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

This standard is issued under the fixed designation D4445; 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.

1. Scope

1.1 This (laboratory) test 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~~

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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.*

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

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2. Referenced Documents

2.1 *ASTM Standards:*[2]

D9 Terminology Relating to Wood and Wood-Based Products

D1165 Nomenclature of Commercial Hardwoods and Softwoods

D1193 Specification for Reagent Water

~~**3.Summary of Method**~~

~~3.1Unseasoned 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.~~

~~3.2The 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**~~

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

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminologies D9 and D1165.

4. Summary of Test Method

4.1 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. Options for testing the toxicity of fungicides include testing against individual fungi or against several fungi by using a mixed spore suspension for the inoculation of the specimens.

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

5. Significance and Use

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

6. Apparatus

~~5.1~~

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

~~5.2~~

6.2 *Steam Sterilizer*.

~~5.3~~

6.3 *Containers*:

~~5.3.1~~6.3.1 *Sterile Petri Dishes*, with minimum size of ~~100~~150 (diameter) by ~~20~~25 mm (height) with lid or,

~~5.3.2~~6.3.2 *Aluminum Pans*, with minimum size of 240 by 100 by 20 mm (height) with aluminum foil cover.

~~6.~~

6.4 *Spacers*:

6.4.1 *U-Shaped Glass Rod*, with 3 mm diameter or,

6.4.2 *Polyethylene Mesh*, cut to cover the bottom of the selected container(s).

7. Reagents

~~6.1~~

7.1 *Purity of Water*—Reference to water shall be understood to mean sterile reagent water conforming to Type IV of Specification D1193.

~~7.~~

8. Wood

~~7.1~~

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* ~~, Vol 11.01,~~ volume information, refer to the standard's Document Summary page on the ASTM website.

8.1 *General Properties*—The wood species to be tested ~~should~~shall 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 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~~

8.2 *Size of Specimens*—Specimens ~~should~~shall be 7 by 20 mm in cross section and 70 mm long.

~~7.3~~8.3 *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, but less than one year, tightly packed specimens ~~may~~shall be ~~kept~~ frozen ~~(–20°C~~(–20°C or lower) in polyethylene bags ~~for up to one year. In this case,~~. For these longer storage cases, the contents of one bag ~~should contain~~ shall be limited to as many specimens as are used for ~~one~~ a single experiment.

~~8.~~9. Test Fungi[3]

~~8.1~~

9.1 *Hardwoods*:

~~8.1.1~~

9.1.1 *Sapstain Fungi*:

~~8.1.1.1~~

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

~~8.1.1.2~~

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

~~8.1.1.3~~

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

~~8.1.2~~

9.1.2 *Mold Fungi*:

~~8.1.2.1*Trichoderm pseudokoningii*~~

9.1.2.1 *Trichoderma pseudokoningii* Rifai (ATCC 26801).

~~8.1.2.2~~

9.1.2.2 *Cephaloascus fragrans* Hanawa (ATCC 12091).

~~8.1.2.3~~

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

~~8.2~~

9.2 *Softwoods*:

~~8.2.1~~

9.2.1 *Sapstain Fungi*:

~~8.2.1.1~~

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

~~8.2.1.2~~

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

~~8.2.1.3~~

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

~~8.2.2~~

9.2.2 *Mold Fungi*:

~~8.2.2.1~~

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

~~8.2.2.2~~

9.2.2.2 *Cephaloascus fragrans* Hanawa (ATCC 12091).

~~8.2.2.3~~

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

~~8.3~~

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

[3] The following numbers refer to standard strains of test fungi maintained in the American Type Collection (ATCC), ~~12301 Parklawn Drive, Rockville, MD 20852.~~P.O. Box 1549, Manassas, VA 20108, www.atcc.org.

sp.; *Penicillium* sp.; *Aspergillis* sp.; *Trichoderma* sp.

~~9.~~

10. 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.~~

10.1 *Agar Substrate*—For both stock culture tube and petri dish cultures of the test fungi, use a nutrient medium: that is, malt extract agar (MEA, 2 % malt extract plus 2 % agar), potato dextrose agar (PDA, 0.4 % potato starch, 2 % dextrose plus 2 % agar), or similar commercial mixtures of MEA or PDA prepared in accordance with manufacturer instructions. PDA stimulates sporulation in some sapstain fungi (for example, *Aureobasidium pullulans*). Sterilize the medium at 121°C, 0.1 MPa, for 20 min.

11. Preparation for Inoculum

~~10.1If~~11.1 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~~For laboratory experiments ~~require~~requiring a relatively small volume (about 100 mL) of ~~inoculum that may be prepared~~ inoculum, preparation using only the stock test tube ~~cultures; prepare~~ cultures is an option. For larger volumes of inoculum, prepare from cultures grown on petri dishes.

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

~~10.2For~~11.2 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~~ MEA or PDA 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,~~ their storage, even without refrigeration, for 2 to 3 weeks is permissible.

~~10.3For~~11.3 For nonsporulating cultures, obtain a mycelial suspension for use by aseptically scraping the surface mycelium off and blending it with sterile water.

~~10.4To~~11.4 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.~~12. Preparation of Test Chambers

~~11.1To~~12.1 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).~~ disks. 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~~*) or polyethylene mesh spacer (Fig. 2) on top of the saturated papers in a sterile petri dish.*

12.2 *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 200 mm long) or a polyethylene mesh spacer on top of the saturated papers. Sterilize if required.

~~12.~~13. Treatment of Specimens

~~12.1~~

13.1 *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),~~ 0.1 MPa, for 20 min.

~~12.2~~13.2 *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~~

13.3 *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 preparing the highest concentration in an amount equal to twice the volume required for treatment of the samples. Then dilute half of this preparation with an equal volume of water to obtain the next preparation. Therefore, a serial set of concentrations is prepared by continuing the dilutions in this way.

~~12.4~~

13.4 *Treating Procedure*—Carry out the treatment in a 600-mL beaker (~~Fig. 2~~Fig. 3). Place two unused test pieces edgewise on the bottom of the beaker, and the specimens, four or five in a layer, also on edge, crosswise on the previous layers until they reach