R56-18

Reprinted from APPLIED MICROBIOLOGY
Vol. 4, No. 6, November 1956
Printed in U.S.A.

A Study of Mixed-Spore Culture and Soil-Burial Procedures in Determining Mildew Resistance of Vinyl-Coated Fabrics[1]

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Received for publication May 18, 1956

Research in these laboratories[2] and by others (Abrams, 1948; Stahl and Pessen, 1953) has shown that fungi and bacteria can deteriorate certain plastic films, whether the films are tested alone (unsupported films) or whether they are evaluated as coatings on fabric (supported films). Films may also be deteriorated by loss of plasticizer through volatilization. However, vinyl film plasticized with equal parts of dioctylphthalate (DOP) and methyl acetylricinoleate (P4) showed no stiffening after autoclaving for 24 hr, or exposure for 8 days to oxygen, or exposure for 30 days to high relative humidity and temperature[2].

Our studies[2] have shown that phthalate and phosphate plasticizers are generally resistant to fungal attack, with the aliphatic-substituted compounds showing a greater degree of resistance than the aromatic. Natural oils, fatty acid derivatives, and certain long-chain dicarboxylates as plasticizers are readily attacked by microflora. Structural modification of the plasticizer may make it fungus-resistant, but not necessarily resistant to bacteria (and *vice versa*). This was demonstrated by the response of *Aspergillus versicolor* and *Pseudomonas aeruginosa* to each of a series of homologous sebacates (Stahl and Pessen, 1953). The same authors (1954) reported that internally plasticized polymers resist degradation by fungi.

Visual rating of growth of microflora and changes in flexibility and breaking strength have been used in these laboratories as indices of deterioration of plastic films and coated fabrics. These observations were made following exposure to soil burial or by single-specie and mixed-spore suspension culture tests. These tests for evaluation of the deterioration of plastic films and coated fabrics on the basis of weight of coating and end use of the fabric have been found to be unreliable. Therefore, laboratory tests are being designed which

[1] Presented at the Twelfth General Meeting, American Institute of Biological Sciences, East Lansing, Michigan, Sept. 7-9, 1955.

[2] Unpublished studies from 1945 through 1953 by L. Boor, Dorothy Beck Daoust, J. V. Harvey, M. Manowitz, and F. Meloro.

will determine the probable effects of several types of exposure on the treatment, rather than the effects of the specific type of exposure under which the fabric might be used.

Methods for exposing coated fabrics are variable with respect to duration of burial in soil and composition of mixed-spore suspensions.

In studies to establish appropriate methods[2], there appeared to be a comparative correlation between results obtained from a 21-day mixed-spore culture exposure and a 14-day soil-burial exposure. The criteria used for deterioration were tensile strength, flexibility, and visual rating of fungus growth. However, no statistical test of correlation was carried out.

The studies reported herein were initiated in 1953 and are the result of a cooperative effort among four industrial laboratories and the Chemicals & Plastics Division, Quartermaster Research & Development Center.

The objectives of these investigations were to determine: (a) adequacy of previously used test procedures and criteria for evaluation of mildew resistance of coated fabrics; (b) whether a base fabric subject to the cellulolytic activity of fungi should be treated with an appropriate fungicide even when coated with a nonsusceptible plasticizer (for example, DOP); and (c) whether partial substitution of DOP by a plasticizer subject to microbial deterioration (for example, P4) would require the addition of a toxicant to the base fabric, the plasticizer, or both.

Experimental Methods

Materials

The coated fabrics tested were as shown in table 1.[3] The fabrics were coated with vinyl organosols formulated as shown in table 2.

The cotton sheeting was coated on one side only with three coats of each of the above formulations. The first two coats were knife-coated with oven heat at 270 F. Oven heat was then raised to 350 F to flux the vinyl.

The cotton duck was coated with three coats of the above formulations. The first two coats were applied to one side of the fabric at an oven temperature of 270 F. The fabric was then reversed, and the other side was given one coat with an oven temperature of 350 F to flux the vinyl.

The cotton sateen was coated with five coats of each of the above formulations on one side only. The first four coats were applied with the oven heat at 270 F and a fifth coat was applied with the oven temperature at 350 F to flux the vinyl coating.

[3] Coated fabrics were prepared by Films & Filaments Section, Chemical & Plastics Division, Quartermaster Research & Development Center.

Table 1

Untreated Cotton Base Fabric	Cu-8-Treated Cotton Base Fabric	Plasticizers & Toxicants
Sheeting		a) DOP
Sheeting		b) DOP-P4
Sheeting		c) DOP-P4 + Cu-8, PMA*
	Sheeting	a) DOP
	Sheeting	b) DOP-P4
	Sheeting	c) DOP-P4 + Cu-8, PMA
Duck		a) DOP
Duck		b) DOP-P4
Duck		c) DOP-P4 + Cu-8, PMA
	Duck	a) DOP
	Duck	b) DOP-P4
	Duck	c) DOP-P4 + Cu-8, PMA
Sateen		a) DOP
Sateen		b) DOP-P4
Sateen		c) DOP-P4 + Cu-8, PMA
	Sateen	a) DOP
	Sateen	b) DOP-P4
	Sateen	c) DOP-P4 + Cu-8, PMA

* Cu-8 (copper-8-quinolinolate) and PMA (phenyl mercuric acetate). 1.0 per cent and 0.5 per cent, respectively, based on weight of plasticizer.

Table 2

Material	Formulation (Parts)		
	A	B	C
Vinyl resin	100	100	100
DOP	70	35	30
Barium ricinoleate stabilizer	1.5	1.5	1.5
$PbSiO_2$ stabilizer	1.5	1.5	1.5
Solvent thinner	As required		
P4		35	35
DOP + Cu-8 (1% Solubilized Cu-8-quinolinolate based on weight of DOP)			7.0
Pigment	As required		
PMA (0.5% PMA based on weight of plasticizer)			0.35

Methods

1. Mixed-spore culture test, 21 days: The mixed spore suspension was comprised of spores of *Penicillium piscarium* (BPI 15.1), *Penicillium luteum* (BPI 66), *Aspergillus flavus* (QM 4m), *Aspergillus niger* (USDA TC-215-4247), and *Trichoderma viride* (BPI T-1). The coated fabrics, prepared as cut-strips (1 in x 6 in) with long dimension parallel to the warp, were placed on mineral salts agar[4] and inoculated with the mixed-spore suspension. After incubation for 21 days the strips were removed, washed, conditioned at 73 ± 2 F and 50 ± 4

[4] NH_4NO_3, 3.0 g; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.50 g; KCl, 0.25 g; agar, 15.0 g; distilled water, 1,000 ml.

per cent relative humidity for 24 hr and broken on the Scott Tester[5] or tested for stiffness in a Clark Flexibility Tester.[6]

2. Soil-burial, 14 days: 1¼ in x 10 in strips were subjected to soil burial in a tropical chamber for 14 days. The tropical chamber was held at 86 ± 2 F and 85 ± 2 per cent relative humidity. Soil burial was followed by determination of breaking strength retained (Scott Tester using the cut-strip method and conditioning the fabric strips at 68 ± 1.8 F and 50 ± 2 per cent relative humidity for 24 hr), and measure of stiffness of the fabric on the Clark Flexibility Tester.

Results

The data are summarized in the scatter diagrams shown in figures 1 through 4. The figures show the average data reported by each laboratory for breaking strength and stiffness of these coated fabrics. The agreement of the data relative to the suggested tolerances for these physical tests may be deduced from these graphs. An increase in stiffness not exceeding 30 per cent, and a loss in breaking strength not in excess of 15 per cent are proposed as tolerances for each of these physical tests. In the graphed data, these tolerances are represented by horizontal lines drawn at 30 and 85 per cent, as required. These allowances are not to be considered final but, rather, are subject to change should other tolerances be considered more characteristic and/or realistic.

The results for each of the five participating laboratories are presented in all cases except, as in figures 3 and 4, where four laboratories reported.

Soil burial and breaking strength retained (*figure 1*). Unprotected duck and sateen coated with vinyl plasticized with DOP are variable in resisting microbiological deterioration. This is evidenced by the wide scatter obtained, showing that some laboratories would pass while others would fail the same fabric relative to the tolerance established. Although all laboratories report more than tolerable loss in breaking strength for a similar coating applied to sheeting, the range of average breaking strength is rather wide.

No substantial difference is indicated for DOP + P4 in vinyl applied to the base fabrics. When the plasticizer protected with Cu-8, and PMA is applied in vinyl coating to unprotected base fabric, or when protected base fabric is coated with DOP; DOP + P4; and DOP + P4 + Cu-8, and PMA, a somewhat larger measure of resistance to mildew is evidenced by this method of test. In these instances, the reproducibility between laboratories, though poor, is enhanced as indicated by the slightly reduced scatter. This method, soil burial, followed by breaking-strength determination, appears to

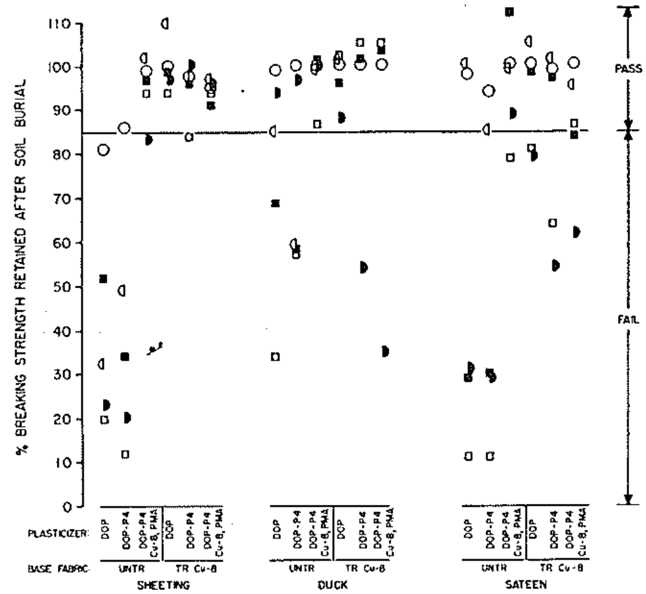

FIG. 1. Scatter-diagram of per cent breaking strength retained after 14-day soil burial for each of 3 coated fabrics reported by 5 laboratories designated as ○, ◐, ◗, □, and ■.

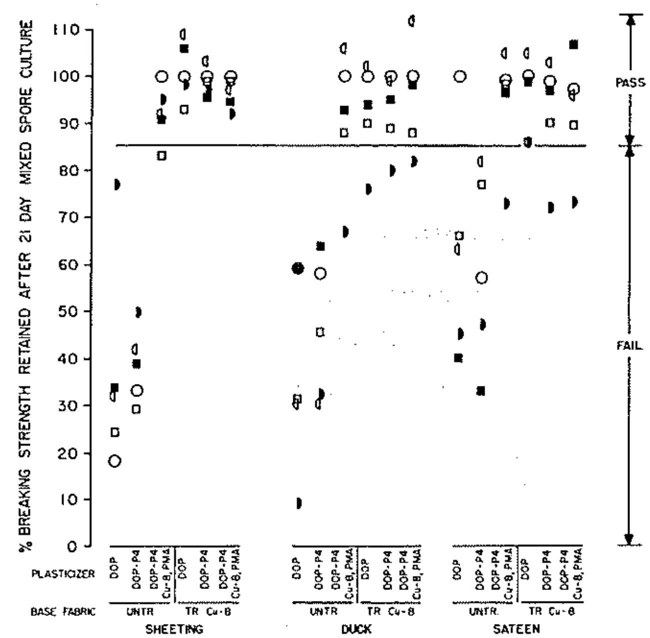

FIG. 2. Scatter-diagram of per cent breaking strength retained for 3 coated fabrics after 21-day mixed spore culture by each of 5 laboratories designated as ○, ◐, ◗, □, and ■.

be poorly reproducible and hence unfeasible as a measure of deterioration for these coated fabrics.

Mixed-spore culture and breaking strength retained (*figure 2*). Unprotected sheeting and duck coated with vinyl plasticized with DOP only were found by all laboratories to deteriorate in excess of tolerance. This was found also to be the case for sateen by all but one laboratory. When P4 supplemented DOP as plasticizer, all laboratories reported greater than tolerable loss in breaking strengths. The breaking strength of unpro-

[5]Scott Testers Inc. Providence, R. I.

[6]Thwing-Albert Instrument Co. Philadelphia 44, Pa.

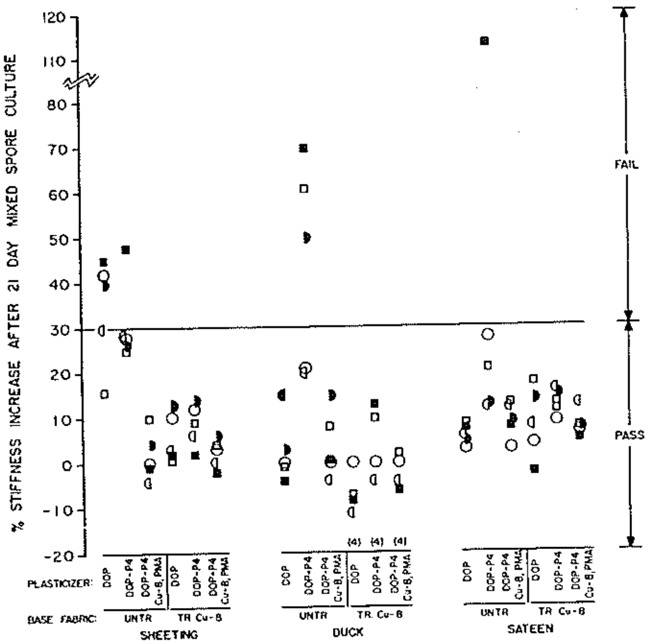

FIG. 3. Scatter-diagram of per cent stiffness increase after 21-day mixed spore culture for 3 coated fabrics by each of 5 laboratories designated as ○, ◐, ◗, □, and ■.

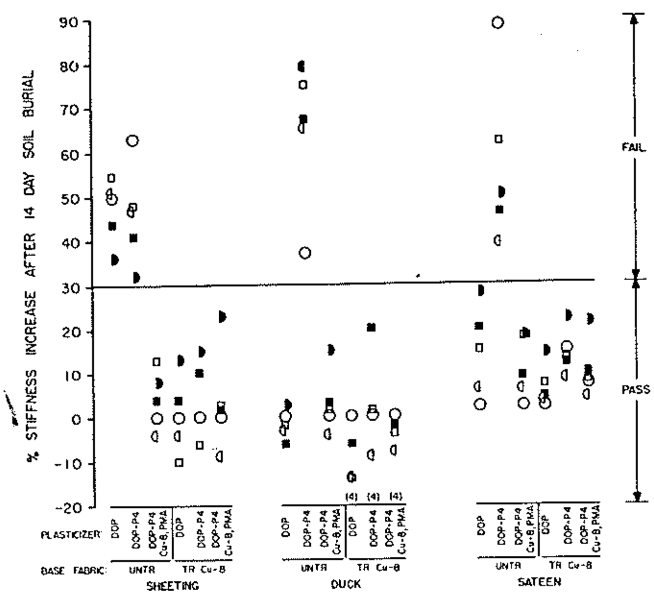

FIG. 4. Scatter diagram of per cent stiffness increase after 14-day soil burial of 3 coated fabrics by each of 5 laboratories designated as ○, ◐, ◗, □, and ■.

tected sheeting coated with vinyl containing DOP + P4 + Cu-8, and PMA was reported by one laboratory to be slightly in excess of tolerance. Similar results for both duck and sateen were reported by one other laboratory.

All coatings applied to protected sheeting resisted deterioration. One laboratory reported more than tolerable loss in breaking strength for protected duck and sateen plasticized with DOP + P4, containing Cu-8 and PMA.

Mixed-spore culture followed by breaking strength retained as the criterion for deterioration is poorly reproducible, as evidenced by this wide scatter. Therefore, this combination of test and criterion is unacceptable as a procedure for test.

Mixed spore culture and stiffness increase (figure 3). Data obtained from 21-day mixed-spore culture followed by flexibility measure afforded better reproducibility than the procedures reviewed previously. It is evident that when Cu-8 and PMA are added to plasticizer of vinyl applied to unprotected base fabrics, or when plasticizer with or without toxicants is applied in coats to Cu-8-treated base fabrics, deterioration does not occur. This is indicated by loss in flexibility not exceeding the tolerance. Further, the reduced scatter of the average data indicates a more consistent performance of these coated fabrics, as evaluated by this procedure. A wide scatter ranging from a tolerable to a greater-than-tolerable change in stiffness is notable when the mixed plasticizer (1:1 of DOP and P4) was used for vinyl coated on unprotected sheeting, duck or sateen. Sheeting, too, coated with vinyl plasticized with DOP alone afforded a wider-than-desirable scatter.

Reproducibility in performance of coated fabrics subjected to mixed-spore culture, followed by determination of stiffness, is better than when soil-burial or mixed-spore culture was followed by breaking-strength determination.

Soil burial and stiffness increase (figure 4). Soil burial followed by flexibility measure afforded the best reproducibility for evaluation of deterioration of the coated fabrics. Although scatter of the data for some of the coated fabrics is inclined to be wide, the data reported are completely consistent and reproducible within the established tolerance.

Therefore, it would be reasonable to assume that these coated fabrics (sheeting, duck, and sateen) and the coating as applied may best be evaluated for microbiological deterioration by using soil burial followed by stiffness measure.

DISCUSSION

Examination of the data indicates that the least variable results, predicated on range of scatter, were obtained with Clark flexibility measure following soil burial of these coated fabrics. Greater variability is evident with flexibility measure after mixed-spore culture and with breaking-strength determinations after either soil-burial or mixed-spore culture.

The particular biological conditions attending fabric deterioration in soil, further complicated by unmeasured integrated physico-chemical processes, have never been fully investigated and, therefore, are not clearly understood. Because of this, spore-culture techniques have been developed to measure deterioration with the thought that such techniques can be closely controlled and replicated. This implies a complete understanding of biological conditions pertaining to degradation of fabric in such test procedures. An understanding of the deteriorative processes in culture, in fact, still remains to be achieved. Mixed-spore cultures have been recognized by some to be more efficacious than single cultures of fungi and these, in turn, more desirable than soil burial. The mixed-spore population was regarded to be more representative of conditions in nature. If "representativeness" of biological material is desirable, then the use of a mixed-spore culture in preference to soil is rather inconsistent since the soil affords the more complete biological spectrum for deterioration evaluation.

Further, the mixed-spore suspension was developed to test treated fabrics against a wider microfloral spectrum. This was believed to afford a more complete evaluation of deterioration than would be possible with single cultures. The culture of even a single fungus on a cellulosic substrate is accompanied by an exceedingly complex and incompletely understood physiologic activity. The influence of several species of fungi each upon the other in competing for this cellulosic substrate is even more complex and not necessarily additive.

In deterioration testing, variation of biological material (substrate and test organism) is axiomatic. The universality of this variation permits no more than an empiric procedure and evaluation at this time. Culture tests, and particularly the mixed-spore suspension, therefore, are no less empiric than soil burial.

The soil-burial procedure was found to be more reproducible than mixed-spore culture and, when coupled with flexibility measure, more diagnostic of deterioration than when coupled with breaking-strength determinations. The dual nature of coated fabrics (base fabric and plastic coating) could possibly influence the criterion to be used for evaluation. The quantitative predominance of either the base fabric or the plastic coating might be the determining factor for the use of either breaking strength or flexibility measure.

Breaking-strength determinations were more variable than flexibility measures following mixed-spore culture or soil burial. It is entirely possible that small deviations (less than 5 per cent) from tolerance for breaking strength or stiffness may not be significant. There still remain, however, a considerable number of breaking-strength determinations in rather serious disagreement. As all fabrics were prepared by one agency, the wide variability as evidenced by the scatter may be ascribed to: (a) those aspects of soil-burial and mixed-spore culture previously discussed; (b) variations in conditions between laboratories for test and procedure; and (c) personal variations in the reading of results.

Most laboratories submitted data in a form which precluded statistical evaluation as it would apply to reproducibility. Although consideration of data in terms of scatter affords an excellent means of appreciating trends for reproducibility, this can be regarded only as a substitute for detailed data.

TABLE 3

Type	Coated Fabric		Method of Test	
	Wt. of base fabric	Thickness of coating	Procedure	Criterion for evaluation
I	Light	Light	Soil burial	Flexibility
II	Light	Heavy	Soil burial	Flexibility
III	Heavy	Light	Soil burial	Breaking strength
IV	Heavy	Heavy	Soil burial	Flexibility

Breaking strength as a measure of degradation of fabrics is a basic determination, and its usefulness should not be discounted for coated fabrics. Compared to flexibility as here tested, however, breaking strength is not quite as reproducible.

Relatively light coatings on light-base fabrics, as here investigated, and heavy coatings on relatively light-base fabrics may be best measured for deterioration by the Clark flexibility measure. However, breaking-strength determination would possibly be more desirable in evaluating degradation of light-coated heavy-base fabric combinations. If relative weights of coating and base fabric are factors in selection of an appropriate measure of deterioration, the test procedures shown in table 3 might be feasible. Further investigation is indicated to determine whether this relationship affords an appropriate means for selection of test and procedure to determine mildew resistance of each type of coated fabric.

CONCLUSIONS

1. Testing for mildew resistance of these coated fabrics prepared with vinyl organosols fluxed on to the base fabric should be predicated on stiffness by the Clark flexibility measure following soil burial for 14 days—incubation at 86 ± 2 F and 85 ± 2 per cent relative humidity. More consistent results were obtained with this procedure and measure than with any other method or criterion investigated.

2. Coated sheeting, duck, and sateen may be protected adequately against microbiological deterioration by addition of a fungicide to the base fabric or to the plastic coating. No evidence was obtained to indicate that both the base fabric and the coating should be protected.

3. In view of the fact that adequate protection against physical deterioration was obtained with copper-8-quinolinolate, which is fungicidal but not bactericidal, it appears that fungi are primary degraders of plastic-coated fabrics and bacteria are of secondary importance. However, the methods used are total in nature and would not characterize deterioration by one kind of microflora or the other.

Summary

A cooperative study was made among four industrial laboratories and these laboratories. The efficacy of mixed-spore culture and soil burial, followed by breaking-strength and flexibility measure, were evaluated as procedures and criteria to determine resistance to microbiological deterioration of variously plasticized vinyl-coated fabrics.

Data presented indicate, for the coated fabrics studied, that soil burial for 14 days, followed by the Clark flexibility measure, afforded best reproducibility for measure of degradation of these materials. Sheeting, duck, and sateen base fabrics may be protected against deterioration when solubilized copper-8-quinolinolate and phenylmercuric acetate at 1.0 per cent and 0.5 per cent, respectively (based on the weight of the plasticizer) are incorporated into the vinyl coat. Coatings based on unprotected duck and sateen evidenced no deterioration when dioctylphthalate, alone, was used as the plasticizer; the same coating based on sheeting, however, did deteriorate. No explanation for this apparent anomaly is offered.

Resistance to biological deterioration was impaired when methyl acetylricinoleate was mixed with dioctylphthalate as the plasticizer. All coated fabrics considered, a larger measure of resistance to the degradative effects of fungi and bacteria is obtained with a fungicide added to the base fabric or to the plasticizer, compared to the response of unprotected base fabric coated with vinyl plasticized with a resistant plasticizer. There is no evidence that addition of fungicide to the base fabric and the addition of bactericide and fungicide to the plasticizer is more efficacious than treatment of either the base fabric or the plastic coating. The feasibility of testing coated fabrics with regard to relative weight of fabric and thickness of coating is suggested by these data, but must be confirmed by intensive study.

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