OUTLINE OF PROPOSED LABORATORY PROCEDURES FOR TESTING MILDEW- AND ROT-PROOFING TREATMENTS

Introductory Note: Although the following procedures need further development to meet special requirements numerous letters of inquiry have indicated a need for releasing what information is available on new developments to improve laboratory tests that may aid in developing or identifying reliable mildew- or rot-proofing treatments. Laboratory work on improved testing methods is continuing and additional modifications or suggestions may be furnished recognized laboratories or organizations dealing with these products. If, for example, the test is found too severe to distinguish between certain treatments that are intended to impart only a moderate amount of protection, several steps are known that might be recommended for decreasing the severity of the test. The Committee will welcome comments as to success or difficulties encountered in using the proposed procedures or any suggestions that may be made for increasing the effectiveness of the method in general or for special materials.

I. General Test

1. Test organism - Metarrhizium sp. (USDA culture No. 1334.2)

2. Preparation of samples for test.

Two sets of controls that should receive all treatments given the test material are recommended. The (a) "untreated control" should be of the original or similar untreated material. The (b) "treated control" should be of the original or similar material that is treated by the preventative method that may be chosen as a standard of comparison. For example, if a treatment for fire hose is being considered the treated check would be fire hose fabric that has received some "standard" treatment in order to determine whether test treatment is better than the one on which the experimentor may already have some data. A third (c) set of controls may be included where percentage of deterioration or actual loss of strength resulting from the test organism is desired. This set of controls, which would probably not be included in most routine tests, would be derived from the treated fabric and would furnish a check on any real or apparent loss of strength resulting from leaching, wetting, etc. instead of from the mildew. It should receive all treatments given the test material except that this (c) set of controls would remain sterile.

Sufficient material of each of the controls and test fabrics should be prepared to provide five strips (10 are recommended for very critical results). Before cutting into strips the fabric should be washed in running water for 24 hours. The washing container should provide about 1 gallon of water for each ounce of fabric. The rate of flow should be gentle but should provide at least three complete changes during 24 hours.

Direct fall of water on fabric should be prevented. If the pH of the tap water is not approximately 7.0 difficulty may be expected in comparing results with other laboratories that have relatively pure tap water.

After drying at room temperature the fabric is cut into strips 6" x 1½" which are then raveled to 1 inch width.

3. Culture medium to be used.

NH_4NO_3	1.0 gms.
K_2HPO_4	1.4 "
$MgSO_4$	1.0 "
$HOOC(CHOH)_2$	2.0 "
Fe, Zn, and Mn as SO_4	0.001 "
Peptone	0.5 "
Distilled water to make	1000 cc.

4. Preparation of inoculum for test.

A convenient method of preparing the inoculum is to use 250 cc. Erlenmeyer flasks containing sufficient glass beads to cover the bottom, one or more discs of filter paper and 15 cc. of culture medium. These flasks are sterilized and inoculated with Metarrhizium spores from pure stock cultures and incubated 5 to 7 days, at which time the filter papers are covered with dark green spores. Harvesting of these spores for the inoculation of the test strips is accomplished by shaking the flask until the inoculum is dispersed by the action of the glass beads, adding sterile distilled water, and pipetting off the suspension.

5. Inoculation and Incubation of test strips.

Place a strip of absorbent cotton batting, 5 inches by 2 inches by 0.5 inch in each culture bottle, add 35 cc. of culture medium and autoclave. When cool inoculate with 2 cc. of spore suspension prepared as directed under 4. The inoculum should be uniformly distributed over the cotton batting strip. A 16 ounce modified screw cap bottle, 2½ inches square by 6 inches high is a convenient culture chamber. The modified screw cap is to be prepared by cutting a circle 4 cm. in diameter, from the center of both the cap and the waxed paper filler. A round piece of glass filter fabric (such as Owens-Corning Fiberglas # CS 30A-20), the diameter of the filler, is to be inserted between the cap and the ring of waxed filler. These culture bottles are similar to those previously described by Greathouse, Klemme and Barker (Ind. and Eng. Chem. 14:614-20. 1942). Incubation of the cotton batting wicks shall be for a period of 5 days at 28°C. At. the end of the 5-day incubation period for the cotton wicks moisten each test strip with culture solution containing a wetting agent (0.05%) such as "Aerosal", "Gardinol", etc.; then lay the test strip on a fruiting cotton batting wick and smooth out to provide complete

contact between the fungus growth and the fabric. At this time add 25 cc. culture medium (minus the Peptone) and incubate for a 7-day period for observation and for breaking strength test determination. To prevent inundation the test strip, that is placed on and adheres to the cotton batting wick, should be incubated with the culture bottle turned on the side so that one edge is in contact with the free nutrient medium.

6. Evaluation of results.

The (a) untreated controls should lose approximately 90% of their breaking strength providing the test has been properly performed. The breaking strength of the (b) and/or (c) treated controls may be used to evaluate the effectiveness of the treatment under test. Am. Soc. Testing Materials, Standards on Textile Materials (1942) is suggested as a guide for obtaining strength determinations.

II. Special adaptation for sandbag fabrics - particularly those containing copper compounds.

All procedures except 2 "Preparation of the sample for test", and 3 "Culture medium to be used" are described under I above.

2. Preparation of samples for test.

A. Moderately severe test - approximating the deterioration caused by 3-6 weeks soil burial for cotton fabrics and 6-9 weeks on jute (as indicated from results at the Southern Regional Research Laboratory of the U. S. Department of Agriculture).

Instead of the 24-hour water wash (I-2) the fabric receives the following preparatory treatment. Leach for 2 hours in an agitated .00316N HNO_3 (pH 2.5) solution. The leaching should involve 3 liters (or 1 gallon) of solution per gram of fabric. Approximately 50 mg. of a commercial wetting agent, is added per 3 liters of water to insure complete wetting of the fabric. The solution is maintained at a temperature of 25°C. by thermostatic control and stirred into vigorous circulation by an electric stirrer. After removal from the acid bath, the sample is rinsed for 30 minutes in gently running water. Following the rinse the sample is shaken for 2 hours in a bentonite suspension. A mechanical apparatus that has been found satisfactory moves through a distance of approximately 1.5 inches in the direction of the long axis, with 150 complete shaking cycles per minute. The bentonite suspension is made by adding 5 gm. of 200 mesh bentonite per liter in water with one liter of suspension being used for each 130 sq. in. of fabric. The sample is rinsed in running water for 30 minutes to remove the adhering bentonite. It is then dryed and the strips are prepared for

inoculation and incubation as described under I-2.

B. Severe test - approximating the deterioration caused by 6-9 weeks soil burial test on cotton fabric and 9-12 weeks on jute.

The procedure differs from 2-A above in that 10 ml. of concentrated NH_4OH is added for each 5 grams of bentonite or for each liter of the bentonite suspension.

3. Culture Medium to be used.

NH_4NO_3	2.0 gms.
K_2HPO_4	2.8 "
$MgSO_4$	11.8 "
$HOOC(CHOH)Z$	2.0 "
Fe, Zn, and Mn as SO_4	0.001 "
Peptone	0.5 "
Distilled water to make	1000 cc.