. Included are a resumé of the limitations imposed by current test-methods and a discussion of the adaptations of this new technique to the numerous variables inherent in laboratory simulations of outdoor exposure.

FUNDAMENTAL scientific discoveries in the biological sciences during the latter part of the nineteenth century slowly brought organized knowledge out of the chaos of conflicting theories as to the character of many natural phenomena. This was especially true in the field of fermentation where these accumulated findings and observations finally served as the basis for the proof that the filamentous fungi were the causal agents in the decay of wood. This knowledge of the decay mechanism, together with increased demand for wood products due to industrial expansion and the concomitant depletion of our best stands of naturally rot-resistant species of timber, served as a stimulus towards organized studies of the physiology of decay organisms and possible means of prophylaxis.

While Nature has been lavish in the supply of fast-growing species, she has also been provident in making such timber more vulnerable to attack by the micro-flora and fauna which act as scavengers for the forests and as conservators for vast quantities of materials which trees take from the soil during their growth period. The necessity of preserving this more easily decayed wood accelerated the search for satisfactory means of protection. This has been especially true in the Bell System where fast-growing but easily rotted southern pine has, to a large extent, been supplanting chestnut and cedar for poles.

In the past the use of certain materials for the preservation of wood was based entirely on their availability or the personal prejudice of proponents for them. This method of selection could result only in widespread waste and oftentimes disastrous consequences, but a wood-preserving industry utilizing certain materials such as coal-tar creosote gradually evolved. The controversies as to what properties of creosote make it an effective preservative still rage, and the problem of choosing and specifying the type of creosote best fitted for the preserva-

tion of timber is still urgent. This is particularly vital in that creosote is a loose term covering a congregation of compounds rarely twice the same in quality or proportion.

When in 1927, research on the development of a rapid means of evaluating wood preservatives was initiated in the Chemical Department of these Laboratories, primary consideration was accorded the selection of the best available method for measuring the toxicity of proposed preservatives against wood-destroying fungi. The technique used was one which had been developed and extended to a considerable degree by the workers at the Forest Products Laboratory in Madison, Wisconsin. This petri dish method, described at some length by Richards in 1923,[1] was standardized in 1929[2] at a conference of American workers in St. Louis. Briefly, the method consists in adding various amounts of the toxic agent under test to a nutrient medium in the form of a hot malt-agar solution which is poured into a petri dish, cooled, and the resulting gel inoculated with small sections of the hyphæ of a wood-destroying fungus (Fig. 1). The organism usually used is culture no. 517 from the Forest Products Laboratory but others may be chosen. The excellence of the preservative is based on the lowest concentration which is able to kill or totally inhibit growth of the test organism.

While the petri dish method can be brought to a high degree of efficiency, accuracy and precision by suitable precautions, it is definitely limited in its practical application. It tells nothing of the permanency of the material under test from the standpoint of leaching, evaporation or chemical instability. Nothing is learned of the possible reaction of the preservative with wood, and the dispersion in warm liquid agar which later gels is a far cry from that obtained in wood. There is an axiom of biological assay, that the substratum for *in vitro* tests be as similar as possible to that encountered in nature. Neglect of this principle in the field of antiseptics and germicides has been responsible for many outstanding failures *in vivo* of materials which had given brilliant promise in the culture tube. Doubt concerning the validity of the petri dish test was substantiated when several preservatives highly toxic according to this method failed in outdoor exposure tests. In many cases such failures could not be ascribed to obvious conditions such as high volatility or solubility. Instances were also met wherein materials of little value according to the petri dish method were able to prevent decay in the field. There is no disposition to advise against all use of this method as it is a valuable tool in making initial judgments on a new material; but it should be verified by other means before the expense of a field trial can be justified, and a

[1] Numbers refer to bibliography at end.

proper degree of skepticism should be exercised before condemning a preservative on the basis of this test alone.

Parallel with the use of nutrient substrata of the malt-agar type in this country, there grew up in Europe a technique which utilized the wood itself as a medium for dispersion of the toxic agent. This kolle flask method (Fig. 2) was standardized and accepted by a conference

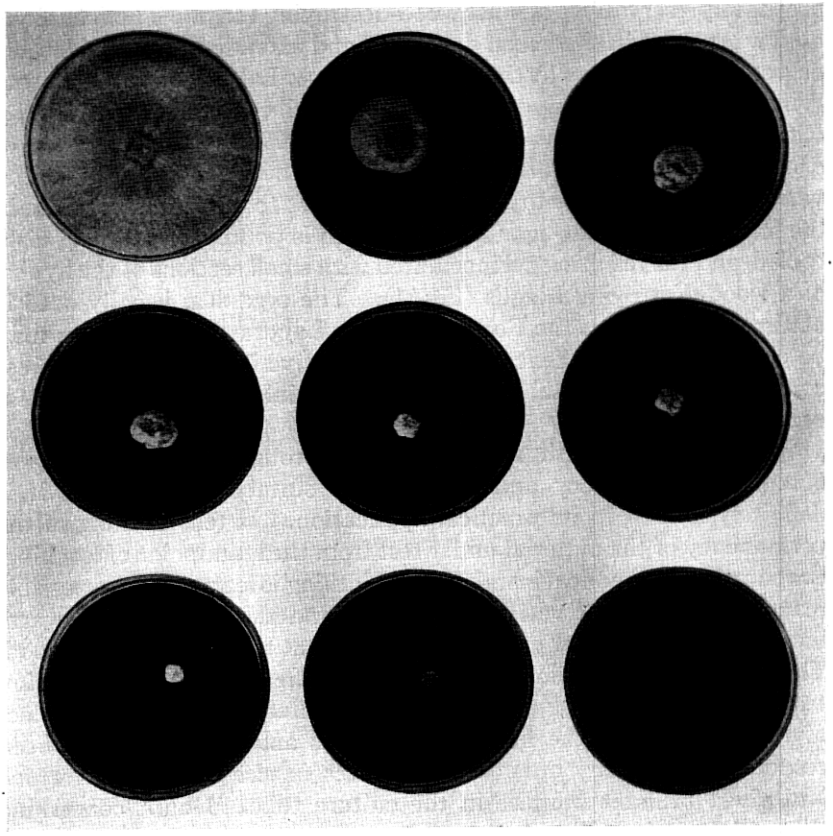

Fig. 1—Assay by petri dish method. Test-fungus No. 517 on increasing amounts of coal-tar creosote.

of European workers at Berlin in 1930.[3] An outline of the method follows: Wood blocks of a convenient size are impregnated with the toxic agent, usually in solution, and after evaporation of the solvent the blocks are placed in kolle flasks and supported on glass rods set in malt-agar covered with the actively growing mycelia of the test fungus. The conference advised the use of *Coniophora cerebella* as the test

fungus, but suggested that at least two species should be used in each test. After three or four months' exposure to the wood-destroying fungi, the blocks are removed from the flasks, freed from adhering mycelium, and the weights taken before and after the test period used as a measure of the amount of decay.

The kolle flask method has much to recommend it, overcoming as it does many of the difficulties inherent in the petri dish technique. However, the test as standardized at Berlin presents serious drawbacks. The kolle flasks are expensive, comparatively fragile, difficult to

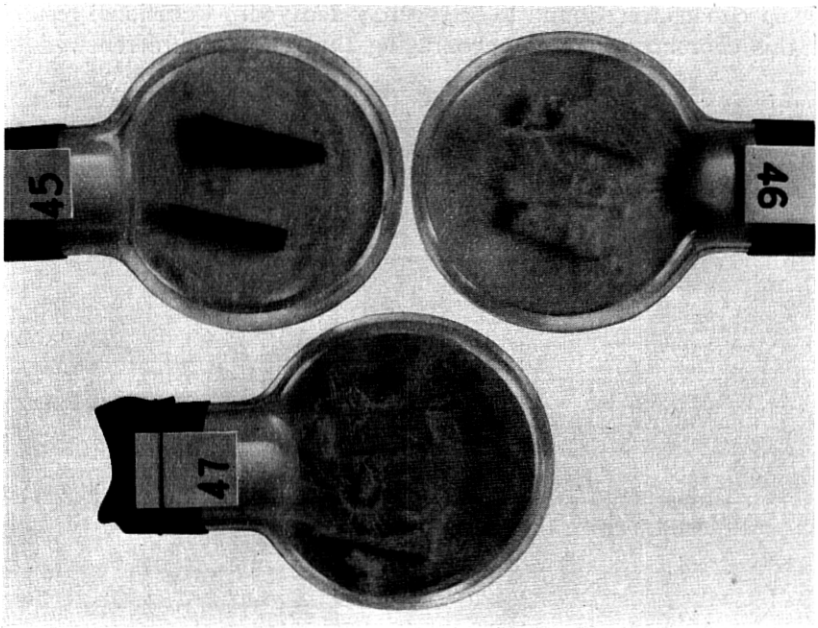

Fig. 2—Assay by kolle flask method. *Poria incrassata* used in comparison of southern pine heartwood versus sapwood.

handle, inconvenient to store, and maintenance of proper moisture conditions is particularly difficult. For really satisfactory results the flasks during the tests should be kept at a constant humidity and temperature. Despite all precautions there is the ever-present danger of excess moisture and resultant lack of decay should the test block touch the agar or any condensed moisture on the flasks. Another unusual problem arose when certain over-ambitious fungi rotted the cotton plugs used to stopper the flasks and even continued to grow into other flasks where they did not belong.

A New Assay Method

Both the petri dish and kolle flask methods had shown definite limitations, and it became apparent that further experimentation on a laboratory assay-method should be directed along somewhat different lines. By chance a few treated pieces which had been removed unscathed after a routine exposure in the kolle flask, were dropped on a beakerful of moist wood heavily infected with a wood-destroying fungus. The beaker was merely covered with a watch-glass and set aside. Growth progressed over the treated blocks with unexpected rapidity and vigor, and when removed at the end of three months, the specimens were found to be severely decayed. Occasional results of this character were so encouraging that efforts were renewed to

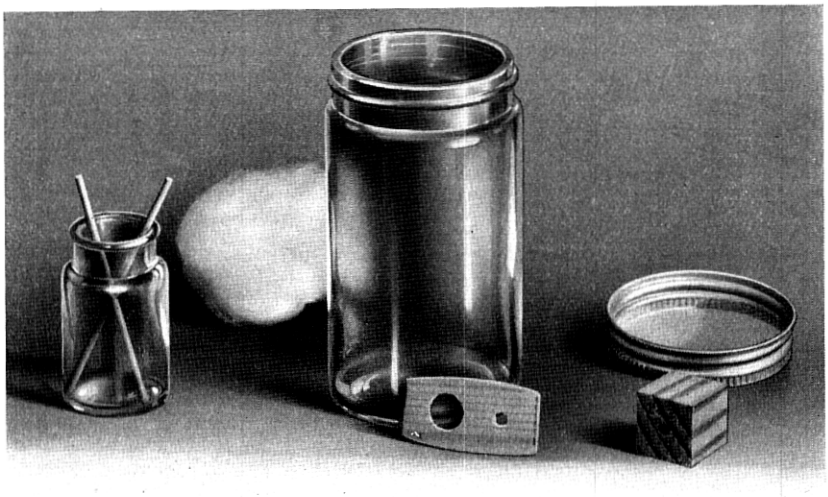

Fig. 3—Apparatus required for modified wood-block method of assay.

develop a technique which would incorporate the use of wood as a secondary substrate together with more favorable moisture control. Experimentation had demonstrated that the amount of moisture in the wood block should be slightly above fibre saturation for optimum growth of the fungus. Inoculated wood placed in air at 100 per cent relative humidity will rot but slowly while too much moisture decelerates and even inactivates the fungal metabolism. The problem therefore was to bring about these optimum decay conditions with low-priced, easily handled equipment. The test in its present state of evolution is inexpensive, easy to manipulate and capable of increased uniformity due to better regulated moisture conditions. The only

equipment necessary consists of a straight-sided screw-capped bottle about 5 inches high and 2 inches in diameter, a smaller bottle 2.5 inches high and 1 inch in diameter, a wad of cotton, a small flat piece of untreated wood and an applicator such as is used by the medical profession for swabs (Fig. 3).

The treated blocks, previously brought to moisture equilibrium, are supported by means of a thin slab of untreated wood on the top of the small bottle which is placed inside the larger screw-topped bottle.

Fig. 4—Assembly of apparatus required for modified wood-block method of assay.

Through holes bored in the test piece and the thin slab of supporting wood are passed the pieces of wooden applicator, which act as a means of anchorage and as wicks for conduction of water to the wood under test. Although not absolutely necessary, cotton is usually wrapped around the small bottle to reduce shock during handling. Water is placed in both bottles and after sterilization of the complete set-up (Fig. 4) the thin slab of wood is inoculated with a portion of

hyphæ of the test-fungus which has been growing on a malt-agar substratum. The bottles are then placed in an incubation room (Fig. 5) at 26–28° C., customarily for a period of 24 weeks. At the end

Fig. 5—Incubator with tests in progress.

of the test period the blocks, freed of adhering mycelium, are again brought to equilibrium at a specified humidity, reweighed and the pieces finally dissected to determine the loss of strength occasioned by the attack of the fungus.

Materials to be tested as possible preservatives are injected in serial concentrations into the blocks of sapwood, commonly southern pine, under conditions simulating as nearly as possible those which would

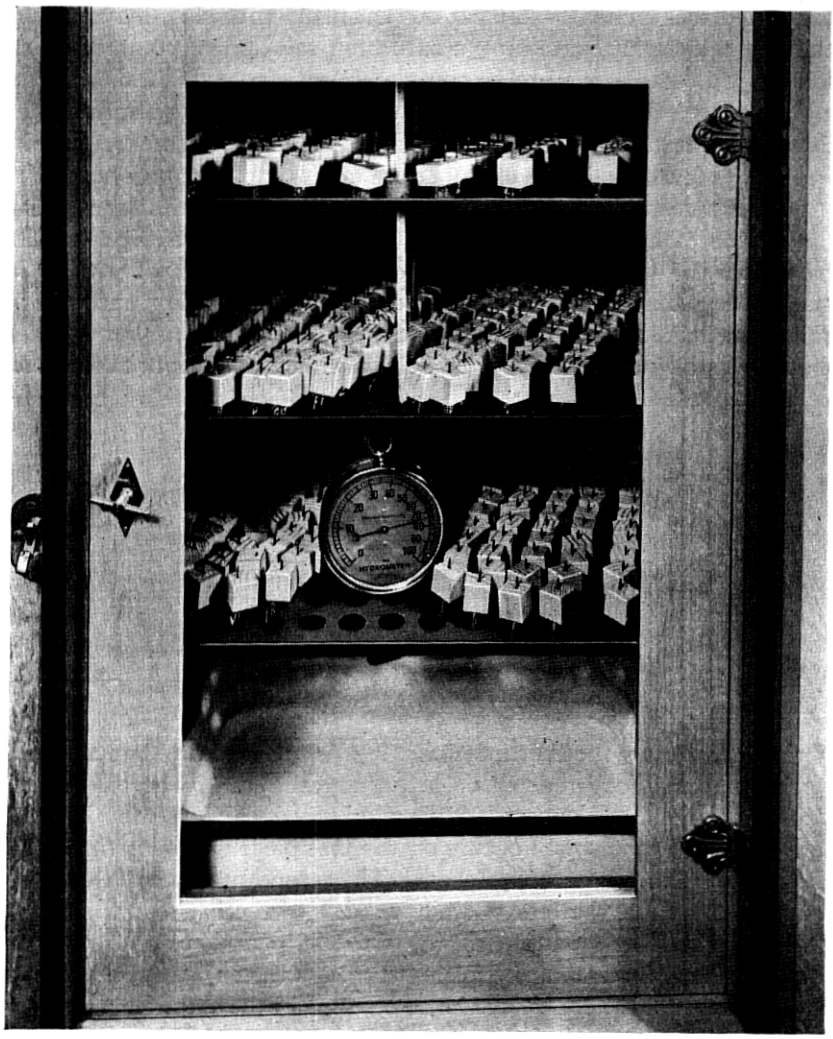

Fig. 6—Constant humidity chamber filled with test blocks.

be used in practice. Due to the variance in the moisture pick-up of wood at different relative humidities, it is necessary to bring the blocks to equilibrium under standard conditions both before and after the

test in order to determine actual weight losses. Since oven-drying is obviously a poor reference standard when volatile materials are under consideration and may also bring about serious changes in the wood, a constant humidity chamber is used for this purpose. An ordinary bacteriological incubator kept at 30° C., fitted with slow-moving fans and a shallow pan containing a saturated solution of common table salt (Fig. 6), has proved to be completely satisfactory in this respect, maintaining a relative humidity of 76 per cent with very little deviation. The test pieces after treatment are placed on racks (Fig. 7) and only a few days in the chamber are necessary for equilibration.

Such a test method allows of three criteria as the basis for judging the degree of attack. First, there is the amount and vigor of the growth of the test-fungus on the wood block, readings of which are made every four weeks. As this is difficult of expression, recourse is had to the classical method of the serologists, wherein plus four denotes the maximum. An attempt is made to evaluate both vigor and extent

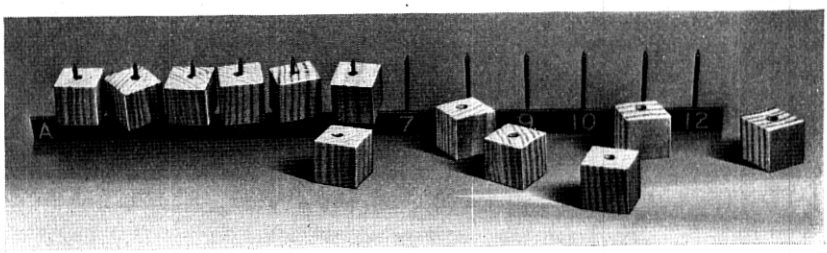

Fig. 7—Test blocks and rack.

of the fungal growth; the notation "2–4" would mean that the test block was partly covered with a heavy mycelial mat, whereas "3–2" would mean almost covered with relatively weak growth (Figs. 8 and 9). Often no growth occurs on the test piece and sometimes the mycelial inoculum is actually killed. The second measure of extent of decay is based on the loss of weight, with corrections for the effect of leaching and evaporation during the test period, computed from data obtained on treated controls put through the entire cycle without inoculation. These controls are also of value in the empirical strength rating made at the end of the test when the pieces are dissected in an effort to judge the remaining strength—a rating of ten indicates no detectable loss in strength as compared to the control and zero denotes complete disintegration.

Long experience with the petri dish method had emphasized the high degree of specificity of the fungi to various toxics. No single

fungus is equally resistant to all preservatives and gross errors are inevitable unless cognizance is taken of this situation. Since it is impossible to use all the organisms which destroy wood, a choice has been made to include genera which are known to be of considerable

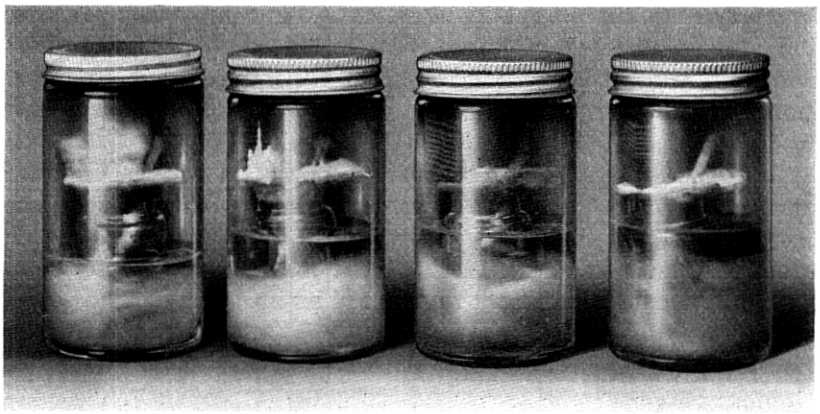

Fig. 8—Assay of a polychlor phenol showing effect of increasing concentration. The test organism *Lentinus lepideus*. The growth ratings from left to right are 4–4, 3–3, 1–2, and √ = no growth on specimen.

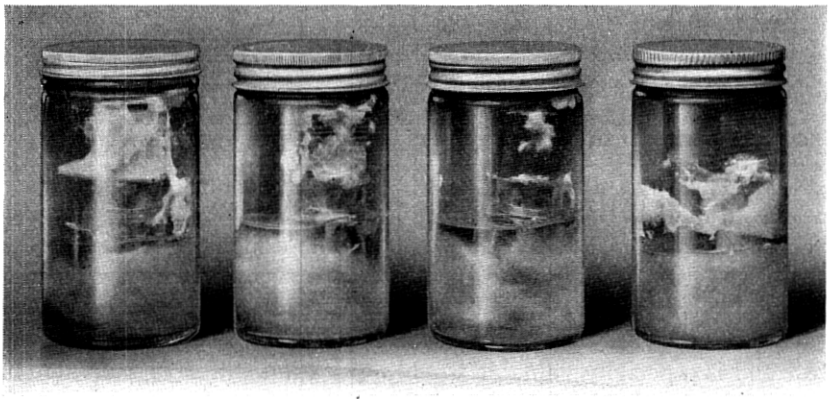

Fig. 9—Same range of concentration as in Figure 8 with U-10 as test fungus. The growth ratings from left to right are 4–4, 3–4, 1–3 and 1–2.

economic importance in the decay of timber or which have been encountered in the actual decay of telephone poles, being sure to include in any given test the fungi which past experience has shown to be resistant to the type of preservative under consideration. Four

organisms in duplicate are used in each test (Fig. 10). *Lentinus lepideus*, cited by Buller,[4] Snell [5] and Humphrey [6] and isolated several times from posts in the Gulfport, Mississippi, test plot,[7] as well as from poles in service, is used in all cases of organic preservatives, but is seldom used against metallic salts, to which it is extremely sensitive. *Lenzites trabea*, another species of great economic importance, Hubert,[8] and also isolated several times from rotted southern pine poles, is somewhat parallel in resistance to *Lentinus lepideus*, but produces a markedly different type of decay. *Polyporus vaporarius*, *Poria incrassata*, and *Coniophora cerebella*, the common "dry rots," although easily killed by many hydrocarbons, are resistant to most inorganic compounds, and at least one of these organisms is included in each test on such materials. *Fomes roseus*, another fungus of wide distribution, reacts in a most inconsistent manner, but its occasional specific virulence is sufficient to warrant its inclusion in all assays of

Fig. 10.—Assay of worthless preservative at maximum concentration. The fungi in duplicate from left to right are *Lenzites trabea*, U-10, *Fomes roseus* and *Lentinus lepideus*.

new and unusual preservatives. Unfortunately the fastest and most versatile decay organism used has no name and masquerades under the designation U (unknown)-10. Isolated several years ago from a decayed pine pole, the identity of U-10 is still a mystery, despite the efforts of many mycological authorities. U-10 is included in every test and is especially valuable when a quick indication of the value of a new preservative is needed, as it is capable of producing an appreciable weight loss in about three months. In addition to the above fungi occasional use is made of such common wood-destroyers as *Trametes serialis*, *Lenzites sepiaria*, *Polystictus versicolor*, *Polyporus sulphureus*, and *Fomes pinicola*.

At the present stage of development this wood block method tells nothing directly about the ability of a wood preservative to resist the action of termites. Most materials which inhibit decay also prevent

termite attack. In addition all promising leads are verified by means of the sapling test [9] at Gulfport, Mississippi, and here Nature has provided a bountiful supply of these industrious insects.

Fig. 11—Growth on southern pine sapwood controls. From left to right; *Polyporus sulphureus*, *Polyporus vaporarius*, *Polystictus hirsutus* and U-10.

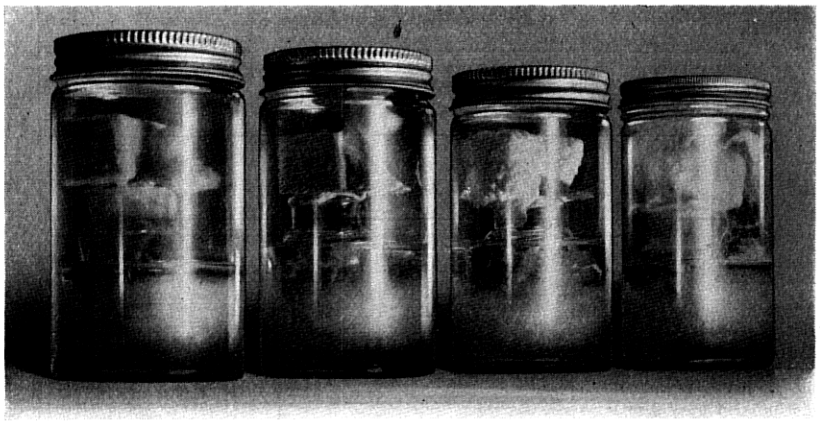

Fig. 12—Growth on southern pine sapwood controls. *Lenzites trabea*, *Fomes roseus*, U-10 and *Lentinus lepideus*.

Experimental Results

The above-mentioned fungi have been tested on untreated southern pine sapwood in order to set up standards of comparison (Figs. 11 and 12). Table I shows individual weight and strength losses effected by our most commonly used organisms in the regular 24-week period. Considerable decay with many of these fungi occurs in a somewhat

TABLE I

WEIGHT LOSSES AND DISSECTION RATINGS ON UNTREATED SOUTHERN PINE BLOCKS EXPOSED TO MOST COMMON TEST FUNGI FOR TWENTY-FOUR WEEKS

Organism	Initial Weight in Grams		Final Weight in Grams		Loss in Per Cent	Empirical Rating Based on Dissection	Description of Decay
	76% Relative Humidity	Oven Dry	76% Relative Humidity	Oven Dry	Based on Oven-Dry Weights		
Lentinus lepideus	2.34	2.05	1.87	1.64	20.0	4	Rather advanced decay throughout
	2.35	2.06	1.87	1.64	20.4	4	–ditto–
	2.25	1.97	1.85	1.62	17.8	5	Moderately advanced decay throughout
	2.26	1.98	1.77	1.55	21.7	4	Rather advanced decay throughout
Fomes roseus	2.25	1.97	1.84	1.61	18.3	4	Rather advanced decay throughout
	2.27	1.99	1.84	1.61	19.1	4	–ditto–
	2.22	1.95	1.78	1.56	20.0	4	–ditto–
	2.21	1.94	1.56	1.37	29.4	1	Thoroughly rotted
U–10	2.33	2.04	1.13	0.99	51.5	0	Complete disintegration
	2.33	2.04	1.37	1.20	41.2	0	–ditto–
	2.20	1.93	1.08	0.95	50.8	0	–ditto–
	2.18	1.91	1.19	1.04	45.5	0	–ditto–
Lenzites trabea	2.32	2.03	1.73	1.52	25.1	2	Deep surface disintegration, advanced decay elsewhere
	2.33	2.04	1.76	1.54	24.5	3	Deep surface disintegration, rather advanced decay elsewhere
	2.07	1.81	1.51	1.32	27.1	2	Advanced decay
	2.09	1.83	1.29	1.13	38.3	1	Thoroughly rotted
Polyporus vaporarius	2.12	1.86	1.69	1.48	20.4	4	Rather advanced decay throughout
	2.01	1.76	1.74	1.53	13.1	6	Mild decay throughout
	2.18	1.91	1.78	1.56	18.3	4	Rather advanced decay throughout
	2.26	1.98	1.82	1.60	19.2	4	–ditto–

Growth rating in all cases was 4–4, signifying that the test blocks were covered with heavy normal growth

shorter time, but experience has shown that more consistent results are obtained with the longer period. This is especially true in the case of materials of moderate toxicity, wherein often little growth is seen for two or three months, after which the fungus may become established and rot the test piece.

Using the routine technique, volatile compounds are often found to be practically worthless. This is true of naphthalene, for instance, but when special precautions are taken to insure its presence during the exposure to the fungus, the effective toxicity of this hydrocarbon cannot be questioned. Analysis of control blocks proved that the naphthalene had evaporated quite completely from the test piece even before sterilization. This difficulty can be surmounted satisfactorily in the case of a single compound by injecting a generous quantity and determining the actual amounts of material present from equilibrium weights of the test pieces before treatment and just prior to inoculation. Steam sterilization, of course, would introduce errors under such circumstances, and while the risk of contamination with foreign organisms is high, satisfactory results have been obtained with unsterilized blocks. In the case of volatile mixtures a similar procedure permits the knowledge of the total evaporation before inoculation, but determination of the loss of the individual constituents is practically impossible.

For all relatively volatile preservatives such as creosotes, the regular method including sterilization can in a way be considered a permanency test. Fortunately this evaporative loss is in the same order of magnitude as that encountered in the field after an exposure of several years, and correlation with outdoor tests is unexpectedly good. Closer control of the amount evaporated would be desirable, but experience has shown this to be difficult of consistent attainment. Artificial weathering machines such as that described by Gillander, Rhodes, King and Roche [10] constitute a reasonably successful attempt to reproduce natural conditions. Leaching is more easily controlled and duplicated than evaporation, and preservatives which by their nature might be considered to be water soluble are subjected to a standard leaching cycle if the initial test has shown them to be promising. Again the close correlation of field results with the laboratory is gratifying.

Of necessity, details of technique have been only sketchily reviewed in this paper; information as to the exact procedure will soon be available in the chemical press together with complete results on several preservatives of interest. Table II contains the results of an assay on a supposedly permanent inorganic preservative before and after

TABLE II

ASSAY BY WOOD-BLOCK METHOD OF "WATER INSOLUBLE" PRESERVATIVE BEFORE AND AFTER LEACHING

Concentration in Pounds per Cubic Foot	*Poria incrassata*				*Coniophora cerebella*				U–10				*Polyporus vaporarius*				Uninoculated	
	Growth Rating		Weight Loss in Per Cent		Dissection Rating		Growth Rating		Weight Loss in Per Cent		Dissection Rating		Growth Rating		Weight Loss in Per Cent		Dissection Rating		Control Weight Loss in Per Cent	
2.24 Original	√	√	3.8	7.3	10	10	√	√	5.3	4.1	10	10	√	√	3.4	8.3	10	10	√	√	5.5	4.6	10	10	3.9	5.4
Leached	4–3	4–4	4.0	1.4	9	10	3–2	3–2	1.8	1.7	10	10	2–2	2–2	2.0	0.8	9	10	2–3	3–2	2.1	1.2	9	10	0.8	1.4
1.24 Original	√	√	2.1	2.4	10	10	√	√	1.3	1.9	10	10	√	√	2.2	2.0	10	10	√	√	0.9	3.1	10	10	2.2	1.9
Leached	3–3	3–3	1.2	7.5	9	8	3–3	3–2	9.7	1.9	7	9	3–3	3–3	15.9	6.1	5	7	4–4	3–4	4.7	7.0	8	8	0.4	0.3
0.60 Original	√	√	0.4	1.3	10	10	√	√	0.4	0.4	10	10	√	√	0.4	0.9	10	10	√	√	0.9	1.2	10	10	0.9	0.4
Leached	4–2	4–2	5.1	10.4	8	6	1–1	4–2	0.8	0.8	10	10	3–4	3–4	16.7	15.8	4	4	3–3	3–3	3.2	11.9	9	6	0.0	0.0
0.28 Original	√	√	0.0	0.4	10	10	√	√	0.0	1.0	10	10	√	√	0.0	0.0	10	10	√	√	0.5	0.0	10	10	0.3	0.1
Leached	4–3	4–4	13.6	30.9	6	2	4–3	4–4	17.6	23.5	4	2	4–4	4–4	50.2	12.4	0	6	4–4	4–4	19.8	15.7	4	4	0.0	0.0
0.14 Original	√	1–1	0.0	0.0	10	10	√	1–1	0.0	0.0	10	10	1–2	√	0.0	0.0	10	10	√	√	0.0	0.0	10	10	0.0	0.0
Leached	4–4	4–4	22.9	32.8	3	1	4–3	4–3	12.8	26.5	6	2	4–4	4–4	46.4	68.2	0	0	4–4	4–4	26.6	32.8	2	1	0.0	0.0

Ratings under each heading represent duplicate determinations
√ = No growth on specimen

TABLE III

ASSAY BY WOOD BLOCK METHOD OF PROPRIETARY PRESERVATIVE

Concentration in Pounds per Cubic Foot	*Lentinus lepideus*			*Fomes roseus*			U–10			*Lenzites trabea*			Uninoculated
	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Control Weight Loss in Per Cent
12.53	4–2 4–1	3.5 1.7	9 10	2–1 √	0.4 0.4	10 10	1–1 √	0.9 0.4	10 10	√ √	0.5 0.0	10 10	1.7 0.8
10.04	4–4 4–4	9.9 7.1	6 7	4–4 4–4	9.3 7.7	5 6	4–3 4–4	24.5 10.4	3 5	4–2 2–1	11.9 3.2	6 8	0.6 0.3
8.06	4–4 4–4	11.1 8.0	5 6	4–4 4–3	10.6 7.7	5 7	4–3 4–3	5.0 4.3	9 9	4–2 4–2	12.6 10.9	6 5	0.2 0.5
6.64	4–4 4–4	13.4 9.6	5 6	4–4 3–1	7.7 0.5	7 10	4–4 4–4	10.1 9.2	6 6	2–2 4–1	4.5 3.7	8 9	0.1 0.0
4.25	3–4 4–3	6.4 6.2	9 9	4–4 4–4	21.1 19.1	4 4	4–4 4–4	27.6 24.2	3 3	4–4 4–3	30.9 12.9	3 6	0.0 0.0
2.60	4–4 4–4	15.6 8.4	4 7	4–4 4–4	12.9 8.7	4 7	4–4 4–4	38.5 30.6	1 1	4–3 4–4	14.7 10.7	5 6	0.0 0.0

Ratings under each heading represent duplicate determinations

√ = no growth on specimen

TABLE IV

Assay by Wood Block Method of Typical Creosote

Concentration in Pounds per Cubic Foot	*Lentinus lepideus*			*Fomes roseus*			U–10			*Trametes serialis*			Uninoculated
	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Control Weight Loss in Per Cent
8.82	2–2 2–2	2.7 1.9	10 10	√ √	1.3 0.9	10 10	1–1 √	0.9 0.9	10 10	√ √	1.3 0.9	10 10	1.4 0.8
4.29	3–1 3–2	2.6 1.8	9 9	3–1 2–1	3.2 1.5	9 10	1–1 √	1.0 0.5	10 10	3–2 2–1	0.0 0.0	10 10	0.6 0.3
2.24	4–2 4–2	2.7 2.7	8 8	2–2 3–2	8.8 6.5	6 7	4–4 4–4	38.9 36.1	1 1	3–2 2–1	2.1 0.0	9 10	0.3 0.1
1.09	4–3 4–3	12.6 11.4	5 5	4–4 4–4	19.4 13.3	4 6	4–4 4–4	44.3 32.1	0 1	4–4 4–4	30.8 12.1	1 6	0.1 0.0

Ratings under each heading represent duplicate determinations
√ = no growth on specimen

TABLE V

Assay by Wood Block Method of Compound Indicated as Worthless According to the Petri Dish Method

Concentration in Pounds per Cubic Foot	*Lentinus lepideus*			*Fomes roseus*			U–10			*Lenzites trabea*			Uninoculated
	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Growth Rating	Weight Loss in Per Cent	Dissection Rating	Control Weight Loss in Per Cent
2.8	√ 1–1	0.0 0.4	10 10	2–1 2–1	0.8 0.0	10 10	3–2 2–2	0.0 0.0	10 10	3–1 3–1	+0.4 +0.4	10 10	+0.4 0.0
2.1	1–1 1–1	+0.4 +0.4	10 10	2–1 3–1	0.0 +0.4	10 10	3–2 3–2	+0.4 +0.4	10 10	3–1 3–1	+0.4 +0.4	10 10	+0.8 +0.4
0.9	1–1 2–1	1.4 1.4	10 10	3–2 4–2	1.4 0.8	10 10	3–1 3–2	0.0 0.9	10 10	3–1 3–1	0.0 0.0	10 10	0.0 0.5

Ratings under each heading represent duplicate determinations
√ = No growth on specimen

leaching. The losses in weight on the unleached specimens are also present in the controls and are presumably due to an extremely soluble non-toxic salt known to be present. Analysis of the leach waters indicates that the toxic substances were also slowly but definitely soluble. Field results on this same preservative were favorable for a year, but considerable decay was found the second year in all but the two highest concentrations. Table III presents the results obtained with a well-known proprietary preservative of the organic type. The concentrations given are for the preservative as purchased, which consists of a 25 per cent solution of solids in a volatile solvent. This solvent was allowed to evaporate completely before exposure of the test blocks to the fungus. For comparative purposes Table IV illustrates a test of a typical coal-tar creosote. Included as a matter of special interest, Table V outlines the wood-block assay on a material which the petri dish method indicated to be worthless.

This adaptation of the kolle flask method has been in constant use more or less in its present form for the past three years. Hundreds of complete assays have been made with results to date in good agreement with the slower and more expensive outdoor tests. By the use of a range of concentrations the relative efficacy of various preservatives can be judged, but definite expressions of the absolute value of any preservative have been avoided. With conditions controlled for maximum decay, this test is admittedly severe. This very severity, however, is probably an asset in the elimination at the outset of the poor and mediocre materials unworthy of further study.

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