Designation: D 4445 – 91 (Reapproved 1996)ε1

Standard Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned Lumber (Laboratory Method)[1]

This standard is issued under the fixed designation D 4445; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Keywords were added editorially in November 1996.

1. Scope

1.1 This (laboratory) method is used for determining the minimum concentration of fungicide, or formulation of fungicides, that is effective in preventing biodeterioration by sapstain fungi and molds in selected species of wood under optimum laboratory conditions.

NOTE 1—From the results of this test, commercial treating solution concentrations cannot be estimated without further field tests.

1.2 The requirements for test materials and procedures are discussed in the following order:

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

3. Summary of Method

3.1 Unseasoned sapwood specimens are treated either by spraying with, or by immersing in, solutions or dispersions of a fungicide formulation prepared at five or more concentration levels. The specimens are exposed to sapstain fungi and molds. The toxicity of fungicides may be tested against individual fungi, in which case sterilization of the samples is necessary, or against several fungi by using a mixed spore suspension for the inoculation of the specimens; in the latter case, sterilization is unnecessary.

3.2 The intensity of surface fungal growth is estimated after incubation and the results used to determine the chemical treatment concentration giving zero growth (CGo).

4. Significance and Use

4.1 This method is useful as a screening procedure for selecting fungicides or formulations for more rigorous field evaluation.

5. Apparatus

5.1 *Incubation Room (or Incubation Cabinet)*, maintained at a temperature of 25 ± 1°C, and relative humidity between 70 and 80 %.

5.2 *Steam Sterilizer*.

5.3 *Containers*:

5.3.1 *Petri Dishes*, with minimum size of 100 (diameter) by 20 mm (height) with lid or,

5.3.2 *Aluminum Pans*, with minimum size of 24 by 10 by 2 cm (height) with aluminum foil cover.

6. Reagents

6.1 *Purity of Water*—Reference to water shall be understood to mean sterile reagent water conforming to Type IV of Specification D 1193.

7. Wood

7.1 *General Properties*—The wood species to be tested should be locally available commercial species selected on the basis of their susceptibility to staining fungi (pine or spruce species are preferred). Sapwood of the selected wood species, unseasoned (moisture content higher than 40 %), free of knots, visible decay, sapstain and mold, shall be used (Note 2). If the fungicide is to be used to protect hardwood, the inclusion of sapwood from a hardwood species is recommended.

NOTE 2—If wood for the test is collected in a sawmill where logs are

[1] This method is under the jurisdiction of ASTM Committee D-7 on Wood and is the direct responsibility of Subcommittee D07.06 on Treatments for Wood Products.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

stored in water, it is necessary to collect lumber from at least three different logs since depletion of nutrients during water storage may strongly affect the growth of molds and staining fungi. Ensure that the lumber collected in a sawmill has not been treated with a sapstain and mold preventive, and if there is any doubt, at least 10 mm of surface wood must be removed and discarded.

7.2 *Size of Specimens*—Specimens should be 7 by 20 mm in cross section and 7 cm long.

7.3 *Preparation of Specimens*—Within two days of collecting, the samples shall be cut from the wood using a sharp saw blade. To prevent drying, the specimens shall be stored in polyethylene bags. For storage longer than one day, tightly packed specimens may be kept frozen (−20°C or lower) in polyethylene bags for up to one year. In this case, one bag should contain as many specimens as are used for one experiment.

8. Test Fungi[3]

8.1 *Hardwoods*:

8.1.1 *Sapstain Fungi*:

8.1.1.1 *Diplodia natalensis* P. Evans (ATCC 34643).

8.1.1.2 *Ceratocystis virescens* (Davidson) C. Moreau (ATCC 11066) a form of *C. coerulescens* found on American hardwoods.

8.1.1.3 *Aureobasidium pullulans* (d. By) Arnaud. (ATCC 16624).

8.1.2 *Mold Fungi*:

8.1.2.1 *Trichoderm pseudokoningii* Rifai (ATCC 26801).

8.1.2.2 *Cephaloascus fragrans* Hanawa (ATCC 12091).

8.1.2.3 *Gliocladium roseum* (Link) Bainier (ATCC 10521).

8.2 *Softwoods*:

8.2.1 *Sapstain Fungi*:

8.2.1.1 *Diplodia natalensis* P. Evans (ATCC 34643).

8.2.1.2 *Ceratocystis pilifera* (Fr.) C. Moreau (ATCC 15457).

8.2.1.3 *Aureobasidium pullulans* (d By) Arnaud (ATCC 16624).

8.2.2 *Mold Fungi*:

8.2.2.1 *Trichoderma pseudokoningii* (Rifai (ATCC 26801).

8.2.2.2 *Cephaloascus fragrans* Hanawa (ATCC 12091).

8.2.2.3 *Gliocladium roseum* (Link) Bainier (ATCC 10521).

8.3 *General Consideration*—In addition to the above fungi, others that are known to cause discoloration on wood species used in test may be included, for example, *Cytospora* sp. (Pine); *Phialophora* sp.; *Graphium* sp.; *Ceratocystis* sp.; *Alternaria* sp.; *Penicillium* sp.; *Aspergillis* sp.; *Trichoderma* sp.

9. Culture Media

9.1 *Malt Agar Substrate*—For both stock culture tube and petri dish cultures of the test fungi, use a nutrient medium consisting of 2 % malt extract and 2 % agar. Sterilize the medium at 121°C, 15 psi (0.1 MPa) for 20 min.

10. Preparation for Inoculum

10.1 If the toxicity of a fungicide is being tested against individual fungi, maintain aseptic conditions when preparing the spore suspension; if the general effectiveness of a fungicide is being tested using a mixed spore supension, aseptic conditions are unnecessary. Most laboratory experiments require a relatively small volume (about 100 mL) of inoculum that may be prepared using only the stock test tube cultures; prepare larger volumes of inoculum from cultures grown on petri dishes.

NOTE 3—Before using any stock test tube culture, reinoculate new tubes for future use.

10.2 For the preparation of a spore suspension, add 5 mL of sterile water to each culture tube or 10 mL to petri dishes, and rub the surface of the malt agar culture with a blunt glass rod to loosen the spores. After collecting the spores and combining them with other similarly collected spores, if desired, adjust the water volume to that required. Although it is a good practice to prepare fresh spore suspensions just before use, they may be kept, even without refrigeration, for 2 to 3 weeks.

10.3 For nonsporulating cultures, obtain a mycelial suspension for use by aseptically scraping the surface mycelium off and blending it with sterile water.

10.4 To evaluate a fungicide use at least six test fungi (three sapstain and three mold) individually, as well as one mixed spore suspension of selected fungi.

11. Preparation of Test Chambers

11.1 To maintain high humidity in the petri dishes during the test period, place eight to ten layers of absorbent paper on the bottom of each dish. Wet the papers with water until free water appears, and press out any air bubbles trapped under and between the paper disks (thoroughly if the dishes are to be sterilized). Place a U-shaped glass rod (3 mm in diameter) on top of the papers and sterilize the petri dishes if required (Fig. 1).

11.2 *Aluminum Containers*—To maintain high humidity in the containers, treat as with the petri dishes. Instead of a U-shaped glass rod however, place two (2) straight rods (3 mm in diameter by 20 cm long) on top of the papers. Sterilize if required.

12. Treatment of Specimens

12.1 *Specimens*—If the wood samples were stored frozen, allow them to thaw in the polyethylene bags. Because of the variation in the susceptibility of wood to fungi, distribute an equal number of specimens from each log, into each treatment per fungus. If specimens were taken from lumber where log identity is not available, select the specimens randomly for testing. Autoclave the specimens before treatment at 121°C, 15 psi (0.1 MPa), for 20 min.

12.2 *Number of Specimens*—Use a minimum of ten specimens per concentration of a fungicide for each fungus tested. Also, use a minimum of ten untreated control specimens for each fungus tested.

12.3 *Preparation of Treating Solution*—Evaluate each fungicide using at least five concentrations. Select the lowest concentration of a fungicide or formulation to be below the expected effective strength and each of the following concentrations shall be twice the previous concentration. Start the preparation of the set of concentrations of each fungicide by

[3] The following numbers refer to standard strains of test fungi maintained in the American Type Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852.