Designation: D7584 – 16

Standard Test Method for Evaluating the Resistance of the Surface of Wet Blue and Wet White to the Growth of Fungi in an Environmental Chamber[1]

This standard is issued under the fixed designation D7584; 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 environmental chamber method measures the resistance of the treated Wet Blue and Wet White to the germination of spores and subsequent vegetative growth over a period of four weeks. The test method is useful in estimating the performance of fungicides and should assist in the prediction of storage time of Wet Blue and Wet White before fungal growth begins. The apparatus is designed so it can be easily built or obtained by any interested party and duplicate the natural environment in which Wet Blue and Wet White is inoculated with fungal spores. Spores that germinate on untreated or treated Wet Blue and Wet White can produce fungal growth, resulting in disfigurement or discoloration, or both, of the Wet Blue and Wet White.

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

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:*[2]

D3273 Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions:*

3.1.1 *fungi*—chemoorganotrophic eukaryotic organisms living mainly under aerobic conditions and generating energy by the oxidation of organic materials.

3.1.2 *mold*—a macroscopic discoloration of the surface of wet blue. Mold also a sign of the presence of microscopic fungal growth in the form of usually clear to white fungal hyphae, spores of various colors, and other structures. Colored spots, probably due to the presence of a colored pigment produced by the fungus, have been observed on the surface of wet blue in places where fungal growth has occurred and then stopped. Fungal structures such as hyphae and spores may be viewed by simply using a 10× hand lens.

3.1.3 *Wet Blue*—hide or skin, or split of a hide or skin, tanned with basic chromium sulfate, containing approximately 50 % moisture and an acidic pH.

3.1.4 *Wet White*—hide or skin that has been processed with tanning as the terminal step by using organic or non-organic tanning agents. Chromium or iron containing agents and vegetable extracts will be excluded from use in Wet White.

3.1.5 *Fungi of Importance in the Tannery:*

3.1.5.1 *Filamentous Fungi:*

(1) A wide variety of fungi have been identified in the tannery, but commonly encountered species include Aspergillus spp., Paecilomyces spp., and Penicillium spp.

(2) Aspergillus niger produces black spores and Penicillium luteum produces yellow-green colored spores.

(3) Trichoderma viride produces green spores.

3.1.5.2 *Yeast*—Many yeasts are cream colored, but pigmented ones may also be encountered including Rhodotorula spp. which is pigmented red.

3.1.5.3 *Factors Favoring the Growth of Fungi in the Tannery:*

(1) Wet Blue and Wet White contain nutrients beneficial to fungal growth.

(2) Favorable environmental factors include a slightly acidic pH, a high moisture content, and warm temperatures.

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.02 on Wet Blue.

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[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* volume information, refer to the standard's Document Summary page on the ASTM website.

(3) Fungal spores are transported by air or on hides and skins into the tannery and distributed within the tannery by physical contact or air currents to favorable substrates for growth including Wet Blue and Wet White.

4. Personal Protective Equipment

4.1 Fungi are opportunistic organisms. For this reason, rubber gloves or powder-free latex examination gloves, safety glasses, and a laboratory coat should be worn whenever handling samples with fungal growth are encountered.

4.2 A dust mask or respirator should be worn whenever the environmental chamber is open or whenever samples with fungal growth are encountered.

4.2.1 *Dust Mask*—Examples, 3M 8210 and 3M 9322 Respirators.

4.2.2 *Half-Mask Respirator*—Equipped with a filter approved 99.97 % efficient against solid or liquid particulates including oil-based particles: For example, the North 7700 Series Half-Mask Respirator equipped with Cartridge PI 00 Filter.

5. Summary of Method of Evaluation

5.1 Wet Blue or Wet White is suspended in a chamber with a warm, moist environment.

5.2 After incubation for seven days, the entire surface area of the grain and flesh sides of the test specimen are examined visually, first one side, for example, the grain side, and then the other, for example, the flesh side, for the presence of fungal growth and rated using a numerical scale from 10 (clean or without any sign of fungal growth) to 0 (completely covered by fungal growth).

5.3 Step B is repeated once per week until ratings have been completed for all samples at 14, 21, and 28 days of environmental chamber exposure.

5.4 After the fourth weekly reading is completed, a report of the results is prepared and delivered to the party requesting the evaluation.

6. Significance and Use

6.1 The environmental chamber method is an accelerated test for determining the resistance of Wet Blue and Wet White to the growth of fungi, the causal agent of mold. See Test Method D3273.[3,4]

6.2 The environmental chamber method is useful in estimating the performance of fungicides and should assist in the prediction of storage time before fungal growth begins.

6.3 The environmental chamber method duplicates the natural environment in which Wet Blue or Wet White is inoculated with fungal spores and subsequently disfigured or discolored by fungi.

[3] Didato, Dean T., Bowen, Judith R., and Hurlow, Elton L., Microorganism Control During Leather Manufacture, Leather Technologists Pocket Book, Chapter 20, Editor M. K. Leafe, The Society of Leather Technologists and Chemists, Withernsea, East Yorkshire, UK, 1999.

[4] Leather Technologists Pocket Book, The Society of Leather Technologists and Chemists, Withernsea, East Yorkshire, UK, 1999, pp. 405.

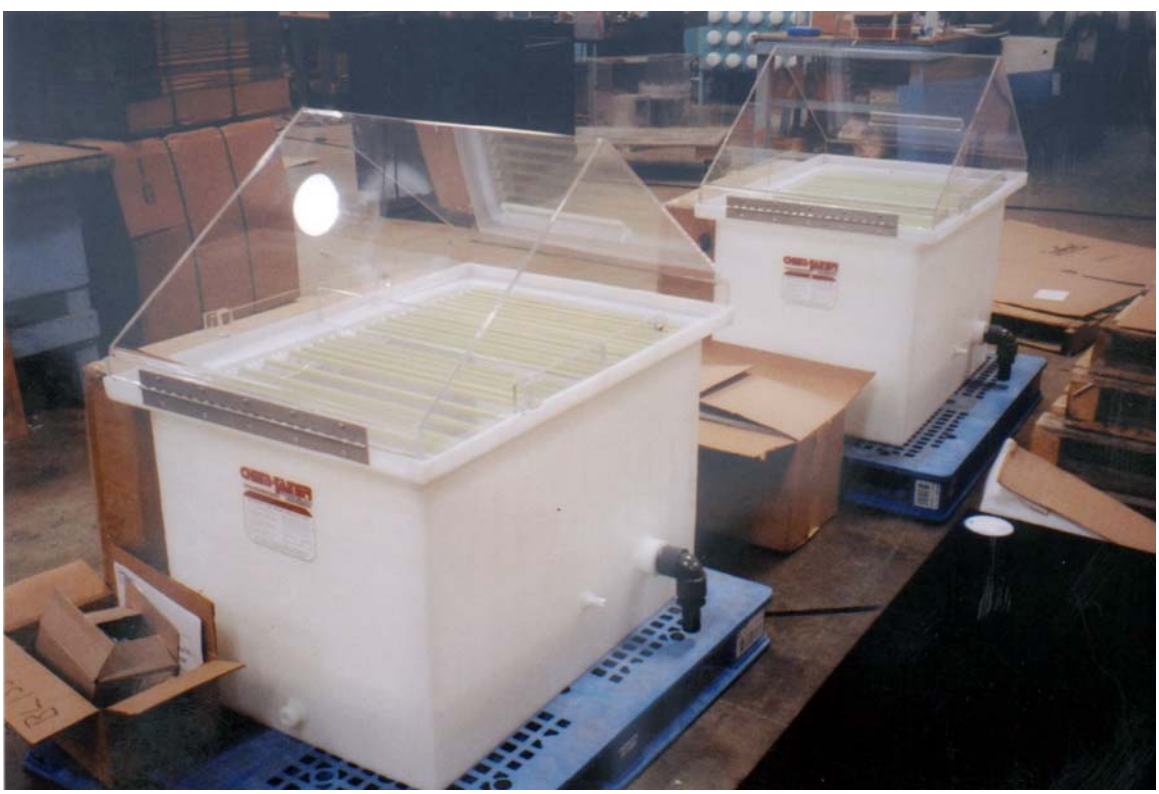

FIG. 1 Environmental Chamber

6.4 The environmental chamber method measures the resistance of the treated Wet Blue or Wet White to the germination of spores and subsequent vegetative growth that spreads over the surface of a comparatively large Wet Blue or Wet White specimen over a period of four weeks.

6.5 The environmental chamber can be kept inoculated with fungi representative of those found in tanneries by adding samples of Wet Blue and Wet White with fungal growth from currently operating tanneries.

6.6 Control specimens of Wet Blue and Wet White without fungicide treatment can be added to the chamber periodically to increase levels of fungal growth in the chamber.

6.7 Leaching of fungicide from the test specimen into the agar often causes a zone of inhibition of fungal growth in the Petri dish test, but in the environmental chamber any leaching of fungicide from the test specimen drips into the water contained in the chamber and thus does not cause the types of false readings observed in the Petri dish test.

7. Interferences

7.1 A common interference is contamination of samples by unwanted organisms, for example arthropods—including culture mites and fungus gnats—that enter the environmental chamber on test specimens or from the laboratory environment.

7.1.1 *Culture Mites (Acari including Tyroglyphus and Tarsonemus):*

7.1.1.1 Culture mites invade the environmental chamber eating the funga hyphae on the test specimens, infecting them with bacteria, and moving from one test specimen to another contaminating them.

7.1.1.2 Mites thrive in environments with high temperature and humidity.

7.1.1.3 Mites are attracted by the odor of fungi and can be also be brought into the environmental chamber on the bodies of flies, organic material, soil, and even test specimens.

7.1.1.4 General hygiene and preventive precautions must be taken to control mites, including examining all new materials entering the laboratory and maintaining separate rooms for initial handling of new samples for testing and a clean room to house the environmental chamber.

7.1.2 *Fungus Gnats (Sciaroidea—including the dark winged fungus flies, Sciaridae):*

7.1.2.1 Larvae of fungus gnats are known to live wherever fungi grow.

7.1.2.2 The same general hygiene and preventive precautions that are used to control mites apply to the control of fungus gnats, especially keeping the room containing the environmental chambers clean.

7.1.3 If culture mites or fungus mites become established in an environmental chamber, terminate work in progress, remove all samples and soil, disinfect all hard surfaces, and begin the chamber startup process again.

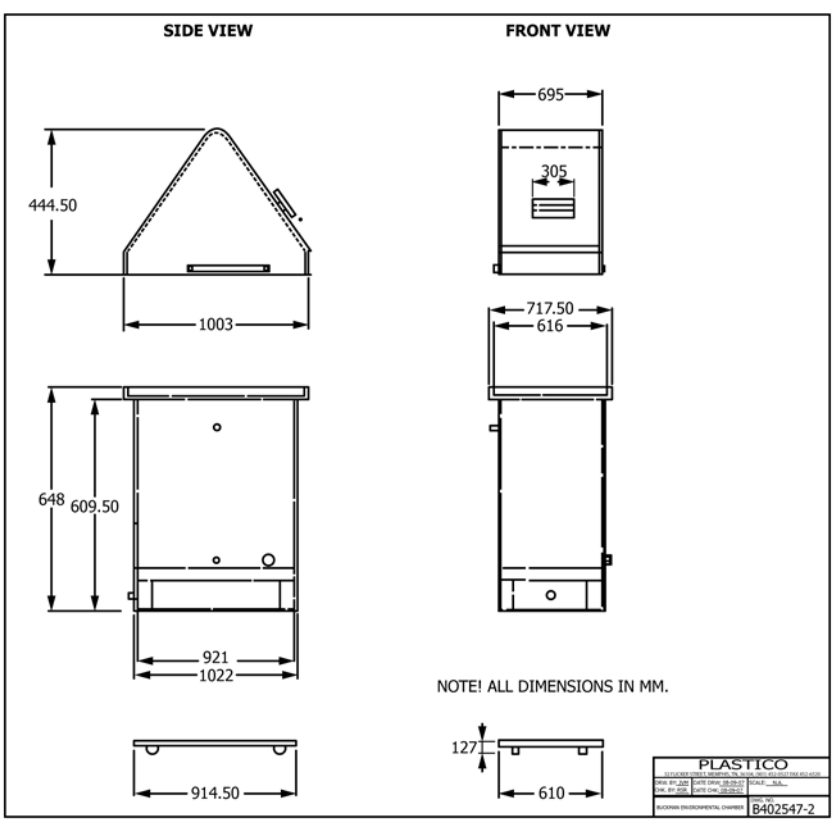

FIG. 2 Environmental Chamber Diagram

7.2 Limit the sunlight entering the chamber room and only have room lights on when working in the room to prevent the growth of algae.

8. Apparatus (see Figs. 1 and 2)

8.1 A typical environmental chamber[5] will have the following components (all measurements rounded to the nearest whole centimeter and may vary in dimensions from one chamber design to another):

8.1.1 The chamber is raised above floor level, made mobile by setting it on a steel platform with casters, and built strong enough to support the weight of the chamber, soil, water, and samples. A typical platform measures 97 cm in length by 66 cm in width and height with steel legs and casters that elevate the chamber an additional 36 cm above the floor.

8.1.2 A rectangular shaped tank is used to contain the water, soil, and samples.

8.1.2.1 A typical tank measures 94 cm in length by 62 cm in width by 62 cm in height with a wall thickness of 1 cm. The tank is built to be watertight. The inside dimensions of the tank are 91 cm in length by 61 cm in width and height. The water level in the bottom of the tank is maintained at a height of 8 to 17 cm.

8.1.2.2 The tank has an offset shoulder at the top rim. This serves to support the chamber cover when in the closed position, to contain water dripping from the chamber cover and to divert the water back to the bottom of the tank without dripping on the Wet Blue or Wet White samples. The rim gives the top of the tank an extended length of 104 cm, an extended width of 72 cm, and a raised outside wall of 3.8 cm in height. The inside of the rim drops 3.6 cm below the surface of the top of the tank to a horizontal shelf measuring 5 cm in width with a curving waterfall that diverts water toward the bottom of the tank.

8.1.3 A rectangular shaped soil tray is used to hold the soil mix and inoculum. See Figs. 3 and 4.

8.1.3.1 The soil tray is seated on top of a table for the purpose of elevating the tray above the level of the water.

[5] The sole source of supply of the complete chamber known to the committee at this time is Indelco Custom Products, 32 Flicker St., Memphis, TN 38182-0183. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

FIG. 3 Rectangular Shaped Soil Tray

FIG. 4 Soil Tray

8.1.3.2 The dimensions of the soil tray are 82 cm in length by 56 cm in width by 5 cm in height.

8.1.3.3 The bottom of the tray consists of a sheet of corrosion-resistant metal mesh. One layer of plastic or fiberglass screening may be placed over the metal mesh to hold the soil in place if necessary.

8.1.3.4 The primary purpose of the soil tray is to help keep the chamber evenly moist.

8.1.4 A rectangular shaped supporting frame is located near the top of the chamber and serves to hold the rods from which the Wet Blue or Wet White samples hang. See Fig. 5. This particular frame will provide enough rod space to hang 100 or more Wet Blue or Wet White samples. Chambers may be designed to provide space for exposure of greater numbers of samples to suit the needs of the testing laboratory.

8.1.4.1 The frame measures 90 cm in length by 58 cm in width by 8 cm in height and is 2 cm in thickness.

8.1.4.2 Four L-shaped brackets hold the frame in place, and each bracket measures 5 cm in length by 2 cm in width by 0.6 cm in thickness on each side. The brackets are fastened to the chamber wall using stainless steel fittings.

8.1.4.3 The top side of the frame on both sides of the chamber running the length of the chamber has U-shaped valleys cut into the top side to hold the rods in place and at a

FIG. 5 Chamber with Rods

right angle to the sides of the chamber. The U-shaped valleys are cut to a maximum depth of 0.6 cm and a width of 1.1 cm.

8.1.4.4 The rods measure 57 cm in length and 1.2 cm in diameter. Ten rods holding 10 to 15 Wet Blue and Wet White samples hung at right angles to the rod can easily be accommodated in the chamber. As an alternative to using rods to hang the samples on, use a wire strung across the top of the chamber.

8.1.4.5 Plastic insulated solid copper wire of 14 gauge (3 mm diameter) is useful in making hangers for Wet Blue and Wet White samples. The wire is bendable into a suitable "S" shape for hanging test samples and retains its shape for multiple uses.

8.1.5 The top of the environmental chamber is constructed of acrylic plastic and is designed to have straight sides and a pitched top so that moisture condensation will run down the sides and be recirculated instead of dripping onto the Wet Blue and Wet White samples. The top features a handle at the front, hinges at the rear, and support struts on each side to allow the lid to be propped in the open position. Typical dimensions include:

8.1.5.1 *Sides*—Two sheets of acrylic cut to 99 cm at base with a 6 cm vertical rise and then at an angle of 40 degrees for 64 cm on each side to the peak.

8.1.5.2 *Front and Rear*—Bottom sheet 69 cm wide by 6 cm on sides rising vertically and attaching to a sheet rising at an angle of 40 degrees measuring 69 cm wide by 64 cm in length.

8.1.5.3 The handle is mounted on the front sheet. The base of the handle measures 3 cm in thickness by 3 cm in width by 30 cm in length and is used to attach the handle (30 cm in length by 5 cm in width by 1 cm in thickness).

8.1.5.4 Single or double support struts are used and measure 69 cm in length by 7 cm in width by 1 cm in thickness.

8.2 The environmental chamber must be capable of maintaining a relative humidity of 95 to 98 % at a temperature of approximately 32°C (90°F) while providing a continuous inoculation of the surface of the test specimens with fungal spores.

8.2.1 Maintaining the room temperature at approximately 24°C is required so that heat loss from the cabinet is insignificant and a relative humidity of 95 to 98 % is readily obtainable in the chamber. Alternatively, the cabinet must be insulated with suitable material to minimize heat loss.

8.2.2 An electric water heater is installed in the bottom of the chamber with watertight connections through the end wall See Figs. 6-8.

8.2.3 The heater should be sized to allow reasonable recovery time and uniform heating of the water when the chamber is opened and closed to place or inspect samples. It is so placed that it is immersed when there are 8 to 17 cm of water in the bottom of the chamber depending on the placement of the heater.

8.2.4 The temperature in the chamber should be monitored and controlled by placing a suitable thermocouple or RTD in an area near the test samples.

8.2.5 The temperature can be displayed and controlled by a solid state proportional controller. See Figs. 9 and 10.

8.2.6 A flow-through water system consisting of a brass 120 VAC solenoid valve with timer, tubing for water supply, and overflow drainpipe is used to prevent accidental overfilling of the tank with water. This system adds water to the tank daily with excess water flowing into the drainpipe with connection to the sewer. See Figs. 11 and 12. For rooms without a drainpipe use a float valve to turn the water on and off.

FIG. 6 Heater Conduit Boc Installed on Tank

FIG. 7 TC Heater Installed in Tank Exterior

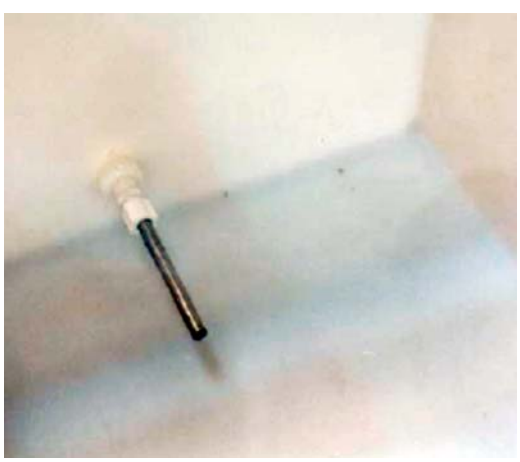

FIG. 8 Heater Installed in Tank Interior

FIG. 9 Controller with connecting Cables: Power Heater Thermocouple

FIG. 12 Solenoid Valve with Tubing Connected

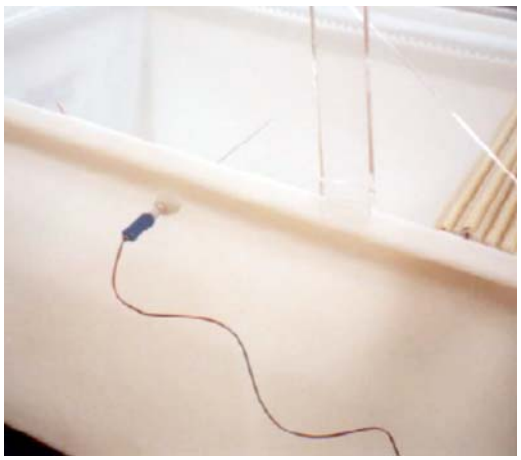

FIG. 10 Thermocouple with Extension Cable

FIG. 11 Solenoid Valve with Timer

8.2.7 A fan is to be used to gently circulate air within the environmental chamber to aid in spore dispersal.

9. Reagents and Materials

9.1 *Potting Soil:*

9.1.1 For the purpose of maintaining a high moisture level in the soil bed and a medium for initial growth of fungal inoculum for the environmental chamber, use a good quality commercial sterilized potting soil due to the risk associated with soil-borne pathogens. The soil should contain at least 25 % peat moss or other suitable organic matter and have a pH of 5.5–7.6. Add 1 part vermiculite or perlite to 3 parts soil, if the commercial potting soil does not contain vermiculite or perlite.

9.2 Use potato dextrose agar for fungal culture from one of the following sources:

9.2.1 Potato dextrose agar, powder (commercially available).

9.2.2 Potato dextrose agar plates (commercially available).

9.2.3 Make your own potato dextrose agar—Formula:

9.2.3.1 Boil 300 g of finely diced potatoes in 500 mL of water until thoroughly cooked. Filter the cooked potatoes through cheesecloth and add water to the filtrate to make up 1.0 L.

9.2.3.2 Add 15 g of agar to the filtrate and dissolve by heating the mixture to boiling while stirring frequently. Add 20 g glucose to the mixture.

9.2.3.3 Dispense the mixture into appropriate vessels and sterilize in an autoclave at 121°C at 15 psi for 20 min. Remove from autoclave and pour into Petri dishes. Allow to cool and harden at room temperature.

9.2.3.4 If the plates are not needed immediately, store them in the refrigerator.

9.3 *Inoculum*—Factors involved in working with fungi include collection, culture, and inoculation of the soil in the environmental chamber.

9.3.1 An inoculum that meets the requirements of this method is available as ATCC (American Type Culture Collection) 16404, and is available from several sources for laboratory supplies.

9.3.2 Obtain samples of Wet Blue or Wet White with fungal growth from tanneries to culture and use to inoculate the soil in your environmental chamber. Success in collecting fungi growing on Wet Blue or Wet White depends upon the proper

selection of Wet Blue or Wet White with active fungal growth and rapid shipment to the laboratory for use in environmental chamber work.

9.3.3 Do your fungal transfer and inoculation work on a disinfected surface in a room that is as clean as possible. Ideally, use a laminar flow hood for culture work if you have access to one.

9.3.4 Use sterile cotton swabs or other suitable inoculation device for transferring fungi from Wet Blue or Wet White to PDA plates or from plate to plate.

9.3.5 Incubate the inoculated plates at room temperature until fungal growth covers the agar surface, or in an incubator at a favorable warm temperature (30 to 32°C).

10. Sampling, Test Specimens, and Test Unit

10.1 Place the sample of Wet Blue or Wet White on a clean cutting board. Using a scalpel equipped with a stainless surgical blade, cut a rectangular test piece of suitable dimensions (5 cm by 10 cm is a typical size). If a test specimen of a different size is used, list the dimensions in your report.

11. Procedure

11.1 Fill the tray to a depth of approximately 6 cm soil, and for best results avoid compacting the soil.

11.2 Lightly cover the surface of the soil with potato dextrose agar powder and gently mix it into the topsail.

11.3 Section the inoculated agar and mix it into the soil in the tray. An alternative is to first mix the sectioned agar into PDA powder and then place it on the top of the soil bed.

11.4 Incubate the inoculated soil in the tray for two weeks under the appropriate temperature (32.5 ± 1°C) and moisture conditions (95 to 98 % relative humidity). Typically, the fungal growth in the inoculated soil declines significantly after two weeks of incubation.

11.5 During this two-week period, start adding untreated Wet Blue (or Wet White) pieces or Wet Blue (or Wet White) with fungal growth from previous tests to the rods in the chamber. From this point on, keep Wet Blue and Wet White specimens with fungal growth hanging in the chamber to continuously provide the spores for inoculation of test specimens. For introduction of a new species of fungi to the chamber, try growing the desired fungal species on a sample of untreated Wet Blue or Wet White in a Petri dish and then transfer the specimen to the environmental chamber.

11.6 Once the chamber is well inoculated, commence your testing.

11.7 Make a vertical incision near the top of the test specimen large enough to insert a coated wire hanger or a stainless steel hook and, using the scalpel, carve a Roman numeral near the bottom of the test piece on the grain side to identify your sample number. For example, for sample number one use "I." Another aid in sample identification is to label at least one sample (usually the first sample in a series) with the type of plastic wire tie that includes a label area. Mark the label area of the tie with all or part of a lot number or other identification number plus the sample number, for example 9265-1.

11.7.1 An alternative method for test specimen preparation is to use a leather hole punch or a hammer and chisel instead of a scalpel to pierce the sample for hanging.

11.8 Place the test piece(s) in a labeled, resealable, poly bag and transport to the environmental chamber. Open the lid of the chamber and remove a hanger rod. If the hanger rod is not labeled, give it an identification number or letter, and then hang the test pieces on the rod. Bracket every three to five test pieces with Wet Blue or Wet White showing 50 % or less clean area to serve as inoculum. Return the rod to the chamber and securely close the chamber lid to avoid loss of humidity during storage of the test pieces.

12. Evaluation Procedure

12.1 Check the test specimen every seven days for fungal growth for a 28-day period. Record the amount of fungal growth on each side of the Wet Blue, chrome crust, or Wet White test piece separately. Rate the fungal growth using the following chart:

10.....No growth
9
8.....Slight growth
7
6.....Medium growth
5
4.....Moderately heavy growth
3
2.....Heavy growth
1
0.....Completely covered

13. Precision and Bias

13.1 The precision of this test method is based on an interlaboratory study of WK18553, Standard Test Method for

TABLE 1 Week 1

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	9.97	0.11	0.18	0.18	0.51	0.51
A - Grain	10.00	0.00	0.00	0.00	0.00	0.00
B - Flesh	9.80	0.42	0.55	0.61	1.53	1.72
B - Grain	10.00	0.00	0.00	0.00	0.00	0.00
C - Flesh	4.57	2.33	1.59	2.67	4.46	7.47
C - Grain	4.53	2.68	0.98	2.80	2.75	7.84

[A] The average of the laboratories' calculated averages.

Resistance to Growth of Fungi on the Surface of Wet Blue in an Environmental Chamber, conducted in 2008. Each of ten laboratories tested the Flesh and Grain sides of three distinct material samples (identified as "A", "B", and "C") for the total resistance of growth of fungi over nine weeks. Every "test result" represents an individual determination by the laboratory analyst. Each laboratory was asked to report triplicate test results (from one operator) for each material. Practice E691 was followed for the design and analysis of the data.[6]

13.1.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "*r*" value for that material; "*r*" is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

13.1.1.1 Repeatability limits are listed in Tables 1-9.

13.1.2 *Reproducibility Limit (R)*—Two test results shall be judged not equivalent if they differ by more than the "*R*" value for that material; "*R*" is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

13.1.2.1 Reproducibility limits are listed in Tables 1-9.

13.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

13.1.4 Any judgment in accordance with 13.1.1 and 13.1.2 would have an approximate 95 % probability of being correct.

13.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

13.3 The precision statement was determined through statistical examination of 512 results, from ten laboratories, on three materials. These three materials were identified as the following:

13.3.1 *Material A*—Wet Blue treated with low concentration of fungicide.

13.3.2 *Material B*—Wet Blue treated with medium concentration of fungicide.

13.3.3 *Material C*—Wet Blue treated with high concentration of fungicide.

13.4 To judge the equivalency of two test results, it is recommended to choose the material closest in characteristics to the test material.

14. Keywords

14.1 basic chromium sulfate; mold resistance; Wet Blue; Wet White

[6] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR: D31–1012.

TABLE 2 Week 2

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	9.03	1.68	1.53	2.09	4.28	5.86
A - Grain	9.70	0.74	0.52	0.86	1.45	2.40
B - Flesh	7.13	2.82	1.68	3.14	4.71	8.79
B - Grain	8.37	1.17	1.10	1.47	3.07	4.12
C - Flesh	0.77	0.97	0.84	1.19	2.34	3.32

[A] The average of the laboratories' calculated averages.

TABLE 3 Week 3

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	8.10	2.54	1.95	3.00	5.46	8.40
A - Grain	9.20	1.12	0.73	1.27	2.04	3.56
B - Flesh	4.33	2.49	2.04	3.00	5.72	8.39
B - Grain	5.13	3.25	1.59	3.50	4.46	9.79
C - Flesh	0.50	0.71	0.63	0.88	1.77	2.45
C - Grain	0.47	0.57	0.37	0.64	1.02	1.80
C - Grain	0.70	0.91	0.32	0.94	0.89	2.65

[A] The average of the laboratories' calculated averages.

TABLE 4 Week 4

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	7.67	3.08	2.18	3.55	6.11	9.95
A - Grain	8.30	2.51	1.56	2.81	4.37	7.87
B - Flesh	3.03	2.59	1.99	3.06	5.58	8.57
B - Grain	3.57	3.60	1.47	3.80	4.12	10.63
C - Flesh	0.17	0.42	0.26	0.47	0.72	1.32
C - Grain	0.17	0.36	0.18	0.39	0.51	1.09

[A] The average of the laboratories' calculated averages.

TABLE 5 Week 5

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	6.96	3.34	2.48	3.91	6.94	10.94
A - Grain	7.52	3.44	1.73	3.72	4.85	10.43
B - Flesh	2.22	2.36	1.78	2.77	5.00	7.76
B - Grain	2.74	3.14	1.15	3.28	3.23	9.19
C - Flesh	0.15	0.44	0.19	0.47	0.54	1.32
C - Grain	0.04	0.11	0.19	0.19	0.54	0.54

[A] The average of the laboratories' calculated averages.

TABLE 6 Week 6

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	6.93	3.39	2.30	3.87	6.45	10.85
A - Grain	7.27	3.51	1.76	3.79	4.93	10.61
B - Flesh	1.97	2.25	1.85	2.71	5.19	7.58
B - Grain	2.03	2.76	1.11	2.91	3.11	8.15
C - Flesh	0.10	0.32	0.00	0.32	0.00	0.89
C - Grain	0.03	0.11	0.18	0.18	0.51	0.51

[A] The average of the laboratories' calculated averages.

TABLE 7 Week 7

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	6.57	3.23	2.45	3.80	6.86	10.63
A - Grain	6.80	3.40	1.62	3.65	4.54	10.22
B - Flesh	1.63	1.91	1.96	2.49	5.48	6.97
B - Grain	1.17	1.67	1.32	1.99	3.69	5.57
C - Flesh	0.00	0.00	0.00	0.00	0.00	0.00
C - Grain	0.03	0.11	0.18	0.18	0.51	0.51

[A] The average of the laboratories' calculated averages.

TABLE 8 Week 8

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	5.97	3.14	2.34	3.67	6.55	10.28
A - Grain	6.17	3.20	1.73	3.50	4.85	9.79
B - Flesh	1.30	1.63	1.91	2.25	5.34	6.31
B - Grain	0.87	1.27	1.18	1.59	3.31	4.47
C - Flesh	0.00	0.00	0.00	0.00	0.00	0.00
C - Grain	0.03	0.11	0.18	0.18	0.51	0.51

[A] The average of the laboratories' calculated averages.

TABLE 9 Week 9

Material	Average[A] $\bar{X}$	Standard Deviation of the Lab Averages $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A - Flesh	5.47	3.19	1.88	3.54	5.26	9.92
A - Grain	5.33	3.03	1.32	3.21	3.69	9.00
B - Flesh	0.00	0.00	0.00	0.00	0.00	0.00
B - Grain	0.13	0.30	0.52	0.52	1.45	1.45
C - Flesh	0.00	0.00	0.00	0.00	0.00	0.00
C - Grain	0.00	0.00	0.00	0.00	0.00	0.00

[A] The average of the laboratories' calculated averages.