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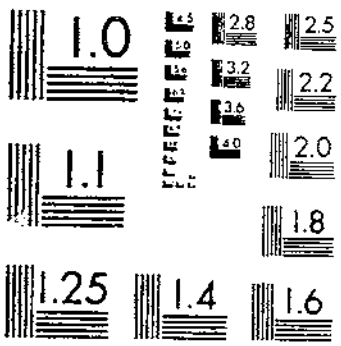
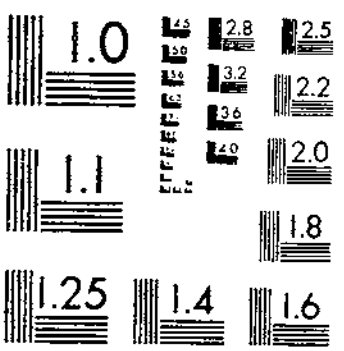


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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NATIONAL BUREAU OF STANDARDS-1963-A

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Testing Fabrics for Resistance to Mildew and Rot[1]

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CONTENTS

One of the wartime problems of our armed forces is the rotting of fabric articles. Tents, sandbags, hammocks, and insect netting, as well as other items used in jungle warfare, are subject to attack by microorganisms. The information here presented concerns the testing of fabric for resistance to such attack and may be summarized as follows.

SUMMARY

A simple apparatus is described for subjecting fabric treated with mildew preventives to water leaching under controlled conditions.

Several fungi have been found capable of tendering treated fabric of higher copper content than that attacked in similar tests by the common test organisms *Chaetomium globosum* and *Metarrhizium* sp. The evidence presented suggests that the copper tolerance of soil fungi contributes to the severity of soil-burial tests on such fabric.

Steam sterilization of fabric containing certain common organic preservatives, even for as short an interval as 15 minutes at 15 pounds' steam pressure, in a number of instances decreased the mildew resistance of the fabric, as indicated by subsequent culture tests. Experience with several fungi indicates that *Chaetomium globosum* is more satisfactory than certain other forms for use in tests on nonsterile fabric.

[1] Submitted for publication December 1944. The authors wish to express their appreciation to H. D. Barker, principal pathologist, of this Bureau, for advice and assistance in planning the experiments and in preparing the manuscript; to M. L. Jaeger, for the photographs; to the Socony-Vacuum Oil Co., the Shepherd Chemical Co., the Nuodex Products Co., the Hercules Powder Co., the Givaudan-Delawanna Co., the Du Pont Co., the Dow Chemical Co., and to Merck & Co., for supplying the mildew-preventive materials; and to the Division of Soil and Fertilizer Investigations, of this Bureau, for soil analyses and advice on soil problems.

A filter-paper-mat technique is described in which the test fabric is planted on a mat of fungus mycelium growing on a filter-paper strip supported on a salt agar medium. When the fungus *Chaetomium globosum* is used in this test it is found to be much more severe on treated fabrics than when it is applied by the pipette-inoculum procedure. The presence of independently nourished mycelial inoculum capable of attacking and reattacking the strip at many points is believed to simulate in part the conditions of soil burial.

Soil-burial tests have been found to be much more drastic on a variety of treated fabrics than the usual pipette-inoculum *Chaetomium* technique.

Data are presented on the minimum air volume required for the break-down of fabric in tightly sealed containers by *Chaetomium globosum*, *Metarrhizium* sp., and *Stemphylium* sp.

It is concluded that the choice of the best test or combination of tests to determine mildew resistance of a fabric depends on the service conditions under which the fabric is to be used. While the tests now available are still admittedly inaccurate in certain respects, they are capable of yielding much useful information when judiciously interpreted.

Data are presented on the fabric-preservative properties of a number of fungicidal compounds. A new material of commercial origin, 2,2′-dihydroxy-5,5′-dichloro diphenylmethane, has unusual fungicidal properties and offers promise as a fabric preservative. Copper naphthenate has been consistently more effective per unit weight on fabric than have several other copper compounds.

REVIEW OF LITERATURE

In 1934 Thom, Humfeld, and Holman (*17*)[2] described a pure-culture test by which the fungus *Chaetomium globosum* G. Kunze could be used to determine the mildew resistance of fabrics. The procedure consisted essentially of placing a small piece of sterilized fabric on a mineral-salts agar medium, inoculating the fabric with spores of the cellulose-decomposing fungus, and determining the residual strength of the fabric after a period of incubation. The test was simple to perform and apparently rested on a more secure scientific basis than previous tests with mixed cultures and natural inoculum. For these reasons it came into widespread use in both commercial and governmental laboratories (*10*, *16*).

Concurrently there developed a common practice known as the "soil-burial" test. It had many variations, but its main feature was that the fabric to be tested was buried in soil and examined at intervals for observation of rotting.

With the greatly increased interest in mildew-resistant fabrics for the armed forces, beginning in this country in 1941, attention was focused on methods for testing such fabrics. The *Chaetomium globosum* test of Thom, Humfeld, and Holman (*17*) quickly found its way into certain fabric-procurement specifications. Shortly thereafter, in 1942, experimental results with an extremely active cellulose-decomposing fungus of the genus *Metarrhizium* were presented by Greathouse, Klemme, and Barker (*8*) and a pure-culture test method with this fungus described. It soon appeared, however, that despite continued use of pure-culture techniques, and in particular of the *C. globosum* technique of Thom,

[2] *Italic* numbers in parentheses refer to Literature Cited, p. 22.

Humfeld, and Holman (*17*), certain of the military agencies placed increasing confidence in soil-burial results. This was true, for example, in the case of copper-treated sandbag fabric; many tests of such fabric were carried out in collaboration with the United States Department of Agriculture. Soil burial provided a more severe and presumably a more reliable test of fabric to be exposed to soil in actual use than did the *Chaetomium* test.

Data from the writers' laboratory, dealing with copper (*14*) and with organic (*9*) fabric protectants and comparing soil-burial results with the results of *Chaetomium* (*17*) and *Metarrhizium* (*8*) tests, have already been presented. Bertolet (*3*) has advocated strongly the use of soil-burial tests. Furry and Zametkin (*5*) have recommended as a drastic test a procedure similar to previous culture-bottle tests in general but employing a thin soil suspension as inoculum. Barker and others (*2*), reviewing the test-method situation in October 1943, stressed particularly the need for experimental analysis of the numerous factors in test procedures. Work toward that end is described in the present bulletin.

TREATMENT OF FABRIC BEFORE BIOLOGICAL TEST

Water Leaching

In most cases a fabric preservative cannot provide lasting protection unless it is resistant to leaching in water. No precise method of testing this property, however, appears to have been widely accepted.

Thom, Humfeld, and Holman (*17*) were concerned primarily with other phases of the problem and suggested merely that the fabric should be soaked in several changes of water or in running water for 2 days, in order to remove water-soluble antiseptics.

The American Society for Testing Materials (commonly referred to as A.S.T.M.) has adopted a tentative method of testing fabrics for resistance to micro-organisms (*1*). It requires the leaching of 10 specimens of the treated and 10 of the untreated fabric on screens under cold running water for 30 hours.

Furry and Zametkin (*5*) recommend that leaching be done as follows:

> Wash approximately one linear yard of each treated fabric to be tested and one-half yard of the same fabric untreated under 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 provide at least 3 complete changes during 24 hours. Direct fall of water on the fabric should be prevented.

Goodavage (*7*) described two types of tests, a spray test and a leaching test, the choice between the two depending on the type of fabric under consideration. The spray test is as follows:

> A sample is mounted on a board which is placed at a 45° angle. The sample is then sprayed for one-half hour with water from a spray nozzle placed vertically above the sample at a distance of 18 inches from the center of the sample. The volume of water sprayed onto the sample shall be approximately 2000 cc. per minute per 60 square inches of sample. The spray nozzle shall contain 19 holes of .035 [inch] diameter, spaced approximately ½ inch apart.

The leaching test is carried out as follows:

> A sample of netting 6×12 inches is immersed in one gallon of water, temperature 65° F. The flow of water is then regulated so as to change the water three times over a period of 24 hours, after which the sample is sterilized and then inoculated for the accelerated mildew test.

Water-Leaching Apparatus

The temperature and pressure of water at the tap are usually variable from time to time, particularly overnight and over week ends in many buildings; leaching procedures depending on these variable factors may be expected to be unsatisfactory. For this reason, it seemed desirable to devise some simple practical method of avoiding these difficulties. A diagram of an apparatus useful for this purpose is shown in figure 1.

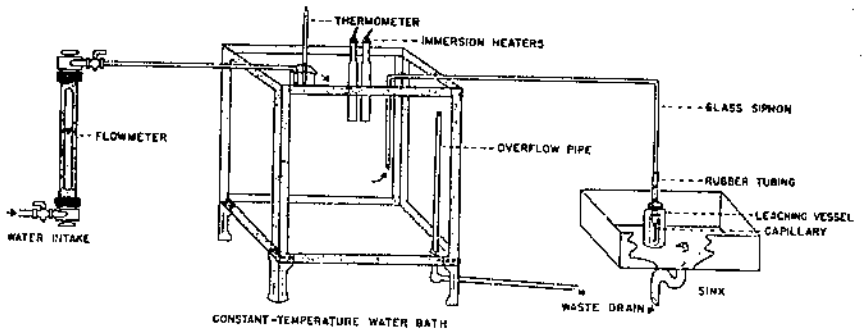


FIGURE 1.—Apparatus for water-leaching mildew- and rot-resistant fabric under conditions of constant temperature and water pressure. Only 1 of a battery of 12 siphons is shown.

Water from a mixing faucet passes through a flowmeter into a small beaker containing a thermometer. The overflow from the beaker passes into a large constant-level water bath in which the temperature is adjusted by immersion heaters that operate in conjunction with a mercury thermoregulator and a tipping mercury relay. The water in the bath is agitated by an electrical stirrer.

Having been adjusted to constant temperature in the bath, the water passes at constant rate to the several individual leaching vessels through glass siphons, connecting rubber tubes, and small glass capillaries. Only 1 siphon and 1 leaching vessel are shown in the diagram; a battery of 8 or 12 was used in the experiments.

The capillary dimensions and the head of water determine the rate of water flow through the siphons. A practical point perhaps worthy of mention is that the glass siphons will not collect air bubbles if their internal diameter is small enough in relation to the volume rate of flow through them. The siphons used in the experiments had an internal diameter of approximately 5 mm. The capillaries are inserted down through the center of wire-mesh cylinders in the leaching vessels and held in position by cross wires (fig. 2). The 6- by 1½-inch samples are fastened in vertical position with rubber bands around the outside of the cylinders, four samples to a cylinder.

In the experiments here reported the bath used had a capacity of 90 gallons and the immersion heaters totaled 1,700 watts. The distance between the water level in the bath and that of the leaching vessels (in this case 1-quart mason jars) was 12 inches. The rate of water flow through each of the leaching vessels was 10 liters per hour ± 2 percent. This flow could be measured readily by opening the connection below the sink into which the water flows after passing from the leaching vessel and collecting this water in a graduated cylinder.

The water in the constant-temperature bath was kept within ±0.05° of 30° C. The output of the immersion heaters was sufficient to maintain this temperature even if that of the incoming water was as low as 20°. Owing to the relatively rapid rate of flow, the temperature of the water in the leaching vessels did not deviate from 30° by more than 0.2°.

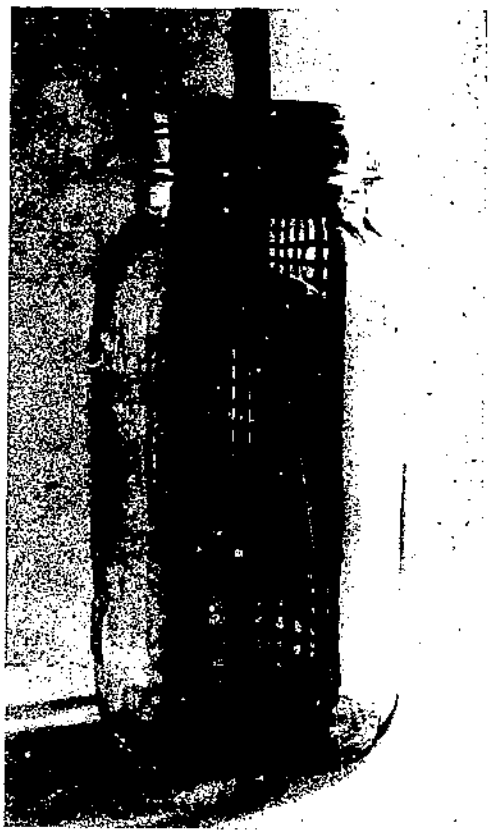


FIGURE 2.—Leaching vessel (1-quart mason jar) containing wire-mesh cylinder (see fig. 1) for supporting fabric during leaching. Part of the cylinder is cut away to show capillary and rubber outlet tube from glass siphon.

WATER-WASH TESTS

In testing the performance of the leaching apparatus described, experiments were conducted with several organic and metal-organic fabric preservatives. Samples of pentachlorophenol, salicylanilide, and 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane were supplied through the courtesy of the manufacturers. These samples had melting points of 188°, 135°, and 179° C., respectively, and in all cases were therefore essentially pure materials. All were applied to 8-ounce cotton duck (original breaking strength, 122 pounds) by dipping the fabric into appropriate solutions of the preservatives in acetone and passing it at once through a household wringer that had been equipped with metal rolls. The wet-weight pick-up of the fabric was kept constant by adjusting the rolls to a constant pressure. Copper naphthenate, copper "tallate" (a copper soap of tall oil), and copper oleate were applied in a similar fashion in a mixture of three parts by volume of Stoddard's solvent and one part of acetone. Copper-hydrogenated resinate was applied in benzene. All fabrics were dried at room temperature.

In several cases the treated samples were then subjected to chemical analysis to check the concentration of the protectant on the fabric. In some instances the percentages of the organic preservatives found on the fabric by analysis differed by as much as 10 percent from the calculated values at the 0.25- and 0.50- percent levels. The importance of this error appears to be minimized, however, by the wide range of concentrations used in the experiments and the large experimental differences noted.

As a background to studies on leaching, determinations of the original fungicidal effectiveness of three phenolic preservatives were made. As shown by the data in table 1, low concentrations of any of the three materials on the fabric—pentachlorophenol, salicylanilide, or 2,2′-dihydroxy-5,5′-dichloro diphenylmethane—were found effective against *Chaetomium globosum*. In these tests the specimens were wet in water, placed on mineral-salts agar in 16-ounce flat-sided glass bottles, inoculated by pipette with 1 cc. of a suspension of spores of *C. globosum*, and incubated at 30° C. for 12 days. The culture medium contained 2.22 gm. $MgSO_4.7H_2O$, 2.09 gm. K_2HPO_4, 2.68 gm. KH_2PO_4, 3 gm. NH_4NO_3, and 20 gm. agar in each liter. In these and all other biological tests here reported the standard A.S.T.M. (*1*) procedures for preparing and breaking raveled strips were followed.

TABLE 1.—*Residual strength of protectant-treated, 8-ounce, nonsterile, unleached cotton duck, after pipette-inoculation and 12-day incubation with Chaetomium globosum*

Fabric protectant	Content (percent) of protectant in fabric				
	0.01	0.02	0.04	0.06	0.08
	Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Pentachlorophenol	7	85	101	100	102
Salicylanilide	5	20	102	100	100
Dihydroxy dichloro diphenylmethane	6	7	8	68	72

Specimens of treated fabric were then similarly tested with *C. globosum* after a 24-hour running-water leach. As shown by the data in table 2, the combined water-wash-mildew test was more severe than the mildew test alone (table 1). It is apparent from the data of tables 1 and 2 that there was leaching loss of each of the compounds, especially pentachlorophenol. Similarly washed samples were tested with *Metarrhizium* sp.; the results of this test are also recorded in table 2.

TABLE 2.—*Residual strength of protectant-treated, 8-ounce cotton duck after water leaching and subsequent culture tests; pipette inoculation of nonsterile fabric; and 12-day incubation at 30° C.*

RUNNING-WATER LEACH[1]

Test organism	Fabric protectant	Content (percent) of compound in fabric before leaching						
		0.2	0.3	0.4	0.5	0.6	0.8	1.0
		Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Chaetomium globosum	Pentachlorophenol	0	-------	0	0	-------	0	0
	Salicylanilide	0	-------	0	60	-------	99	98
	Dihydroxy dichloro diphenylmethane.	7	-------	89	88	-------	99	99
Metarrhizium sp.	Pentachlorophenol	0	-------	0	0	-------	2	9
	Salicylanilide	0	-------	2	0	-------	98	98
	Dihydroxy dichloro diphenylmethane.	52	-------	99	101	-------	101	95
STATIC-WATER LEACH[2]								
Chaetomium globosum	Pentachlorophenol	0	0	0	-------	1	98	-------
	Salicylanilide	0	1	43	-------	108	107	-------
	Dihydroxy dichloro diphenylmethane.	7	33	37	-------	110	111	-------

[1] 10 liters of water per hour at 30° C. passing over 4 experimental strips; each breaking-strength figure is an average of 4 replicates.
[2] 3 successive 24-hour leaches, each with a 250-to-1 weight ratio of water to fabric; each breaking-strength figure is an average of 3 replicates.

The water-wash-biological tests mentioned were carried out on fabric unprotected by waterproofing or water-repellent treatments. They therefore do not represent the behavior of mill-run commercial samples containing waterproofing materials. It was noted, however, that even a static-water leach with small volumes of water brought about major losses of the preservatives from the laboratory-treated fabric. In an experiment of this kind, 8-ounce cotton duck samples, already cut and raveled for biological test, were placed in distilled water in 2-liter glass vessels at 20° C. A weight ratio of 250 parts of distilled water to 1 part of fabric was used, and the water was drained and replaced at the end of 24 and 48 hours, providing 3 days of leaching and a total water-to-fabric ratio of 750 to 1. The unexpectedly high leaching losses that obviously occurred in the static-water leach may have been due largely to poor adherence of the crystalline organic protectants to the fabric and consequent mechanical loss.

Chemical analyses of fabrics after leaching were carried out to check the results of the water-leach-biological tests. The results of such analyses are shown in table 3.

TABLE 3.—*Percentage of 3 organic fabric protectants remaining on 8-ounce cotton duck after standard running-water leach, at a flow of 10 liters per hour and a water temperature of 30° C.; determinations on duplicate samples*

Fabric protectant	Content (percent) of protectant in fabric					
	Before leaching		After leaching—			
			24 hours		48 hours	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Pentachlorophenol	0.54	0.55	0.016	0.016	(1)	(1)
Salicylanilide	.48	.51	.07	.07	0.02	0.02
Dihydroxy dichloro diphenylmethane	.48	.49	.23	.22	.10	.10

[1] Trace.

Fabric to be analyzed for organic mildew preventives was thoroughly acetone-extracted before application of the preventive. After leaching, it was air-dried and then extracted with several portions of acetone, the extracts were combined, the acetone was evaporated off at low temperature, and the analyses were carried out on the residue.

The pentachlorophenol analyses were carried out by a procedure recommended by one of the manufacturers. In this procedure there were added to the acetone extract in a beaker 30 cc. of benzene and 10 cc. of 2-to-1 nitric acid. The beaker was heated on a steam bath for 12 minutes. The benzene layer was then washed with water in a separatory funnel, dried with anhydrous sodium sulfate, diluted with further benzene, and its color measured in a photoelectric colorimeter.

Analyses for 2,2′-dihydroxy-5,5′-dichloro diphenylmethane were carried out on the acetone extract by the semimicro method of Willard and Thompson (*18*) for determination of halogens in organic halogen compounds. Salicylanilide was determined by the micro-Kjeldahl method for nitrogen, as described by Niederl and Niederl (*15, p. 54*); a few drops of 60-percent perchloric acid were found to aid in the digestion of the sample.

When samples of four different copper-treated fabrics were leached for 72 hours under the conditions specified for the running-water leach, the percentages of copper in the fabrics were found to have changed less proportionately than had been the case with the pure organic preservatives. The copper-leaching analytical data are shown in table 4. Copper analyses were made by the iodometric method as described by Kolthoff and Sandell (*11, p. 604*), ashing of the fabric being carried out in a muffle furnace at a temperature below 475° C.

TABLE 4.—*Copper content of treated fabrics before and after 72-hour water leaching, at a flow of 10 liters per hour and a water temperature of 30° C.*

Treatment	Method of application	Fabric	Copper in fabric	
			Before leaching	After leaching
			Percent	*Percent*
Copper naphthenate	Commercial	Osnaburg	0.94	0.95
	Laboratory	8-ounce duck	.41	.34
	Commercial, applied with asphalt bentonite from ammonia solution.	do	1.09	.99
Cuprammonium hydroxide	Commercial	6-ounce duck	2.19	2.00

A number of different commercially treated fabrics have been tested with the leaching apparatus described. Although the complete composition of such samples is seldom known, the test results are nevertheless important for comparison with laboratory-treated samples. Results of a few tests of such fabrics are discussed in connection with experiments in a later section and are presented in table 15 (p. 15).

STEAM STERILIZATION

Steam sterilization is a feature of most pure-culture experiments with micro-organisms. Thom, Humfeld, and Holman (*17*), for example, used steam sterilization and laid particular emphasis on the maintenance of pure-culture conditions in their mildew-resistance tests. Bertolet (*3*) and many other workers, however, believe that steam sterilization may represent an abnormal hazard for certain fabric preservatives, a hazard having no counterpart in actual service.

In order to determine the magnitude of any influence that steam sterilization might have on test results, two similar sets of laboratory-treated fabrics containing organic preservatives were tested. The usual *Chaetomium* test (p. 6) was employed, both with and without sterilization. The test results are shown in table 5. The shift in minimum protective concentration caused by steam sterilization actually turned out in this experiment to be less than had been anticipated for the first three treatments listed. It is seen, however, that in certain cases steam sterilization made the difference between complete resistance to growth of *C. globosum* and little or no resistance.

TABLE 5.—*Residual strength of protectant-treated, 8-ounce cotton duck, after pipette-inoculation and 12-day incubation with Chaetomium globosum; steam sterilization under 15 pounds' pressure for 15 minutes; 3 replicates*

Fabric protectant	Sterilization of fabric	Content (percent) of compound in fabric						
		0.02	0.04	0.06	0.08	0.12	0.16	0.24
		Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Pentachlorophenol	Sterile	2	9	92	94	88	90	84
	Nonsterile	34	92	101	93	91	93	93
Salicylanilide	Sterile	3	5	57	97	101	84	101
	Nonsterile	36	79	95	93	102	94	102
Dihydroxy dichloro diphenylmethane.	Sterile	3	5	3	7	34	93	80
	Nonsterile	2	6	61	89	105	102	93
Tetrabrom-o-cresol	Sterile	0	0	0	0	7	4	0
	Nonsterile	0	2	29	61	89	97	94

Chaetomium globosum appears to be better adapted to use under nonsterile test conditions than any of the several other fungi that have been tried. Tests of nonsterile fabric by the pipette-inoculum technique, described on page 6, with representatives of the genera *Hormodendrum, Stemphylium, Cladosporium, Cephalosporium,* and *Graphium,* have proved rather unsatisfactory. This is apparently due in large measure to the fact that the growth of these fungi on the fabric is inhibited by bacteria during the first 24 to 48 hours of incubation.

FACTORS IN BIOLOGICAL TESTS

SENSITIVITY OF TEST ORGANISMS TO FUNGICIDES

Previous work has shown (*14*) that as little as 0.1 percent of copper on an 8-ounce cotton duck is sufficient to provide a considerable degree of protection against *Chaetomium globosum* in the test procedure of Thom, Humfeld, and Holman (*17*) or against *Metarrhizium* sp. in the method of Greathouse, Klemme, and Barker (*8*). The copper in these experiments was applied as copper naphthenate, copper oleate, copper tallate, and copper-hydrogenated resinate. Jarrell and others (*10*) had previously reported 0.11 percent as the minimum concentration of copper required for protection in the *C. globosum* test (*17*).

The soil-burial procedure, on the other hand, is much more severe, causing break-down of certain copper-treated fabrics containing as much as 0.8 percent of copper. It seemed possible that the severity of the soil-burial procedure might have been due in part to the presence in soils of copper-tolerant cellulose-decomposing fungi. The copper tolerance of *Aspergillus niger* has been mentioned previously (*14*), and, although the several isolates of this fungus used in the experiments failed to decompose cellulose, it seemed that among the numerous related forms in soils copper-tolerant cellulose decomposers might be found. A few experiments to investigate this possibility have shown that such fungi do exist. Pure-culture tests of copper-treated fabrics with these fungi have yielded results more closely approximating soil-burial results than are obtained with either the *Chaetomium* (*17*) or the *Metarrhizium* (*8*) procedures.

Table 6 shows the results of soil-burial tests on copper-treated fabric strips buried in four different soils at 30° C. for 2 weeks. In these and all other burial tests here reported the soil was placed in enameled containers the size of ordinary dishpans. The soil moisture was maintained at such a level as might be used in the growing of plants. The samples, cut and raveled as for culture tests, were wet in water and planted in a vertical position with about ¾ inch projecting above the soil line.

TABLE 6.—*Residual strength of copper-treated, 8-ounce cotton duck remaining after 14-day soil burial in 4 different soils at 30° C.*

Soil series	Fabric protectant	Content (percent) of copper in fabric			
		0.1	0.2	0.4	0.8
		Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Carrington	Copper naphthenate	29	91	108	116
	Copper oleate	0	14	46	81
	Copper tallate	0	2	39	76
	Copper-hydrogenated resinate	0	0	28	24
Wooster	Copper naphthenate	25	77	94	91
	Copper oleate	0	21	75	89
	Copper tallate	0	16	59	98
	Copper-hydrogenated resinate	0	7	52	83
Davidson	Copper naphthenate	84	102	104	109
	Copper oleate	0	19	91	97
	Copper tallate	0	16	68	108
	Copper-hydrogenated resinate	0	0	7	8
Norfolk	Copper naphthenate	69	103	110	114
	Copper oleate	13	39	82	92
	Copper tallate	4	22	80	99
	Copper-hydrogenated resinate	20	77	107	105

Physical and chemical properties of the soils used are shown in table 7. The test results of table 6 may be contrasted with the *Chaetomium* and *Metarrhizium* results previously reported (*14*). When, however, a *Trichoderma* isolated from soil was used instead of *Chaetomium* or *Metarrhizium* in the regular pure-culture method (p. 6), entirely different results were obtained, as may be seen in table 8. These and all other copper-treated fabrics were moistened in water and steam-sterilized at 15 pounds' pressure for 10 minutes before culture tests. Fabrics Nos. 1 and 2 were both 8-ounce cotton duck.

TABLE 7.—*Properties of soils used in soil-burial tests*

Soil series	Soil particles with diameter (in millimeters) of—				pH	Organic matter [1]
	2.0–0.2	0.2–0.02	0.02–0.002	<0.002		
	Percent	*Percent*	*Percent*	*Percent*		*Percent*
Carrington	1.3	48.3	23.5	26.9	6.0	4.4
Davidson	8.1	13.1	37.9	40.9	6.0	1.8
Wooster	3.6	43.2	38.2	15.0	6.3	1.6
Norfolk	48.0	35.4	12.3	4.3	5.5	.8

[1] By hydrogen peroxide method.

TABLE 8.—*Residual strength of copper-treated, 8-ounce cotton duck, after pure-culture test with Trichoderma sp., pipette-inoculated, and 14-day incubation at 30° C.; 5 replicates*

FABRIC No. 1

Fabric protectant	Content (percent) of copper in fabric		
	0.1	0.2	0.4
	Residual strength percent	*Residual strength percent*	*Residual strength percent*
Copper naphthenate	24	69	84
Copper oleate	12	16	32
Copper tallate	18	20	29
Copper-hydrogenated resinate	17	20	42

FABRIC No. 2

Copper naphthenate	45	70	73
Copper oleate	12	17	29
Copper tallate	15	16	31
Copper-hydrogenated resinate	14	17	12

The data in table 9 show the severity of a *Stemphylium* pipette-inoculum test, as compared with a similar *Chaetomium globosum* test (p. 6) on copper-treated osnaburg.

TABLE 9.—*Residual strength of copper-treated osnaburg cloth after 12-day pure-culture tests with Stemphylium sp. incubated at 20° C. and Chaetomium globosum at 30° C.; 5 replicates*

TEST ORGANISM: STEMPHYLIUM SP.

Fabric protectant	Content (percent) of copper in fabric				
	0.06	0.12	0.25	0.50	1.00
	Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Copper naphthenate	7	14	51	77	77
Copper fluoride	6	6	8	13	17

TEST ORGANISM: CHAETOMIUM GLOBOSUM

Copper naphthenate	59	68	71	77	83
Copper fluoride	0	0	41	75	81

Table 10 presents results obtained in an experiment in which a copper-treated 8-ounce cotton duck was tested by the regular pure-culture procedure (p. 6) with *Chaetomium globosum*, *Hormodendrum* sp., and *Stemphylium* sp. It is obvious from these data that quite different results may be obtained in pure-culture tests according to the fungus used. Isolation from the soil yielded in addition to the above-mentioned *Trichoderma* several copper-tolerant species of *Aspergillus* and *Penicillium*. The isolations were carried out simply by streaking a dilute soil suspension on copper-oleate-treated filter paper resting on a glucose mineral-salts agar medium. One *Aspergillus* in the standard culture test at 30° C. for 8 days deteriorated 8-ounce cotton duck treated to contain 0.4 percent copper as copper-hydrogenated resinate until only

34 percent of the original strength remained; the value for the untreated control was 20 percent and for a 0.4 percent-copper as copper naphthenate, 78 percent. Parallel values with another soil-isolated *Aspergillus* were 51, 37, and 83 percent, and for still a third *Aspergillus* 48, 21, and 85 percent.

TABLE 10.—*Residual strength of copper-treated 8-ounce cotton duck after 12-day pure-culture tests with 3 different organisms—Stemphylium and Hormodendrum spp. incubated at 20° C. and Chaetomium globosum at 30° C.; 5 replicates*

TEST ORGANISM: STEMPHYLIUM SP.

Fabric protectant	Content (percent) of copper in fabric				
	0.0	0.1	0.2	0.3	0.4
	Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Copper naphthenate		34	80	103	102
Copper tallate		19	18	25	23
Copper-hydrogenated resinate		11	26	29	30
None (control)	2				

TEST ORGANISM: HORMODENDRUM SP.

Copper naphthenate		42	77	89	82
Copper tallate		31	39	52	62
Copper-hydrogenated resinate		16	25	57	46
None (control)	10				

TEST ORGANISM: CHAETOMIUM GLOBOSUM

Copper naphthenate		92	83	106	94
Copper-hydrogenated resinate		18	60	90	90
None (control)	0				

The loss of copper from copper-treated fabric in soil-burial tests and the chemical inactivation of copper in situ on the fabric have been subjects of previous investigation (*14*). The presence in soils of copper-tolerant fungi serves further to explain the severity of soil-burial tests on copper-treated fabrics.

In an attempt to determine whether large differences in sensitivity to phenolic preservatives exist among three of the common test fungi, another experiment was carried out, the results of which are shown in table 11. Tests with each of the three fungi used indicated that the first three compounds are distinctly more effective per unit weight on the fabric than the fourth, tetrabrom-*o*-cresol. The differences in sensitivity of the three fungi in table 11 are perhaps not large in terms of the percentages of these preservatives customarily used on fabrics in commercial practice. It is clear, however, that in certain cases success or failure of a treated fabric in a culture test may depend entirely on the test organism used. For a closer interpretation of the data of table 11 in regard to tetrabrom-*o*-cresol, the data from table 5, showing that steam sterilization decreases the fungicidal effectiveness of this compound on fabric, should be considered.

TABLE 11.—*Residual strength of organic-protectant-treated 8-ounce cotton duck after 2-week pure-culture tests with 3 different fungi; fabric steam-sterilized for 15 minutes at 15 pounds' pressure, pipette-inoculated, 3 replicates*

Compound	Fungus [1]	Content (percent) of compound in fabric							
		0.01	0.02	0.04	0.06	0.08	0.12	0.16	0.24
		Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Pentachlorophenol	*Chaetomium globosum*	0	0	0	101	100	78	97	101
	Stemphylium sp.	0	0	15	42	88	85	108	105
	Hormodendrum sp.	10	11	42	58	97	88	88	91
Salicylanilide	*Chaetomium globosum*	0	0	1	80	52	71	94	102
	Stemphylium sp.	1	0	65	58	33	105	100	100
	Hormodendrum sp.	11	13	17	24	82	100	102	99
Dihydroxy dichloro diphenylmethane.	*Chaetomium globosum*	0	0	0	0	0	14	63	79
	Stemphylium sp.	0	0	4	5	14	104	97	105
	Hormodendrum sp.	15	16	20	32	56	91	97	97
Tetrabrom-o-cresol	*Chaetomium globosum*	0	0	0	0	0	0	6	0
	Stemphylium sp.	0	0	0	1	2	0	20	13
	Hormodendrum sp.	12	14	16	18	19	26	30	29

[1] Untreated control: *Chaetomium globosum*, 0; *Stemphylium* sp., 0; *Hormodendrum* sp., 13.

PLANTING FABRIC ON A GROWING-FUNGUS MAT

Under certain conditions fabrics are subject to attack by fungus hyphae growing directly from a good fungus-growth medium. In composted soils or other soils high in organic matter, for example, cellulose-destroying fungi are commonly in an active condition of growth and are able to attack and reattack the test strip by growth from the soil. Owing to the large number of fungi in some soils, the number of points of fungus attack per unit area of fabric may be very large. In an attempt at partial imitation of this situation, attention was given to culture procedures providing a large quantity of independently nourished mycelial inoculum.

Fabric strips treated with organic protectants were moistened in water and then placed without sterilization on 2-day-old mats of *Chaetomium globosum*. These mats had been prepared by inoculation of sterile 5- by 1½-inch filter-paper strips lying on the surface of the usual mineral-salts agar medium, followed by incubation at 30° C. for 12 days. Parallel tests were carried out by the usual pipette-inoculation procedure with nonsterile strips. As indicated by the data given in table 12, the filter-paper mat, or preinoculation technique, was decidedly the more severe of the two types of test.

TABLE 12.—*Residual strength of protectant-treated nonsterile cotton duck after pipette-inoculation with Chaetomium globosum and 12-day incubation at 30° C., as compared with same duck after preinoculation filter-paper-mat procedure; 3 replicates*

Fabric protectant	Inoculation technique	Content (percent) of compound in fabric						
		0.02	0.04	0.06	0.08	0.12	0.16	0.24
		Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Pentachlorophenol	Pipette [1]	68	101	97	102	99	94	95
	Mat [2]	0	0	0	7	51	38	66
Salicylanilide	Pipette	11	86	99	93	89	91	95
	Mat	0	0	0	2	3	7	3
Dihydroxy dichloro diphenylmethane.	Pipette	2	10	94	95	98	98	107
	Mat	0	0	0	0	0	0	19

[1] Direct-pipette inoculation, 1 ml. of heavy spore suspension.
[2] Preinoculation filter-paper mat, incubated at 30° C. for 48 hours prior to planting the fabric specimen on it.

Filter-paper-mat tests of a copper-hydrogenated resinate-treated 8-ounce cotton duck brought out the interesting circumstance that the severity of the test may be definitely influenced by the salt composition of the test medium. When mats were grown on a 2-percent agar medium containing salts at three different concentrations (p. 6) the strength losses of the treated fabric were greater on the two media of higher salt content than on that of low salt content (table 13). The same salts were used in the same relative concentrations one to another in all three cases, the total concentration of the low salt medium being one-third that of the standard medium (p. 6) and the total concentration in the high salt medium being five-thirds that of the standard medium.

TABLE 13.—*Residual strength of copper-hydrogenated-resinate-treated, sterile, 8-ounce cotton duck, after inoculation with Stemphylium sp., on media of different salt content, by filter-paper-mat procedure*

Percentage of copper in fabric	Content of salt in medium		
	1/3 standard	Standard [1]	5/3 standard
	Residual strength percent	*Residual strength percent*	*Residual strength percent*
0.1	20	2	7
.2	59	18	12
.3	76	26	23
.4	77	25	19

[1] See p. 6 for composition of standard medium.

It is noteworthy that in this experiment all the untreated control strips had a low residual strength; the salt effect observed is apparently not a simple matter of limitation of total fungus growth in the less concentrated media. A possible relation with ion-antagonism phenomena is discussed on page 20.

SOIL-BURIAL COMPARED WITH PURE-CULTURE TESTS

The results of soil-burial tests may be expected to vary from one soil to another and from one time to another in the same soil because of their differences and changes in physical, chemical, and biological make-up. For this reason significance can be attached to soil-burial results only when the experimental differences observed are large and are observed fairly consistently for a number of different soils. Actually such large differences are often observed. When, for example, five different synthetic fabrics were subjected to a burial test in four different soils, the results shown in table 14 were obtained. The data indicate clearly that synthetic fibers differ markedly in their resistance to rotting in soils. While this conclusion might have been suggested by the results of pure-culture tests, such as are shown in the same table, the soil-burial data obviously indicate more general resistance to micro-organisms on the part of the resistant fabrics than could be predicted from a limited number of pure-culture tests. Borlaug (*4*) has reported tests on several synthetic fabrics in pure-culture and in soil contact.

TABLE 14.—*Residual strength of 6 synthetic fabrics remaining after soil burial for 11 days at 30° C., and nonsterile culture tests by pipette inoculation*

Fabric	Burial in—					Culture tests	
	Norfolk soil	Wooster soil	Carrington soil	Davidson soil	Compost	*Chaetomium globosum*	*Metarhizium* sp.
	Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Nylon	97	92	97	95	98	92	100
Fortisan	11	10	0	22	0	2	8
Bemberg rayon	0	0	0	6	0	0	0
Cellulose acetate rayon.	98	84	97	93	94	87	100
Tenasco	0	0	0	0	0	0	0

Another case in which large and consistent differences have been observed by the burial method concerns the relative merits in soil contact of copper naphthenate and other copper soaps. Table 6 and a previous article (*14*) bring out the fact that tests in eight different soils have all indicated that copper naphthenate provides protection at lower copper concentrations on fabrics than three other copper compounds.

Table 15 brings out the striking differences observed among different commercially treated fabrics in soil-burial tests and the severity of the soil-burial as compared with culture tests, including even the *Chaetomium* filter-paper-mat test.

TABLE 15.—*Residual strength of commercial mildew-resistant fabrics after leaching for different time periods and 12-day culture and 18-day soil-burial tests*

Fabric protectant		Cotton fabric		Time leached	Culture tests (12 days)			Soil-burial tests (18 days)		
Kind	Content	Type	Original strength		*Chaetomium* sp., direct inoculation, nonsterile	*Chaetomium* sp., pre-inoculation, nonsterile	*Stemphylium* sp., direct inoculation, sterile	Carrington soil	Compost	Wooster soil
	Percent		*Pounds*	*Hours*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Phenylmercury oleate	0.25	Duck	111	0	98	95	95	0	0	2
				24	97	98	100	0	0	1
				48	85					
				72	25					
Copper hydroxy naphthenate	[1] .50	do	114	0	100	94	96	104	95	95
				24	104	107	101	100	90	102
				48	95					
				72	82					
Salicylanilide	.48	Balloon cloth	60	0	100	0	83	0	0	0
				24	32					
				48	2					
				72	5					
Phenylmercury triethanolammonium lactate	.72	Duck	140	0	103	100	101	0	0	6
				24	106	83	94	0	0	1
				48	96					
				72	83					
Copper fluoride	[1] 1.00	Balloon cloth	60	0	95			0		
				24	93			0		
				48	97			0		
				0	58			3		
Tetrabrom-o-cresol	2.00	do	64	24	0			0		
				48	0			0		
Dihydroxy dichloro diphenylmethane	.32	Duck	57	0	108			34		
				24	0			0		
				48	0			0		
Do	1.00	Sheeting	55	0	102			4		
				24	22			0		
				48	0			0		

[1] Percentage of copper on fabric; all other figures refer to percentage of total compound on fabric.

Results on laboratory-treated fabric with three phenolic protectants are shown in table 16. Although large absolute differences are observed between the results in different soils in these tests, the same general relative order of effectiveness of the three compounds is found in each case; the results with Norfolk soil are the only exception, in that pentachlorophenol appears to be no more effective in this soil than dihydroxy dichloro diphenylmethane. Table 16 shows that the soil-burial results are very much more severe than the results of pure-culture tests (table 1).

TABLE 16.—*Residual strength of organic protectant-treated 8-ounce cotton duck after 14-day soil-burial test in 5 different soils*

Soil and protectant	Content (percent) of protectant in fabric					
	0.2	0.3	0.4	0.6	0.8	1.2
	Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
Compost:						
Pentachlorophenol	2	96	88	100	99	104
Salicylanilide	0	0	0	0	0	3
Dihydroxy dichloro diphenylmethane	0	38	57	100	92	103
Carrington:						
Pentachlorophenol	65	74	38	96	101	101
Salicylanilide	0	0	0	0	4	15
Dihydroxy dichloro diphenylmethane	0	0	0	22	46	83
Davidson:						
Pentachlorophenol	54	96	94	96	99	102
Salicylanilide	1	2	8	36	39	76
Dihydroxy dichloro diphenylmethane	5	15	25	34	73	102
Wooster:						
Pentachlorophenol	21	69	38	105	96	99
Salicylanilide	0	0	0	4	1	16
Dihydroxy dichloro diphenylmethane	17	34	34	69	74	74
Norfolk:						
Pentachlorophenol	96	96	101	104	94	108
Salicylanilide	4	16	15	21	36	65
Dihydroxy dichloro diphenylmethane	96	101	92	96	102	95

In view of the complexity of soils, results with the soil-burial test in the laboratory were rather more consistent than had been expected prior to carrying out the tests. The use of several different soil types insures against incorrect interpretations arising from tests in a single soil that might for some reason give an aberrant or uncommon type of response to some particular protective agent. Composting yields soils high in cellulose-decomposing organisms. It is felt by some that the high biological activity of compost is accompanied by such rapid changes in the biological and chemical components that field soils, presumably much more stable in this respect, are to be preferred. Attention is being directed to this question, no conclusive experimental evidence being available at present.

Two further experiments were carried out to test the comparative severity of a pipette-inoculum *Chaetomium* test as compared with soil burial. In the first experiment a miscellaneous group of phenolic and other organic compounds were selected, applied to 8-ounce cotton duck, and tested by the two methods. Although several of the compounds afforded good protection against *Chaetomium*, not one sample out of the 210 tested had sufficient strength after 12 days in the soil to be removed without falling to pieces. The results of this experiment are shown in table 17.

TABLE 17.—*Residual strength of protectant-treated, 8-ounce cotton duck after 12-day nonsterile pipette-inoculum test with Chaetomium globosum* [1]

Compound	Content (percent) of compound in fabric					
	0.2	0.3	0.4	0.6	0.8	1.2
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Sym.-tetrachloronitrobenzene	10	20	128	123	113	118
8-hydroxyquinoline	113	127	125	133	137	133
Tri-p-cresyl phosphate	11	20	13	8	129	126
Beta naphthol	0	18	120	126	130	131
2,5-dihydroxydiphenyl	97	119	135	130	132	136
2,4,6-tribromophenol	137	127	135	130	139	130
Acenaphthene	0	0	94	128	122	127

[1] After a 12-day soil-burial test all samples were completely deteriorated; 0, breaking strength.

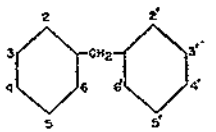
In the second experiment, comparative tests were carried out on 8-ounce cotton duck treated in the laboratory with a group of compounds related to 2,2′-dihydroxy-5,5′-dichloro diphenylmethane. As may be seen from table 18, the soil-burial test was again much more severe than the *Chaetomium* test. Selection of promising fungicidal and fabric-protective materials is often carried out in commercial laboratories by the use of routine biological tests, often called screening tests. The importance of choice of method for such tests is well illustrated by the results shown in tables 17 and 18.

TABLE 18.—*Residual strength after 12-day soil-burial test and 12-day test with Chaetomium globosum pipette-inoculated of unsterilized 8-ounce cotton duck treated with various dihydroxy diphenylmethane derivatives*

Compound	Diphenylmethane [1] with substituents on carbons numbered—									
	2	3	4	5	6	2'	3'	4'	5'	6'
11	OH			Cl		OH			Cl	
13	OH	Cl		Cl		OH	Cl		Cl	
15	OH	Cl		Cl	Cl	OH	Cl		Cl	Cl
17	OH	Cl	Cl	Cl	Cl	OH	Cl	Cl	Cl	Cl
12	OH			Br		OH			Br	
14	OH	Br		Br		OH	Br		Br	
16	OH	Br		Br	Br	OH	Br		Br	Br
18	OCH [3]			Cl		OCH [3]			Cl	
20	OCH [3]	Cl		Cl	Cl	OCH [3]	Cl		Cl	Cl
2	OH							OH		
3			OH					OH		

Compound	Content (percent) of compound in fabric and original strength after—							
	Soil-burial test [2]				*Chaetomium* test [3]			
	0.1	0.2	0.4	0.8	0.1	0.2	0.4	0.8
	Residual strength percent	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*	*Residual strength percent*
11	3	12	20	83	93	105	100	96
13	0	0	0	0	99	102	105	101
15	2	16	40	37	95	108	94	107
17	0	2	4	0	4	93	55	80
12	5	13	51	52	100	98	108	99
14	0	0	0	0	0	4	54	46
16	0	0	0	2	60	94	99	97
18	0	0	0	0	0	0	0	34
20	0	0	0	0	0	0	0	0
2	0	0	0	0	0	2	86	102
3	0	0	0	0	0	0	76	102

[1] Basic structure of diphenylmethane:



[2] In Carrington soil.
[3] Nonsterile.

AERATION REQUIREMENTS

The customary practice in the laboratory was to use culture bottle tops in which a circular hole, 1¼ inches in diameter, was cut and a piece of glass cloth inserted to provide for gas exchange (*8*). In case it is difficult to obtain such cloth or if a completely closed container is desired, a question arises regarding the quantity of air space necessary inside the container to provide adequate oxygen for fungus growth and complete break-down of the fabric. While the answer to this question is in all probability dependent on the weight of fabric used, the data in table 19 provide a satisfactory answer in the case of 8-ounce cotton duck.

TABLE 19.—*Residual strength of untreated 8-ounce cotton duck after 12-day incubation in pure-culture tests with 3 fungi in tightly closed containers*

Test organisms and container	Content of salt in medium		
	1/3 standard	Standard [1]	5/3 standard
	Residual strength *percent*	*Residual strength* *percent*	*Residual strength* *percent*
Metarrhizium sp.:			
500-cc. bottle	11	14	21
Quart jar	0	0	0
Chaetomium globosum:			
500-cc. bottle	16	21	16
Quart jar	0	0	0
Stemphylium sp.:			
500-cc. bottle	25	27	27
Quart jar	11	17	17

[1] See p. 6 for composition of standard medium.

The 500-cc. bottles used in this experiment were the same as those used in all other experiments here reported, except that solid caps were used and the bottles were sealed with paraffin. Ordinary 1-quart mason jars were used; they were sealed tightly with the usual jar rubbers. Three different levels of mineral-salt content were used in the agar medium, since it was considered possible that this factor might influence the efficiency of utilization of oxygen. Table 19 shows that none of the fungi completely deteriorated the strips in the 500-cc. bottles and that the quart-bottle air space was adequate for *Metarrhizium* sp. and *Chaetomium globosum*, but not for *Stemphylium* sp.

DISCUSSION

Interpretation of much of the experimental evidence in the literature regarding water leaching of mildew-proofed fabric is difficult. In many cases the original quantity of the preventive on the fabric was not accurately known, no chemical analyses to determine the extent of loss in leaching had been made, and the minimum quantity of the preservative that had to remain on the fabric to prevent fungus growth was not determined. In these circumstances, the information that a treated fabric has passed a combined water-wash-biological test leaves one without certain knowledge of whether the result observed is attributable (1) to good retention of the preservative on the fabric or (2) to high fungicidal effectiveness of the preservative, counterbalancing losses by leaching.

The method here described for standardization of water leaching is obviously only one of several techniques that might be used. The apparatus described has proved simple to operate.

It has been shown in a previous article (*14*) that fabric may contain high concentrations of copper in certain forms and still be rotting badly in soil; this was especially true with copper-hydrogenated resinate-treated fabric. The data here reported show that under pure-culture conditions, any of several fungi may grow on fabric high in copper content and deteriorate it. Copper tolerance of soil fungi is believed to be a factor contributing to the severity of soil-burial exposure of copper-treated fabric. It is thought, however, that there are many other reasons for the extraordinary severity of soil-burial exposure—direct chemical reactions between soil constituents and the fabric protectant (*14*), adsorption of the protectant, the large number of micro-organisms

in the soil, the independent nourishment of the attacking organisms, enabling them to attack and reattack by growth from the soil to the fabric, and other factors.

The factors influencing copper tolerance of fungi have not been investigated in detail; for example, the difference in degree of break-down of the 0.4-percent copper treatment as hydrogenated resinate on fabric No. 1 as compared with similar treatment for fabric No. 2 (table 8) is not known. It is clear, however, that striking inherent differences in copper tolerance do occur in fungi.

In the absence of conclusive data, there remains some doubt whether fungi will grow on fabric in above-ground service-condition exposures at copper concentrations as high as may be attacked in pure-culture experiments with copper-tolerant fungi. Lin (*12*) has shown that the toxicity of copper sulfate to the conidia of *Sclerotinia fructicola* may be strikingly decreased by any of several common electrolytes, and Marsh (*13*) has found that under certain conditions copper absorption by the spores of this fungus may be greatly decreased in the presence of electrolytes, accounting for the decrease in fungicidal activity. Such antidoting action of electrolytes might occur in pure-culture tests and in soil-burial exposure, but not in above-ground exposures. The data of table 13 are pertinent and suggestive, but admittedly leave the question unanswered.

A question may arise concerning the moderate but significant strength losses of osnaburg fabric of high copper content in the *Chaetomium* results of table 9. Such losses have not occurred with *Chaetomium* in similar tests on 8-ounce cotton duck; it is believed that the total quantity of copper present in the strip, which is obviously related to the weight of the strip, may play a role in determining the percentage of copper tolerated on a fabric by *C. globosum*. The general experience of other workers seems to be in agreement with this concept. The osnaburg cloth used for the experiments of table 9 was considerably lighter in weight than the 8-ounce cotton duck used in other experiments.

A second question that may arise in connection with the same table concerns the validity of comparisons of copper tolerance of fungi growing at different temperatures. It might be suggested that *Chaetomium globosum* growing at 20° C. would be as copper-tolerant as *Stemphylium* at that temperature. Actually this does not turn out to be the case. A set of copper-hydrogenated resinate-treated 8-ounce cotton duck samples were pipette-inoculated with *C. globosum* and incubated at 30° C., and a similar set inoculated and incubated at 20°. The breaking strengths, as percentages of the original break for the 20° samples at 0.1, 0.2, 0.3, and 0.4 percent of copper on the fabric, were 39, 90, 101, and 97 percent, as compared with 27, 85, 98, and 95 percent, respectively, for the 30° strips.

The filter-paper-mat technique as here described is obviously a severe biological test and can be tentatively recommended for fabrics to be exposed to soil. Its use on above-ground fabrics may be justifiable if the results obtained are interpreted with a realization of the severity of the test. In cases in which nonsterile tests with pipette-inoculum technique fail because of contaminant organisms and when steam sterilization is especially to be avoided, the filter-paper-mat technique may be useful.

The development of highly satisfactory test methods for determining mildew and rot resistance of fabrics obviously will depend on the accumu-

lation of data from the use of many different methods with many different test fabrics. It is recognized that certain of the factors involved in the mildew and decay of textiles (*6, pp. 129-138*) have received scant attention and that progress in test methods is dependent upon further investigation. In the meantime a few short comments on the various procedures now in use should be valuable.

Many workers (*1, 2, 3, 14*), including the present writers, have adhered to the use of the soil-burial test in spite of its well-known weaknesses. A fabric that consistently survives prolonged contact with a number of different microbiologically active soils has survived several severe hazards. The conditions of the test are not all adequately understood; however, the test is generally recognized to simulate the severe conditions experienced in service by certain fabrics. For this reason and because of its simplicity of performance, the soil-burial test may be expected to continue in use for some time and not to be displaced entirely by other methods. The control of temperature and soil moisture and the use of several different soil types (*14*) would appear to be highly desirable in soil-burial tests.

Preliminary tests appear to substantiate the claims of Furry and Zametkin (*5*) in regard to the severity of their soil-suspension test procedure; further work is in progress relative to that method and to similar procedures.

Exposure on above-ground weathering racks has been used in many instances as a practical measure of the ability of fabrics to retain their original properties in service. Under conditions favorable to mildew growth, loss in fabric strength is often due to the action of micro-organisms. While great variations are found with this type of procedure, there is no doubt that its use has yielded much valuable information.

The *Chaetomium* procedure of Thom, Humfeld, and Holman (*17*) has been much criticized, primarily on the ground that it is not severe enough. It is clear that the soil-burial procedure may, and often does, represent a much more severe test than this particular pure-culture procedure. Bertolet (*3*) points out, for example, that copper resinate passes the *Chaetomium* test (*17*), even though it is unsatisfactory in soil contact. The data here reported show that other pure-culture techniques may more closely approximate the soil-burial test. Pure-culture procedures have the immense advantage that the conditions of the tests may be reproduced from one time to another and from one laboratory to another. When discrepancies appear between different soil-burial results the cause may be difficult or impossible to determine experimentally, or even to guess. The variables in pure-culture tests are less numerous and are more readily subjected to experimental control.

The pure-culture technique of Greathouse and others (*8*) has been found particularly adaptable to tests in which carbon sources external o the test fabric are to be rigidly excluded. The fungus *Metarrhizium*, introduced in connection with this test procedure, has been used in many laboratories.

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