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Laboratory and Field Exposure Studies of Leather Fungicides†

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ABSTRACT

The effectiveness of seven experimental leather fungicides that were previously evaluated in laboratory tests has been studied under field conditions together with 4-nitrophenol as the comparison standard. In addition to 4-nitrophenol, it was found that 2-chloro-4-nitrophenol, 4-thiocyanophenol, and bis(4-nitrophenyl) carbonate prevent mildew growth on leathers under tropical jungle conditions at concentrations no higher than 0.60 per cent and are adequately stable under severe weathering conditions. N-(trichloromethylthio) tetrahydrophthalimide and tetrachlorohydroquinone inhibited mildew growth at 0.60 per cent concentration in some cases, but are not dependable leather fungicides. 2-Phenylphenol and 4-chloro-m-xyleneol are not effective leather fungicides even at concentrations well over one per cent.

1. INTRODUCTION

Prevention of mildew growth on military leather items has been an important problem for the United States Armed Forces since World War II. Most tanning agents and leather lubricants required for the production of satisfactory military leathers are mildew susceptible. It is, therefore, necessary to incorporate a fungicide* into the leather to make it mildew resistant in hot and humid areas. The fungicide now being used for that purpose by the Quartermaster Corps is 4-nitrophenol. A comprehensive review on the use of this compound as a leather fungicide has recently appeared in the literature.¹

†These studies were sponsored by the Quartermaster Corps of the Department of the Army as a part of research and development project 7-65-01-003B.

*Throughout this paper the term fungicide is applied to any compound that prevents or inhibits mildew growth.

Although 4-nitrophenol is the preferred leather fungicide at the present time, new and possibly better ones are being sought. In time of national emergency any one fungicide might be unavailable. This was true of 4-nitrophenol during World War II. The availability of suitable alternates is consequently required.

The Quartermaster Corps is supporting a program at the National Bureau of Standards for the investigation of compounds that are known to be fungitoxic* and that might be useful as leather fungicides. Part of this program has consisted of screening numerous compounds in order to determine those most effective in inhibiting mildew growth in leather.

A field exposure study of some of the compounds evaluated in screening tests was undertaken for two reasons: (1) in order to test further the weathering stability and fungicidal efficiency of those compounds that had been found promising in the earlier screening tests, and (2) in order to establish the reliability of the screening test as a method for predicting the efficiency of fungicides in field tests.

2. DESCRIPTION OF THE QMC SCREENING PROGRAM FOR LEATHER FUNGICIDES AT NBS

As previously mentioned, all the compounds that have been submitted for evaluation as leather fungicides at the National Bureau of Standards possessed fungitoxic properties. This was known from fungitoxicity tests, such as that employed by the Prevention of Deterioration Center of the National Research Council², or the fact that the compounds had already been used as fungicides for protection of materials other than leather.

It is generally recognized that the fungitoxicity exhibited by a given compound against a specific organism is greatly influenced by the substrate and by other environmental factors³. Leather is a complex material that supports the growth of a great variety of organisms, most of which have been studied to only a limited extent. Compounds that are found to be effective fungicides for other materials, such as textiles or paints, or that exhibit fungicidal activity in tests using pure cultures and nutrient agar medium are not necessarily good leather fungicides. From these considerations it is obviously desirable to evaluate leather fungicides on a leather substrate and preferably under the conditions and against microflora typical of a tropical jungle. Such conditions and flora are approximated in a tropical room at the Engineering Center of the U. S. Army at Fort Belvoir, Virginia. This room, which is referred to only as the tropical room in the remainder of this paper, was made available for use in the Quartermaster Corps screening program of leather fungicides at the National Bureau of Standards through the cooperation of the Engineering Center. Some details of the procedures used in the screening program are given as a background for the present field exposure study.

*No attempt has been made to distinguish between fungicidal and fungistatic activity.

2.1 Test Leather

After evaluating the fungus resistance of different leathers by exposure in the tropical room, a vegetable-tanned sole leather crust was chosen as the test leather. This leather contains less filler and oil than finished sole leather and is not compressed by rolling. It is very susceptible to mildew growth and is uniformly so from batch to batch. Many other types of vegetable-tanned leathers vary considerably in this respect. Vegetable-tanned leathers are more susceptible to mildew growth than leathers of other types of tannages⁵.

2.2 Incorporation of Fungicides into the Leather

Leather specimens were dipped into a suitable organic solvent-type formulation of the fungicide. The choice of solvents apparently does not affect the fungitoxic properties of the compound. This conclusion has been reported by others⁴ and assumes no reaction between solvent and fungicide. Whenever possible, a solvent mixture of cyclohexanone (10 per cent), mineral oil (20 per cent), and trichloroethylene (65-70 per cent according to per cent fungicide, by weight) was used for incorporation of the fungicide into the test leather. This mixture penetrates quickly through the leather. Analysis of leather treated with 4-nitrophenol in this solvent mixture indicated that the deposition of the fungicide was uniform throughout the leather. The amount of fungicide deposited, as found by analysis, agreed generally with the amount calculated from the formulation absorption by the leather. Apparently, there is no preferential absorption of the components of the formulation.

2.3 Tests for Mildew Resistance

(a) Initial screening test

Fungicides submitted for test were exposed to an initial screening test as follows: The fungicide was dissolved in an organic solvent such as trichloroethylene, cyclohexanone, Stoddard solvent, or combinations of these. The solution was then applied to 2- by 4-inch vegetable-tanned sole leather specimens by immersing the leather in the solution. The pickup of fungicide was determined by differences in weight before and after immersion. The specimens were dried for a minimum of 24 hours and cut into four specimens 1 inch by 2 inches.

Testing was done according to Federal Specification KK-L-311a, Leather; Methods of Sampling and Testing, Methods 5011 and 5021, as follows: Mycelial mats were prepared by suspending the spores of a ripe, fruiting agar culture of *Aspergillus niger* (ATCC-6275) in 100 ml. of distilled water containing a small amount of wetting agent. Using a sterile camel's hair brush, the spore suspension was brushed uniformly over the surface of a hardened, sterile nutrient agar medium. Prior to inoculation, the culture medium was

sterilized in an autoclave for 20 minutes at 122°C., poured into sterile 3-inch Petri dishes under aseptic conditions and allowed to harden. After inoculation, the Petri dishes were incubated for approximately 48 hours at 29°C., relative humidity 85-90 per cent. At the end of the incubation period, the surface of the agar medium was covered with a white mycelial mat of the test organism.

Two of the 1-by 2-inch leather specimens were tumbled for three hours in shake bottles containing distilled water. These specimens plus a third non-tumbled specimen were placed on the mycelial mats of *A. niger*. The specimens were incubated for seven days at 29°C. at a relative humidity of 85 to 90 percent. A leather specimen free of fungicides was exposed concurrently as a control. If the control failed to show a heavy growth of mildew the test was repeated. The results of the test were determined by visual examination of the specimens.

Other specimens were tested for resistance to mildew growth by the American Leather Chemists' Association sand spore mixture. These specimens were incubated for 21 days under the same conditions described previously. Visual examination for mildew growth was made on the seventh, fourteenth, and twenty-first day of the incubation period.

(b) Second stage screening test or tropical room exposure test

Fungicides that were found to prevent or to a considerable extent inhibit mildew growth in the initial screening test were tested further by the tropical room exposure test. Leather specimens containing definite percentages of the fungicides were exposed for five weeks in the tropical room. The specimens were inspected for mildew growth once each week. In many cases a treatment that made the test leather mildew-proof in the initial screening test failed even to inhibit mildew growth in the tropical room exposure; no treatment shown to be ineffective by the initial screening test has been found effective by the tropical room test. Treatments that merely inhibit mildew growth in the initial screening test are always less effective in the tropical room exposure. Tropical room exposure appears to be a more severe test for leather fungicides.

The minimum percentage of a given fungicide required to make the test leather mildew resistant in the tropical room exposure test is assumed to be the percentage required for protection under the most severe conditions military leather items will encounter during service. 4-Nitrophenol, present in the leather at 0.3 per cent concentration, prevents mildew growth under the tropical room conditions. Generally, no higher concentration than 0.6 per cent has been used in the screening program since the search has been directed towards finding fungicides as effective as or more effective than 4-nitro-

phenol. If a compound did not exhibit at least some inhibition of mildew growth at 0.6 per cent, it was considered too ineffective to be of value. Others were unsuitable as leather fungicides because of obvious defects from the standpoint of color, stability, and solubility characteristics, or were known to possess properties considered harmful to human health. Consequently, only a few compounds out of those screened to date can be considered as substitutes or alternates for 4-nitrophenol as a leather fungicide in Quarter-master items. The more promising ones found in the screening program are reported upon in this paper.

3. FIELD EXPOSURE STUDY

3.1 Compounds

Eight fungicides were included in the field exposure study. All of these had prevented or greatly inhibited mildew growth at no more than 0.3 per cent in the initial screening test described in 2.3 (a). One of the fungicides was 4-nitrophenol, which was used as the comparison standard. The fungicides and their relative mildew-preventive efficiencies as found in the tropical room screening test described in 2.3 (b) are listed in table 1. The borderline concentrations referred to in the table are the minimum percentages of the fungicides required in the leather to make it mildew resistant under the tropical room conditions. With two exceptions, the fungicides prevented or greatly inhibited mildew growth at no more than 0.3 per cent as shown in table 1. A concentration of 0.6 per cent of 2-phenylphenol or 4-chloro-m-xyleneol was

TABLE 1
Screening Test Results (Tropical Room) of Fungicides used in Field
Exposure Study

Fungicide No.	Name	Borderline concentration Per Cent
1	4-thiocyanophenol	0.15
2	2-chloro-4-nitrophenol	0.15
3	4-nitrophenol (comparison standard)	0.30
4	bis(4-nitrophenyl) carbonate	0.30
5	tetrachlorohydroquinone	0.30
6	N-(trichloromethylthio) tetrahydrophthalimide	0.30*
7	2-phenylphenol	0.60**
8	4-chloro-m-xyleneol	0.60***

*Leather specimens containing 0.15 per cent of the fungicide were mildew resistant if the specimens had not been exposed to heat or light. Specimens heated for 1 week at 80° C. or exposed to sunlight for 1 week were not completely mildew resistant even when containing 0.60 per cent of the fungicide. A concentration of 0.30 per cent was taken as the borderline concentration for storage conditions.

**0.60 per cent did not prevent mildew growth. Nearly total inhibition of mildew growth required a minimum of 1.5 per cent deposition in subsequent tests.

***0.60 per cent did not prevent mildew growth. This compound was not tested at concentrations greater than 0.60 per cent.

not sufficient to prevent mildew growth. Subsequent testing has shown that a minimum concentration of 1.5 per cent of 2-phenylphenol was required for nearly total inhibition of mildew growth in the tropical room screening test. 4-Chloro-m-xyleneol was not tested at concentrations greater than 0.6 per cent. Leather specimens containing 0.15 per cent of N-(trichloromethylthio) tetrahydrophthalimide were mildew resistant in the tropical room screening test if the specimens had not been exposed to heat or light. Specimens heated for one week at 80°C. or exposed to direct sunlight for a week were not completely mildew resistant even when containing 0.6 per cent of this fungicide.

2-Phenylphenol and 4-chloro-m-xyleneol were included in the present study even though they had been found comparatively ineffective in the tropical room screening test. The reasons for this were: (1) to permit comparison of field exposure results and screening test results on some relatively ineffective fungicides as well as on relatively effective ones, and (2) because price and availability were thought to be factors potentially favorable to these fungicides if they were effective at higher concentrations than had been used in the screening tests. However, most of the compounds listed are not yet commercially available and no complete comparison as to cost can be made at this time. The National Bureau of Standards has not investigated methods of tannery applications and has not made tests for properties relating to human health hazards of any of the fungicides included in this study.

3.2 Preparation of Leather Samples

Three types of leathers were used in this study and are referred to as leathers A, B, and C:

A—Vegetable-tanned sole leather crust. This is the same type of leather that had been used in the screening test described previously.

B—Vegetable-tanned strap leather.

C—Chrome-retanned military-type upper leather.

All of these leathers were obtained free of 4-nitrophenol or any other fungicide. Four bends of leather A, four sides (half hides) of leather B, and two hides of leather C were used. Four like sets of leather samples were prepared, one each for the four exposure sites used (see section 3.6). Each of the eight fungicides was tested at three concentration levels, and three specimens of each type leather were used for each level. In addition, three controls (specimens containing no fungicide) of each type leather were included in each set of samples. The total number of specimens sent to each exposure site was therefore 225, and this amount constituted one set of samples. The size of each specimen was 2 by 4 inches. Only those portions of the bends, sides, or hides that were free from obvious flaws, such as scars and creases, were used. All specimens of each leather were cut out, mixed, and assigned to

fungicide treatments and to set of samples at random, and marked for identification.

3.3 Formulations

A 5-per cent (by weight) organic solvent stock formulation of each fungicide was first prepared. Portions of the stock formulation were then diluted to the concentrations required to deposit the desired percentage of fungicide in the leather. All dilutions of the stock formulation of a given fungicide were made with the same solvent mixture as was used in that stock formulation, with the exception of the formulation of 2-phenylphenol (see (d), this section).

The formulation compositions were as follows:

(a) For 4-thiocyanophenol, 2-chloro-4-nitrophenol, 4-nitrophenol, and 4-chloro-m-xyleneol: 10 per cent cyclohexanone, 20 per cent mineral oil, and trichloroethylene to make to 100 per cent.

(b) For bis(4-nitrophenyl) carbonate and N-(trichloromethylthio) tetrahydrophthalimide: 50 per cent dioxane, 10 per cent neat's-foot oil, and chloroform to make to 100 per cent.

(c) For tetrachlorohydroquinone: 30 per cent cyclohexanone, 20 per cent mineral oil, and trichloroethylene to make to 100 per cent.

(d) For 2-phenylphenol: A 5-per cent formulation of this compound was supplied by the manufacturer and used as supplied for the treatment of all specimens receiving the highest concentration of 2-phenylphenol. For the treatment of specimens receiving lower concentrations, the 5 per cent formulation was diluted with the solvent mixture shown under (a).

3.4 Concentration Levels of the Fungicides in the Leather

As has been mentioned, each fungicide was tested at three levels of concentration. (a) When the approximate borderline concentration had been established in screening tests (see the table in section 3.1), the three levels were: *Highest level*: Twice the borderline concentration; *Intermediate level*: Borderline concentration; *Lowest level*: One-half the borderline concentration. (b) For 2-phenylphenol and 4-chloro-m-xyleneol where the borderline concentrations had not been established in screening tests, the three levels were: *Highest level*: For 2-phenylphenol, the per cent deposition obtained by treatment of the leathers with the 5-per cent formulation supplied by the manufacturer was used. For leathers A, B, and C these depositions became, respectively, 1.5 per cent, 1.7 per cent, and 1.1 per cent, corresponding to differences in absorptivity of the leathers. For 4-chloro-m-xyleneol, 1.2 per cent was used, or twice the highest concentration used in the previous screening tests: *Intermediate level*: 0.60 per cent; *Lowest level*: 0.30 per cent.

3.5 Treatment of Leather Specimens

The leather specimens were treated by dipping into formulations of the required concentrations to give the desired per cent depositions of the fungicides in the leather. The required formulation concentrations were calculated from the average per cent of the formulation absorbed by the respective leathers during the treatment, determined in preliminary experiments. The calculated per cent depositions of the fungicides in the leathers were checked by analyses in the cases of 4-nitrophenol, tetrachlorohydroquinone, and 2-phenylphenol, for which analytical methods were available^{5, 6, 7}. The agreement between the calculated fungicide percentages and those found by analyses was of the order that could be expected from the variability in formulation absorption by individual specimens. This variability is shown in the following table.

Leather	No. of specimens tested	Formulation absorption	
		Average	Range*
		Per cent	Per cent
A	24	45.0	15.9
B	24	53.0	11.7
C	24	38.0	14.8

*Largest minus smallest.

All specimens of one leather receiving the same treatment were treated simultaneously. The controls (specimens containing no fungicide) were treated with the solvent mixture described in (a), section 3.3. After treatment, the specimens were placed in an oven at 80°C. for 6 hours in order to remove the volatile solvents.

3.6 Exposure Conditions

Four like sets of leather samples, prepared and treated as described, were exposed as follows:

Sample set 1—Exposed at Yuma, Arizona (desert weathering, hot-dry environment). The samples were exposed to direct sunlight but were sheltered from desert storms. They were reversed twice during the exposure period so that grain and flesh surfaces received about equal amounts of sunlight. The exposure lasted for three months (from July 6 to October 6, 1954) and the samples were subsequently tested for mildew resistance by storage in the tropical room at Fort Belvoir, Virginia.

Sample set 2—Exposed on a roof at the National Bureau of Standards, Washington, D. C. (weathering, moderate hot-dry environment). The samples were exposed in the open during working hours, but sheltered the rest of the

time and during rainstorms. Grain and flesh surfaces received about equal sunlight exposure. The exposure period and subsequent mildew-resistance test were the same as described for sample set 1.

Sample set 3—Not exposed to weathering. The samples were stored in the laboratory and were not exposed to any direct weathering before they were tested for mildew resistance by exposure in the tropical room.

Sample set 4—Exposed in Panama (mildew-growing conditions, hot-humid environment). The samples were protected from direct sunlight and rain, but were otherwise exposed to an ambient environment at a site located in the jungle on the Atlantic side of the Isthmus of Panama. Weekly observations of mildew growth on these samples were made for a period of 12 weeks (from August 3 to October 25, 1954) after growth began to appear on some specimens. Previous to this period, the samples had been exposed at two other Panama sites for a total of five weeks, during which time no mildew growth appeared even on untreated specimens. The environmental conditions at the first two sites apparently were unsuitable for rapid mildew growth and made relocation necessary. Summaries of the climatological data collected at the various exposure sites, and the daily cycle of temperature and per cent relative humidity of the tropical room, are given in an appendix to this paper, although no discussion of the effect of the climatological conditions on mildew growth is included in this study.

It may be observed that none of the exposure conditions was such that specific information about the effect of leaching by water on the permanency of the fungicidal treatments could be obtained. However, both in the Panama exposure and in the tropical room exposure the samples were wetted to the point of dripping during repeated condensations of the humid atmospheres. Some leaching effect is, therefore, considered to be included in the over-all effectiveness of the fungicides under these conditions.

All four sets of samples were prepared during the first part of May 1954 and sent to the various exposure sites during the following month. The samples exposed at Yuma, Ariz., (sample set 1) were returned to the National Bureau of Standards about the middle of October 1954, and sets 1, 2, and 3 were then tested for mildew resistance in the tropical room simultaneously.

The exposures of the samples at Yuma, Ariz., and in Panama as well as the observations on mildew growth on the latter samples were conducted by the Quartermaster Research and Development Field Evaluation Agency, Fort Lee, Virginia.

3.7 Collection and Treatment of Test Data on Mildew Growth

Weekly observations on mildew growth were made and recorded separately for the grain and flesh areas and the edges of each specimen. The amount of mildew growth was indicated by the percentage of the given area covered with it and was designated by the following code numbers:

0—No mildew growth.

1—From scattered colonies to 25 per cent of the area covered with mildew growth.

2—From 26 per cent to 50 per cent of the area covered with mildew growth.

3—From 51 per cent to 75 per cent of the area covered with mildew growth.

4—From 76 per cent to 100 per cent of the area covered with mildew growth.

The treatment of the original test data to obtain the data shown in figures 1 - 4 was performed as follows:

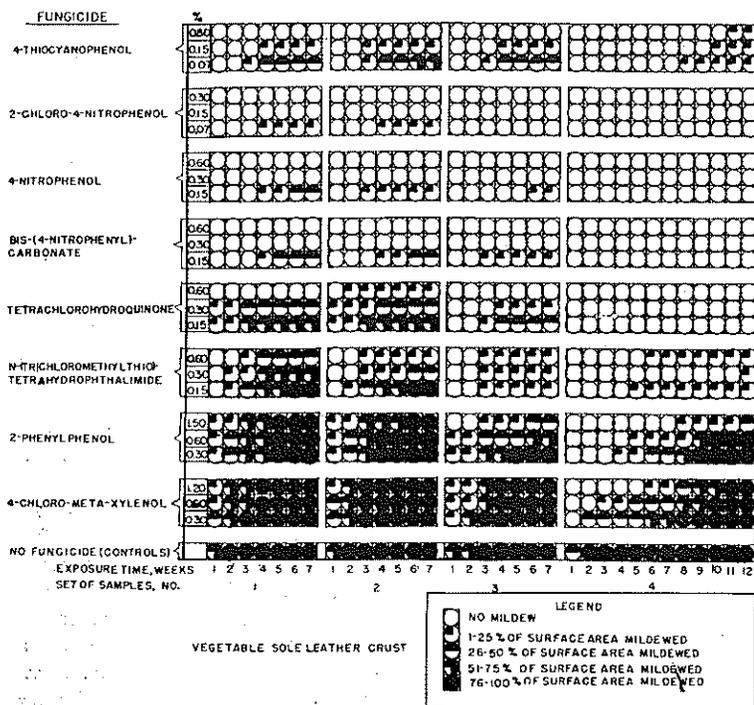


FIGURE 1.—Weekly observations on mildew growth on vegetable-tanned sole leather crust.

Sample set 1 was exposed to weathering at Yuma, Ariz.

Sample set 2 was exposed to weathering on NBS roof.

Sample set 3 was not exposed to weathering (stored in NBS lab.)

These samples were tested for mildew resistance by exposure in a tropical room.

Sample set 4 was not exposed to weathering prior to being tested for mildew resistance by exposure at a Panama jungle site.

(a) Derivation of a single value for each specimen

If the values recorded for grain and flesh on a given specimen were the same, that value was taken as the value for the specimen. If they were not the same, the higher value was taken as the specimen value rather than the average. It is assumed that mildew growth is equally objectionable whether it grows on grain or flesh. If a fungicide does not give the same protection to both sides of the leather, it should be rated according to the area on which it is least effective.

Mildew growth on the edges was neglected. The sizes of edge areas are negligible compared to grain or flesh areas. Furthermore, the cut and loose

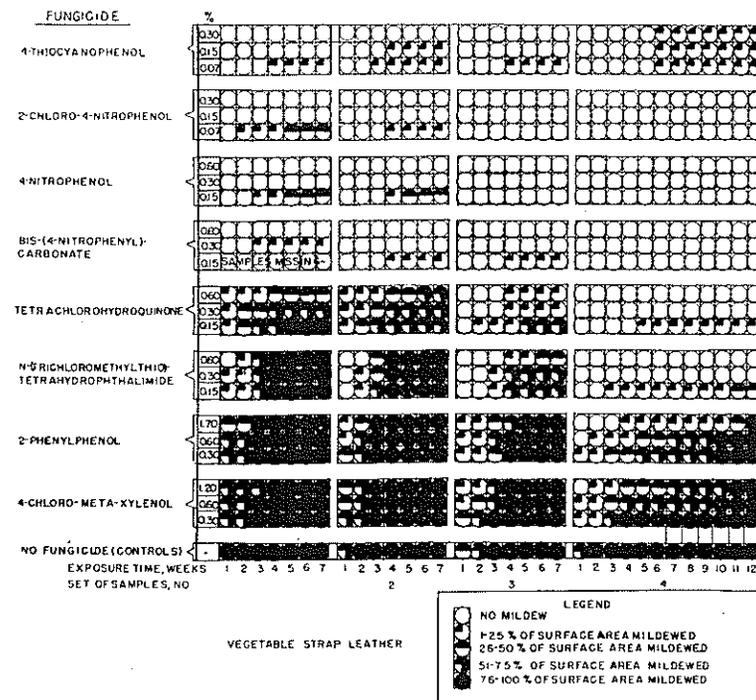


FIGURE 2.—Weekly observations on mildew growth on vegetable-tanned strap leather.

Sample set 1 was exposed to weathering at Yuma, Ariz.

Sample set 2 was exposed to weathering on NBS roof.

Sample set 3 was not exposed to weathering (stored in NBS lab.)

These samples were tested for mildew resistance by exposure in a tropical room.

Sample set 4 was not exposed to weathering prior to being tested for mildew resistance by exposure at a Panama jungle site.

fibers of edge areas are not typical of the surfaces that are of most concern in leather mildew prevention.

(b) Derivation of a single value for each treatment

After a single value for each of the three specimens receiving the same treatment was obtained as explained in (a), the average of the three values was taken as the mildew growth for that treatment. This average was rounded off to the nearest whole number.

3.8 Results

Figures 1, 2, and 3 are graphs of all data on observations of mildew growth

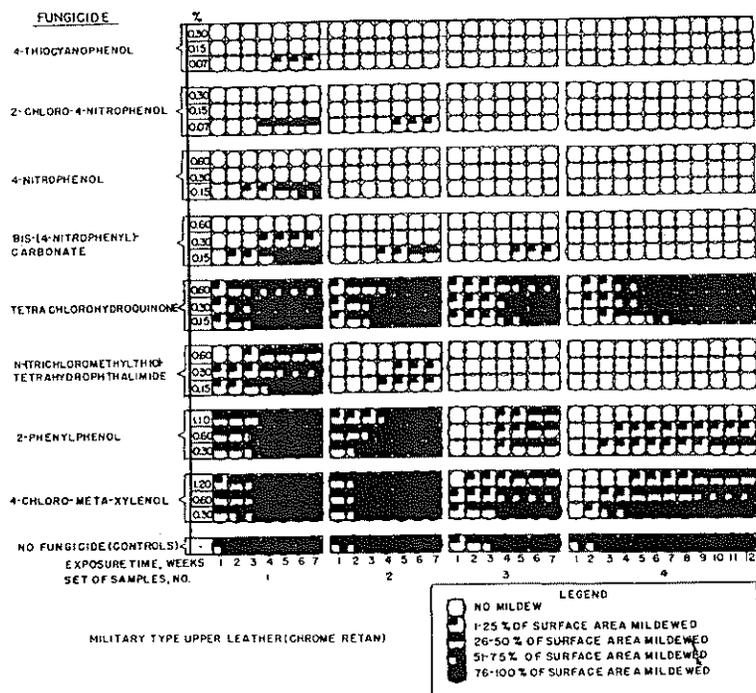


FIGURE 3.—Weekly observations on mildew growth on chrome-retained military-type upper leather.

Sample set 1 was exposed to weathering at Yuma, Ariz.

Sample set 2 was exposed to weathering on NBS roof.

Sample set 3 was not exposed to weathering (stored in NBS lab.).

These samples were tested for mildew resistance by exposure in a tropical room.

Sample set 4 was not exposed to weathering prior to being tested for mildew resistance by exposure at a Panama jungle site.

after the original data have been treated as described in section 3.7; they show the results for sole leather crust, vegetable strap leather, and military-type upper leather, respectively. Weekly accumulations of mildew growth on leathers containing the various concentrations of each of the eight fungi-

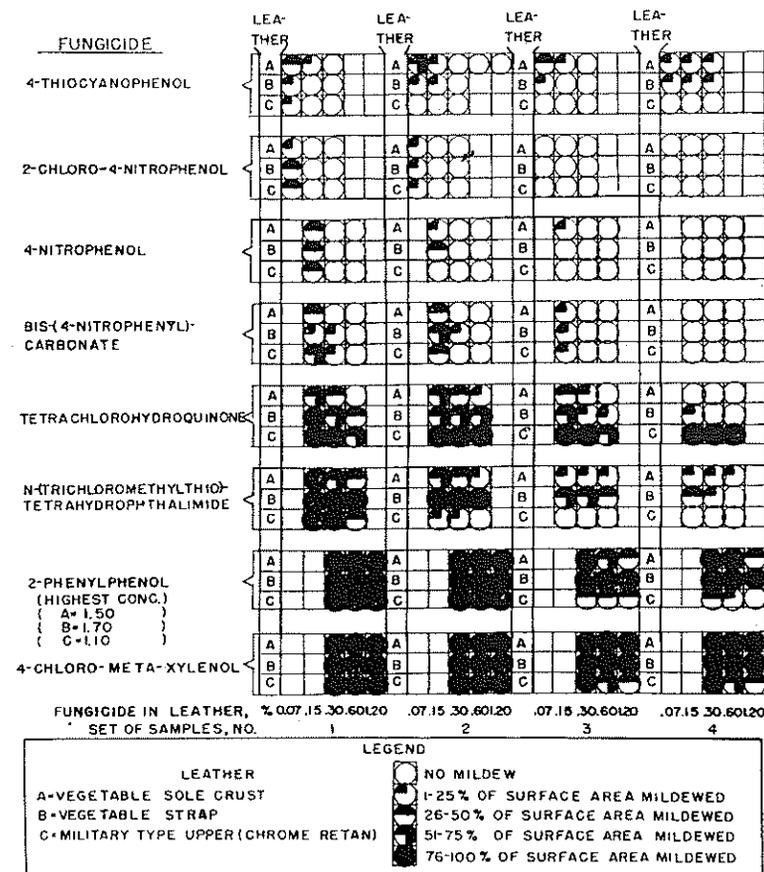


FIGURE 4.—Final mildew growth on leather after completion of mildew resistance tests. Sample set 1 was exposed to weathering at Yuma, Ariz. Sample set 2 was exposed to weathering on NBS roof. Sample set 3 was not exposed to weathering (stored in NBS lab.). These samples were tested for mildew resistance by exposure in a tropical room. Sample set 4 was not exposed to weathering prior to being tested for mildew resistance by exposure at a Panama jungle site.

cides are shown. The tropical room exposure was terminated after five weeks. The Panama exposure was terminated after 12 weeks at the final exposure site. Figure 4 is a compilation of information taken from figures 1, 2, and 3; it shows the final accumulations of mildew growth on all leathers of the four sets of samples after completion of the mildew-resistance tests.

3.9 Discussion

(a) Tropical room exposure versus Panama exposure

Examination of the data for the two sets of samples not exposed to weathering prior to the mildew growth exposure (sample sets 3 and 4) shows that initial mildew growth generally appeared on corresponding specimens after shorter exposure times in the tropical room than at the Panama site (figures 1, 2, and 3). This is to be expected since the tropical room is controlled so as to produce continuous optimum conditions for mildew growth, while at the Panama site natural atmospheric conditions prevailed. The latter conditions were not uniformly optimum for mildew growth. Figure 4 shows that the two sets of samples yielded final data that are in good agreement, except those for tetrachlorohydroquinone in leathers A and B. However, there are clear indications that a 5-week exposure period in the tropical room is at least as severe a test for mildew resistance as a 12-week exposure period at the Panama jungle site.

(b) Effect of weathering

Comparison of the three sets of samples exposed to mildew growth in the tropical room, but previously exposed to different weathering conditions (sample sets 1, 2, and 3), shows that weathering appears to make the leathers more susceptible to mildew growth. From figures 1, 2, and 3 it is seen that mildew generally appeared first on weathered samples. This difference is persistently reflected in the final data also, as may be observed from figure 4. The greater mildew susceptibility of weathered samples may be due to greater accumulation of dirt and spores on them compared to samples that were stored in the laboratory. It may also be due to fungicide removal or degradation caused by weathering.

The results of this study show no appreciable differences among the fungicides in sensitivity to weathering. In the screening test evaluation (see the table in section 3.1), N-(trichloromethylthio)tetrahydrophthalimide exhibited fungicidal effectiveness when leather specimens containing it were not exposed to heat or light, and decreased effectiveness after exposure to these conditions. In the field exposure study the instability of this fungicide when exposed to heat and light (weathering) was not so marked. Possibly the long storage period of the unweathered treated samples (about 6 months) before they were tested for mildew resistance had an effect similar to direct weather-

ing and so obscured the characteristics observed in the screening tests. In the screening tests the samples were tested for mildew resistance within about one month after treatment.

There is no appreciable difference in mildew resistance between the samples exposed to weathering at Yuma, Ariz., and those exposed on a roof at the National Bureau of Standards, Washington, D. C. There was, however, a surprising contrast between the two exposures in their effects on the color of the two vegetable-tanned leathers. The Yuma exposure of these leathers made them lighter in color than they were originally, while the NBS roof exposure made them darker. The explanation for this phenomenon presumably is associated with differences in atmospheric conditions at the two locations. During the exposure in the tropical room, the color differences between the two sets of samples disappeared.

Both weathering exposures produced stiffening and curling of the leather specimens. Vegetable strap leather was affected the most in these respects and military upper leather with high grease content was affected the least. The stiffening and curling were more marked on the samples exposed at Yuma than on those exposed at NBS.

(c) Relative effectiveness of the fungicides used in this investigation

The following ranking according to decreasing effectiveness is derived from the over-all results shown in figure 4.

(1) 2-Chloro-4-nitrophenol: No more than 0.15 per cent was required to make all leathers of all sets of samples mildew resistant. This fungicide appears to be slightly more effective than 4-nitrophenol and is the only one of those investigated that is ranked more effective than 4-nitrophenol.

(2) 4-Thiocyanophenol and 4-nitrophenol: No more than 0.30 per cent was required to make all leathers of all sets of samples mildew resistant except for a very slight amount of mildew found on leathers A and B, Panama samples, that contained 0.30 per cent of 4-thiocyanophenol.

(3) Bis(4-nitrophenyl) carbonate: No more than 0.60 per cent was required to make all leathers of all sets of samples mildew resistant, and this concentration was required only for leathers B and C of the set of samples exposed to weathering at Yuma, Ariz., and for leather B of the set of samples exposed to weathering at NBS. In all other cases, no more than 0.30 per cent was required for prevention of mildew growth.

(4) N-(trichloromethylthio)tetrahydrophthalimide: This fungicide produced somewhat erratic results. The highest concentration used, 0.60 per cent, mildewproofed only one weathered sample, which was leather C of the samples exposed to weathering at NBS. Three unweathered samples were mildewproofed. These samples and the lowest concentrations required for

mildewproofness in each case were: Leather C, samples kept in NBS laboratory until tested for mildew resistance, 0.15 per cent; leather B, Panama samples, 0.60 per cent; and leather C, Panama samples, 0.15 per cent.

The fungicide apparently is sufficiently effective to mildewproof less mildew-susceptible leathers, such as leather C, under storage conditions at a concentration of about 0.60 per cent. Mildew growth on the more susceptible vegetable-tanned leathers can also be expected to be considerably inhibited by this concentration under storage conditions. The fungicide may be somewhat sensitive to weathering exposure. Although this characteristic was not clearly indicated in the field exposure study, it was very noticeable in the preliminary screening tests where the time interval between treatment of leather and test for mildew-resistance was much shorter [see section 3.9 (b)].

(5) Tetrachlorohydroquinone: The highest concentration used, 0.60 per cent, mildewproofed only leather A of the Yuma samples, leather A of the NBS unweathered samples, and leathers A and B of the Panama samples. The concentration levels required for mildew prevention in these four cases were 0.60, 0.60, 0.15, and 0.30 per cent, respectively.

This fungicide showed poor and erratic effectiveness in this field exposure test compared to the effectiveness shown in the tropical room screening tests in which 0.30 per cent had consistently provided mildewproofness of leather A (table 1). However, in earlier screening tests erratic behavior of this fungicide had been observed. It was thought then that it was due to an unsuitable formulation used, from which the fungicide had a tendency to precipitate. The formulation used in this investigation appeared to be stable for several months and, therefore, cannot be responsible for the erratic behavior.

Apparently tetrachlorohydroquinone is especially ineffective with leather C. Since this leather has a much higher oil content than leathers A and B, and tetrachlorohydroquinone is relatively insoluble in oil, it is suggested that these combined factors may be responsible for the observed behavior. It is possible that fluctuations in surrounding conditions bring about slow migration of the oil in a leather of high oil content. Through this process, after a considerable time the oil that comes to the surface of the leather may contain little, if any, of the oil-insoluble fungicide. The leather surfaces would then become mildew susceptible. If this is what happens, the oil solubility of a leather fungicide is a very important property from the standpoint of the fungicide's usefulness.

The possibility that tetrachlorohydroquinone is particularly ineffective with leather C because of the presence of chromium in this leather, contrasting to leathers A and B, seems unlikely but probably should not be excluded.

(6) 2-Phenylphenol and 4-chloro-m-xyleneol: Only one case of mildewproofness was obtained with 2-phenylphenol. This was leather C of the Panama samples. None of the samples treated with 4-chloro-m-xyleneol was mildew-

proofed. In view of the high concentration levels used, up to 1.70 per cent for 2-phenylphenol and up to 1.20 per cent for 4-chloro-m-xyleneol, both fungicides are judged to be relatively ineffective mildewproofing agents for leather.

(d) *Correlation between field evaluation and screening tests*

Generally the results of this field exposure study show the same relative effectiveness of the fungicides as was previously found in screening tests where the tropical room exposure test was used (see section 3.1, table). However, tetrachlorohydroquinone proved less effective than was expected from the screening tests, mainly because it was so ineffective in the case of leather C, which was not used in screening tests. It has been assumed that a fungicide treatment that mildewproofs a vegetable-tanned leather will also mildewproof any other type of leather, since the former is more mildew susceptible. Apparently, there are exceptions to this generalization.

According to the initial screening test, as has already been indicated (section 3.1), leather containing 0.30 per cent of any of the fungicides included in this study was found to be mildew resistant, or very nearly so. The field performance cannot be correctly predicted from this test, except that all treatments failing to prevent mildew growth in the initial screening test can safely be assumed to fail under tropical jungle conditions. The tropical room exposure test may be assumed to predict fairly correctly the field performance under tropical jungle conditions and is the most dependable test available for evaluation of efficiencies of leather fungicides.

3.10 *Conclusions*

(a) In addition to 4-nitrophenol, it was found that 2-chloro-4-nitrophenol, 4-thiocyanophenol, and bis(4-nitrophenyl) carbonate prevent mildew growth on leathers under tropical jungle conditions at concentrations no higher than 0.60 per cent and are adequately stable under severe weathering conditions.

Compared to the effectiveness of 4-nitrophenol, 2-chloro-4-nitrophenol is slightly more effective, 4-thiocyanophenol is about equally effective, and bis(4-nitrophenyl) carbonate is slightly less effective, considering the over-all results of this study.

(b) N-(trichloromethylthio)tetrahydrophthalimide is moderately effective under storage conditions, but may be somewhat sensitive to severe weathering. At a concentration of 0.60 per cent this fungicide may be expected to mildewproof chrome retan leather and to considerably inhibit mildew growth on vegetable leathers under storage conditions.

(c) Tetrachlorohydroquinone gives erratic results and probably will not be dependable as a leather fungicide unless the causes of its erratic behavior are found and corrected.

(d) 2-Phenylphenol and 4-chloro-m-xylene do not prevent or to any considerable extent inhibit mildew growth on leathers under severe mildew-growing conditions even at high concentrations (up to 1.70 per cent for 2-phenylphenol and up to 1.2 per cent for 4-chloro-m-xylene).

(e) The tropical room exposure test for evaluation of leather fungicides predicts quite correctly the performance of the fungicides under the most severe mildew-growing conditions that may be encountered by military leather items. The agar plate test with *A. niger* and the ALCA mixed spores method (Methods 5011 and 5021 of Fed. Spec. KK-L-311a) are useful only as initial screening tests for leather fungicides. A fungicide treatment failing in these tests can safely be rejected. A fungicide treatment passing these tests will not necessarily be satisfactory under severe mildew-growing conditions in tropical jungles.

ACKNOWLEDGMENT

The authors acknowledge the cooperation by staff members of the Quartermaster Research and Development Field Evaluation Agency, Fort Lee, Virginia. The permission of the Office of the Chief of Engineers, U. S. Army, to use the tropical room at the Engineering Center, Fort Belvoir, Va., is greatly appreciated.

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Received August 20, 1955.

Appendix—Climatological Conditions

1. Yuma, Arizona (observations at Yuma Test Station, Yuma, Ariz.)

Period	No. of daily observations made	Temperature			Relative humidity			Precipitation		
		Mean °F	Max-imum °F	Min-imum °F	Mean Per cent	Max-imum Per cent	Min-imum Per cent	Total in.	Fre- quency days	Daily average in.
1954										
July 6-31	26	93.5	116.0	75	47.2	96	15	0.19	3	0.007
Aug. 1-31	30	90.4	115.0	56	41.2	82	10	0.11	1	0.004
Sept. 1-30	30	89.4	108.0	69	35.9	90	5	0.24	2	0.008
Oct. 1-6	6	81.5	100.0	64	40.8	80	10	0.00	0	0.000
Average for total period		88.7	109.8	66	41.3	87	10			0.006

2. Washington, D. C. (observations at National Airport)

July 6-31	26	78.7	103.0	62	62	94	24.0	1.09	7	0.16
Aug. 1-31	31	75.4	94.0	58	68	96	34.0	3.15	11	0.29
Sept. 1-30	30	73.5	99.0	50	67	95	32.0	0.63	8	0.08
Oct. 1-6	6	76.5	94.0	54	75	95	40.0	0.45	3	0.15
Average for Total period		76.0	97.5	56	68	95	32.5			0.17

3. Panama Test Sites (temperature and relative humidity observations were obtained with a hygrothermograph located in the shelter with the leather samples. The observations on precipitation were obtained from the Naval Research Laboratory Tropical Experimental Station, Coco Solo Naval Station, Panama Canal Zone.)

Site	Period	No. of daily observations made	Temperature			Relative humidity			Precipitation		
			Mean °F	Max-imum °F	Min-imum °F	Mean Per cent	Max-imum Per cent	Min-imum Per cent	Total in.	Fre- quency days	Daily average in.
1*	June 30- July 15	15	81.0	108	59	62.5	97	30	7.34	9	0.49
2**	July 16- Aug. 3	19	80.0	90	71	83.4	100	58	5.70	14	0.30
3***	Aug. 4- Oct. 28	86	78.7	90	72	86.5	99	51	35.75	67	0.53

* Corozal General Depot, U. S. Army, cleared area.

** Corozal General Depot, U. S. Army, thin jungle area.

*** Jungle Hut of the Naval Research Laboratory Tropical Exposure Station, Galata Road, near Coco Solo Naval Station.

No mildew grew on any of the samples during exposure at sites 1 and 2. The data on mildew growth were obtained during the exposure at site 3.

4. Tropical room at the Engineering Center, U. S. Army, Fort Belvoir, Virginia.

Daily cycle of temperature and relative humidity.

0400—0800 hours	75°F, 95 per cent relative humidity.
0800—1200 hours	Temperature increasing from 75° F to 85° F and relative humidity decreasing from 95 per cent to 90 per cent.
1200—2000 hours	85° F, 90 per cent relative humidity.
2000—0400 hours	Temperature decreasing from 85° F to 75° F and relative humidity increasing from 90 per cent to 95 per cent.