A Wood Soil Contact Culture Technique for Laboratory Study of Wood-Destroying Fungi, Wood Decay and Wood Preservation

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Limitations imposed by other biological test methods have largely been overcome by using autoclaved top soil for the substrate and pure cultures of the decay organisms. The use of soil was the direct result of observations on the rapid decay of wood in contact with soil in laboratory termite colonies.

Development of a wood-soil contact culture technique as a result of these observations furnished an excellent laboratory tool for further research on the biological factors promoting and the preservative compounds proposed for preventing decay. Research on the factors promoting decay showed not only that the average top soil furnishes nutrients and nutrilites in the quantity and proportion highly favorable to many decay organisms but also an effective means of regulating the water content of wood or cellulose during the decay period.

Comparisons between laboratory and field results showed the amount of decay obtained by the wood soil contact technique to be more rapid and uniform than decay in the field. The severity of the exposure in the laboratory ensures immediate eliminations of compounds unworthy of further more expensive field studies and evaluates compounds in the same order of effectiveness.

Comparisons and evaluation of wood and cellulose preservatives plus artificial weathering cycles followed by exposure to the method will provide valuable information on initial toxicity and permanence thereby affording a sound basis for the engineering selection of preservatives for a variety of purposes.

LABORATORY tests for evaluating fungicides are often used as a means of predicting field results and for investigating the action of cellulose and wood-destroying fungi. Of the several laboratory procedures hitherto devised for these purposes, however, none has been entirely adequate. This has led to incorrect interpretation of laboratory assays of fungicidal compounds, with attendant misapplication of preservatives. The confusion and misunderstanding concerning the use of preservatives have been further increased by the misapplication of the laboratory procedures themselves. A brief review and explanation of some procedures and their application will clarify these statements.

Minute quantities of toxic agents and growth-promoting substances which are not readily detected by known chemical analyses may be determined by bio assay methods, the value of which depends upon a prior determination of the reaction of one or more organisms to known quantities of these substances. Another bio assay is the so-called "acceptance test" for fungicides, by which the fungus resistant qualities of materials impregnated with fungicides may be determined. Since fungus resistant qualities are the primary concern in such a test, the identity and quantity of the preservative are of only incidental interest. However, the identity, fungus-proof qualities and quantity of fungicidal compounds are important when

laboratory procedures are devised for comparing effectiveness in the development of different preservatives. In addition, the chemical and physical properties of the different preservatives must be considered for the determination of their subsequent behavior when exposed to a variety of environmental conditions. Bio assays may thus be used for quantitative, qualitative, comparative, or predictive purposes.

In order to survey existing tests, it may be helpful to classify them. There are three groups of rather ill-defined laboratory methods based on the nutrient and physical properties of the substrate. The first group is comprised of those methods in which an agar or similar base is used. Various nutrients or nutrilites* may be added to this base[1], and prior to inoculation with one or more fungi the preservative may also be added. This group includes the standard petri dish test described by Richards[2], 1923, which has had extensive use in the field of wood preservation. The carbohydrate source in the standard petri dish method was malt sugar. Later, in response to the requests by industry, Richards attempted to substitute wood flour as the nutrient. However, the radial fungus growth used as the criterion of toxicity was very sparse and thin and the substitution of wood flour for sugar was discarded. It is of interest to record here that Richards also summarized the previous work on toximetric tests of wood preservatives.

The second group includes those methods in which the preservative is added directly to a cellulose material before exposure to organisms. The preserved material may be the only source of nutrient for the fungi, or a piece of similar untreated material may be provided. Such a method is described in a paper by Waterman, Leutritz and Hill[3], 1938. No agar is used, and the untreated wood is supported over water by mechanical means. When agar is used to support the preserved material and to supply water, nutrients, nutrilites or combinations of each of these may be added to the agar. This may be done in several ways, among which are the kolle flask method for wood preservatives described by Falck[4], 1927, the standard method of the American Society for Testing Materials for testing fabrics[5], 1942, and the present Signal Corps test of fungicidal coatings[6], 1943. Of these, the first two methods are used chiefly as "acceptance" tests by determining the fungus-proof qualities of fungicidally treated wood and fabrics. They are also used in development work for comparison and for predicting the field behavior of preservatives when supplemented by artificial weathering cycles. The Signal Corps test is used as an acceptance test of fungicidal coatings which are sprayed on electical equipment. Since the criterion is the inhibition of fungus growth at some distance from a paper impreg-

* Nutrients here include the sugars and compounds used by the fungi for food purposes, and nutrilites will be referred to in this paper as those compounds necessary to fungus nutrition, such as vitamins, growth substances and minerals, Williams, R. J., 1928. (See Bibliography at end of this paper.)

nated with the fungicidal coating it is fundamentally a quantitative measure of the amount of fungicide which diffuses into the agar from the impregnated paper specimen.

A third group of test procedures employs soil or soil suspension in conjunction with the preservative materials. Here the soil furnishes an active microbial culture and supplementary nutrients and nutrilites. The soil suspension method has been described by Furry, and Zametkin[7], 1943, and the soil burial method by the American Society for Testing Materials.

The techniques included in the first group are time saving, permit of replication, and are readily duplicated by other investigators. However, the results in the agar-fungicide system do not apply to a cellulose-fungicide system and are therefore a source of confusion resulting from their misinterpretation when so used. Agar-fungicide systems as originally described by Richards are quantitative tests and have been used principally for comparative toxicity studies. From such comparative studies attempts to predict the behavior of a preservative in subsequent field tests have been generally unsuccessful. Examples of the discrepancies between the results from field and petri dish tests will be discussed later in this paper.

In general, the second group of methods takes a longer time, and replication leaves much to be desired. Since the preserved material is the same for laboratory and field tests, better agreement between field and laboratory results should be obtained with the kolle flask-wood block method and the A.S.T.M. fabric methods. However, the Signal Corps method for testing fungicidal coatings used on electrical equipment is not a a true test of the coating material per se.

The third group of methods introduces a large number of variables through the use of soil. Previously, replication of results and concomitant duplication by other investigators had been lacking, due to microbial activity, physical properties, nutrient properties, and moisture variations of the soil. However, during experimental work with termites, the author[8] made certain observations on the various factors involved in the decay process. These led to an intensive study of the problem resulting in the development of a test method for wood preservatives which overcomes many of the limitations of earlier methods. The soil burial method is a severe test of fungicide treated material, and, with the modification to be discussed in this paper, it is anticipated that the variables which cause non-uniformity of results can be eliminated. The method is also evaluated by comparison between the results obtained in the laboratory and those obtained from parallel field tests.

Rapid decay of wood in contact with soil was observed during an attempt to establish experimental termite colonies in the laboratory (Leutritz[8], 1939). Instead of becoming infested by the termites, nearly all the blocks

decayed more rapidly and more completely than in any previous laboratory test. Preliminary experiments were devised to ascertain the factors responsible for the accelerated decay and to establish optimum conditions for growth of fungi in laboratory tests of wood preservatives. As a result of this exploratory work a laboratory technique was devised which permitted study of these factors and which offered a convenient means of evaluating toxicity and preservative properties of chemical compounds. Further investigation was made on the effect of nutrients and nutrilites in the soil, temperature, and the moisture content of the wood. Parallel with this laboratory investigation, a study was made of the fungus attack on wood under climatic conditions very favorable for decay at Gulfport, Mississippi.

Initial Experiments and Results

As a preliminary step, the moisture content of the soil from the termite colonies was determined by oven-drying 100-gram samples. This was found to average 22% of the oven-dry weight of the soil. Tests with several soils showed that approximately the same moisture content could be obtained by merely adding to dry soil just enough water to make the mixture cohere when squeezed in the hand.

A one-hundred-gram sample of moist soil was placed in each of 24 large-mouthed, eight-ounce, screw-capped bottles (12 cm. high and 6 cm. in diameter). A weighed oven-dry block of southern pine sapwood, 2 x 2 x 2 cm., was pushed to a depth of about 2 cm. into the soil in each bottle. The caps were put on, and the preparations were sterilized for 30 minutes at 15 pounds' pressure in an autoclave. After cooling, the block in each of twelve of the bottles was inoculated with a pure culture of one of seven common wood-destorying fungi—*Lentinus lepideus*, *Fomes roseus*, *Poria microspora*,* *Polyporus vaporarius*, *Coniophora cerebella*, *Poria incrassata*, and *Lenzites trabea*. Twelve bottles, not inoculated, were used for moisture determinations.

The bottles were then placed in an incubator maintained at 26°–28°C. At the end of each month three of the bottles inoculated with each fungus were taken from the incubator. Each block was removed from the soil and weighed immediately; it was then allowed to dry in an oven at 105°–110°C. to a constant weight. The average percentage loss in dry weight due to decay was calculated. The results, recorded in Fig. 1, show that the very rapid decay of wooden blocks in contact with the soil is not the result of any one particularly active fungus. Each of the seven species produced exceedingly rapid decay under the conditions of the soil assay.

* This fungus was designated BTL U-10 until recently identified as *Poria microspora* by Miss Mildred K. Nobles, Dept. of Agriculture, Ottawa, Canada, 1943. (See Bibliography at end of paper.)

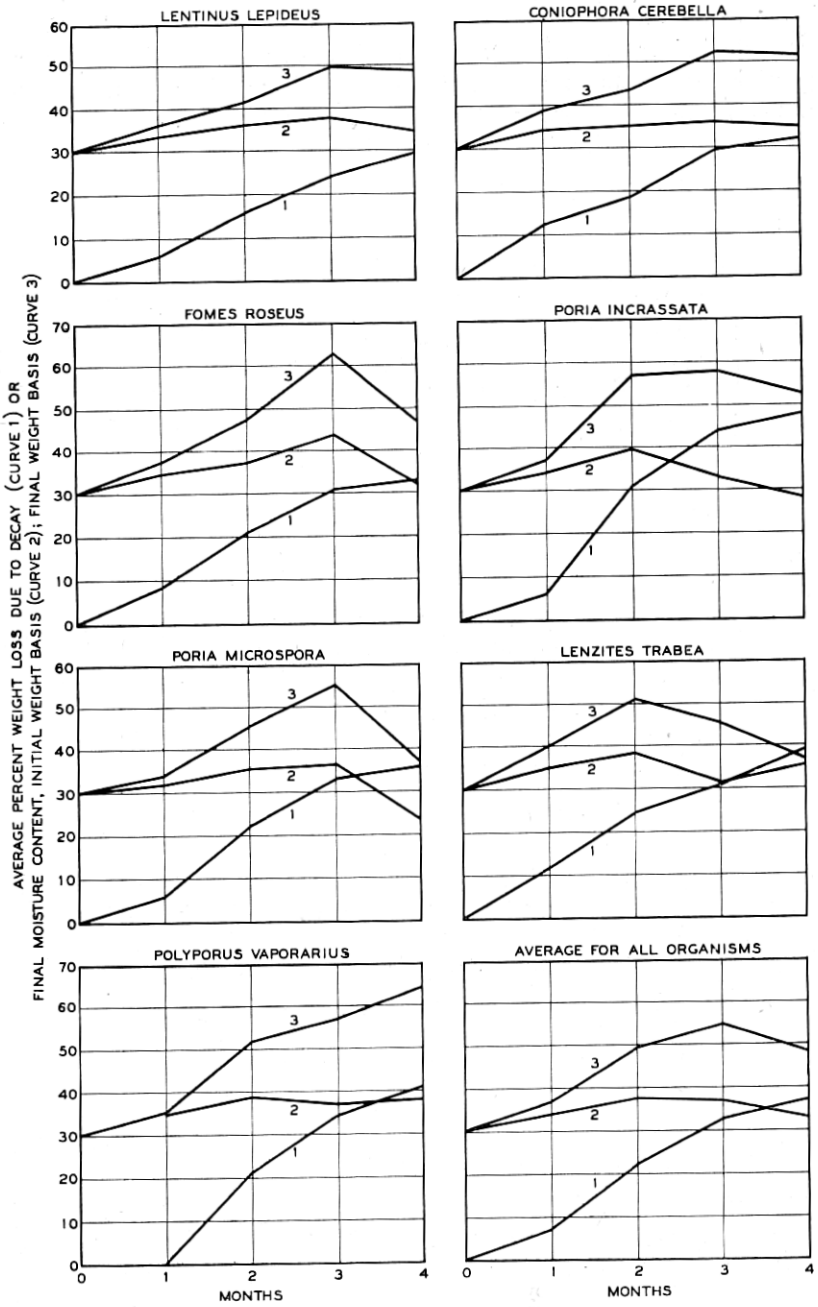

Fig. 1—Wood soil contact technique

For comparison, a similar test was made according to the method described by Waterman, Leutritz, and Hill[8], 1938, in which the test blocks are placed on inoculated sapwood slabs supported over water in capped wide-mouthed bottles. Comparison of the average percent weight loss due to decay for all organisms by both methods, Fig. 2, shows that the water-wood method is far less effective in producing decay than the soil method.

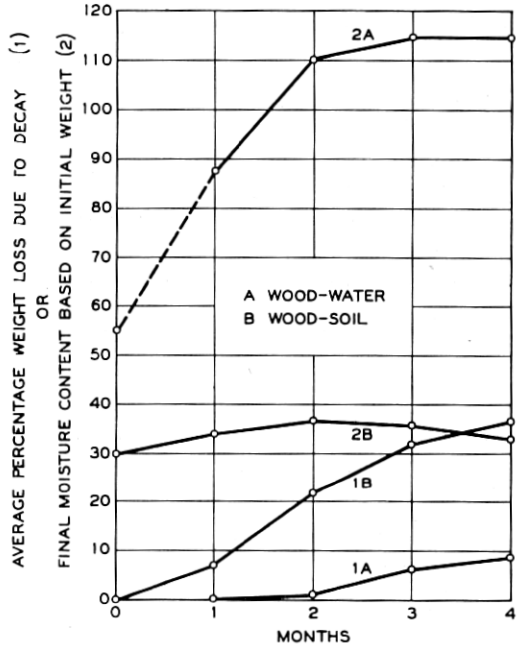

Fig. 2—Comparison of average weight loss and final moisture content by wood-water and wood-soil techniques

An additional experiment was conducted with several strains of two of the fungi previously used, *Coniophora cerebella* and *Lentinus lepideus*. The *Coniophora cerebella* strains were as follows:

Baarn, from Dr. Johanna Westerdyjk, Holland

Liese, from Dr. Liese, Germany

Idaweiche, from Dr. Idaweiche, Germany

Madison, from Forest Products Laboratory, Madison, Wisconsin, isolated from oak, November 13, 1919

BTL, also from Forest Products Laboratory, Madison, Wisconsin, 1930

The *Lentinus lepideus* strains were from the following sources:

No. 534, from Forest Products Laboratory, Madison (No. 534)

BTL U-1, U-13, U-14, and U-32, from creosoted pine telephone poles which had failed in service

Gulfport, from a test post in Gulfport, Mississippi, used for our assay work

The results of the assay with these strains of *Coniophora cerebella* and *Lentinus lepideus* showed the average weight loss in percent due to decay to be 32.0 and 27.3 percent respectively which was as great as that in the previous soil tests with the single representative of these species. A greater amount of decay was obtained with one strain of Coniophora cerebella due to a slight change in technique, i.e., the fungus was first established on small slabs of southern pine sapwood, and then sterile oven-dry blocks were dropped on the vigorously growing fungus. The large amount of decay (60%) which resulted led to the adoption of this modification in all subsequent tests.

The foregoing tests may be regarded as supporting the use of the criteria previously employed in the selection of fungi for laboratory tests—namely, their occurrence as saprophytes of wood, their isolation from service materials for example, pine telephone poles or tests posts, and their demonstrated ability to bring about decay of wood in the laboratory.

As a result of these preliminary experiments, the use of soil as the medium in testing procedure was adopted.

Soil Contact Technique

On the basis of the foregoing experiments and in view of the rapidity of the decay occurring on test blocks in the soil-contact test, the following method is described as a means of evaluating the effectiveness of preservatives or toxic materials which are recommended for the protection of wood or other cellulosic materials. The method may also be used to study environmental factors which affect decay or it may be adapted to the study of fungi other than the wood-destroying fungi of the Basidiomycetes.

Ordinary top soil, such as a florist would use for potted plants, is satisfactory for the test. While experience has shown that top soil from a number of different sources may be used without materially affecting the results, standardization would be desirable. Therefore the term "soil" will be defined as a sandy loam type which contains 4–6 percent of organic matter and a pH originally between 5–7. The soil is passed through a coarse-mesh screen to remove rubble, stones and other debris; this is most easily accomplished when the soil is dry. The screened soil is moistened with just enough water to effect cohesion into a soft ball when squeezed in the hand, and a check may then be made by determining the moisture content of the soil. When prepared in this manner the moisture content of the soil should be 20–25% on an oven-dry weight basis. In an alternative procedure, the moisture content of the dry soil is ascertained and then sufficient water is added to give a moisture content of 20–25%.

Bottles, 12 cm. high and 6 cm. in diameter, are half filled (60–100 grams)

with the moistened soil. Two pieces of southern pine sapwood "feeder strips" (3.5 x 2.0 x 0.3 cm.) are placed on the soil in each bottle, Fig. 3. The bottles are closed tightly with screw caps and then autoclaved for 30 minutes at 15 pounds' pressure. When the bottles have cooled, a small inoculum (a few millimeters square) cut from a pure culture of a suitable wood-destroying fungus is placed on the sapwood substrate. Each bottle contains a single dominant fungus culture. It is best to use at least four to eight selected species of fungi for an assay. The bottles are again capped and placed in an incubator, or a controlled temperature room, held at 26°–28°C., for at least one month. Any contaminated or weak cultures are

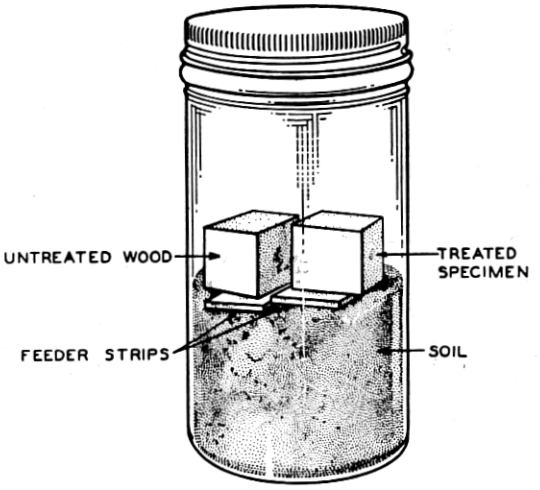

Fig. 3—Schematic diagram of wood soil contact method

discarded. This completes the preparation of the pure fungus cultures, Fig. 4, and they are now ready to receive the test blocks.

To each bottle containing a culture established on sapwood substrate are added an untreated control block and a block treated with a preservative according to the following method:

The required number of $\frac{3}{4}$" cubes of sapwood blocks are placed in a humidity chamber at 30°C. and 76% relative humidity until the blocks have reached a constant weight. Then the necessary number of weighed blocks, weighted to ensure immersion, are placed in a container of convenient size under a bell jar fitted with a separatory funnel. After evacuation of the bell jar to a pressure not greater than 2 cm. as measured by a mercury manometer, the vacuum is held for 5 minutes. The stopcock in the pump line is then closed, and sufficient solution is admitted from the separatory funnel

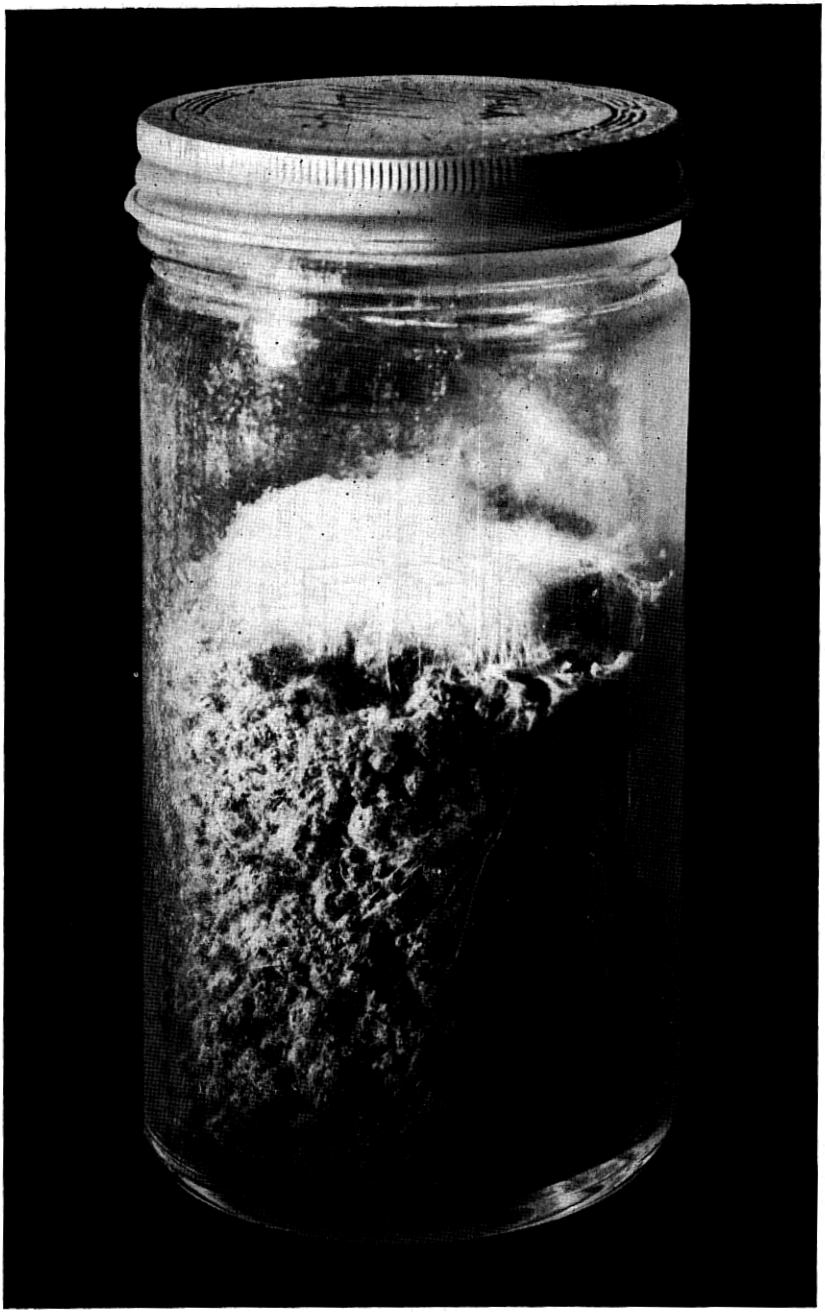

Fig. 4—Pure fungus culture of Poria incrassata

to submerge the blocks completely when the air is admitted. After remaining in the solution for 5 minutes, the blocks are wiped superficially and and weighed. This treated weight is used for calculation of the theoretical retention according to the following formula:

$$R = \frac{GC\,(62.4)}{100\ V}$$

in which R* = pounds of preservative per cubic foot of wood, G = gain in weight in grams, C= grams of the preservative in 100 grams of solution, and V = volume of the test piece in cubic centimeters. When the solvent has evaporated from the blocks, they are placed on racks, returned to the humidity chamber and brought to constant weight. The difference between the humidity weights before and after treatment serves as the basis for calculating the actual retention, and the final equilibrium weight is used also as the initial weight of the treated block before exposure to the fungus.

The cross-section side of the blocks is placed in contact with the vigorously growing mycelia of the sapwood culture which in turn is in contact with the soil. For each concentration of preservative, three treated blocks and three untreated control blocks are exposed to each species of fungus. The bottles are recapped and place in an incubator or constant temperature room at 26°–28° C. with a relative humidity of 85–95%.

Exposure of the blocks to the fungus for from twelve to twenty-four weeks gives satisfactory results. If a sufficient number of treated specimens is exposed to the same organism, one or two specimens may be removed at the end of twelve weeks; and if considerable decay has taken place, the test may be concluded. At the end of the exposure period the blocks are brushed free of mycelia and immediately weighed to determine their moisture content. The blocks are then allowed to stand in the room until dry, after which they are again transferred to the humid chamber (temperature 30°C., relative humidity 76%) for two-three days until a constant weight is attained.

In the toxicity work reported in this paper an untreated reference block was added to each test bottle. If a large number of assays are contemplated, the number of weighings may be reduced by eliminating the untreated blocks except for occasional reference purposes. The reduction in the number of reference blocks may be accomplished by establishing a decay norm for each test organism. This norm would be based on data similar to that used in Fig. 1 except that the procedure would be the same as that described for treated blocks. Comparison of the percentage weight loss due to decay

* To express the retention metrically $R \times 16.018 = \frac{\text{Kilograms}}{\text{Cubic meter}}$.

of the norm with the percentage weight loss due to decay of the test block may be used as a measure of the effectiveness of the preservative or toxic material. An index of the value of a preservative treatment may be obtained from the following computation:

$$\frac{\%\text{ loss of norm} - \%\text{ loss of treated block}}{\%\text{ loss of norm}} \times 100$$

Values of the index would range from 100, representing complete protection against decay, to 0, representing no protection whatever.

In most cases, especially when no volatile preservative is present, the untreated reference block disintegrates completely within twelve to sixteen weeks. An inspection rating based on strength may be used to supplement weight loss due to decay. The rating is made on the basis of appearance and strength: 10 denotes a sound condition, 9 superficial decay, 8 superficial decay in spots or streaks, 7 general surface decay, 6 considerable decay but not enough to allow specimen to be broken easily, 5 advanced decay, and 4, 3, 2, and 1 different stages of advanced decay, determined primarily by the ease with which the specimen is broken; 0 denotes complete disintegration. Ratings of 5 and below are considered failures. Similar ratings have been used for sticks in field work. This method of rating was used in the field trials of preservatives which appear later in this paper. Although the system is an arbitrary one, considerable correlation has been shown between these dissection ratings and the weight loss in percentage. With a series of field sticks or blocks treated with the same low amount of a preservative, ratings based on strength for the series are found to be very closely correlated with weight losses, even when the ratings are made by different workers.

The Influence of Moisture on Decay

The first experimental factor studied was the moisture content of the wood preceding and during the time that decay took place. Statements in the literature concerning the optimum moisture content for the decay of wood have placed the figure variously from fiber saturation, 27–30% to 60% (Schorger, 1926)[10] and 150% (Benton & Ehrlich, 1940)[11] of the wood substance based on the oven-dry weight.

Figure 1 gives data on the moisture content of blocks exposed to the seven species of fungi for periods of one, two, three, and four months. Uninoculated control blocks, removed at the end of each of these periods, were found to be at fiber saturation, indicating 100% relative humidity in the bottles and little or no migration of liquid water.

During the progress of decay there is a rapid decrease in the weight of the wood substance. But the amount of water present in each block does

not decrease and at all stages of decay corresponds to about 35% of the original dry weight of the block. Since the amount of water does not decrease as the amount of wood substance decreases, there is an increase in the percentage of water expressed in terms of dry weight as shown by curves labeled 3 in Fig. 1. Such an increase in the amount of water relative to the remaining wood substance would tend to limit decay if the optimum moisture content for initiating decay is considered to be at or near fiber saturation.

If the absolute amount of water in the blocks does not change during the progress of decay, then the final amount of moisture divided by the initial weight of the blocks should give a percentage figure fairly close to the initial fiber saturation of approximately 30%. The curves labeled 2 in Fig. 1 showing the moisture content based on the initial weight indicate that this is the case. For example, Fig. 1 shows that the average for all organisms is 4% greater than 30% after one month, 7% greater after two, 6.3 after three, and only 2.1 greater after four months. Between the third and fourth months the soil showed signs of drying out and examination of all of the moisture curves, Fig. 1, indicates a loss of water through the bottle caps, which accounts partially for the discrepancy. The 5–10% increase in water over the original fiber saturation may be due to slight condensation on the blocks or to the respiratory activity of the fungi in breaking down carbohydrates into CO_2 and water.

The average amount of decay (curve 1) and the final moisture content referred to the initial weight (curve 2) for all the organisms obtained by the wood-water (A) and wood-soil (B) assays are compared in Fig. 2. The water content of the blocks in the water test varied from 55–165% based on the oven-dry weight of wood and the decay was much less in amount and uniformity than that obtained by the wood-soil technique with the same organisms. From the results for individual blocks, the limiting water content at which no decay took place was determined as 78% for *Poria incrassata*, 84% for *Coniophora cerebella*, and 66% for *Polyporus vaporarius*. In a few instances, despite the full cell saturated conditions, decay did take place. Examination of these blocks indicated that most of the decay was confined to the surfaces of the blocks. This indicates that lack of oxygen was the limiting factor.

When wood was supported on glass rods over agar (kolle flash technique) full cell saturation of the blocks often occurs due to capillarity of the glass, condensation of water, accidental contact between wood and agar, and conduction by the fungus filaments. That the water content of the wood in the kolle flask technique is also too high is indicated by the "optimum" moisture content of 150% and the relatively small weight losses due to decay, less than 10%, cited in the experiments of Benton and Ehrlich.[11] The amount of decay was again shown to be affected by the water content

of the wood. If decay is to be used as a criterion of toxic effectiveness, the importance of eliminating variations in the water content of the block can be fully realized. The wood soil technique offers an excellent means of controlling moisture for studies of wood decay.

Sand, cotton, sawdust, wood flour, and soils with varying moisture contents were also used as supporting substrates, but in no case was the amount of decay as great as that with the same technique using soil of 20–25% moisture described above. When soils with water contents of 5%, 10%, 20–25% and 30% were compared, the moisture contents of blocks in contact with them were 12.8%, 23.9%, 27–30% and 73.9%, respectively. Decay of the blocks was adversely affected by lack of moisture in the first two cases and by full cell saturation of the wood in the last instance. However, if moisture were the only controlling factor the amount of decay of wood in contact with sand should be comparable to that in soil, but this was not the case.

The Influence of Soil Nutrient or Nutrilites

Nitrogen in the form of asparagine has been shown by Schmitz and Kaufert[12] (1936) to cause an increase in the amount of decay of *Pinus resinosa* by *Lenzites trabea*. Since wood contains only about 0.1% to 0.3% of nitrogen, any additional nitrogen received from the soil should promote decay. It might be expected that the soil supplies nitrogenous and other nutrients, nutrilites, vitamins, etc., that accelerate decay. Evidence for this was obtained by comparing the decay of blocks in contact with (1) top soil, (2) top soil that had been leached for several days with hot water, and (3) three artificial soils composed of washed sand and fuller's earth. In this experiment *Poria incrassata* was used as the inoculum for a test period of 12 weeks. The moisture content of the uninoculated control blocks was 27–30 percent and of the substrate for each series 22 percent; the temperature and time were constant. The average weight loss for the blocks in contact with top soil was 54%, with water extracted top soil 45.4% and the three mixtures of sand-fuller's earth 24.7%.

It is apparent from these data that the top soil promotes decay to a far greater extent than the sand-fuller's earth mixtures and slightly more than the water extracted top soil, despite the same moisture (fiber saturation) content of the blocks. The actual rate of decay of blocks in contact with the top soil was more than double that of the other mixtures. Therefore, the conclusion may be drawn that nutrients or nutrilites are present in the top soil which stimulate growth of the fungus and promote decay.

Since the water-extracted soil proved not so favorable for decay as the original top soil, some of the growth-promoting substances must have been soluble in water. The greater decay of wood in contact with the extracted

top soil than of that on the mixtures of sand and fuller's earth indicates that the nutrients present in the soil were not all removed by the water extraction. Further information was obtained by adding soil extract and other nutrient solutions to the sand-fuller's earth mixtures. The following materials were used:

4500 grams of washed beach sand
900 grams of fuller's earth
300 ml. of soil extract
60 ml. of malt extract (2% water solution)
50 leached blocks
vitamins B_1, B_6 and biotin (free acid)
stock mineral solution of the following composition:
1.5 grams per liter of KH_2PO_4
1.0 gram per liter of $MgSO_4 7H_2O$
plus the following elements in parts per million:
0.02 Cu, 0.01 Mn, 0.005 B, 0.10 Fe, 0.01 Mo, 0.09 Zn
pure cultures of the fungus *Poria incrassata*
42 eight-ounce bottles, screw capped (12 cm. high by 6 cm. diameter)
maltose

Procedure:

The sand and fuller's earth in the proportions mentioned above were mixed in a porcelain jar on a ball mill for six hours. The blocks were leached for two years with weekly changes of distilled water, using 50 ml. of distilled water for each block. Before the test, the blocks were oven-dried to constant weight at 105°C. and a volume measurement was made by mercury displacement method. The volume at fiber saturation was calculated from the oven-dry volume according to the formula: Volume at fiber saturation = Oven-dry volume + 0.25 × oven-dry weight.

One hundred grams of the soil moistened with 20 ml. of the appropriate nutrient solution (Table 1) was placed in an eight-ounce bottle for each block. The weighed block was pushed into the soil with a cross-section of the block facing upward until the top of the block was level with the soil.

The bottles were capped and autoclaved for 20 minutes at 20 pounds' pressure. After sterilization and cooling, an inoculum from a pure culture of the fungus *Poria incrassata* was placed on the top of each block. The bottles were then placed in a controlled temperature room (26°–28°C., relative humidity 90–95%) for 16 weeks.

At the end of this time the blocks, brushed free of soil and mycelia, were weighed immediately, and the volume was measured. Finally, the blocks were again oven-dried to constant weight and the volume was measured again.

The average volume at fiber saturation of the 42 blocks calculated from the initial volume when oven-dry was found to be 7.24 cc., and the final average volume when removed from the test was 7.26 cc. No shrinkage took place until the blocks were oven-dried, and then the distortion became

permanent. This constancy of the volume during the decay period indicates the mechanism by which the water is held practically constant during the decay period. Any loss of water would result in a shrinkage from which there would be no recovery.

Table 1 gives a list of the solutions used to moisten the artificial soil and the average weight loss for 3 blocks in percentage for each variation. Several

TABLE 1

EFFECT ON THE DECAY OF WOOD IN CONTACT WITH SAND AND FULLER'S EARTH MIXTURES MOISTENED WITH VARIOUS NUTRIENTS AND NUTRILITES

Organism *Poria Incrassata*. Time 12 Weeks

Solution Used to Moisten Artificial Soil	Average Weight Loss in Per Cent Due to Decay
Top Soil (Control)	67.0
M.S.* + 0.2% Ammonium Nitrate + 1% Maltose + Vitamins B_1, B_6 and Biotin†	62.7
2% Malt Extract	59.3
M.S. + 2% Ammonium Nitrate + Vitamins	51.3
M.S. + 2% Ammonium Nitrate	47.9
M.S. + Various Combinations of Vitamins‡	29.1
Distilled Water	28.4
M.S. + Vitamins + 1% Maltose	11.6
M.S. + 1% Maltose	12.9

* M.S. = mineral solution containing the following minerals:

Potassium Dihydrogen Phosphate	1.5 grams per liter
Magnesium Sulfate	1.0 gram per liter
Copper	0.02 parts per million
Manganese	0.01 parts per million
Boron	0.005 parts per million
Iron	0.10 parts per million
Molybdenum	0.10 parts per million
Zinc	0.09 parts per million

† The vitamins used and the concentrations per liter were as follows:

B_1	0.1 milligrams per liter
B_6	0.1 milligrams per liter
Biotin	0.02 milligrams per liter

‡ The vitamins were added singly and in the following combinations:

B_1 + B_6 + Biotin—Concentration of each vitamin as listed above.
B_1 + B_6
B_1 + Biotin
B_6 + Biotin

conclusions may be drawn. It is evident that the soil greatly accelerates decay, and that the soil extract contains a large portion of the nutrients and nutrilites which accelerate decay. Malt extract, which contains proteins and sugars, and the mineral solution fortified with ammonium nitrate also stimulate decay. The effect of nitrogen in increasing decay confirms the experiments made by Schmitz and Kaufert,[12] 1936. When nitrogen is lacking and a simple sugar is present, the fungus consumes the simpler sugar instead of the more complex carbohydrate cellulose. This preference is not evident if sufficient nitrogen is present since both carbohy-

drates are destroyed. There is a slight indication that the vitamin mixtures promote decay but the effect as measured by the weight loss is not very pronounced. The basic mineral solution which was used in this experiment promoted only slightly more decay than the distilled water. While the results are not included in the table, it may be stated that leaching of the blocks had no apparent effect on decay when compared with unleached blocks.

Influence of Temperature on Decay

Most of the early work in the Bell Telephone Laboratories was conducted by the petri dish method at temperatures in the range 26°–28°C., following the recommendations of Richards, 1923. But certain fungi, including *Merulius lachrymans*, failed to grow at this temperature. When several inocula of *Merulius lachrymans* that had failed to grow at 26°–28°C. were transplanted to sterile blocks, according to the earlier sapwood-water technique, the loss in weight due to decay after six months averaged 34% at 21°C. and only 7% at 26°–28°C.

An experiment was planned to test the influence of a wide range of temperatures on the decay of wood in the soil contact assay method. Four kinds of fungi were established under sterile conditions on untreated wood slabs laid on moist garden soil. An abundant growth of the fungi was secured within one to two months. Cubes of sapwood were placed on the vigorously growing mycelia, both of which had been conditioned by exposure overnight to the various temperatures. Sterile soil, also conditioned to the temperatures, was used to cover the blocks. After 15 weeks' exposure, the results were as follows:

	Average Weight Loss in Per Cent				
	0°C.	21°C.	26–28°C.	30°C.	35°C.
Poria incrassata	0.0	35.6	58.2	34.7	0.7
Polyporus vaporarius	0.0	42.7	58.9	1.0	0.8
Poria microspora	0.0	53.3	59.0	42.4	2.4
BTL-U-11	0.0	27.1	62.7	60.5	1.3

The results indicate that the standard temperature, 26°–28°C., was optimum for the four fungi tested. No decay was produced by any of the fungi at 0°C. A temperature of 35°C. was too high for active decay; in the case of *Poria microspora*, for instance, only a single block was attacked. The series at 35°C. was repeated because the soil in some of the bottles seemed to have become rather dry, although the blocks contained 30% moisture. In the new series the humidity was maintained at 76% around the bottles to reduce loss of water, and three more organisms were used. The results

of the previous temperature tests were confirmed and the weight losses due to decay by the three additional fungi, which are known to tolerate higher temperatures, were as follows:

Organism	Percentage Weight Loss Due to Decay
Lentinus lepideus	21.8
Lenzites sepiaria	21.3
Lenzites trabea (BTL U-40)	44.0

These results indicate that certain fungi are able to bring about decay of wood over a wider temperature range than others. It is clear that a complete statement cannot be made until the effects of various temperatures between 0°C. and 21°C. have been ascertained. In the light of results with *Lentinus lepideus*, *Lenzites sepiaria*, and *Lenzites trabea* showing considerable decay at 35°C., the upper limits of temperature should be determined for these fungi.

Humphrey and Siggers,[13] 1933, studied the effects of different temperatures on the growth of sixty-four fungi. Two different nutrient substrates were used, but the optimum temperature with these rarely differed by more than 2°C. The following summary shows a comparison of their results with those obtained in the above tests:

	Optimum, °C.		Upper Limit, °C.	
	H&S	BTL	H&S	BTL
Merulius lachrymans	20	21*	28	>28
Poria incrassata	24–30	26–28	34	34
Lentinus lepideus	28	28	36	>35
Lenzites trabea	28–36	28–35	40	>35
Lenzites sepiaria	28–36	28		>35

* Bottle method used; no test has been made yet with soil.

Two of the fungi, *Merulius lachrymans* and *Lentinus lepideus*, brought about decay at limits higher than those reported for cessation of growth by Humphrey and Siggers. *Poria incrassata* had the same limiting temperature in both tests. The temperatures for maximum growth and maximum decay check rather well in both tests.

Field Studies

The rapid decay obtained in the foregoing laboratory experiments based on the soil technique was further evaluated by investigating the rapidity of decay in the field.

Selection of Wood:

For both laboratory and field assays care is exercised in selecting the wood. Boards of southern pine sapwood of the shortleaf type, which includes *Pinus echinata*, and *Pinus taeda*, are obtained from local lumber dealers and are cut into sticks $\frac{3}{4}$ x $\frac{3}{4}$ x 32 inches. Since the square sticks facilitate calculation of volume and retention of toxics or preservatives, they have superseded the round saplings cited by Waterman and Williams,[14] 1934. The sticks are selected on the basis of uniformity of growth, density and ratio of springwood to summerwood. The presence of any heartwood,

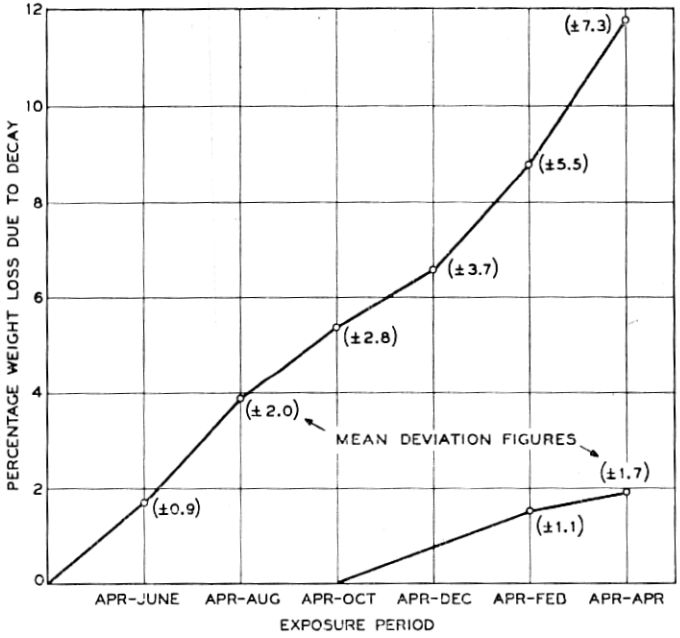

Fig. 5—Field test of untreated sapwood squares exposed at Gulfport, Mississippi, 1941-42

sap stain or other indication of incipient invasion by fungi is cause for rejection. After classification into piles according to arbitrarily chosen weight increments, twenty to twenty-five 32″ sticks, for each concentration of preservative used in field studies are selected by taking the appropriate number of specimens from each pile to give a representative distribution based on density. Since the specimens are subsequently cut in half each individual treatment is represented by 40–50 specimens. Sticks in the median range of density are generally used for laboratory studies after they have been cut into $\frac{3}{4}$″ cubes (8 cc. volume).

An experiment with untreated sticks was carried out at Gulfport, Mississippi, where the climatic conditions are very favorable for decay and also

for termite attack. Six hundred 8-inch lengths were dried in the oven in the laboratory at 105°–110°C. and then weighed. The specimens were then shipped to Gulfport, Mississippi, and distributed throughout the test plot in April. Each eight-inch specimen was buried in the soil until the end of the specimen was even with the level of the soil. At the end of each two-month period subsequent to exposure about 80 specimens were removed, brushed free of dirt and mycelia, oven-dried, and reweighed. In order to study decay during the winter months, 150 additional pieces were planted in October; seventy-five of these were removed after four months and the rest after six months exposure.

From Fig. 5 it is evident that in the field test the loss in weight due to decay was far less than that obtained in the soil test in the laboratory. The maximum amount of decay in the field after two months was as high as 10% in only two out of the 81 samples exposed; after four months it was 19%, after six months 30%, after eight months 20%, after 10 months 30%, and after 12 months 50%. While the maximum percentage loss applies only to one specimen in each case, in general the average percentage losses noted in the figure were far below these figures. Therefore, decay in the field does not approach in uniformity and rapidity that occurring under controlled conditions in the laboratory.

Experiment at Chester, New Jersey, Using Various Nutrients:

Another field experiment was devised in which an attempt was made to increase the rate of decay by using nutrient materials and salts which would change the pH of the soil. Sixteen-inch untreated sticks were selected for uniformity within a very narrow density range and exposed in each of six specially prepared plots in northern New Jersey. The ground was first plowed, then harrowed and raked free of stones so that the soil in all plots was nearly uniform before treatment. Then fifty sticks were buried to a depth of 7 inches in each plot in rows of five, with two feet between each row and one foot between the sticks in each row. The plots were treated as follows:

Plot No.	Treatment
1	Control
2	Barnyard manure
3	5 pounds lime
4	5 pounds commercial fertilizer (5–10–5)
5	5 pounds aluminum sulfate
6	Nutrient solution*

* Containing the following minerals dissolved in ten gallons of water, then sprinkled over the entire plot:

		grams
A)	$Ca(NO_3)_2 \cdot 4H_2O$	297.0
	$MgSO_4 \cdot 7H_2O$	118.8
	KH_2PO_4	83.6
B)	$ZnSO_4$	0.176
	$MnSO_4 \cdot 7H_2O$	0.572
	Boric Acid	0.704
	$Al_2(SO_4)_3$	0.176
C)	$FeSO_4$	5.0

Note: The salts in A, B, and C were dissolved separately and then the three parts mixed.

The sticks were removed and examined at intervals of three, twelve, and fifteen months. The percentage failure, as determined by the ease with which the specimen could be broken, is shown in the following:

	Percentage Failure		
	3 mo.	12 mo.	15 mo.
Control	2	8	72
Manure	10	12	96
Lime	2	10	80
Fertilizer	4	8	94
Aluminum sulfate	16	36	100
Nutrient solution	4	12	100

At the end of 12 months the greatest number of failures due to decay was observed in the plot treated with the acid salt (aluminum sulfate). Colorimetric determinations of the pH of the soil showed it to be between 5.8 to 6.0 for the soil treated with the acid salt and about 6.6 to 6.8 for the control plot. This experiment needs to be repeated for confirmation of results, but the present indications are that the acid soil was much more favorable for decay than the soil in the control plot. Limed and fertilized soils gave results comparable to those of the control plot, with an indication that the fertilizer increased the decay. The complete disintegration of the sticks in the acid treated soil was particularly noticeable, whereas the sticks in the soil treated with manure were intact, though easily broken. The plot treated with the nutrient solution showed the same rate of decay as the manure plot at the end of the twelve-month period. Ten pounds of aluminum sulfate were then added to the nutrient plot, and three months later all the sticks were completely disintegrated. The rapid disintegration of the sticks in the plot treated with the acid salt points to the importance of further work on the effect of the pH on the rate of decay.

Comparison of Soil Technique With Other Toxicity Assays

In the selection of a method for testing relative toxicity of chemicals to microorganisms, the rapidity of test, the standardization of the medium,

the choice of test organisms, the ease of manipulation, the replication of results, and the duplication by other investigators have been the paramount objectives.

A hypothetical test which would meet these requirements could be performed with distilled water to which could be added increasing concentrations of the chemical to be tested. A known amount of fungus mycelium or spores could be shaken with the toxic solution and left for several different time intervals. The fungus filaments or spores could then be removed to a nutrient agar, and the viability of the fungal filaments or percentage of spores germinating could be readily determined. Comparative toxicity of a large number of compounds could be quickly and easily ascertained. The results, however, would be applicable only to a distilled water-poison system, and the concentration of most toxic materials necessary to inhibit growth would be very low.

The addition of nutrients would necessitate larger amounts of the toxic materials (Van den Berge,[15] 1935). Therefore the mineral solutions—nutrient agar, soil extract agar, soil or wood substrate would in general necessitate an increase in toxic material, the amount of increase depending upon which substrate best meets the nutritional requirements of any particular fungus. Some toxicity values would also be affected by chemical or certain physical changes resulting from interaction between the toxic material and the substrate.

In the petri dish method, the fungi selected for studies of wood destruction grow well on the nutrient substrate containing 1.5% malt extract and 2% agar. Although such a mixture has been recommended as a standard substrate,[2] it should be pointed out that the malt syrup is somewhat variable in composition and constituents and that even the agar varies in the amounts of various growth substances present, Robbins and Ma,[16] 1941. The interpretation of results obtained by the assay of a fungicide when dispersed in an agar system should be restricted to that specific system and not applied to a wood-fungicide system.

When wood preservation studies are carried out, reliance cannot be placed on the results of petri dish tests. The use of wood permits the testing of a large variety of the more common preservatives and fungus-proofing agents, many of which may react with the wood or are precipitated in the wood upon loss of solvent. Organic preservatives which are relatively insoluble in water are not readily tested by petri dish assay.

Comparison of the wood-soil contact method with the wood-water method when untreated wood blocks are used is shown in Fig. 2. The greater uniformity in the amount and rapidity of decay and the better control of moisture showed the soil technique to be superior. It is obvious that if the amount of decay is variable and adversely affected by other factors,

the effect of the preservative or fungicide will be obscured. When the sapwood-water method[3] was published, comparison between it and the kolle flask method showed that the wood-water method had certain advantages.

Comparing the method of soil contact with that of soil burial, the principal point of difference is that a pure culture is used in the soil contact method and a mixed culture is used in the soil burial method. Common to both are the moisture-regulating and nutrient properties of the soil. Since the microbial activity of unsterile soil is diverse, depending on the type and source of soil, uniform results from soil burial could not be expected. When wood specimens were exposed individually in bottles of non-sterile soil in the laboratory, the amount of decay after 12 weeks' exposure was less than 10% for all specimens. The results were similar to those obtained by the exposure of untreated wood out-of-doors at Gulfport, Mississippi, for the two-month period (Fig. 5). Since decay-producing organisms were shown to be present, the other organisms in the soil must have interfered with the growth of the wood-destroying fungi. The antagonism between the wood-destroying fungus *Lentinus lepideus* and a contamination is shown in Fig. 6.

The soil-contact technique instead of the soil burial method has been used extensively to test cotton fabric, thread, paper, jute, fibers, and a variety of other materials. The organisms have been varied according to their occurrence on the particular substrate in nature. The fungi *Chaetomium globosum*, *Aspergillus niger*, *Stachybotrys atra*, *Stysanus media*, and *Metarrhizium* have been established with excellent results on a substrate of cloth when testing fabric. The loss in tensile strength of an unprotected cotton thread which had an initial absolute pull of 30 pounds was 90–100% after two weeks' exposure to *Chaetomium globosum*. Treated threads or other cellulosic materials may be tested as satisfactorily as treated wood.

Examination of numerous reports from soil burial studies of treated textiles indicates that organisms which tolerate certain types of chemicals become dominant in the test beds. As a result, a preservative which shows great promise initially may suddenly fail when the test is repeated. If a pure culture technique were used, a better evaluation of the preservative would be possible.

Similarly the controversies which have arisen over the ability of certain fungi to destroy cellulose could be resolved by using the suitable cellulose soil technique. At least there is very good evidence that many of the environmental variations affecting decay are at or near the optimum.

Toxicity Tests

In the toxicity tests which follow, petri dish results are given for several compounds, soil contact test results are given for compounds not readily

assayed by the petri dish method, and the field test results are included for comparison with the results of the soil contact test method.

Fig. 6—Antagonism which has persisted for over one year between the wood-destroying fungus, Lentinus lepideus (outer portion) and contaminating fungus (inner portion) in a soil culture.

During the initial stages of this research on the evaluation of toxic properties of various compounds for wood preservation, the petri dish method was used for assay studies in the laboratory and the modified sapling method

of Waterman and Williams[14] (1934) was used for the field tests at Gulfport, Mississippi. Table 2 shows the results obtained with the petri dish method on the toxicity of four common inorganic salts and a creosote to several of the usual test fungi.

TABLE 2
TOXICITY EXPRESSED IN PER CENT TOXIC AGENT PRESENT IN NUTRIENT AGAR AS DETERMINED BY PETRI DISH ASSAY

Compound	Fungi	Inhibition Point	Killing Point
Arsenic Trioxide	Madison # 517	0.04	0.064
	Poria incrassata	0.10	0.10
	Lentinus lepideus	0.30	0.30
	Fomes roseus	0.30	0.30
	Poria microspora	0.30	0.30
	Polyporus vaporarius	0.49	0.49
Zinc Chloride	*Poria incrassata*	0.16	0.16
	Madison # 517	0.15	0.23
	Lentinus lepideus	0.16	0.40
	Fomes roseus	0.64	0.64
	Poria microspora	1.40	1.90
	Polyporus vaporarius	1.40	1.90
Mercuric Chloride	Madison # 517	0.0012	0.0012
	Lentinus lepideus	0.002	0.002
	Polyporus vaporarius	0.002	0.002
	Poria incrassata	0.005	0.005
	Poria microspora	0.01	0.01
	Fomes roseus	0.01	0.01
Copper Sulfate	*Lentinus lepideus*	0.06	0.16
	Madison # 517	0.10	0.16
	Lenziles sepiaria	0.30	0.30
	Fomes roseus	0.24	0.36
	Poria incrassata	0.50	0.50
	Polyporus vaporarius	1.00	1.00
	Poria microspora	1.0	1.00
Creosote	*Poria incrassata*	0.012	0.096
	Polyporus vaporarius	0.024	0.12
	Lenziles sepiaria	0.96	1.00
	Poria microspora	0.20	1.40
	Lentinus lepideus	0.96	1.60

Resistance of the fungi to the four salts is variable, but it will be noted that *Lentinus lepideus*, which is most sensitive to copper sulfate, tolerates the highest concentration of creosote. *Poria incrassata*, which is fairly tolerant of copper salts by petri dish test, is the most sensitive to zinc chloride and creosote. *Poria microspora* tolerates relatively greater concentrations of all the compounds than any of the fungi tested.

The four salts assayed can be easily dissolved in an agar medium in concentrations high enough to be toxic, but uniform dispersal of insoluble salts

in the agar is not so easily accomplished. Many salts may be made soluble by dissolving them in dilute ammonia or acetic acid solutions. For example, copper arsenate or zinc meta-arsenite are soluble in ammonia or acetic acid, and by evaporation of the volatile portions of the solvent the salts are precipitated. When precipitation of the salts from ammoniacal or acetic acid solution is carried out in treatments of wood, subsequent evaporation of ammonia or acetic acid from the wood is rather rapid. In agar solutions, uniform precipitation of the salts through evaporation of the ammonia and acetic acid is not easily attained.

TABLE 3

TWENTY FOUR WEEK SOIL ASSAY OF WOOD PRESERVATIVE COMPOUNDS NOT READILY ASSAYABLE BY PETRI DISH METHODS

Mixture #1	Average Weight Loss in Per Cent			
	1.60 lbs/cu.ft.	0.80 lbs/cu.ft.	0.41 lbs/cu.ft.	Untreated*
Poria incrassata	0.0	10.1	10.1	61.8
Polyporus vaporarius	0.0	0.7	0.6	34.1
B.T.L. U-11	0.0	1.8	6.4	56.3
Mixture #2	2.79 lbs/cu.ft.	1.40 lbs/cu.ft.	0.68 lbs/cu.ft.	
Poria incrassata	40.9	31.9	38.8	52.0
Polyporus vaporarius	45.9	30.6		48.6
B.T.L. U-11	21.3	57.0	31.8	48.5
Mixture #3	0.35 lbs/cu.ft.	0.15 lbs/cu.ft.		
Poria incrassata	46.7	22.9		52.4
Polyporus vaporarius	0.0	6.1		63.8
B.T.L. U-11	0.0	5.9		56.4
Mixture #4	0.72 lbs/cu.ft.	0.36 lbs/cu.ft.		
Poria incrassata	1.0	13.8		50.7
Polyporus vaporarius	0.0			55.4
B.T.L. U-11	1.0	14.5		53.6

* Average per cent weight loss of untreated blocks in the same bottles with the treated blocks.

Agar cannot readily be used for assays of two other types of compounds used as wood preservatives. The first type depends on chemical reactions with and also within the wood. Specific examples of this type are the series of compounds fixed in the wood by the reduction of chromium salts which was first studied by Kamesam,[17] 1934. It is now the generally accepted view that the reduction of the chromium is brought about by various sugars in the wood. Subsequent research led to the use of Ascu (Kamesam) or Greensalt K and to the later development of Greensalt "O" by the Bell Telephone Laboratories in the United States and the Bolidens' salts in Sweden. These inorganic salt mixtures were developed in the search for preservatives which

would be fixed in the wood and thus resist leaching when exposed to the action of ground waters.

The second type is comprised of organic compounds or mixtures of organic compounds, such as creosote, which has had an excellent service record as a preservative. Also included in this type of compounds (which are insoluble in water and have a relatively low vapor pressure) are certain chlorinated phenols and cresols. Because of the low water solubility or immiscibility with agar solutions, the uniform dispersal of the toxic agents in the agar system, which is essential to reproducibility of results, is almost impossible. Uniform injection of these materials into wood, however, presents no particular problem.

Assays of four representative mixtures not readily assayable in the petri dish are included in the results of Table 3. The composition of the treating solutions of the mixtures 1, 2, 3, and 4 is given below:

Mixture 1	Zinc oxide	46.6%
	Chromic acid	3.8%
	Arsenic acid	49.6% dissolved in a 10% ammonia solution
Mixture 2	Copper phenolate	4%
	Zinc phenolates	1%
Mixture 3	Sodium fluoride	25%
	Disodium hydrogen arsenate	25%
	Sodium chromate	37.5%
	Dinitrophenol	12.5%
Mixture 4	Zinc oxide	22.5%
	Arsenic oxide	35.5%
	Sodium carbonate	1.0%
	Acetic acid	41.0%

In the assays shown in Table 3 the maximum retention of the mixture by the wood is that recommended for the treatment of wood that is not to be used in contact with the ground. To make the test as severe as possible, organisms were selected which were known from petri dish, kolle flask, and wood-water assays to have a high tolerance for various inorganic salts.

Examination of the data in Table 3 shows that the compounds produced in the wood by mixture 4 afford almost complete protection to the wood which was treated with 0.72 pound of the salt per cubic foot. The vigorous attack on the untreated blocks is evidence of the severity of the test. Similarly, the comparable treatment of the wood with 0.8 pound of mixture 1 per cubic foot was very effective in protecting the wood against fungus attack. If the amount of mixture 1 in the wood is doubled, almost perfect protection against decay may be obtained. Wood treated with 0.35 pound of mixture 3 per cubic foot is protected against *Polyporus vaporarius* and BTL U-11 but

not against *Poria incrassata*. The latter fungus completely disintegrates both the treated and the untreated wood. Despite the fact that the wood treated with mixture 2 represented the highest concentration of any preservative used (2.8 pounds per cubic foot), complete disintegration of the wood results from the action of the three fungi just mentioned.

Compounds of the Greensalt type were also assayed by means of the soil-contact method. The solution commonly used for treatment of wood with Greensalt K contains three chemicals in the following proportions:

Potassium dichromate	$K_2Cr_2O_7$	55%
Copper sulfate	$CuSO_4 \cdot 5H_2O$	33%
Arsenic acid	$As_2O_5 \cdot 2H_2O$	11%

After treatment of the wood with this solution, reduction of the chromium by the sugars in the wood together with evaporation of water precipitates in the wood fibers several complex insoluble salts, among which presumably

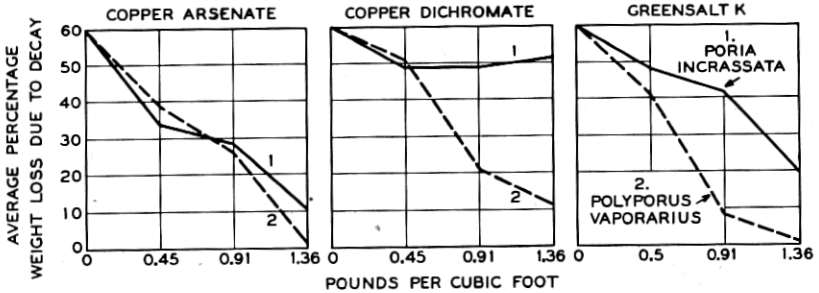

Fig. 7—Comparison by wood-soil assay of components with the whole salt of Greensalt K. Organisms Poria incrassata and Polyporous vaporarius. 24 weeks' time.

are copper arsenate and copper dichromate. Results of soil-contact assay of wood treated separately with solutions of these two components and with the whole Greensalt K complex are given in Fig. 7. Before exposure to the fungi, the wood specimens were leached by a diffusion method described by Waterman, Leutritz and Hill[3], 1938. No untreated control blocks were included in the bottles with this test, which was conducted for 24 weeks. The copper arsenate component of the Greensalt K complex is shown to be much more effective as a preservative than the copper dichromate component but not as effective as the whole K salt complex. Figure 8 shows the setup for the wood-soil assay of 0.75 lbs/cu. ft. of Greensalt K from a recent series of cooperative experiments conducted by the Forest Products Laboratory, Madison, Wisconsin. Figure 9 is a comparison of the Greensalt K treated and untreated reference blocks in the same bottles after exposure to the following fungi:

Blocks	Fungi
A	*Lenzites trabea* #617 F.P.L.
B	*Poria incrassata* #563 F.P.L.
D	*Lentinus lepideus* #534 F.P.L.
E	*Poria microspora* #106 F.P.L.
F	*Poria luteofibrata* (Baxter)

The distortion and shrinkage of the blocks can be used as a visual confirmation of the weight loss due to decay.

The more recently developed Greensalt O is similar to the K salt. The treating solution of this salt mixture is composed of copper oxide, hydroxide or carbonate, chromic acid anhydride, and arsenic acid in percentages based on the chemical equivalents of the copper, chromate and arsenic salts in the K salt solution. The toxicity from the wood-soil assay of Greensalt O is given in Table 4 for fourteen fungi and for three concentrations of the preservative. The effectiveness of the Greensalt O treatment of wood is apparent from examination of the toxicity index. The weight losses due to decay of the untreated reference blocks indicated in general that conditions for decay were again very severe, but the reference blocks exposed to the fungus *Lentinus lepideus* were protected by their proximity to the treated specimens. As previously pointed out, the fungus *Lentinus lepideus* does not tolerate even slight concentrations of copper, which is a major component of the Greensalt complex.

The fungus *Poria incrassata* was again shown to be the least affected by the toxicity of the preservative. The toxicity index for the blocks treated with the highest concentration of the preservative was 94% after 16 weeks' exposure to this organism. In view of the excellent field record for the equivalent K salt preservatives ratings 90 to 100% by the toxicity index would be satisfactory. Additional data will undoubtedly determine the limits of the toxicity index.

At the end of the 24-week period, the fungi BTL U-11, *Lenzites trabea*, *Trametes serialis*, *Polyporus vaporarius*, and one strain of *Coniophora cerebella* caused slight decay of one or more blocks treated with the maximum concentration of preservative, the fungi *Lenzites trabea*, *Coniophora cerebella*, and *Trametes serialis* were still capable of causing only slight losses. Exposure of the blocks treated with the low contentration of preservative to *Polyporus vaporarius* and BTL U-11 resulted in an increase in the amount of decay.

Since the resultant salts of the Greensalt O reaction should be similar to those produced by Greensalt K, field trials of these materials would be expected to give comparable results. Extended field tests of wood treated with one pound of Greensalt K per cubic foot have been in progress for ten years without a single failure having occurred in more than 40 specimens. Specimens treated with Greensalt O have been tested in the field for only

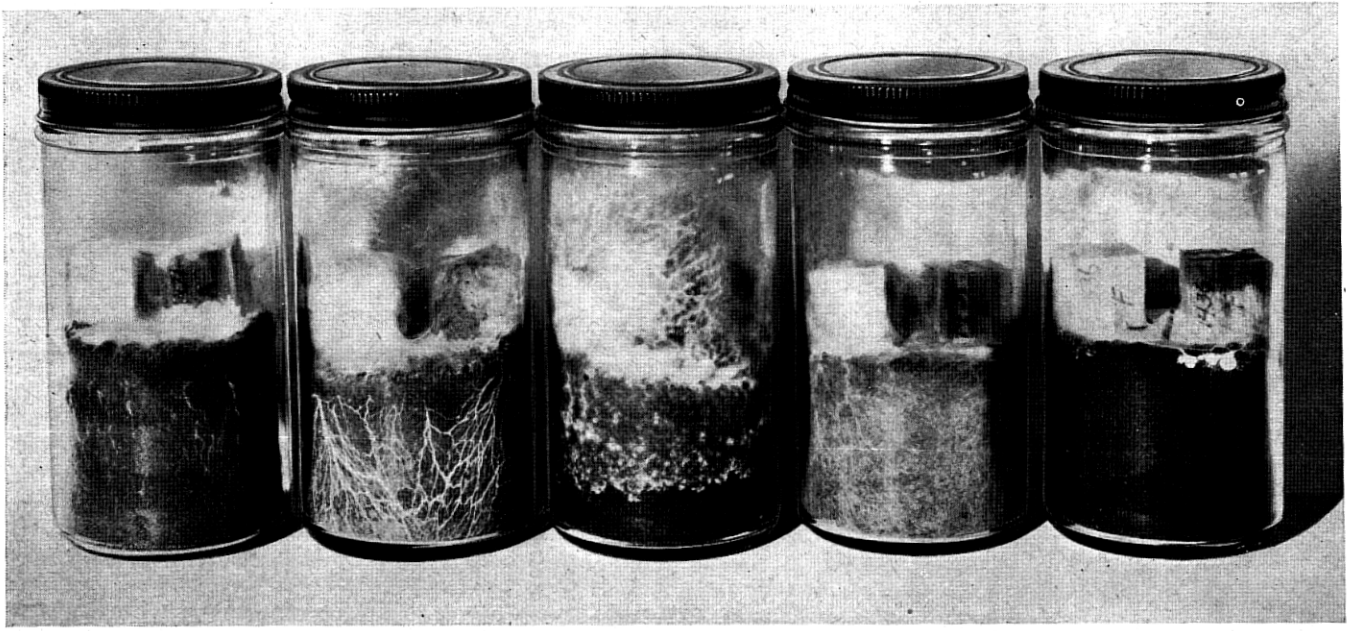

Fig. 8—Wood-soil assay of 0.75 lbs/cu. ft. of Greensalt K (courtesy of Forest Products Laboratory, Madison, Wisconsin). Reading left to right the samples are:

Blocks	*Fungi*
A—	*Lenzites trabea* #617 F.P.L.
B—	*Poria incrassata* #563 F.P.L.
D—	*Lentinus lepideus* #534 F.P.L.
E—	*Poria microspora* #106 F.P.L.
F—	*Poria luteofibrata* (Baxter)

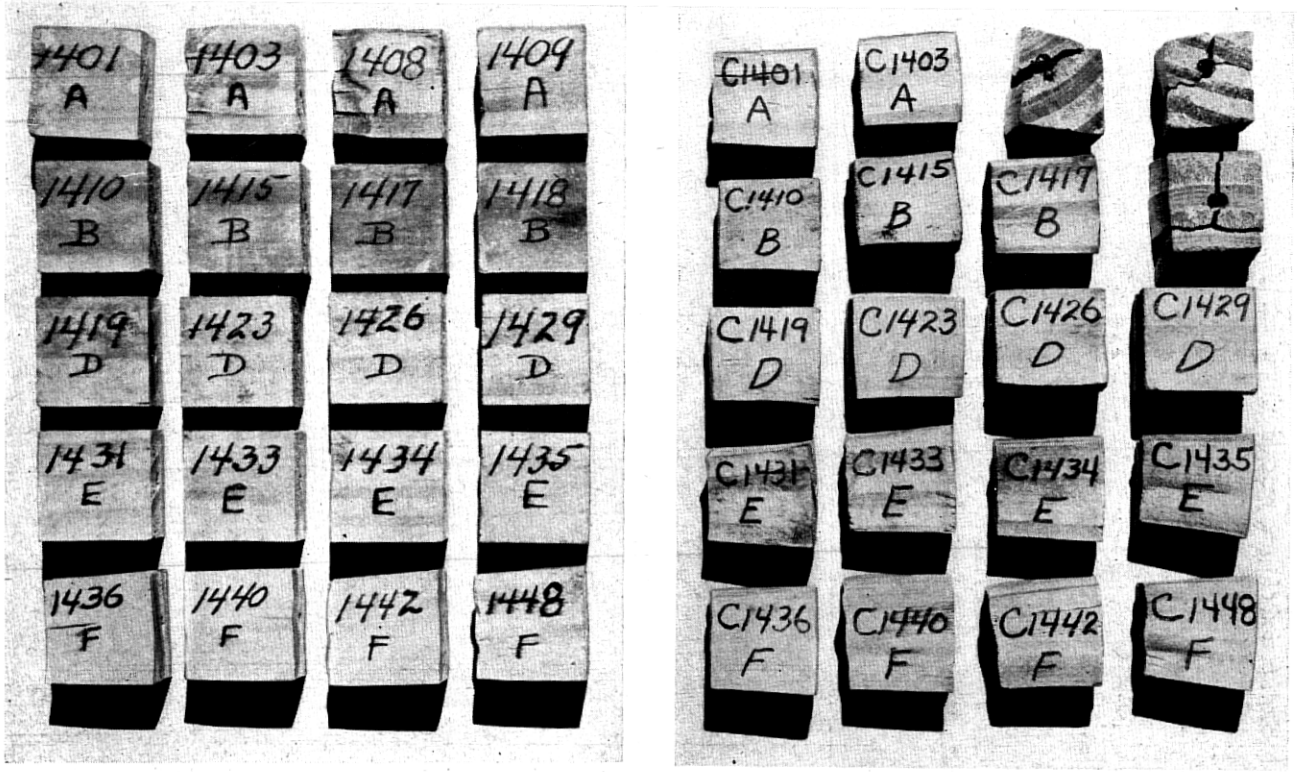

Fig. 9—A comparison of the Greensalt K treated and untreated reference blocks after exposure to fungi. (Treated on the left—untreated on the right side of picture.)

Blocks	*Fungi*
A—	*Lenzites trabea* ⁂617 F.P.L.
B—	*Poria incrassata* ⁂563 F.P.L.
D—	*Lentinus lepideus* ⁂534 F.P.L.
E—	*Poria microspora* ⁂106 F.P.L.
F—	*Poria luteofibrata* (Baxter)

the relatively short period of three years, during which time all the specimens have remained sound.

Results of other field trials for a three-year period with the same mixtures 1, 2, 3, and 4, listed previously, copper arsenate, Greensalt O, and a creosote are given in Fig. 10. Twenty-five untreated controls showed 84% failure, 4% sound, and 12% badly infected in one year. All had failed at the end of the second year. Twenty specimens were used for each retention of the individual preservatives, with the exception of the creosote. The reason for fewer creosote specimens within the correct retention is that the empty-cell treatments of wood with creosote give a wider range of retention than the full-cell treatments of the wood with water solutions of the salts.

TABLE 4

TWENTY FOUR WEEK—WOOD SOIL ASSAY OF GREENSALT "O" USING 13 SPECIES OF WOOD DESTROYING FUNGI

	Toxicity Index*		
	1.17 lbs/cu.ft.	0.96 lbs/cu.ft.	0.476 lbs/cu.ft.
Poria incrassata (16 wks)	94	68	25
B.T.L. U-11	98	88	62
Polyporus vaporarius	98	93	55
Lenzites trabea	98	96	94
Coniophora cerebella	98	98	98
Trametes serialis	98	98	98
B.T.L. U-4	100	98	98
Polyporus anceps	100	98	98
B.T.L. U-53	100	99	98
B.T.L. U-24	100	99	98
Lenzites sepiaria	100	100	98
Poria microspora	100	100	98
Fomes roseus	100	100	100
Lentinus lepideus	100	100	100

* Toxicity Index $= \frac{\%\text{ loss of norm} - \%\text{ loss of treated block}}{\%\text{ loss of norm}} \times 100.$

In the three-year period of exposure only the treatments of wood with 1.3 pounds of copper arsenate and 7 pounds of creosote per cubic foot showed a perfect record. Copper arsenate had previously been shown to be a very effective component of the Greensalt complexes when tested by the soil-contact method. Mixture 2 was found to be the poorest by soil-contact assay and also in the field trials. Mixtures 4, 1, and 3 were rated in that order of decreasing effectiveness in the field test. In the soil-contact assays at comparable retention, 0.72 and 0.80 pound of salt per cubic foot of wood, respectively, mixture 4 was better than 1; and at 0.41 and 0.35 pound of salt per cubic foot of wood, respectively, compound 1 was slightly better than 3, especially against *Poria incrassata*.

Results from field and laboratory tests show good agreement in the evalu-

ation of the compounds. The advantage of the laboratory method in the matter of time is a decided one, but the field trial is valuable for testing the permanence of the preservative. For example, mixture 4 was found in the laboratory test to be a very effective preservative, but the initial preservative properties were dissipated by exposure to the weather, since 50% of the

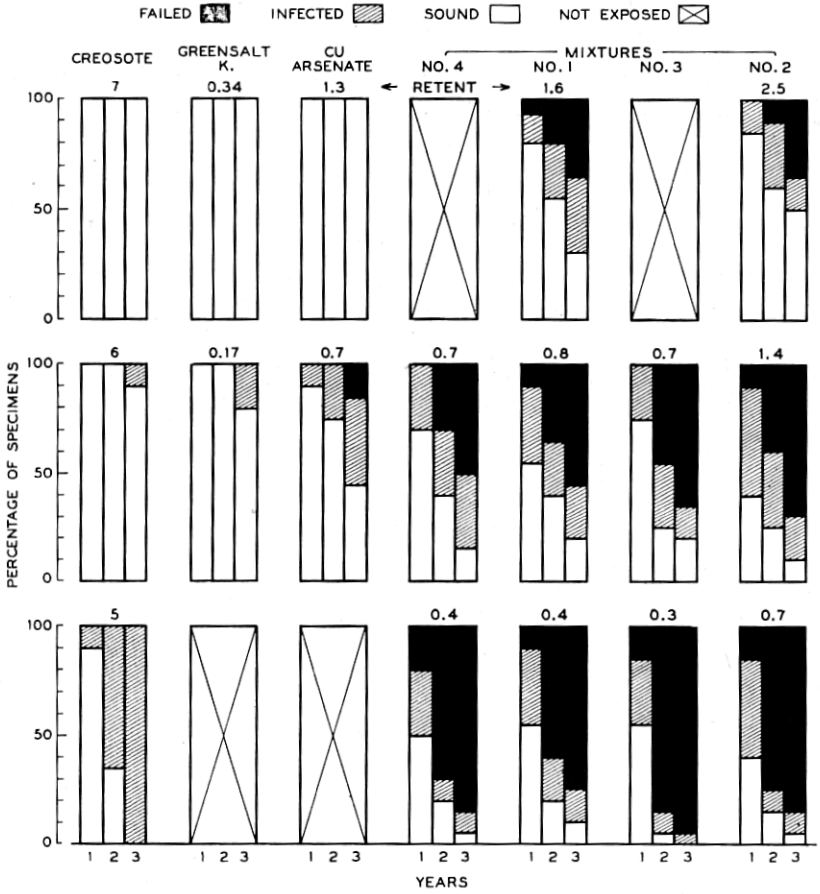

Fig. 10—Results of field exposures on the four mixtures of Table 3 and other compounds creosote, Greensalt K, copper arsenate (retent in pounds per cubic foot).

specimens had to be removed because of failure within three years. When a compound is a poor preservative, as in the case of mixture 2, both laboratory and field trials serve to eliminate it from further consideration. By the soil-contact method every specimen treated with mixture 2 was badly attacked by all the organisms used, whereas at a comparable retention only 35% of the specimens in the field trials had failed after three years' exposure.

Since these results indicate that the soil-contact provides optimum conditions for decay, the laboratory method serves as a means of quickly eliminating inferior preservatives and minimizing the number for field studies.

Summary

The soil-contact method described in this paper has been shown to be a valuable laboratory tool for the study of fungus destruction of cellulose and wood and for the determination of the value of wood and cellulose preservatives.

Top soil containing 20 to 25% moisture on a dry-weight basis, when used as a supporting substrate for decaying wood, proved to be an excellent means controlling the moisture content of wood during the decay process. Investigation showed that the optimum moisture content for initiating the decay of wood was fiber saturation. It also was found that during decay the initial water content of the wood remained constant, through maintenance of a constant volume of the wood structure despite loss of wood substance.

Experiments with various combinations of nutrients and nutrilites added to artificial soil showed the importance of these materials in decay studies. The need for nitrogen in the destruction of cellulose by fungi was confirmed. Lack of wood decay in the presence of a sugar when there is also a deficiency of nitrogen presents an interesting problem the explanation of which may throw considerable light on discrepancies in many test procedures. Comparison of results with nutrient artificial soils and an average top soil indicates the possibility of employing a standard artificial soil in the contact test method.

The optimum temperature for most wood-destroying fungi tested was found to be 26°–28°C. Decay occurred over a wider range of temperature in soil-contact tests than in petri dish tests.

It was found that decay was much more uniform and more rapid in the soil-contact method than in other laboratory methods or in field trials. There is a large, single, vigorous inoculum in the soil-contact laboratory method, while in the field antagonism between wood-destroying organisms and the other flora and fauna of the soil frequently checks the decay process.

Toxicity studies based on petri dish assays showed that the amount of a compound tolerated by several fungi varies considerably. Petri dish assays of toxic materials are often misleading. Generally, higher retentions of the preservatives are needed to prevent decay than are indicated by petri dish assay. Occasionally, a material which performs poorly in the petri dish test will, however, act as a satisfactory preservative of cellulosic derivatives in both soil-contact and field tests.

Field trials of preservatives, though in general less rapid, confirm the results of the soil-contact method and in addition determine the degree of

permanence of the preservative. However, heating, leaching and other simulated weathering cycles may be used in conjunction with the soil-contact method to determine the stability of a preservative to evaporation, to the solvent action of ground water, and to chemical deterioration by ultra-violet light. Futher comparisons between soil-contact assay and field test of a wood preservative such as Greensalt confirms the fact that conditions for decay are more nearly optimum in the former and result in an unusually severe test of the preservatives.

The soil-contact method is an excellent laboratory tool easily adapted to fundamental studies of the decay process and to evaluation of preservatives. The method has shown considerable promise in evaluating preservatives for a wide variety of materials, including leather, cotton, felt, paper and jute. Since the factors influencing decay are very near optimum in the soil-contact method, any preservative that prevents decay in this laboratory test and is permanently retained will be effective under any climatic conditions.

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BIBLIOGRAPHY

1. Williams, R. J., 1928, "Nutrilites," *Science 67*, 607.
2. Richards, C. A., 1923, "Methods of testing relative toxicity," *Proc. Amer. Wood-Pres. Assoc., 19.*
3. Waterman, R. E., Leutritz, John, and Hill, C. M., 1937, "A Laboratory Evaluation of Wood Preservatives, *Bell Sys. Tech. Jour., 16*, 194–211.
4. Falck, R., 1927, "Merkblätter zur Holz-Schutzfrage," *Hausschwammforschungen begrundet, von A Möller, Heft* 8, p. 17, *Jena.*
5. *American Society for Testing Materials*, 1942, "Tentative Methods of Test for Resistance of Textile Fabrics to Microorganisms," *A.S.T.M. Designation:* D684–42T.
6. *Signal Corps Tentative Specification* No. 71-2202A, April 12, 1944, "Moisture and Fungus-Resistant Treatment of Signal Corps Ground Signal Equipment."
7. Furry, Margaret S. and Zametkin, Marion, 1943, "Soil-suspension Method for Testing Mildew Resistance of Treated Fabrics," *AM Dyestuffs Reptr., 32*, 395–8.
8. Leutritz, John, Jr., 1939. "Acceleration of Toximetric Tests of Wood Preservatives by Use of Soil as a Medium," *Phytopathology, 29* (10) 901–903.
9. Nobles, Mildred K., 1943, "A Contribution Toward a Clarification of the *Trametes Serialis* Complex." *Canadian Jour. of Res., 21*, 211–234.
10. Schorger, A. W., 1926, "The Chemistry of Cellulose and Wood," McGraw, New York.
11. Benton, V. L. and Ehrlich, John, 1941, "Variation in Culture of Several Isolates of *Armillaria Mellea* from Western White Pine," *Phytopathology, 31*, (9) 803–811.
12. Schmitz, Henry and Kaufert, Frank, 1936, "The Effect of Certain Nitrogenous Compounds on the Rate of Decay of Wood," *Amer. Jour. of Botany, 23*, (10) 635–8.
13. Humphrey, C. J. and Siggers, P. V., 1933, "Temperature Relations of Wood-Destroying Fungi," *Jour. of Agr. Res., 47*, (12) 997–1008.
14. Waterman, R. E. and Williams, R. R., 1934, "Small Sapling Method of Evaluating Wood Preservatives," *Ind. and Engr. Chem., Analytical Ed., 6*, 413–419.
15. Van den Berge, J., 1935, "Testing and Suitability of Fungicides for Wood Preservatives." Unpublished manuscript. Translation from the Dutch.
16. Robbins, W. J. and Ma, Roberta, 1941, "Biotin and the Growth of *Fusarium Avenaceum*, *Bull. Torrey Bot. Club, 68* (7) 446–462.
17. Kamesam, Sonti, 1934, *British Patent* 404,855.