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Procedures for Laboratory Studies on Wood Decay Resistance

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Procedures for Laboratory Studies on Wood Decay Resistance[1,2]

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Laboratory studies on wood decay resistance are useful in both research and teaching. These studies have been used extensively to determine decay resistance of tropical woods to wood rot fungi found in the temperate regions of North America (9, 16, 20, 25, 26, 27). The methods have been used or suggested as tools in basic research on fungal ecology (4, 13, 14, 17), abnormal wood (5, 6, 12, 24) and wood variability (8, 21, 22, 28). The techniques also are adapted for use as laboratory exercises in a Plant Pathology course, especially if conducted in conjunction with other laboratory deterioration procedures (18, 23, 29). Because of these uses, the suggestion was made that these laboratory procedures be presented in their entirety. This paper is an attempt to achieve this purpose.

There are two general procedures for laboratory study of wood decay resistance. These are the agar-block (10, 20) and soil-block methods (3, 10, 11). Because procedures are duplicated partially in these two methods, they will be presented together.

PREPARATION OF TEST WOOD

If possible, complete records should be kept on the origin of the wood for study. These include data on geographical location of the original tree and the position of the wood within the tree. The position code adopted by the American Society for Testing Materials is recommended (1).

Small test blocks are cut from the north and south radial series (Figure 1) thus allowing for complete radial sampling of the tree from pith to bark. In tests representing only heartwood, samples

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[2]The procedures reported herein have evolved since 1947 as a part of the Tropical Woods Study at the Yale University School of Forestry in cooperation with the Office of Naval Research and the Bureau of Ships, United States Navy Department, under Contracts N6-ori-44, Task Order XV (Project Designation No. NR-033-020) and N 7-onr-28810 (Project Designation No. NR-335-001, NS-032-001.) The author wishes to acknowledge the indebtedness to Prof. J. S. Boyce of Yale University under whose direction these decay resistance tests have been conducted, Jack R. Mulholland for valuable comments and suggestions and the many other associates who have had a part in these tests.

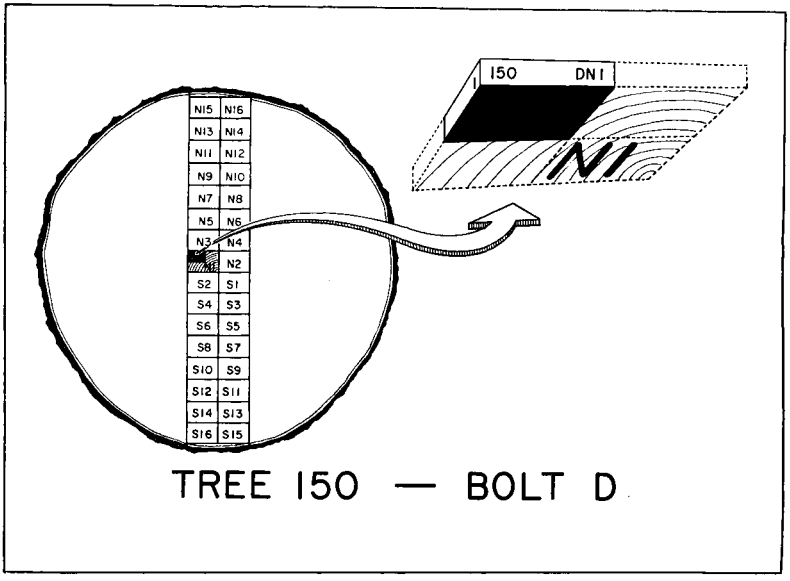

Figure 1. Location code of test wood blocks (1) (Courtesy R. Albertson)

should be divided evenly between inner, middle and outer heartwood. Where the amount of heartwood is small, often only two divisions can be made.

Test blocks are either 1 inch x 1 inch x ¼ inch or ¾ inch x ¾ inch x ⅜ inch, with the short dimension being along the grain. Smaller blocks may be used but changes in exposure time and interpretation of data result (2). Block edges are sanded so that no fragments of wood remain. The codes designating tree and wood positions are written on one edge of the block and the block number on an adjacent edge (Figure 1). Block numbers run consecutively, starting with inner heartwood, for each tree.

Six test blocks are needed per wood position for each test fungus. Five of these blocks are exposed to the fungus and one is a control. In the overall test the control blocks are divided into two groups. One-half of them are subjected to the same treatment, except for fungus exposure, as the actual test blocks. They will be referred to as moisture control blocks. The remaining blocks are stored for future reference and therefore, are referred to as reference blocks.

Test and moisture control blocks are conditioned to an equilibrium weight before exposure. This conditioning can be achieved by placing the blocks in a drying oven set at 40° C. for two weeks. If available, a constant humidity, temperature chamber can be used. At the end of the two-week conditioning period, the blocks are removed from the

oven and weighed. During the weighing, care must be taken that only a minimum amount of moisture is absorbed by the blocks from the atmosphere. They should be placed in a desiccator upon removal from the oven. Each block is kept in the desiccator until it is taken out and weighed. A small beaker of $CaCl_2$ should be placed within the glass enclosure of the balance in order to maintain as dry an atmosphere as possible during weighing. The beaker of $CaCl_2$ should be placed in a drying oven when the balance is not in use. The weighing data and block weights, taken to the nearest 0.0001 gm, are recorded on a data sheet.

Preparation of Test Fungi

The preparation of test fungi should be done simultaneously with that of test blocks. In this manner, both will be ready for exposure at the same time.

The test fungi should represent species causing both white and brown rot. The most common white rot fungus used is *Polyporus versicolor*. Other members of this group that have been used include *Daedalea confragosa, Polyporus gilvus, Stereum frustulosum* and *Stereum hirsutum*. *Poria monticola* is the most common brown rot fungus used. Others in this group include *Lentinus lepideus, Lenzites trabea, Poria incrassata* and *Stereum sanguinolentum*. Cultures of these fungi are available from the Forest Products Laboratory at Madison, Wisconsin.

Petri-plates containing malt agar (see next section) are used in producing fungus inoculum. These petri-plates are inoculated from stock cultures of the desired fungi. A two- to three-week growth period at 25° C. is adequate for the fungi to cover the agar surface. The culture surface is cut aseptically into one-fourth inch squares by means of a sterile scalpel. These squares serve as inocula for the decay chambers.

Preparation of Decay Chambers

The decay chambers are 6-oz. French square glass bottles. They have metal screw caps from which paper insets have been removed because chemicals in the pulp from which the insets are made can retard the growth of fungi. Use of plastic caps should be avoided because chemical vapors from them can have an inhibitory effect on fungus growth.

Agar-block Method

Each bottle contains 25 ml of malt-agar medium. This medium contains 30 gm of Difco Malt Extract and 15 gm of Difco Bacto Agar

dissolved in one liter of distilled water. The capped bottles containing media are sterilized by autoclaving in a vertical position for 15 minutes under 15 lbs. pressure. After autoclaving, the bottles are placed in a horizontal position until the medium has hardened.

When the medium has hardened, inoculation of the decay chambers with the test fungi can proceed. The one-fourth inch squares of inoculum are transferred aseptically by a sterile transfer needle to the agar surface in the bottles. The inoculum square is placed in the center of the agar surface so that a small, V-shaped, solid, glass rod can be placed around the inoculum (Figure 2-1). These rods have been previously sterilized by autoclaving for 20 minutes under 15 lbs. pressure. They are sterilized in petri-plates and transferred singly, aseptically, with sterile forceps to the decay chambers. The metal caps are replaced loosely enough so as to allow air passage for good growth of the fungi. If a large number of decay chambers are to be prepared, it is easier to complete either the inoculation or the glass rod operation for all the bottles before doing the other of these two operations.

Although transferring procedures used in microbiology are well known, a note on the above inoculations should be made. The preparation of decay chambers should be done in a room which has a

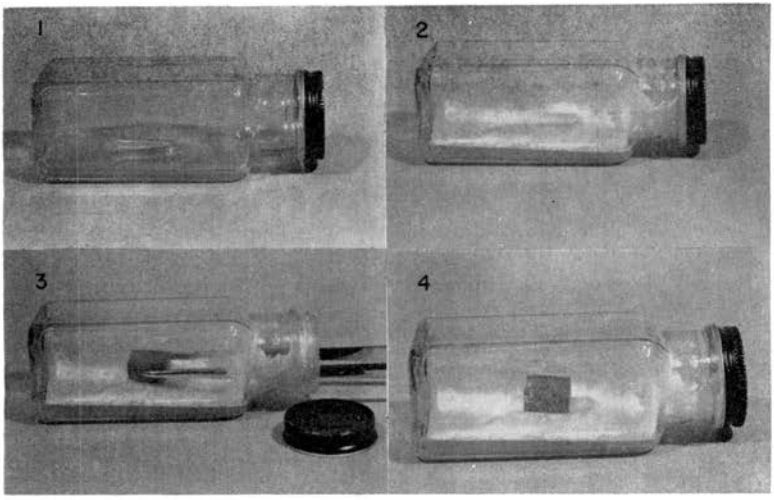

Figure 2. Agar-block method. 1. Placement of inoculum square and glass rod. 2. Well formed fungus mat of *Poria monticola*. 3. Placement of test wood block on fungus covered glass rod. 4. Decay chamber with test wood block in position. (Courtesy L. A. Facto)

minimum of air movement. The working surface should be covered with a damp piece of cheese cloth. The transfer needle or forceps are heat-sterilized after each inoculation. Although all these precautions keep contamination to a minimum, a number of extra chambers should be prepared in case problems do arise. The number of extras needed depends upon individual conditions. The author found that more contamination can be expected when the operation is done in a large room, during the summer months, and near research activities that deal with common contaminating fungi (corn storage studies, *Neurospora* genetics).

The inoculated decay chambers are incubated at 25° C. until well formed fungus mats are present on the medium surfaces. (Figure 2-2). This usually takes a period of two weeks. The chambers are incubated in horizontal positions in cardboard or wooden boxes to facilitate handling. The name of the test fungus and the date of inoculation are written on the outside of the container.

Soil-block Method

Each bottle contains approximately 100 gm of soil at approximately 42 per cent moisture content and a wood feeder block. The soil should be a loam with a pH of between 5 and 6. A supply of soil can be stored in the laboratory in sealed garbage cans.

The moisture percentage, determined on an oven-dry basis, of the soil is calculated each time a new series of decay chambers are prepared. The amount of water needed to raise the 100-gm soil amount per decay chamber to approximately 42 per cent moisture is determined in the following manner:

Weight of metal cup used for drying soil	– 16.4 gm
Weight of soil direct from can	– 50.0 gm
Total weight of soil and cup	– 66.4 gm
Weight of oven-dried soil and cup	– 54.4 gm
Weight of metal cup	– 16.4 gm
Weight of oven-dried soil	– 38.0 gm
Weight of 100 gm of oven-dried soil	– 76.0 gm

$$\text{Formula for percentage moisture, oven-dry basis} = \frac{\text{Wet wt.} - \text{Dry wt.}}{\text{Dry wt.}} \times 100$$

$$\text{Substituting} - \frac{\text{Wet wt.} - 76.0}{76.0} \times 100 = 42\%; \text{ Wet wt.} = 107.0 \text{ gm.}$$

Weight of water to be added to raise 100 gm of the original soil to 42 per cent (107.9—100.0)	– 7.9 gm
Volume of water needed per decay chamber	– 7.9 ml

The amount of distilled water needed per bottle is added by means of a pipette or burette *before* the soil. The approximately 100 gm. of

soil is added to each bottle by means of a metal funnel. The soil amount is roughly determined on a trip balance or by lining the level of soil so that it is even with the soil level in a bottle containing a known 100-gm sample. The soil is settled by tapping and the sur-

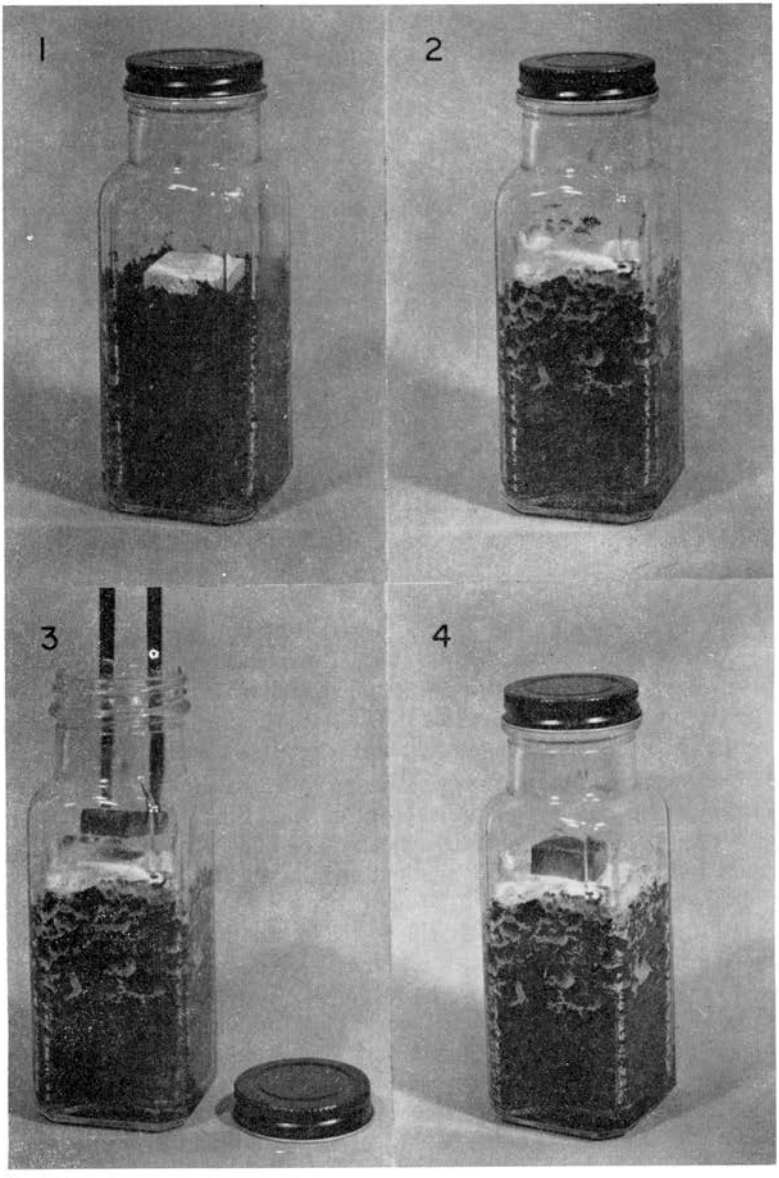

Figure 3. Soil-block method. 1. Placement of feeder block and inoculum square. 2. Well formed fungus mat of *Poria maticola*. 3. Placement of test wood block on fungus covered feeder block. 4. Decay chamber with test wood block in position (Courtesy L. A. Facto)

face leveled by a small wooden tamper. A feeder block of aspen or spruce, 1 x 1/8 inch in cross section and 1 inch along the grain, is placed on the soil surface and pressed gently into place (Figure 3-1). The capped bottles are sterilized by autoclaving in a vertical position for 30 minutes under 15 lbs. pressure.

When the soil has cooled, inoculation of the decay chambers with the test fungi can proceed. The one-fourth inch squares of inoculum are placed aseptically by a sterile transfer needle adjacent to the cross-sectional edge of the feeder blocks in the decay chambers. The metal caps are replaced loosely enough so as to allow air passage for good growth of the fungi.

The same precautions against contaminating fungi should be observed during the above procedures as was outlined under the agar-block method.

The inoculated decay chambers are incubated at 25° C. until well formed fungus mats are present on the feeder block surfaces (Figure 3-2). This incubation period varies from two to three weeks depending upon the rate of growth of the fungus species used. The fungi with slower rates of growth should be started in their decay chambers before others. The chambers are incubated in horizontal positions in cardboard or wooden boxes to facilitate handling. The name of the test fungus and the date of inoculation are written on the outside of the container.

Exposure of Test Wood to Test Fungi

The weighed test wood blocks are sterilized by steam before being placed in the decay chambers (7, 15). The blocks are placed in single layers in petri-plates which are steamed under atmospheric pressure for 15 minutes. Later handling is facilitated if the blocks are placed in the petri-plates in a manner to allow for easy identification.

The sterilized test blocks are transferred aseptically into the decay chambers by means of large, sterile forceps (Figures 2-3 and 3-3). Each chamber receives one test block. The blocks are placed on the glass rod in the agar-block method and on the feeder block in the soil-block method (Figures 2-4 and 3-4). Observations have indicated that possible block-weight increases are produced when test wood comes into contact with agar media. The capped decay chambers are incubated for 16 weeks at 25° C. Handling is again facilitated if the bottles which have the test block number written in waxed pencil on their sides are stored during incubation in cardboard or wooden boxes. Tree number and exposure period dates should be indicated on the outside of the container.

Care of Test Wood at End of Exposure Periods

The decay chambers are removed from incubation at the end of the 16-week exposure period. The block number should be checked

against the number on the side of the bottle as each test block is removed from its chamber. Those numbers that are not readable should be touched up with soft pencil. As the blocks are removed from exposure, they are brushed gently with a piece of cheese cloth which has been moistened with a Zephiran chloride[3] solution. This removes all traces of fungus mat material from the wood. Care should be exercised so as to not break off bits of decayed wood.

In order to attempt to determine reasons for possible extreme values in the final calculations, certain observations are recorded on the data sheet when the blocks are removed from the bottles. These include presence or absence of mites in the decay chamber, presence or absence of contamination of the test block by other fungi and a visual observation on the amount of fungus mycelium on the test block. The latter is usually expressed as heavy, medium, light or none.

The cleaned test blocks and the moisture control blocks are again conditioned to an equilibrium weight. This procedure is the same as that outlined under Preparation of Test Wood. These final weights and weighing date are recorded on the data sheet.

Computation of Data

The data sheets contain eleven columns. The column headings are the following: Block Number, Initial Weight and Date, Final Weight and Date, Adjusted Initial Weight, Weight Difference, Percentage Weight Loss (dry weight basis), Average Percentage for Heartwood Positions, Average Percentage for Test Fungus, Mites (presence or absence), Contamination of Test Block (presence or absence) and Visible Amount of Mycelium on Test Block.

An equilibrium factor is applied to all initial block weights in order to minimize errors in weighing due to atmospheric changes. The resulting adjusted initial weights are used in the final percentage weight loss calculations. Calculations for determining the equilibrium factor and its use are the following:

$$\text{Equilibrium factor} = \frac{\text{Final weight of moisture control block}}{\text{Initial weight of moisture control block}}$$

$$\text{Adjusted initial weight} = \text{Equilibrium factor} \times \text{Initial weight}$$

The equilibrium factor used for a given initial weight adjustment is that whose moisture control block weighing dates correspond to the weighing dates of the test wood block. This may be just one factor or an average of several.

When the initial weights are adjusted, percentage weight loss can be calculated for each block by the following method:

[3]A trade brand name for Benzalkonium chloride produced by Winthrop Laboratories, 1450 Broadway, New York 18, New York.

$$\text{Adjusted initial weight} - \text{Final weight} = \text{Difference}$$

$$\frac{\text{Difference}}{\text{Adjusted initial weight}} \times 100 = \text{Percentage weight loss}$$

The percentage figure is expressed to hundredths. Average percentages are determined for each wood position group. The mean of these wood position averages for each test fungus represents the percentage value used for expressing the relative decay resistance of the wood under test. Thus, this test basically indicates the relative decay resistances of wood species that have undergone the same laboratory decay treatment. Care must be exercised in comparing percentage weight loss and loss of strength properties in woods (19).

The final reporting of decay resistance data is illustrated in many publications (8, 9, 16, 20, 21, 22, 25, 26, 27, 28).

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