SOIL-BLOCK AND AGAR-BLOCK TECHNIQUES FOR EVALUATION OF
OIL-TYPE WOOD PRESERVATIVES: CReOSOTE, COPPER
NAPHTHENATE AND PENTACHLOROPHENOL

By

CATHERINE G. DUNCAN1, Pathologist

INTRODUCTION

A laboratory method for evaluating wood preservatives should provide in a comparatively short time an integrated measure of the relative permanence and fungus-inhibiting capacity of a product. There is as yet no generally acceptable laboratory method of this type. Systematic service records serve to bolster the reputation of established preservatives. Accelerated field tests provide a basis for recognizing new preservatives. However, if it were not for basic technical information, supplied through laboratory experiments, changes in specifications which might further improve such preservatives are delayed. Furthermore, the development of new and promising preservative materials is retarded if evaluation must depend entirely on years of service. Such retardation complicates the consumer’s problem of procuring suitable substitute preservatives when material shortages arise during emergencies.

Various methods, summarized in recent extensive literature reviews (3, 4, 28),2 have been employed for evaluating wood preservatives. Reference to pertinent publications will be made at appropriate points in the paper. The Petri-dish, flask, or toximetric test (37) in which the preservative material is dispersed in a malt-agar medium, should not be underrated in its own field, namely, in the determination of relative toxicities. However, a more realistic laboratory test is to expose treated wood blocks to the action of wood-destroying fungi. The agar-block or soil-block culture techniques can be used to screen out the less effective and to test, under laboratory conditions, the relative permanence of the more effective preservative materials. The soil-block test is the more severe of the two, demanding in general higher preservative retentions for inhibition of decay in the blocks. It also permits better control of moisture content than the agar-block method. Therefore, it is believed that the soil-block technique as now used at Madison approaches the ideal laboratory test more closely than does the agar-block procedure.

1 In cooperation with the Forest Products Laboratory, maintained by the Forest Service, U.S. Department of Agriculture, at Madison, Wis., in cooperation with the University of Wisconsin.

2 Italic numbers in parentheses refer to Literature Cited, p. 35.
The experimental data presented here are based on work that was started in 1946 and essentially finished in 1948 in cooperation with Bell Telephone Laboratories, Inc. The materials tested were a creosote, a solution of pentachlorophenol, and a solution of copper naphthenate in a light petroleum oil, and mixtures of these three. Five test fungi were employed.

The object of the paper is to evaluate the agar-block and soil-block methods for testing treated blocks, both before and after weathering of the test specimens, with particular emphasis on oil-type preservatives. The presentation and discussion will show the modifications in technique developed by other published (10, 11, 12, 13, 14, 15) and unpublished work on the soil-block method carried on either simultaneously or since the tests in question were completed. Lines along which current and future work are developing to resolve controversial problems will be indicated.

DESCRIPTION AND EXPLANATION OF TEST METHODS

MATERIALS TESTED

The materials tested are listed in table 1. The analytical data\(^3\) for the coal-tar creosote and petroleum are shown in tables 2 and 3, respectively.

PREPARATION OF TEST BLOCKS

Longleaf pine, *Pinus palustris*, was chosen as the source for the test blocks because it has wide use in the form of preserved ties, timbers, poles, and piles. Logs from freshly felled trees were selected for uniformity and growth rate. Boards were sawed from these logs and then kiln-dried immediately to prevent mold and stain development. The kiln-dried boards were conditioned to approximately 6 percent moisture content before the test blocks were cut to size. Only the sapwood was used because it is highly decay-susceptible and easy to impregnate under laboratory conditions.

The blocks for the soil-block method\(^4\) were 3/4 by 3/4 by 3/4 inch with a 1/8-inch hole drilled radially through the center of a tangential face. Those for the agar-block method\(^4\) were 1-1/2 by 3/4 by 3/8 (along the grain) inches and without a hole. Although all test blocks were of the same volume, except for the hole in the 3/4-inch cube, those for the agar-block method had twice the transverse surface area. In a previous study (34) of the agar- and soil-block methods a 3/4-inch cube, with a hole drilled through the center of a transverse surface, was used in both techniques. It was found that the cube specimen readily slipped off the glass support and came in contact with the malt-agar medium. In the weathering process the soil blocks were strung on nylon threads and the agar blocks were placed on trays. The change in direction of drilling the hole in the soil blocks was made to facilitate production of the blocks, and at the same time the radial hole facilitated distribution of the preservative. The weight of the blocks (oven-dry basis) varied from 3.3 to 5.2 grams. The shape of the test blocks permitted rapid absorption as well as rapid loss of preservative.

\(^3\) Supplied by Bell Telephone Laboratories, Inc.

\(^4\) Test blocks in the soil-block method will be referred to as "soil blocks"; those in the agar-block method as "agar blocks."
Table 1.—Materials tested

<table>
<thead>
<tr>
<th>Test series</th>
<th>Materials</th>
<th>Component parts (Percent by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Coal-tar creosote</td>
<td></td>
<td>Creosote: 100.00</td>
</tr>
<tr>
<td>B: Penta-petroleum: a 5 percent solution by weight</td>
<td></td>
<td>Penta-chlorophenol: 5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copper: 95.00</td>
</tr>
<tr>
<td>C: Copper-petroleum: a solution of copper naphthenate equivalent to 0.525 percent copper by weight in a light naphthenic-base</td>
<td></td>
<td>Petroleum oil: 0.525</td>
</tr>
<tr>
<td>D: Penta-copper-petroleum: a blend of equal parts by volume (50-50) of B and C</td>
<td></td>
<td>Penta-chlorophenol: 2.50</td>
</tr>
<tr>
<td>E: Penta-petroleum-creosote; a blend of equal parts by volume (50-50) of A and B</td>
<td></td>
<td>Copper: 42.00</td>
</tr>
<tr>
<td>F: Copper-petroleum-creosote; a blend of equal parts by volume (50-50) of A and C</td>
<td></td>
<td>55.79</td>
</tr>
<tr>
<td>G: Penta-copper-petroleum-creosote; a blend of equal parts by volume (50-50) of A and D</td>
<td></td>
<td>55.79</td>
</tr>
<tr>
<td>H: Petroleum-creosote; a blend of equal parts by volume (50-50) of A and I</td>
<td></td>
<td>55.79</td>
</tr>
<tr>
<td>I: Petroleum oil; a light naphthenic-base</td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

1. The materials tested were furnished by Bell Telephone Laboratories, Inc. A, B, C, and I are basic materials and D, E, F, G, and H were made by blending. All treating solutions were made by dissolving the materials tested in toluene in sufficient range of concentrations to insure a gradient in the retention of the material in the treated blocks.

2. Added as copper naphthenate.

3. The percent by weight of petroleum wherever copper is present includes the naphthenate.

4. Materials tested only by the soil-block method.
### Table 2. -- Analytical data for the creosote used

<table>
<thead>
<tr>
<th>Distillation fraction</th>
<th>Specific gravity at 38° C</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0° - 210° C.</td>
<td>1.088</td>
<td></td>
</tr>
<tr>
<td>210° - 235° C.</td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>235° - 270° C.</td>
<td></td>
<td>12.07</td>
</tr>
<tr>
<td>270° - 300° C.</td>
<td></td>
<td>29.25</td>
</tr>
<tr>
<td>300° - 315° C.</td>
<td></td>
<td>12.18</td>
</tr>
<tr>
<td>315° - 355° C.</td>
<td></td>
<td>24.80</td>
</tr>
<tr>
<td>Residue above 355° C</td>
<td></td>
<td>20.90</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

- Sulphonation residue: .51
- Tar acids: 4.10
- Benzol insoluble: .07

A domestic coal-tar creosote secured directly from the creosote producer by Bell Telephone Laboratories, Inc.

### Table 3. -- Characteristics of the straight-run light naphthenic-base petroleum oil used

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity</td>
<td>29.8</td>
</tr>
<tr>
<td>Specific gravity at 60° F/60° F</td>
<td>.977</td>
</tr>
<tr>
<td>Pounds per gallon at 60° F</td>
<td>7.30</td>
</tr>
<tr>
<td>Specific gravity at 38° C/15.5° C</td>
<td>.862</td>
</tr>
<tr>
<td>Distillation (ASTM D-156)</td>
<td></td>
</tr>
<tr>
<td>Initial boiling point</td>
<td></td>
</tr>
<tr>
<td>5 percent</td>
<td>421</td>
</tr>
<tr>
<td>10 percent</td>
<td>462</td>
</tr>
<tr>
<td>50 percent</td>
<td>511</td>
</tr>
<tr>
<td>75 percent</td>
<td>550</td>
</tr>
<tr>
<td>97 percent</td>
<td>570</td>
</tr>
<tr>
<td>Final boiling point</td>
<td></td>
</tr>
<tr>
<td>Residue 2 percent</td>
<td></td>
</tr>
<tr>
<td>Flash point (closed cup)</td>
<td>592</td>
</tr>
<tr>
<td>Viscosity (S.U.S. 100° F)</td>
<td>192</td>
</tr>
<tr>
<td>Aniline point</td>
<td>33.0</td>
</tr>
<tr>
<td>Aromatics, by volume</td>
<td>16.0</td>
</tr>
<tr>
<td>Unsaturates, by volume</td>
<td>25.0</td>
</tr>
<tr>
<td>Dimethyl sulphate value</td>
<td>14.0</td>
</tr>
</tbody>
</table>
After the blocks were cut they were marked for identification, brought to constant weight (approximately 6 percent moisture) in a conditioning room maintained at 30 percent relative humidity and 80° F., and then weighed individually ($T_1$). (See table 4 for symbols used in recording weights and calculations.)

**IMPREGNATION OF TEST BLOCKS**

A single concentration of preservative was used to treat 35 blocks for testing, 4 blocks for future chemical assay, and 4 for moisture determinations for each of the two methods. Approximately one-half of the blocks were tested after conditioning, the other half after conditioning and weathering. Only the blocks to be weathered were treated with the higher concentrations of some of the materials. Three of the materials were not tested by the agar-block method (see table 1). A total of approximately 6,000 blocks were treated for use in the two test methods.

The blocks were placed in a beaker contained in a desiccator attached to a suction pump and to a manometer. The pressure in the desiccator was reduced to a differential value of 6 centimeters of mercury and held for approximately 15 minutes. Each of the materials was diluted with toluene, to obtain a gradient series of retentions in the blocks above and below that at which decay might be expected to occur. The dilution of a material, made just prior to its use, was poured into a 1,000-milliliter globe separatory funnel with the stem extending through the top of the desiccator and into the beaker containing the blocks. The suction pump then was turned off and the separatory funnel stopcock opened to let the diluted preservative material run directly into the beaker in the evacuated desiccator. Enough preservative was used to cover the blocks completely. After the vacuum was released, the beaker containing the blocks was removed and covered. The blocks were taken out individually, the excess liquid was wiped off, and the block weighed ($T_2$, table 4). They were then placed on galvanized wire trays in the laboratory for 1 day and the solvent allowed to go off.

In subsequent tests the blocks, held down by a glass weight, were left under the reduced pressure for 30 minutes before the treating solution was added; after the solution was added the blocks were left covered by the solution in a tightly closed container for at least 30 minutes before they were weighed. This was done to assure complete absorption in all blocks after it was found that some materials, especially the oil preservatives, were absorbed slowly even after most of the air was removed from the wood. With this method, absorptions have been higher and more uniform in blocks of comparable specific gravity.

**CALCULATION OF RETENTIONS**

The retention ($R$) in the blocks in terms of pounds of the preservative material (table 1) per cubic foot was calculated by the following formula:

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

$G$ - grams of treating solution (material plus toluene) absorbed by the blocks.

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

$V$ - volume of test block (6.76 cubic centimeters for the soil block and 6.91 cubic centimeters for the agar block).

62.4 - factor for converting grams per cubic centimeter to pounds per cubic foot.
Table 4.—Sample of data recorded for test blocks

<table>
<thead>
<tr>
<th>Block No.</th>
<th>Fungus</th>
<th>T1</th>
<th>T2</th>
<th>G</th>
<th>C</th>
<th>CXS</th>
<th>R</th>
<th>T3</th>
<th>T4-T1</th>
<th>F1</th>
<th>T3w</th>
<th>T3w-T1</th>
<th>R</th>
<th>Tm</th>
<th>T4</th>
<th>Weight %</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>131A</td>
<td>534</td>
<td>4.36</td>
<td>7.44</td>
<td>3.08</td>
<td>10.6</td>
<td>0.236</td>
<td>3.01</td>
<td>6.58</td>
<td>0.22</td>
<td>2.03</td>
<td>5.27</td>
<td>4.25</td>
<td>5.07</td>
<td>4.07</td>
<td>7.2</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>135A</td>
<td>617</td>
<td>5.36</td>
<td>7.79</td>
<td>2.43</td>
<td>10.6</td>
<td>0.258</td>
<td>2.38</td>
<td>5.94</td>
<td>0.18</td>
<td>1.66</td>
<td>6.56</td>
<td>5.40</td>
<td>2.5</td>
<td>28.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>137A</td>
<td>604</td>
<td>4.15</td>
<td>7.42</td>
<td>3.27</td>
<td>10.6</td>
<td>0.294</td>
<td>3.20</td>
<td>4.39</td>
<td>0.22</td>
<td>2.22</td>
<td>5.22</td>
<td>4.30</td>
<td>2.1</td>
<td>28.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>139A</td>
<td>617</td>
<td>5.11</td>
<td>7.68</td>
<td>2.57</td>
<td>10.6</td>
<td>0.272</td>
<td>2.51</td>
<td>5.30</td>
<td>0.19</td>
<td>1.75</td>
<td>6.30</td>
<td>5.20</td>
<td>1.9</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>139B</td>
<td>698</td>
<td>4.16</td>
<td>7.41</td>
<td>3.25</td>
<td>10.6</td>
<td>0.344</td>
<td>3.18</td>
<td>4.40</td>
<td>0.24</td>
<td>2.22</td>
<td>5.20</td>
<td>4.30</td>
<td>2.3</td>
<td>26.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>112A</td>
<td>534</td>
<td>4.15</td>
<td>7.44</td>
<td>3.29</td>
<td>10.6</td>
<td>0.349</td>
<td>3.22</td>
<td>4.40</td>
<td>0.25</td>
<td>2.31</td>
<td>4.32</td>
<td>0.17</td>
<td>1.57</td>
<td>4.71</td>
<td>3.38</td>
<td>21.8</td>
<td>47.6</td>
</tr>
<tr>
<td>124A</td>
<td>617</td>
<td>4.92</td>
<td>7.58</td>
<td>2.60</td>
<td>10.6</td>
<td>0.276</td>
<td>2.55</td>
<td>5.11</td>
<td>0.19</td>
<td>1.75</td>
<td>5.05</td>
<td>0.13</td>
<td>1.20</td>
<td>5.91</td>
<td>4.61</td>
<td>2.7</td>
<td>35.9</td>
</tr>
<tr>
<td>116A</td>
<td>604</td>
<td>4.50</td>
<td>7.35</td>
<td>2.85</td>
<td>10.6</td>
<td>0.302</td>
<td>2.79</td>
<td>4.72</td>
<td>0.22</td>
<td>2.03</td>
<td>4.65</td>
<td>0.15</td>
<td>1.38</td>
<td>5.53</td>
<td>4.55</td>
<td>2.2</td>
<td>28.9</td>
</tr>
<tr>
<td>126A</td>
<td>517</td>
<td>4.58</td>
<td>7.43</td>
<td>2.85</td>
<td>10.6</td>
<td>0.302</td>
<td>2.79</td>
<td>4.79</td>
<td>0.21</td>
<td>1.94</td>
<td>4.73</td>
<td>0.15</td>
<td>1.38</td>
<td>5.61</td>
<td>4.62</td>
<td>2.3</td>
<td>26.7</td>
</tr>
<tr>
<td>130A</td>
<td>604</td>
<td>3.62</td>
<td>3.48</td>
<td>3.87</td>
<td>10.6</td>
<td>0.304</td>
<td>2.61</td>
<td>4.82</td>
<td>0.21</td>
<td>1.94</td>
<td>4.76</td>
<td>0.15</td>
<td>1.38</td>
<td>5.58</td>
<td>4.53</td>
<td>4.8</td>
<td>30.7</td>
</tr>
</tbody>
</table>

Weights

- **T1** = weight of the test block, after reaching an approximate 6 percent moisture equilibrium in the 30 percent relative humidity, 60°F conditioning room, before testing.
- **T2** = weight of the test block immediately after impregnation. (T1 plus grams of treating solutions absorbed).
- **T3** = weight of the test block plus residual material, after conditioning to an approximate 6 percent moisture equilibrium before testing.
- **T3w** = weight of the block plus residual material, after conditioning, weathering, and reconditioning to an approximate 6 percent moisture equilibrium, before testing.
- **T4** = weight of block just after removal from test bottle and after adhering fungus mycelium was brushed off.
- **Tm** = weight of the block after test and reconditioning to an approximate 6 percent moisture equilibrium.

Calculations

- $\Delta = \text{grams of treating solution absorbed by the block (T2-T1)}$.
- $\Delta = \text{grams of material in 100 grams of treating solution}$.
- $\Delta = \text{grams of material absorbed by the block during impregnation}$.
- $\Delta = \text{grams of residual material in the block after conditioning, but before testing}$.
- $\Delta = \text{grams of residual material in the block, after conditioning, weathering, and reconditioning, but before testing}$.
- $\Delta = \text{pounds of material per cubic foot of wood in the block at treatment}$.
- $\Delta = \text{pounds of residual material per cubic feet of wood in the block after conditioning, but before testing}$.
- $\Delta = \text{pounds of residual material per cubic feet of wood in the block after conditioning, weathering, and reconditioning, but before testing}$.

Weight loss expressed in percent is the loss of material plus wood substances when decay occurs.

\[
100 \times \frac{\Delta}{\Delta} = \text{percent loss in weight}
\]

Moisture is the percent moisture in the blocks after the test period based on the calculated oven-dry weight of the block at the end of the test period.

\[
\frac{\Delta}{\Delta} = \text{calculated oven-dry weight of wood containing residual material, after the test period.}
\]

\[
100 \times \frac{\Delta}{\Delta} = \text{percent moisture}
\]
If $G$ is first calculated to be only the grams of material that are absorbed ($C$ times $G$) the formula would be:

$$R = \frac{G (62.4)}{V}$$

CONDITIONING THE TREATED BLOCKS

All the treated blocks were conditioned for 2 to 3 weeks to an approximate moisture equilibrium of 6 percent. After weighing (Table 4, $T_3$), approximately one-half the blocks, or those to be tested without weathering, were then ready for the culture test.

The use of toluene, a toxic material, as a diluent for the preservative materials made it necessary to allow time for toluene evaporation from the blocks. Weight calculations indicate that, in less than 24 hours, all of the toluene is evaporated from blocks impregnated with toluene only. Several days must elapse before it can be assumed that all or most of the toluene has evaporated from blocks treated with a material made up in a toluene solution. During the conditioning period some of the volatile components of a preservative material also are lost. This means that when the blocks finally are tested they do not contain the full amount of preservative with which they were impregnated. The conditioning period, therefore, becomes a phase of the permanence test for volatile preservative materials.

WEATHERING OF TEST BLOCKS

The soil blocks to be weathered were strung on nylon threads, each block separated by a glass bead. The strung blocks were supported horizontally on a rack. The agar blocks to be weathered were placed between plastic screens attached to a wooden frame. This assembly with the agar blocks was turned three times a week. All the blocks were exposed on the south roof of the U. S. Forest Products Laboratory for 60 days, beginning May 15, 1947. During this period there were 33 clear days, and 27 during which some rain fell, the total precipitation for the entire period being 10.7 inches. The minimum temperature of the period was 32° F. and the high 91° F., with a mean minimum-maximum of 51° - 74° F. The blocks were thoroughly sprayed for 5 minutes with water three times a week, provided there was no heavy rain on these days.

After the weathering period, the blocks were returned to the conditioning room for 2 weeks and brought to an approximate moisture equilibrium. The blocks were then weighed (Table 4, $T_{3w}$), and were ready for the culture test.

CONTROL BLOCKS

Ten blocks were left untreated and tested against each fungus in bottles containing no treated blocks. Approximately 25 blocks were left untreated and placed in culture bottles with blocks treated with each material. A similar number of untreated blocks were weathered before they were tested. Before and after testing, all untreated blocks were conditioned to the approximate 6 percent moisture.

Additional blocks were treated with toluene alone and tested against each fungus.
CULTURE METHODS

Test fungi.—The fungi used were Lentinus lepideus (Madison 534), Lenzites trabea (Madison 617), Lenzites saepiaria (Madison 604), Poria monticola (Madison 698), and Polyporus tulipiferus (Madison 517).

The first four of the fungi were selected because they are common wood destroyers under a wide range of conditions, cause fairly rapid decay in pine sapwood, are easily cultivated under laboratory conditions, and have a relatively high resistance to the materials tested. Lent. lepideus frequently attacks coniferous woods used in contact with the ground, such as poles, ties, and foundation timbers; it also has more tolerance against creosote than any of the other fungi used in accelerated laboratory testing both in this country and in Europe. L. saepiaria causes extensive damage to coniferous timbers in service. L. trabea often is the cause of decay in treated coniferous woods, especially above the ground line; in the laboratory it shows considerable tolerance to pentachlorophenol. P. monticola, which attacks a number of softwoods, is tolerant to both pentachlorophenol and copper compounds under laboratory conditions. Madison 517, at one time called Fomes annosus (33) but now identified as Polyporus tulipiferus (7), was added to the tests because of its extensive use for more than 30 years as one of the standard test organisms for determining toxicities of various wood preservatives by the Petri-dish or agar-flask method. Little is known about its part as a destroyer of wood in structural timbers.

Preparation of cultures.—For the agar-block method, 30 milliliters of medium containing 2.5 percent Trommer’s malt extract5 and 1.5 percent Difco Bacto agar in distilled water were placed in 8-ounce French square bottles with screw caps, without the screw cap liners. The bottles were steam sterilized for 20 minutes at 15 pounds pressure, removed from the autoclave while hot and placed in a horizontal position so that the agar would harden on one of the long flat sides. The medium was then inoculated near the neck of the bottle with a test fungus, which had been growing for 2 to 3 weeks on malt agar in a Petri dish. The inoculum was small, so that there was little adhering malt agar. The cultures were incubated at 70 percent relative humidity and 80°F. and allowed to grow until the fungus mat practically covered the agar surface, the time varying with the fungus. None of the cultures was more than 3 weeks old when used. Each test block was supported on a sterile V-shaped glass rod that was carefully placed on the fungus mat in such a way as to avoid the spot where the inoculum was introduced. A preservative is more apt to be leached when the block is in contact with the inoculum and its adhering malt agar. As a result, some fungi are killed early in the test period; others only inhibited may be able to cause decay in the block when the amount of preservative is reduced.

For the soil-block method, approximately 100 grams (oven-dry basis) of soil (Miami silt loam), previously sifted through a 6-mesh screen, were put into each 8-ounce bottle. Each bottle contained enough water to bring the moisture content of the soil to 25 percent (oven-dry basis) for the testing of the conditioned blocks and to 30 percent for the weathered blocks. (In subsequent tests 40 percent moisture has been used.) Two longleaf pine sapwood feeder blocks 2.0 by 0.2 by 3.5 (along the grain) centimeters (in subsequent tests the feeder block thickness has been 0.4

5 Ingredients (percent): maltose, 51.02; dextrine, 10.94; albuminates, 3.11; glycerine, 9.27; free acid, 0.43; inorganic substances, 1.16; water, 24.07.
were then centimeters) were placed side by side on the leveled surface of the soil. The bottles were then loosely capped and sterilized for 30 minutes at 15 pounds pressure. The following day an inoculum that was about a centimeter square and cut from a not more than 3-week-old Petri-dish culture was placed at about the centers of the two feeder blocks. When the cultures were made for testing conditioned blocks, the liners were left in the lids of the culture jars and the lids screwed on tightly after inoculation. Fifty percent of these cultures showed very poor growth, and preliminary studies had indicated that the oxygen supply in some of the jars was inadequate for good growth of the test fungus. Unfortunately, some of these cultures that showed poor growth were used. The effect on the results of the conditioned block test is not known. When the cultures were prepared for the weathered blocks, the liners were removed from the lids and the lids were left loose. This procedure has been followed in subsequent tests, resulting in uniform and good growth in all cultures.

The inoculated bottles were then incubated at 80° F. and 70 percent relative humidity for 3 weeks. By that time the feeder blocks were covered and the fungus had grown down into the soil.

Setting up the tests.--Certain molds, commonly present, are able to grow on blocks treated with concentrations of preservative materials that are effective in preventing decay by wood-destroying fungi. Hormodendrum resinæ (5, 29) not only tolerates a far higher concentration of creosote than wood destroyers, but also is able to grow and reproduce with no other source of nourishment than occurs in creosote. Although a mold probably does not affect the strength of wood, its presence could prevent the growth of a test fungus. To kill any surface contaminants, the conditioned and weathered blocks were placed by retention groups in large tightly plugged test tubes (6-ounce bottles with tightly fitting lids are now used) and steamed for 20 minutes at atmospheric pressure. It is possible that the treated conditioned blocks lose some of the volatile part of a preservative material during steaming. Little if any loss would be expected from steaming blocks that had been subjected to a permanence test.

Two treated blocks of approximately the same retention were placed on the feeder blocks in each soil-block bottle in such a way that they did not touch each other or the sides of the test bottle. For each retention group three or four replicate blocks were tested against a single fungus. Whenever there were three blocks, two were tested together and the third was paired with an untreated block. Ten untreated blocks tested against each fungus were placed singly in bottles. In subsequent tests all bottles have contained either two treated blocks of approximately the same retention or two untreated ones.

Each agar-block culture bottle contained one treated block or one untreated block.

Incubation.--The bottles containing the test blocks were placed in a culture room with a constant temperature of 80° F. and 70 percent relative humidity for 16 weeks. (The incubation time in subsequent tests has been only 12 weeks.)

RECONDITIONING AND DETERMINATION OF WEIGHT LOSS AND MOISTURE CONTENT

At the end of the incubation period the blocks were removed from the culture bottles, the fungus brushed off, and the blocks weighed (Table 4, Tm). The blocks
were then reconditioned to approximate moisture equilibrium in the controlled humidity room, and the final weights taken (Table 4, \( T_4 \)).

The percent loss in weight was computed from this weight and the weight of the conditioned block immediately prior to testing (Table 4, \( T_3 \) or \( T_{3w} \)).

The percent moisture content of the blocks at the end of the test period was computed from the weight of the block at the end of the test period, immediately after the fungus was brushed off (Table 4, \( T_m \)), and the final computed oven-dry weight of the test block \( T_4 \) after testing. In computing this oven-dry weight it was assumed that a treated block after testing had come to about the same moisture equilibrium in the conditioning room as an untreated one before testing, namely, 6 percent. The moisture content determination indicated whether the moisture conditions had been satisfactory for decay.

EVALUATION OF RESULTS

The purpose, when testing preservatives by the soil- or agar-block method, is to determine the minimum amount of preservative material, based on initial retentions in the wood, that will be effective in preventing decay by a particular test fungus. This amount of preservative material, in terms of pounds per cubic foot, is the threshold or threshold retention. To establish a threshold, two criteria were used, one visual and the other based on percentage weight losses in the test blocks.

By visual inspection. The test blocks were examined when removed from the test bottles and after subsequent drying in the conditioning room. Distortion, shrinkage, and softening of the blocks were considered evidence of decay. These abnormalities are pronounced in blocks with the lower retentions of preservative but become progressively less evident with higher retentions until they are no longer apparent. According to visual evidence, this point would be considered the threshold retention.

A threshold based entirely on visual inspection is subject to considerable experimental error because the decision rests on the judgment of the experimenter and on evidence furnished by a few blocks near the estimated threshold. Where abnormalities produced by decay are slight in wood tested against certain fungi, which cause deformation only at a late stage in decay, visual determinations are difficult. It becomes necessary, therefore, to determine whether a weight loss has occurred before it is possible to state whether the blocks have been attacked.

Use of percentage weight losses. Weight loss alone does not necessarily mean that decay has occurred. Part or all of the weight loss is primarily due to evaporation of the preservative material during the test period (operational losses). It is quite possible when testing blocks containing the higher retentions of some preservatives to have a weight loss of 10 percent with no visible attack by the fungus. On the other hand, decay may be clearly present, when testing other preservatives, when there is a weight loss of only 2 percent. Therefore, to say that there is no decay below or above a set percentage weight loss would lead to error when comparing a number of materials.

Losses that are not due to decay may be provided for by applying a correction factor. Such a factor is based on an average loss in weight of blocks, containing similar retentions to those tested, which have been held over sterile agar or soil
for the period of the test. Recent tests have shown that treated blocks, however, do not necessarily lose the same amount of preservative over a sterile medium as they do when in contact with a fungus. Furthermore, it was impracticable in these tests to select the test blocks for all categories within a narrow specific gravity range. The absorption of a preservative material varies inversely with the specific gravity of the test block. It is not likely, therefore, that the use of an average factor could be justified when blocks containing a gradient series of retentions are used.

It is felt that a threshold based on the results of more of the test blocks is more reliable and reproducible than one that is based on only those blocks at the threshold. To be more objective, the percentage weight loss was plotted against the corresponding retention of preservative in each test block (see fig. 1). Previous reports (13, 15) have shown the retention on the ordinate axis, and the weight loss on the abscissa. Two distinct relations between weight loss and retentions were apparent. The blocks toward the upper end of the absorption scale lay close to a straight line, horizontal or sloping. The slope of this line depended on the material tested and whether or not the blocks had been through a weathering phase. This line represented only losses of the preservative material (operational losses) and served as the adjusted line of zero weight loss (no decay line). The blocks with the lower absorptions, or at least for those having weight losses no more than one-third to two-thirds of the untreated ones, lay close to a straight line sloping upward. This line represented losses of both the preservative tested and wood substance, the latter by decay. The point of intersection of these lines established the zero point for decay losses, and by definition the corresponding retention was the threshold value. Such a method eliminated the need to apply a correction factor to take care of the loss in weight of materials alone, or the error in weight loss that could have resulted from differences in moisture equilibrium between the blocks before and after testing.

The accuracy of determining a threshold in this manner depends on the use of a range of retentions spaced more or less uniformly above and below the anticipated threshold. It is not easy to anticipate approximate thresholds when unknown preservatives are being tested unless a preliminary range-finding test has been run. If blocks containing a wide range of retentions are tested, those with retentions considerably below the threshold for a fungus are likely to show weight losses similar to the untreated test blocks. For example, *Lent. lepideus* caused approximately 30 percent weight loss in untreated blocks and in weathered blocks treated with 1 pound or less of creosote (fig. 1). In some cases even more decay may occur in the treated than in the untreated blocks. Minute quantities of toxic materials have been shown to favor decay (1, 25). Such weight losses do not bear a linear relation to the threshold retention and are not considered in drawing the decay line. Weight loss values near the point of intersection of the two lines are better bases for judging the thresholds than those too far from it.

If time is not a determining factor, a range-finding preliminary test in which the threshold is tentatively determined often is advisable. A second test is then run with retentions at close intervals near the approximate threshold. The indications are that tentative thresholds can be determined in a preliminary test of only 3 to 4 weeks. This procedure insures a closer estimate of the threshold, which is also verified by two independent experiments.

The decay and no-decay lines may be located by inspection from the graph with reasonable accuracy. Since the relation between weight loss and retention is usually
Figure 1.--Graphical representation of the line intersection method for locating the threshold in soil-block tests; weathered blocks; Material E, penta-petroleum-creosote; test fungus, Lentinus lepidews. The indicated threshold retention at treatment is 3.4 pounds per cubic foot.
linear through most of the retention range, it also is possible to locate the lines mathematically by the method of least squares (45). An estimate of a threshold by line intersection is free from bias except that a decision must be made in doubtful cases as to whether to use all the weight loss values or to ignore low-retention values that do not appear to fall around a straight line.

The drawing of lines to establish the threshold was done mathematically in this test. In our experience thresholds obtained mathematically or those located by inspection of weight loss graphs have not differed greatly from those determined visually. When decay by visual inspection is questionable, the threshold may be somewhat higher if determined by line intersection. On the other hand, a threshold determined by line intersection may be slightly lower than a visual threshold if the latter has been based on a few blocks not representative of other blocks in the group.

The German standard (8) adheres to the principle of reporting two threshold values, namely, the largest amount of preservative that permitted a small amount of decay, and the smallest amount that did not permit any decay. Sometimes these two reported values are quite different from each other and the reader is given no idea of whether the threshold is nearer the lower or higher value. The line intersection method makes effective use of the weight loss data, thereby providing a closer estimate of the threshold.

**DISCUSSION OF RESULTS**

**POLYPORUS TULIPIFERUS (MADISON 517) AS A TEST ORGANISM**

*Polyporus tulipiferus* (Madison 517) has been commonly used in the United States as a test fungus in the Petri-dish or agar-flask test. This test (37) consists of planting the fungus on malt-agar media containing gradient concentrations of a given preservative. The concentrations at which the growth of the fungus is inhibited or killed are determined and used as measures of the toxicity of the preservative. The inhibition and killing points of Madison 517 are known for many preservatives. The Petri-dish test, of course, differs from the agar-block test in that the former (1) does not test the preservative in wood, and (2) permits little if any evaporation of the preservative before it is tested. A considerable amount of volatile preservative may be lost from treated blocks before testing, either in the conditioning or weathering for the soil-block or agar-block tests. It is to be expected, therefore, that more preservative would be required in impregnated wood to inhibit a fungus than if the preservative were dispersed in malt-agar. By including *Poly. tulipiferus* as a test fungus it was hoped that a general degree of difference might be found between the Petri-dish method and the soil- or agar-block methods.

*Poly. tulipiferus* grew luxuriantly and covered the malt-agar medium faster than the other test fungi. In the agar-block method, it was less susceptible than the other four fungi to pentachlorophenol, but similar to the other fungi in its susceptibility to the other materials tested.

Little tolerance to any of the preservative materials, however, was indicated by *Poly. tulipiferus* in the soil-block tests. In tests of conditioned blocks it grew poorly in the soil, with little visible attack of the feeder blocks. There was less
than 3 percent weight loss from decay in the untreated blocks and in blocks with the lowest retentions of the preservative materials. Consequently, it appeared that some necessary growth factor was present in the agar that was lacking or unavailable in the soil.

Trommer's malt extract, equal to 2.5 percent by weight of the soil moisture, was added to the cultures in which the weathered blocks were tested, to supply this extra growth factor. The malt was mixed in the approximately 10 cubic centimeters of water added to the top of the soil, after the feeder blocks were in position, to bring the moisture content of the soil up to 30 percent. As indicated earlier, the water was ordinarily added first and the soil second in making up the soil bottles. It is probable that the first effects of adding the malt and water were an uneven distribution of the malt in the soil, as well as an increase in the moisture content of the feeder blocks. There was better growth in the soil and on the feeder blocks in these cultures than those used for testing the conditioned blocks. However, even then there was only 10 percent weight loss in untreated blocks or in blocks with the lowest retentions of any of the materials tested.

Two English creosotes were subsequently tested (10) by the soil- and agar-block methods, with Madison 517 as one of the test fungi. The water containing 2.5 percent malt, which was added to bring the soil moisture in this case up to 40 percent, was placed on top of the soil for testing the conditioned blocks; it was placed in the bottle before the soil was added for testing the weathered blocks. In the conditioned-block tests the untreated blocks and those with retentions of the creosotes below the threshold showed considerably more decay in the soil blocks than in similar agar blocks. *Poly. tulipiferus* proved approximately as tolerant to the oils, in either the agar- or soil-block method, as *L. trabea*, *L. saepiaria*, and *P. monticola*.

The erratic results with *Poly. tulipiferus* as a test organism in the soil-block test are not yet fully explained. The culture and moisture requirements should be further studied, if it should appear desirable to use *Poly. tulipiferus* in any future soil-block tests. Therefore, in the interpretation of the results of the two test methods *Poly. tulipiferus* is excluded, and only the results of the other four fungi, which grew equally well on soil and agar, are compared. The approximate preservative thresholds are presented in table 5 for reference.

**EFFECT OF PETROLEUM CARRIER AND TOLUENE ON THE FUNGI**

*Petroleum oil*—The petroleum oil (1) used as a carrier for the pentachlorophenol or copper naphthenate and as part of the mixture with creosote retarded the rate of decay but was not in itself sufficiently effective to prevent decay by the fungi in either of the test methods. This was indicated by the presence of decay in all of the blocks containing 8 to 10 pounds of the petroleum oil per cubic foot, the highest retention used, but the amount of decay in the blocks was less than in untreated blocks. Therefore, the petroleum was not an inactive part of the material tested.

Generally less, but no more creosote, was required for the thresholds of the fungi for the petroleum-creosote (II) than when the creosote (A) was used alone (table 5). It has been reported (14, 15) that the thresholds obtained for certain petroleum-creosote mixtures contain more creosote than would be needed if the creosote were used alone. In these cases the petroleum apparently caused a blanketing of the toxic
### Table 5.--Threshold retentions -- pounds per cubic foot at treatment

<table>
<thead>
<tr>
<th>Material: Test</th>
<th>Conditioned blocks</th>
<th>Weathered blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>tested fungus</td>
<td>Agar-block</td>
<td>Soil-block</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>B</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1. A, Coal-tar creosote: B, penta-petroleum: a 5 percent solution by weight of pentachlorophenol in a light naphthenic-base petroleum oil (I); C, copper-petroleum: a solution of copper naphthenate equivalent to 0.525 percent copper metal by weight in a light naphthenic-base petroleum oil (I); D, penta-copper-petroleum: a blend of equal parts by volume (50-50) of A and C; E, penta-petroleum-creosote: a blend of equal parts by volume (50-50) of A and D; F, copper-petroleum-creosote: a blend of equal parts by volume (50-50) of A and C; G, penta-petroleum-creosote: a blend of equal parts by volume (50-50) of A and F; H, penta-petroleum-creosote: a blend of equal parts by volume (50-50) of A and D; I, petroleum oil: a light naphthenic-base. (No petroleum, I, thresholds were found. See Discussion of Results: Petroleum oil).

components. In 1950 (13) it was shown that the petroleum used as a carrier for pentachlorophenol may influence the effectiveness of the solution of which it is a part, although alone it did not prevent, but only retarded decay.

Toluene.--The toluene used as a diluent in making up the treating solutions for all the materials tested was not effective in preventing decay under the conditions in either test method. Blocks treated with 100 percent toluene, and conditioned before testing, were decayed similarly to untreated blocks in both tests. Toluene has been shown to be toxic by the Petri-dish agar method. However, the weights indicated that all of the toluene had evaporated within 24 hours from blocks treated with toluene alone. When blocks contained a preservative material applied in a toluene solution, a loss in weight corresponding to the amount of toluene absorbed did not occur for 2 weeks. The conditioning of all the blocks for 2 to 3 weeks before testing allowed time for the evaporation of most of the toluene. Volatiles in the materials tested were also being lost during the conditioning period. It is possible, therefore, that some of the toluene remained in the blocks at the time they were placed in test. Any toluene left in the blocks could evaporate during the test period, since the lids on the bottles were left loose.

When using any diluent such as toluene, or acetone or chloroform used by European investigators, it may be necessary to reckon with its effect on the evaporability of the material being tested. Schultz and Becker (40) show that from a free surface certain components of creosote evaporate more readily and others are not affected when mixed with either acetone or chloroform. Whether this actually happens when creosote is introduced into wood is not known. At present the use of a diluent in the laboratory testing is desirable for oil-type materials such as creosote, in order to get uniform distribution throughout the test specimen and at low enough concentrations to obtain thresholds for individual fungi. It has been shown (14) that thresholds for pentachlorophenol were approximately the same whether a 5 percent pentachlorophenol in heavy gas oil was diluted with toluene or the pentachlorophenol carried in heavy gas oil alone was used. Checks on the effect of toluene on the creosote thresholds for the various fungi are now being made to determine whether similar results are obtained.

TOLERANCE OF THE FUNGI TO THE PRESERVATIVE MATERIALS

The minimum amounts (thresholds) of preservative materials, and of each component, required in the blocks to inhibit decay by each test fungus are shown in table 5. Three of the materials were tested by the soil-block method only. The relative tolerance of the different test fungi to the preservative materials is indicated by the position of their index numbers on conditioned and weathered block bars in figure 2. A comparison of those materials tested by both methods shows that:

1. In general, the same fungus was most tolerant to a given preservative whether the results were obtained on soil or agar.

   Lent. lepideus was more tolerant than the other fungi to creosote (A) or the mixture of petroleum-creosote (H).

   L. trabea and P. monticola were about equally susceptible to pentachlorophenol, before the blocks were weathered. L. trabea was less susceptible than P. monticola, or the other test fungi, to pentachlorophenol in the blocks that were weathered.
Figure 2.—Threshold retentions at treatment. Materials tested: A, Coal-tar creosote; B, penta-petroleum: a 5 percent solution by weight of pentachlorophenol in a light naphthenic-base petroleum oil (I); C, copper-petroleum: a solution of copper naphthenate equivalent to 0.525 percent copper metal by weight in a light naphthenic-base petroleum oil (I); D, penta-copper-petroleum: a blend of equal parts by volume (50-50) of B and C; E, penta-petroleum-creosote: a blend of equal parts by volume (50-50) of A and B; F, copper-petroleum-creosote: a blend of equal parts by volume (50-50) of A and C; G, penta-copper-petroleum-creosote: a blend of equal parts by volume (50-50) of A and D; H, petroleum-creosote: a blend of equal parts by volume (50-50) of A and I; I, petroleum-oil: a light naphthenic base (no petroleum) (I) thresholds were found. (See discussion of results.)
P. monticola was less susceptible than the other fungi to copper naphthenate in all the blocks except those in the weathered soil-block test. *Lent. lepideus* attacked the weathered soil blocks with higher initial retentions of the copper naphthenate than did *P. monticola*.

All the fungi were about equally tolerant of penta-petroleum-creosote (E) before the blocks were weathered. After weathering, *Lent. trabea* was most tolerant in the soil-block tests and *P. monticola* in the agar-block tests.

*L. saepiaria* was less tolerant than the other fungi to all the preservative materials.

2. The minimum amount of preservative required to prevent decay by the fungus most tolerant to it was higher by the soil-block than the agar-block method, but the order of preservative effectiveness was similar (fig. 2).

The preservatives would not be rated in exactly the same order by each of the test fungi regardless of the method used. Figure 2 shows that the fungi react differently to the preservative materials and their component parts. It is not possible, therefore, to draw conclusions from one fungus. The most significant results are those obtained with the fungus most tolerant to the preservative under test, provided that fungus is known to be of economic importance. Unfortunately, information on the species of decay fungi that cause failure of treated wood in service is comparatively sparse. Recently *Poria radiculosa* has been reported (24) to cause decay in creosoted wood. As such information is obtained, the fungi should be considered as possible test organisms. An attempt is now being made to learn what fungi tend to attack wood treated with known retentions of different preservatives and exposed in different soil and ground cover environments. Such information will provide a sounder basis for the selection of test fungi, especially as new preservative types are tested.

The order of preservative effectiveness, based on the results of the most tolerant fungus, was the same as when threshold values of *Lent. lepideus*, *L. saepiaria*, and *P. monticola* were averaged. *L. saepiaria*, the least tolerant fungus, could be excluded without changing the relative order of the averages. The order of effectiveness of the preservative materials was not changed by weathering. The wider spread and higher threshold values obtained in the soil-block test gave clearer indications of the reactions of the test organisms toward the different preservatives.

**MOISTURE CONTENT OF BLOCKS IN THE AGAR- AND SOIL-BLOCK TESTS**

Soil and agar blocks were removed at intervals throughout the test period for moisture content determinations, based on the calculated oven-dry weight of the treated blocks. The treated blocks contained varied amounts of preservatives so that the data were not strictly comparable in the different periods. The relationship of moisture to weight loss is best illustrated at the end of the 3-month test by blocks containing a series of retentions of one preservative material tested against each fungus (figs. 3 and 4).
Figure 3.—Relation between final weight loss and moisture content in individual blocks after 3 months' exposure to Lentinus lepideus and L. trabea in the soil- and agar-block tests. The blocks were treated with coal-tar creosote (Material A).
Figure 4.--Relation between final weight loss and moisture content in individual blocks after 3 months' exposure to L. saepiaria, P. monticola, and Poly. pulipiferus in the soil- and agar-block tests. The blocks were treated with coal-tar creosote (Material A).
The moisture content in the treated soil blocks remained near the fiber saturation for untreated pine sapwood (approximately 27 percent) until decay was initiated. The fungi produce water as they destroy the wood substance and decrease its weight. Thus, as weight losses due to decay increased there was a concomitant increase in moisture content. The moisture, depending on the fungus and its attack, ranged from fiber saturation to approximately 80 percent in soil blocks containing high to low retentions of the materials tested. Since the blocks having retentions above the threshold remained at fiber saturation, there were clear indications that the relative humidity in the test bottle was approximately 100 percent throughout the test; also, that there was little or no migration of liquid water from the soil to the block. It might be added that this was generally true in all subsequent soil-block tests even though 40 percent moisture was present in the soil. Occasionally, it was found that a test fungus was able to grow on or over blocks treated with certain materials without causing decay. The moisture was raised slightly above fiber saturation in these cases. It has also been noted in subsequent tests that the moisture content in blocks with the higher concentrations of certain materials was slightly below fiber saturation. There is the possibility that oil-type preservatives retard absorption of moisture to a slight degree.

In the case of the agar blocks, the moisture content varied from 30 to approximately 100 percent in blocks showing no loss in weight due to decay. The relationship between moisture content and weight loss varied considerably. It has been suggested that high moisture in the agar blocks may be due to capillary movement of water from the agar to the wood via the glass rods or mycelium, accidental contact between the agar and the wood, and condensation of water. Care in handling the test bottle and special attention to assure that the block was not placed over the inoculum generally prevented accidental touching of the block to the agar. Condensation occurred on the glass bottles in both methods. One side of the test bottle in the agar-block method was above the block, so that as moisture collected, drops could fall on the blocks. In the soil-block method the test bottle was upright so that any water that condensed ran down the sides of the bottle. In subsequent tests, it has been observed that when the soil is placed horizontally in the bottle, the moisture in the blocks is increased.

Most of the moisture studies of blocks under culture test conditions have been made in tests with the agar-block method, or variations of it. These have indicated that the moisture for decay range from fiber saturation to over 200 percent of the wood substance, based on the oven-dry weight, when different fungi and woods were used. Snell (46) stated that when the presence of water in the wood reduced the percent of air to less than 20 percent of the volume of wood, decay did not take place. Snell, Howard, and Lamb (47) found that rates of decay produced in small blocks of nondense wood were about the same over a broad moisture range from somewhat above fiber saturation (± 30 percent) to over 100 percent. The relationship of moisture content to decay at the end of 4 months' exposure in several agar-block tests was summarized in 1946 (16). The general conclusion was that moisture content tended to increase as the amount of decay increased, but for the most part the relationship was rather a broad one, and in some tests for reasons not clear there was no relationship. However, there was no evidence that moisture might have prevented decay. Highly resistant woods generally showed moisture contents disproportionate to the amount of decay. Also, the representative rates of decay in fir and oak were found (17) to be maintained even when high moisture contents were present. European investigators only occasionally mention cases where blocks, which should show decay, remain unattacked because of a high moisture content (1).
The possibility that decay of wood by some test fungi may be retarded by low or high moisture contents, depending on the wood or preservative in it, should not be disregarded in these block tests. The moisture content at which the maximum amount of decay occurs is not necessarily the same for all fungi, species of wood, or wood treated with different preservatives. Increasing the moisture is known to increase the amount of decay by some fungi or cause the same weight loss in a shorter time.

Any laboratory method should provide an initial moisture in the test block which will allow the fungus to attack, and secondly, a sufficient supply of moisture in the substrate to maintain that attack. The soil- or agar-block method provides enough moisture in the block for most fungi to initiate decay. The close correlation between the amount of decay and the moisture content in the block in the soil-block method suggests that the moisture is controlled by the activity of the fungus. The general lack of such relationship in the agar-block method suggests that sometimes there is more moisture in the test block than would be associated with the presence of the fungus. How much this additional moisture is an influencing factor for the fungi considered cannot be stated definitely at present.

LOSS OF PRESERVATIVE MATERIAL DURING CONDITIONING AND WEATHERING

The loss of preservative materials during conditioning and weathering was determined by weight. The original weight of the block (Table 4, T1) subtracted from the treated and conditioned weight of the block (T2), and the treated and reconditioned weight of the block after weathering (T3w), gives the grams of residual preservative remaining after conditioning and weathering, respectively. The figures in grams are then converted to pounds per cubic foot by the same formula used to convert the initial gain in grams of preservative.

Table 6 shows the calculated average pounds of preservative material per cubic foot in blocks for each concentration of treating solution (1) at the time of treatment, (2) the amount of residual preservative material after conditioning, and (3) the amount of residual material after weathering. One-half of the blocks of the given group are involved in the weathering.

Three possible variables must be considered in calculating retention at time of treatment. First, the absorption of preservative material is based on the weight of an untreated block that has approximately 6 percent moisture. This is assuming that no moisture is lost from the block under reduced pressure, before the preservative is added. It has recently been found that the reduced pressure does cause a slight loss of moisture, but this does not greatly affect the calculated retention. Secondly, there is the loss of volatile components from the treating solution in the evacuated desiccator, such as the toluene diluent, at the time of treatment. Such a loss would tend to increase the concentration of the preservative material in the treating solution and would result in raising the calculated retention figures slightly over those reported. To prevent loss during treatment the vacuum was drawn on the blocks; the preservative was then dropped on the blocks in the evacuated jar from the separatory funnel as quickly as possible; the vacuum was broken at once and the beaker containing the blocks, which were completely submerged in the treating solution, was covered immediately and kept covered while the blocks were held in solution. An analysis of a pentachlorophenol solution, by Dr. R. H. Baechler of the Forest Products Laboratory, showed the percent pentachlorophenol to be 1.097 before and 1.10
after treatment. Thirdly, any evaporation from the surface of treated blocks immediately after removal of the blocks from the treating solution and before the treated weights were taken would tend to lower the gain in weight or preservative pickup and so bring about a lowering of the calculated retention at the time of treatment. To avoid loss after impregnation, the treated blocks were quickly wiped off individually and weighed immediately. In an earlier paper (14) it was shown that the amount of pentachlorophenol calculated from the grams of solution absorbed by the blocks at treatment checked closely the amount calculated from a chemical determination of the amounts of chlorides present.

It is assumed that the treated blocks are brought back to the same moisture equilibrium after conditioning and weathering as that of untreated blocks. This assumption may not be completely applicable to all types of preservatives, since certain oils absorb and hold more moisture than others. Recent experience has shown that, when the loss by evaporation of such oils is slight, blocks treated with them may weigh more after conditioning than the original conditioned weight of the untreated block plus the known weight of the oil.

The calculated figures for residual preservative are at best an imperfect indication of what portions of a preservative material have been lost. The amount of residual preservative based on weight losses is not a true measure of the toxic or preservative components remaining in the blocks. However, the bioassay is an accurate index of the preservative value of the remaining components. Fungi react to small changes in a preservative which are not indicated by test-block weight, and which may not be clearly shown by a chemical assay. The threshold values appearing in table 5 and figure 2 clearly indicate that effective preservative elements of either pentachlorophenol or copper naphthenate remain in the wood, although the petroleum carrier has almost completely disappeared. It was hoped that information based on loss of preservative material by weight might be supplemented by chemical assays. These assays were not completed and cannot be embodied in this report.

Blocks can be analyzed for residual copper without, however, providing a true measure of the preservative value of the naphthenate factor. Assay methods for determining chlorides permit the calculation of residual pentachlorophenol. At the present time, weight loss is the most convenient way of determining residual creosote. Micro-extraction techniques and micro-distillation methods have been used (20) for determining the character and total recoverable amount of the remaining creosote. It has been shown (15) that the residual creosote by weight remaining after leaching is about the same for several creosotes, but the effectiveness of the residual creosotes is quite different.

It is obvious that the actual amount of preservative material in the blocks was less at the time of testing than at the time of treatment. This was expected and was especially true for the volatile preservative materials. The indicated losses are important because enough must be put into the wood originally to insure an effective amount of residual preservative during long-time service. Therefore, it is necessary to state thresholds in terms of the retention at time of treatment. Required threshold retentions are given throughout this paper and previous reports in terms of original treatment retentions.

Part of the material in table 6 is incorporated in figure 5 and illustrates the losses for creosote (A) from the soil blocks. A slight change in the conditioning
<table>
<thead>
<tr>
<th>Material: Concentration:</th>
<th>Soil-block tests</th>
<th>Agar-block tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention:</td>
<td>Residual:</td>
</tr>
<tr>
<td></td>
<td>At :</td>
<td>After :</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent</td>
<td>Lb. per cu.ft.</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.0</td>
<td>10.4</td>
<td>5.4</td>
</tr>
<tr>
<td>28.2</td>
<td>7.4</td>
<td>5.4</td>
</tr>
<tr>
<td>20.6</td>
<td>5.3</td>
<td>5.4</td>
</tr>
<tr>
<td>18.7</td>
<td>3.6</td>
<td>5.4</td>
</tr>
<tr>
<td>10.6</td>
<td>2.8</td>
<td>1.9</td>
</tr>
<tr>
<td>7.6</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>5.4</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>3.9</td>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>2.8</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>1.0</td>
<td>0.7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 6. -- Loss of material tested
<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28.7</td>
<td>20.6</td>
<td>14.7</td>
<td>20.6</td>
</tr>
<tr>
<td>B</td>
<td>5.4</td>
<td>3.9</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>C</td>
<td>3.6</td>
<td>7.6</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>D</td>
<td>5.6</td>
<td>7.6</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>E</td>
<td>3.9</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>F</td>
<td>10.8</td>
<td>5.3</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>G</td>
<td>8.2</td>
<td>6.4</td>
<td>2.4</td>
<td>7.7</td>
</tr>
<tr>
<td>H</td>
<td>4.6</td>
<td>1.2</td>
<td>1.4</td>
<td>3.7</td>
</tr>
<tr>
<td>I</td>
<td>24</td>
<td>29</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>J</td>
<td>57</td>
<td>65</td>
<td>69</td>
<td>86</td>
</tr>
<tr>
<td>K</td>
<td>6.9</td>
<td>6.9</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>L</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>M</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>N</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>O</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>P</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Q</td>
<td>67</td>
<td>67</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>R</td>
<td>57</td>
<td>57</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>S</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>T</td>
<td>61</td>
<td>61</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>U</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>V</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>W</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>X</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Y</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Z</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

1. Loss of material tested determined on basis of weight changes in test blocks as a result of conditioning and weathering procedures.
2. Materials tested: A. coal-tar creosote; B. penta-petroleum: a 5 percent solution by weight of penta-chlorophenol in a light naphthenic-base petroleum oil (1); C. copper-petroleum: a solution of copper napthenate equivalent to 0.5 percent copper metal by weight in a light naphthenic-base petroleum oil (1); D. penta-copper-petroleum: a blend of equal parts by volume (50-50) of B and C; E. penta-petroleum-creosote: a blend of equal parts by volume (50-50) of A and B; F. copper-petroleum-creosote: a blend of equal parts by volume (50-50) of A and C; G. penta-copper-petroleum-creosote: a blend of equal parts by volume (50-50) of A and D; H. petroleum-creosote: a blend of equal parts by volume (50-50) of A and I; I. petroleum oil, a light naphthenic base.
3. Concentration of material tested in the treating solution in which toluene was the diluent.
4. Indicates approximate residual threshold for the most tolerant test fungus.
5. Amount remaining not enough to determine under accuracy of test.
Figure 5. -- Retention of coal-tar creosote (Material A) at time of treatment, after conditioning, and after weathering and reconditioning.
period would influence the rate of evaporation of the preservative material, thereby changing the percent loss and the slope of the line representing the conditioned blocks. Similarly, the line representing the residual preservative after weathering and reconditioning might be changed by subjecting the blocks to another type of permanence test or to a longer or shorter weathering period. A straight line relationship (fig. 5) indicated that the treatment of the blocks and subsequent loss of preservative material were consistent for a given concentration of treating solution, the losses showing a direct relationship to the amount of material retained by the blocks at treatment.

When treating to refusal under vacuum, as in treating these blocks, there is a definite relationship between specific gravity and absorption, the low-density blocks absorbing more, and the higher density blocks less than the average for the given treatment. Since the blocks were accurately cut to size and volume, their weight variation at conditioned moisture equilibrium was a reasonably precise index of their variation in specific gravity. The data so far indicate there is very little correlation between density in pine sapwood and threshold retention. It is true that when blocks of varying specific gravity have been treated with a given preservative material, those of low specific gravity generally show less weight loss by decay than those of a higher specific gravity; however, they also have a somewhat higher retention. In the test reported no attempt was made to select for density or ring count, but variations generally were small. Such selection has been employed since and later results should indicate the significance of density variation.

A variation in the size of the test block itself changes the percentage of material lost. The agar and the soil blocks are of the same volume, if the hole in the latter is disregarded. There is, however, twice the end grain area in the agar block than in the soil block. As would be expected, the percentage loss in the agar block was higher than the loss in the soil block, as shown by table 6. It is evident that block size, shape, and direction of grain should be controlled carefully in any systematic comparison of laboratory techniques by different investigators.

Both the soil- and agar-block methods give an accelerated test of the loss of preservative material. It would require months or years for the same relative losses in treated wood in service. The blocks have a disproportionate amount of end grain in comparison to such products as telephone poles. Another factor that must be considered in such comparisons is that the preservative is uniformly distributed in the blocks, whereas it decreases in amount from the surface inward in large commercially treated products.

A PERMANENCE TEST

High initial toxicity is one of the requirements of a good preservative, but it is of little importance if the toxic components are soon lost in wood exposed to field conditions. The toxicity factor of a preservative is not static, but tends to vary with changes in composition of the preservative that eventually take place in the wood. Such changes are brought about by a loss of the preservative material, through leaching or volatilization or both, as the wood is subjected to currents of air, sunlight, heat, cold, and rain or soil water. Chemical changes and polymerization, in addition, may take place as the preservative is aged in the wood, which may either increase or decrease toxicity. Soil organisms may change or destroy the toxicant, thereby making it possible for wood destroyers to attack the wood. Therefore,
weathering changes quality as well as quantity of the preservative material through the more rapid loss of some components than others. The active components of a chemically complex preservative material, such as creosote, differ not only in toxicity to fungi, but also in vapor pressure, water solubility, and perhaps in the way they are absorbed or adsorbed by the treated wood.

Nontoxic components of a preservative cannot be ignored; for instance, they may slow down or prevent the loss of toxic portions of a preservative. Laboratory tests (14) have indicated that nontoxic constituents may tie up or inactivate toxic portions. They may also serve to decrease absorption of moisture by the wood, with the result that wood-destroying fungi are retarded because the moisture content is too low.

The soil- or agar-block methods should not be regarded as a means of confirming a toximetric rating, as determined by the agar-flask or Petri-dish test, but rather as a means of securing better indications of the relative effectiveness of preservatives in use. In any such evaluation, the preservative material in wood must be subjected to some sort of weathering cycle indicating permanence. Permanence of an oil-type preservative may be tested in the laboratory by subjecting the treated wood to leaching or evaporation. However, in actual service treated wood is used in such a variety of ways and locations that it is impracticable to attempt to simulate all of the conditions to which treated wood may be subjected. Service data on how weathering actually affects a preservative in wood over a long period of time would be a basis for establishing laboratory permanence tests. It is necessary in accelerating the loss of a preservative that approximately the same end result is achieved as is obtained in a larger piece of wood over an undetermined number of years.

A variety of methods for assessing permanence in the laboratory has been used by different investigators. A summary of these methods may be found in "Wood Preservation During the Last 50 Years" (3), or in the Third Edition of "Holzkonservierung" (28). Generally, the loss of preservative has been determined either by chemical assay of the leach water or of the blocks themselves, by mycological testing of the blocks before and after a permanence test, or both. The majority of the methods have subjected the blocks to various schedules of leaching and drying in alternate periods. A variety of block sizes and shapes has been used also.

Gillander et al. (20) placed treated blocks on a wheel, and during each rotation of the wheel the blocks passed successively through running water, an air stream for drying, and a heating hood in which the blocks were exposed to radiant heat at approximately 62°C. The current method used in the Forest Products Laboratory in England (35) consists in placing the blocks in a modified Soxhlet extractor apparatus that allows a regulated amount of liquid to drip onto the wood during the day. Overnight the blocks remain in the apparatus without water. The extraction water is collected and analyzed at specific times. The standard method used in Germany (9) consists in impregnating the treated blocks with water under vacuum and allowing them to remain in the water-filled flasks from Monday to Friday at room temperature. The water is changed daily, morning and night, with frequent shaking. From Friday to Monday the blocks are allowed to dry in the air. The blocks are again saturated with water on each Monday morning, and the cycle is continued for 4 weeks. At the end of this period the blocks are allowed to dry and then are exposed to fungus action. A few investigators have subjected blocks to leaching only for different periods of time and at different temperatures. Few attempts have been made to determine the effects
of sunlight, cold, or the pH of the leach water, which may have considerable effect on some preservative materials. Natural out-of-door weathering has been employed by Curtin, Kline, and Thordarson (6), Gillander et al. (20), and by this Laboratory since 1944.

The permanence test used in this report consisted of two phases: (1) a conditioning period of 3 weeks in a controlled 80°F. and 30 percent relative humidity room; (2) a 60-day period of natural exposure out of doors. Blocks from each phase were subjected to mycological testing. In the controlled conditioning room preservative material was lost if volatiles were present, some of which probably possessed considerable toxicity. While these evaporation losses were slowed down greatly by the end of a 3-week period, they had not stopped. The degree to which volatilization was slowed down or stopped depended almost entirely on the preservative material under test. Subsequent tests have shown a variation in thresholds in similar groups of blocks held for the same period in the same room (15). Obviously, thresholds obtained by testing conditioned blocks that are still losing preservative material are likely to be somewhat less reproducible than thresholds determined after the residual components have become more or less stable in the wood, as indicated by weight loss curves that show negligible changes from day to day.

Although it is not possible to reproduce actual field conditions in the laboratory, a closer approach to them can be made by a permanence test that includes such factors as air currents, sunlight, heat, cold, and rain. The weathering phase of the permanence test used in this and all subsequent soil-block tests, with one exception (15), has been criticized because of the possible effect on the results of variation in site, season, and climatic conditions. However, there is no evidence to indicate that these outdoor conditions need to be strictly controlled or duplicated, provided the blocks are exposed long enough each time to deplete the preservative materials to about the same degree. The bioassay appears to be the only way to determine whether duplication of the depletion has been attained or approximated. The out-of-door weathering at least brings into play the elements of natural exposure that affect treated wood above the ground. Whether such weathering is the closest approach to actual conditions to which wood in service is subjected at the ground line remains to be seen. Part of the answer will be obtained from the correlation of laboratory tests with stake tests that are already underway in several field test plots.

A number of creosotes were tested recently (15) after conditioning and after leaching in daily changes of distilled water for 3 weeks. The lowest threshold values were obtained for the blocks that were leached, which was contrary to expectation. Blocks treated to the same retentions with the same or similar creosotes were then weathered out of doors for 2 months, with the result that the effectiveness of the creosote was found to be greatly reduced. It seems improbable that soluble sugars or growth substances necessary for the test fungi were lost in the leaching process, since the decay in untreated leached or weathered blocks was approximately the same as in untreated not leached or weathered ones. The similarity in decay of leached and not leached untreated blocks also indicated that antibiotic substances produced by microorganisms growing in the leach water probably were not involved. Suolahti (48) surmised that antibiotic substances produced by microorganisms growing in standing leach water increase the resistance of untreated *Pinus sylvestris* sapwood to *Lentinus lepideus*. He stated that the effects of submersion in water that is changed daily are insignificant. Varner and Krause (50) reported that in both decay and stain tests the untreated and treated blocks were less subject to fungus attack after
heating and leaching. Verrall and Mook (51) found that leaching progressively decreased stainability of untreated pine sapwood.

A permanence test that is designed to remove as much of the preservative material as is often lost after long-time field exposure would serve to differentiate between good and poor preservatives. Some of the preservatives eliminated might be effective for shorter periods of time or for exposures in which the wood was never subjected to severe ground-line conditions in service. It may be that several permanence tests will have to be applied before one can completely evaluate a preservative for use in both aboveground and ground-contact environments. Comparisons are now underway that involve out-of-door weathering and various schedules of leaching, wet-dry cycles, exposure to heat and ultra-violet light.

**MALT AGAR VERSUS SOIL AS A CULTURE MEDIUM**

Variations in the manner in which a preservative is put into wood and in the further treatment of the wood before testing may result in different threshold values. The blocks for the soil- and agar-block methods herein reported were treated and handled similarly, but threshold retentions in the latter were lower. The values obtained by the Europeans, summarized by Schulze, Theden, and Starfinger (41), with malt agar, also appear lower than those found by the soil-block test for similar preservatives and test fungi species. The reason for the difference must lie in part in the two types of culture media on which the test fungi were growing.

The Europeans since 1920 have used a standard method for testing impregnated wood blocks in which malt agar is employed for growing the test fungi. However, Breazzano (2), in 1922, and Rabanus (32), in 1931, advocated the use of moist sand. In 1933 Flerov and Popov (19) and in 1939 Leutritz (26) suggested the use of soil covered with thin sections of sapwood. A comparative study made by Richards and Addoms (34) and Varner and Krause (50) indicated that both soil, covered by feeder strips, and malt agar gave comparable relative ratings, but that the soil was the more severe test. Harrow (21) has now tested several water-soluble preservatives, following the method of Leutritz (27). The culture method described in this report is similar in principal to that of Flerov and Popov or Leutritz. Others such as Zycha (52), in 1939, and Moore (30), in 1944, followed by Sedziak (42, 43, 44), used soil but suggested that the test block be buried and they used no feeder strip.

A standard laboratory method should yield reproducible results when similar preservatives are tested in the same or in different laboratories. For the growth of fungi, a medium that is not composed of chemically pure ingredients could conceivably give a variation in results. Natural substances as soil, malt, or agar are not always the same in composition. Malt varies in the composition of its constituents, including the amount of various growth substances present. Both malt and agar vary in kinds and amounts of extraneous materials they contain. Such differences can be reduced to a minimum by obtaining the malt and agar from the same source of supply.

The loam soil used in the soil-block test at Madison was more or less uniform in composition. However, an analysis of soil may vary in samples taken from a small area and it is likely to differ considerably if taken from different localities. Because of the effect that this variation may have on the test results, the use of soil as a substrate would be questionable if factors other than nutrients were not further studied. Even so, the comparison of a number of potential preservatives in
one laboratory by the soil-block method should not be discouraged. It is only when comparisons are made between laboratories using different soil types that particular attention must be given to results that may be altered by substrate variations.

There is a question whether soil or any substrate that offers adequate moisture control in the test block and permits viable cultures throughout the test period needs to be an exact composition in the testing of wood preservatives. A fungus that causes decay in wood in service uses the wood as its main source of food. The feeder block placed on top of the soil provides a uniform and favorable food supply. We have found that some wood destroyers grow poorly if at all in the soil when the feeder block is absent. With the feeder block, these fungi rapidly attack the feeder block. This observation is a point against the burial of test blocks in the soil without a feeder block. It seems desirable to establish the test fungus in the soil, as we would find it in nature, before the test block is added.

Soils especially rich in carbon and nitrogen sources or containing special nutrients might enable the fungus to produce more decay or the same amount of decay in a shorter time. Schmitz (36) and Schmitz and Kauvert (38, 39) found that alkaline salts or organic nitrogen compounds in some cases increased the amount of decay on sawdust, but inorganic nitrogen compounds and sugars in many cases decreased the intensity of attack. Zycha (52, 53) found that salts containing nitrogen increased the intensity of fungus attack on wood blocks half buried in the loam soil. Leutritz (27) demonstrated the importance of nutrients in the soil in the production of a certain percentage of decay in wood. Findlay (18) reported a decrease in the intensity of fungal attack in wood treated with sugar solutions. However, as soon as the added sugars were used the decay of wood took its normal course. He suggests that the fungus prefers the easily digested sugars to the less digestible polysaccharides of wood. In the testing of wood preservatives, however, the amount of decay is secondary to the question of whether the fungus will or will not attack the wood treated with a preservative.

No reference has been found in literature on the question of whether different nutrients in a given substrate can change a threshold value. In the present study the threshold values obtained by using soil and feeder blocks are higher than those when malt agar was the medium. This does not necessarily indicate that the nutrients alone in the soil contributed to this difference. A supplementary test on untreated blocks and blocks treated with a creosote indicated that decay by Lentinus lepideus and Poria radiculosa generally was not increased by the addition of malt to clay, sand, vermiculite or the loam with varying percentages of moisture, when a feeder block was present. However, the total amount of decay was usually less when the moisture percentage of the substrate was lower. Sand and vermiculite could contribute very little to the nutrient demands of these fungi; consequently, it would seem that the feeder block served as the all-important source of food. The respective thresholds of the creosote tested against Lent. lepideus and P. radiculosa were similar, regardless of the various substrates or the presence of malt. Except for the sand containing only 10 percent moisture, the moisture itself was not a factor in establishing a threshold. Other supplementary tests have shown that blocks buried in the soil absorb a higher percentage of moisture than is added to the soil, whereas test blocks placed on feeder strips resting on the soil reach approximately fiber saturation before decay is initiated. Blocks buried in soil that is not so wet that adequate aeration is prevented will decay faster than blocks on top of the soil. However, threshold values of a creosote and pentachlorophenol solution were not changed by this difference in moisture content of the blocks.
Liese (28) stated that a treated test block separated from soil by only a thin feeder strip is subject to leaching. Until decay is initiated the soil block contains approximately 30 percent moisture, which would be unlikely to permit leaching. Furthermore, such a criticism is not warranted if one accepts the agar-block test where the moisture content is likely to be considerably higher. The weight losses for the unattacked conditioned soil blocks during the test period are usually proportional to the amount of volatile material present.

Variations that might occur in the pH of the soil have not been checked. The pH of the loam soil used was readily changed from a pH of 5.5 to 6.0 to a pH of 3.0 to 3.5 by the test fungi in 3 weeks' time.

Sand, a substrate that could be more easily standardized than loam, has the disadvantage of not holding enough water to prevent possible drying out of the culture during the 3-month test period. Quartz sand becomes saturated with only 20 percent moisture. Water can be added to the cultures during the test period, but this is time consuming if contamination is to be avoided. Sealing the test jar to prevent loss of water has been demonstrated to completely inhibit decay by some test fungi. If the sand is saturated with water there is little available oxygen for the fungus except on the surface. Sand also lacks particles that might absorb toxic vapors given off by high concentrations of some preservatives. It is important in any laboratory test that the fungus remain viable throughout the test period. It is known that fungi growing on malt agar are often killed by toxic vapors that are absorbed by the malt-agar medium. In the present tests the untreated blocks in the same soil-culture bottle with the treated blocks were attacked as much as untreated blocks tested alone. It always has been possible to isolate the fungus at the end of the test period from the soil cultures in which the highest concentration of the various preservatives were tested. These results indicate the value of the ability of the soil to absorb volatile toxicants.

Vermiculite varies considerably so that problems of standardization also arise in its use. It has the advantage of holding a large amount of moisture while maintaining adequate aeration for the fungus. Enough comparative studies have not been made to determine whether all test fungi would remain viable in vermiculite if toxic vapors from the test block were present. Vermiculite with the use of a feeder strip has definite possibilities and further comparative studies of it should be made.

If further work now underway confirms the observations to date that threshold values for standard test fungi and other preservative materials are not changed by additional organic material in the soil or a variation in pH, it should be possible to standardize soil sufficiently on the basis of water-holding capacity and a pH range for general use. However, the search for a suitable substrate of more constant composition will be continued.

**REPRODUCIBILITY OF RESULTS BY THE SOIL-BLOCK METHOD**

Reproducibility of threshold values is a requisite of a standard method for the laboratory evaluation of preservatives. It has been possible to reproduce both the conditioned and weathered thresholds of a pentachlorophenol solution by the soil-block method. The 5 percent pentachlorophenol in petroleum (material B) has been included as a test material in six other soil-block series run at different times...
over a period of 5 years. The threshold for L. trabea for this solution has not varied more than ± 0.2 pound (± 0.01 pound pentachlorophenol) from 2.2 pounds in the conditioned and 4.8 pounds in the weathered tests. Similarly, thresholds for Lent. lepideus and P. monticola have not varied in these other tests more than 0.2 pound from those given in this report (table 5).

Threshold values on conditioned but unweathered blocks were found to vary when the same creosotes were tested at different times by the soil-block method (15). A close examination of these results, however, disclosed that this variation was not caused by the culture method. The indications were that in testing of volatile preservatives, the conditioning of the blocks was not done under sufficiently controlled conditions.

Since the aim of a block method should be to secure better laboratory indications of the relative effectiveness to be expected of preservatives in use, it is imperative that the blocks be subjected to a permanence test involving other factors as well as evaporation. A threshold for conditioned blocks does not represent the initial toxicity of a volatile preservative. For instance, blocks treated with a creosote and conditioned for 3 weeks already have been subjected to a type of permanence test. On the other hand, a threshold after conditioning for preservative material in which the toxic components are not volatile does represent an initial toxicity. In view of this, efforts to develop a method that will give reproducible threshold values should be more successful if the permanence test involves more than one factor. Much attention is now being given to a permanence test in which the factors of leaching, evaporation, and heat will be controlled. The indications are that such a test will lead to similar threshold values even though the work is done at different times or places.

The use of "a preservative of reference" in testing treated blocks is a desirable check on the uniformity of test methods. It is true that the amount of decay in untreated blocks or the feeder blocks is an indication of such uniformity. However, a fungus might produce the same amount of decay in untreated blocks at different times even though it had changed somewhat in tolerance to a given preservative component. In Canada copper pentachlorophenate has been used as a reference preservative. The penta-petroleum solution that has been included with most of the soil-block tests at Madison is not an ideal reference material for several reasons. Both the toxic component and the carrier in a reference preservative should be chemically pure materials that will be readily available at all times. It probably will be necessary to select several materials as representative of different preservative types.

TIME REQUIRED TO DETERMINE THRESHOLDS
BY SOIL-BLOCK METHOD

The determination of thresholds for a wood preservative by the soil- or agar-block method described requires 4 to 6 months, depending on whether conditioning or conditioning and weathering of the blocks is done. No difference has been noted in the tests between the agar- and soil-block method in ease of manipulation or the time required. Even 6 months is a short period as compared to that required for stake tests, but the block method is by no means a "quick test."

For the present it is felt that improvements in reproducibility and sensitivity of the present soil-block method are more important than attempts to shorten it. It
is obvious that without some standard test the merits of a modified method cannot be determined anyway. Trendelenburg (49), Hopkins and Coldwell (23), and Pechmann and Schäle (31) have indicated that the amount of fungal infection in a piece of wood, subjected to only a 1- to 2-month test period, can be shown by the breaking strength of the test specimen. A test involving breaking strength demands the use of closely matched pieces of wood.

Preliminary study has shown that threshold values for at least the oil-type preservatives usually can be determined by the present soil-block method after only a 2-month culture period. Also, threshold values have been approximately the same when treated blocks were weathered for 2 months or subjected in the laboratory to 2 weeks of leaching and drying with the application of heat. Thus, by using an accelerated laboratory permanence test and a shorter culture period, threshold values based on permanence may eventually be determined by the soil-block test in approximately 3 months.

CONCLUSIONS

The following conclusions are based on the results of a comparative study of the soil- and agar-block techniques and of soil-block tests made on oil-type preservatives. The studies with the soil-block method have involved approximately 40,000 blocks treated with 75 oil-type preservative materials.

The soil culture technique was found to have certain advantages over the use of malt agar, namely,

1. The soil maintains, until decay is initiated, an approximate moisture content equal to fiber saturation in the test block. The close correlation between moisture and decay, once decay is initiated, suggests that the additional moisture from the soil is obtained by the fungus as it is needed for the metabolic processes.

2. The soil has the capacity to absorb certain quantities of toxic material that might kill the fungus culture during the test period. The volatile components of a preservative, therefore, are kept from exerting a greater effect than they would in service.

3. The sapwood feeder strips on top of the soil provide a natural source of food for the test fungi. Preliminary tests indicate that additional nutrients may speed up metabolic activity, causing more decay, but that threshold values are not thereby changed.

4. The threshold values obtained more closely approach those of actual field experience. Whether the method will give the same order of preservative effectiveness as shown in field exposure will be evident from the results of field tests now in progress.

5. The soil culture responds to a wider range of solution strengths and, therefore, affords a broader and more reliable basis on which to establish threshold values based on weight-loss trends.

The block method of testing wood preservatives is directed at improving laboratory indications of the relative effectiveness to be expected of preservatives in use. Therefore, emphasis should be placed on the results obtained from treated blocks subjected to permanence tests that include at least the factors of evaporation, leaching, and heat.
Current work on the soil-block method is being conducted on (1) the development of laboratory permanence tests that will show practical differences in leachability and volatility of preservatives; (2) improvements in the test, to insure reproducibility of results, leading to standardization and use by different laboratories; and (3) making it adaptable for a variety of preservative materials, species of wood other than pine, and economically important test fungi that are particularly tolerant to preservatives.

LITERATURE CITED


(2) BREAZZANO, A. 1922. METODI NORMALI DI PROVO SULLA PUTRESCIBILITA DEI LEGNANI. Rivista Tecnica Delle Ferrovie Italiane. Turin.


DUNCAN, C. G. \[1952\] EVALUATING WOOD PRESERVATIVES BY SOIL-BLOCK TESTS: 5. LIGNITE-TAR AND OIL-TAR CREOSOTE. Amer. Wood Preservers' Assn. Proc. 48. [In press.]


______ and T. C. SCHEFFER. 1946. MOISTURE CONTENT OF WOOD TESTED FOR DECAY RESISTANCE IN GLASS BOTTLES. Office Report. USDA, Div. of Forest Pathology, Madison, Wis.

______ and T. C. SCHEFFER. 1950. RELATION OF WEIGHT LOSS TO PERIOD OF EXPOSURE IN LABORATORY TESTS OF THE NATURAL DECAY RESISTANCE OF WOOD. Office Report. USDA, Div. of Forest Pathology, Madison, Wis.


(35) RICHARDSON, N. A., and E. E. LARNER.

(36) SCHMITZ, H.

(37) _______ et al.

(38) SCHMITZ, H., and F. KAUFERT.

(39) _______ and F. KAUFERT.

(40) SCHULZE, B., and G. BECKER.
1948. UNTERSUCHUNGEN ÜBER DIE PILZWIDRIGE UND INSEKTENTÖTENDE WIRKUNG VON FRAXIONEN UND EINZELSTOFFEN DES STEINKOHLENLEITERÖLS. Holzforschung 2:97-118.

(41) SCHULZE, B., G. THEDEN, and K. STARFINGER.

(42) SEDZIAK, H. P.
1949. ACCELERATED TESTING OF WOOD PRESERVATIVES INCLUDING WOOD BLOCK SOIL TECHNIQUE. Forest Products Laboratory, Ottawa, Canada. Mimeo. O-149.

(43) _______.

(44) _______.
[1952.] THE EVALUATION OF TWO MODERN WOOD PRESERVATIVES. Forest Products Research Society Proc. 6. [In press.]

(45) SNEDECOR, G. W.
1946. STATISTICAL METHODS. Iowa College Press.

(46) SNELL, WALTER H.
(47) SNELL, W. H., N. O. HOWARD, and M. U. LAMB.  
1925. THE RELATION OF MOISTURE CONTENTS OF WOOD TO ITS DECAY. II. Science  

(48) SUOLAHTI, O.  

(49) TRENDLENBURG, R.  
1940. ÜBER DIE ABKÜRZUNG DER ZEITDAUER VON PILZVERSUCHEN AN HOLZ MIT HILFE DER SCHLAGBIEGEPRÜFUNG. Holz als Roh- und Werkstoff 3: 397-407.

(50) VARNER, R. W., and R. L. KRAUSE.  

(51) VERRALL, A. F., and P. V. MOOK.  

(52) ZYCHA, H.  

(53)  