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ANTIFUNGAL ANTIBIOTICS

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FOREWORD

This report was prepared by the University of Rhode Island under USAF Contract No. AF 33(616)-154. The contract was initiated under Task No. 73124, "Preservative Chemicals", formerly RDO No. 611-15, "Preservative Chemicals", and was administered under the direction of the Materials Laboratory, Directorate of Research, Wright Air Development Center, with Dr. Alton E. Prince acting as project engineer.

The purpose of this report is to evaluate the performance of a number of products for a specific application. Many of the materials evaluated were not developed or intended by the manufacturer for the conditions to which they have been subjected. Any failure or poor performance of a material therefore is not necessarily indicative of the utility of the material under less stringent conditions or for other applications.

ACKNOWLEDGEMENT

The initial phase of this investigation was conducted by Mr. W. Keith Smith, a visiting research professor from the University of Bristol, England, from 1 May 1952 until December 1952. Then Dr. C. A. Apostolides, formerly of the University of Oklahoma and the University of Athens, Greece, was engaged to carry forward the research from January 1953 until 30 June 1953. Miss Barbara Champlin has faithfully and accurately carried out the technical phases of the project since June 1952. She deserves the most credit for the experimental results obtained. Supervision and interpretation of data have been the responsibility of Dr. Frank L. Howard, Head, Department of Plant Pathology-Entomology. The willingness of industrial and governmental laboratories to cooperate by providing candidate materials gratis has made the research possible.

ABSTRACT

One hundred forty-three candidate antibiotics furnished by twenty-one agencies were tested for their fungicidal activity. The molds specified and used were Aspergillus niger, A. terreus, Myrothecium verrucaria, and Chaetomium globosum. The difficult to inhibit spores of Alternaria solani, Helminthosporium carbonum and Curvularia lunata were used for germination tests. While most of the compounds were natural antibiotics, others were synthetic organic chemicals. Evaluation of antifungal action was obtained by four techniques: toxic agar in petri plates, spore germination inhibition on glass slides, impregnated cellulose pads on seeded agar, and retention of tensile strength of impregnated thread after exposure to molds.

The five most antifungal antibiotics were found to be: comirin, benzyl mucochlorate, endomycin, netropsin sulfate, and rimocidin. The Squibb compound, 2-pyridinethiol 1-oxide and its twelve salts, compared favorably with standard copper 8-quinolinolate as a mildew inhibitor without necessarily staining the cloth. A furfural derivative exhibited evidence of high fungicidal action.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDER:

M. R. WHITMORE
Technical Director
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INTRODUCTION

This research was undertaken to investigate the antifungal properties of antibiotics. It was felt that among the several hundred substances synthesized by living matter reported in the literature, which inhibit the development of competing organisms, some would stop the growth of fungi that cause deterioration of material. At least, fungitoxic chemical structures might be uncovered which would permit the synthetic production of antifungal compounds with more desirable characteristics than those currently available commercially.

Fungi are known to produce a great variety of specialized metabolic products. For example, a few fungi produce enzymes which destroy cellulosic fibers. Other organisms, it is believed, have survived by competitive selection through the development of antagonistic or antidotal metabolites which are called antibiotics. Although antibiotics were first conceived of as biostatic substances derived from fungous metabolism, three additional origins may now be recognized; bacterial, green plants, and synthetic. Examples of all of these categories have been examined in this investigation. Among the chemical metabolic products recognized as synthesized by protoplasm are quinones and xanthenes which are specifically toxic. Due to the wide variation in metabolites utilized by fungi, no universally antifungal substance can be anticipated.

In evaluating the data presented it must be kept in mind that the candidate materials were tested on the basis of 100 percent active ingredient or purity as received. Since most antibiotics are unknown in composition, the purity of the sample supplied was unknown. Many were available in only small quantities.

MATERIALS

Antifungal Agents. One hundred forty-three candidate compounds were supplied by twenty-one sources. Table 1 is a list of the name, date of receipt and supplier of the materials examined. Each was given a R. I. accession number and a data sheet filled out for inclusion in a loose-leaf compilation of potential antifungal antibiotics with notes on their properties. The chemical and biological characteristics of some of the products tested are summarized in Table 2 to indicate their varied producing agent, chemical composition and toxic action. Physical properties have a direct bearing on the potential usefulness and adaptation of fungitoxic agents and therefore these are shown in Table 3 for the materials evaluated. Solubility particularly governs the methods that may be employed to determine inherent antifungal action, the category of test organism that the compound will inhibit, and ultimately the means of application and use.

As a reference standard for comparison copper 8-quinolinolate (Milmer 1) was obtained from the Monsanto Chemical Company. Later it was learned that the Scientific Oil Compounding Co., Inc., had improved formulations known as Cunilates 2174, 2174-WP, and 2419 containing 10 percent solubilized copper 8-quinolinolate and samples were obtained and compared. This product is known as "oxine" in Great Britain and products containing the copper salt have been used successfully for plant disease control.

Glassware. Pyrex glassware was used throughout the screening program for keeping stock cultures of the test fungi, preparing and measuring media, and pouring agar surfaces in petri plates. Non-corrosive micro slides were used in spore inhibition studies.

Chemicals. CP grade dextrose and reagent grade inorganic salts were used in preparing standard nutrient agar. Bacto-yeast extract and Bacto-tryptone, which are standard Difco products, were employed in the preparation of special media. The "Bacto" agar and potato dextrose agar used were standard Difco products.

Test Fungi. The four fungi stipulated by the contract and required in the "Official Screening Test Method" were supplied by the Materials Laboratory, Directorate of Research, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio, and are listed as follows:

Aspergillus niger TC-215-4247
Aspergillus terreus PQMD 82j
Myrothecium verrucaria USDA 1334.2
Chaetomium globosum USDA 1042.4

In spore inhibition studies *Alternaria solani*, *Helminthosporium carbonum*, and *Curvularia lunata* were employed as test fungi; these three organisms were obtained from stock cultures in the University of Rhode Island, Department of Plant Pathology.

Table 1.--List of Candidate Antibiotics with Date of Receipt and Source.

No.	Name of Antibiotic	Date Received	Source
1	*Puromycin	10/14/52	Lederle
2	Actidione	6/5/52	Upjohn Co.
3	Albidin	6/5/52	Imperial Chem.
4	Alternaric acid	6/5/52	" "
5	Alternaric acid	5/ /52	" "
6	Aspergillic acid	6/9/52	U.S.D.A., N.R.R.L.
7	Aureothricin	10/6/52	U.S.I. Chem.
8	Bacitracin, Zn salt	10/14/52	Comm. Solv.
9	Candicidin A	1/30/53	Heyden Chem.
10	Candicidin B	1/30/53	" "
11	Clavacin	6/9/52	U.S.D.A., N.R.R.L.
12	Comirin	6/25/52	Colonial Res. Inst.
13	Endomycin	6/3/52	Upjohn Co.
14	Fradicin	10/14/52	Comm. Solv.
15	Frequentin	6/5/52	Imperial Chem.
16	Gladiolic acid	6/5/52	" "
17	Gliotoxin	6/5/52	" "
18	Griseofulvin	6/5/52	" "
19	Kojic acid	6/9/52	U.S.D.A., N.R.R.L.
20	Methyl gallate	7/28/52	Mallinckrodt Chem.
21	Musarin	10/6/52	U.S.I. Chem.
22	Mycophenolic acid	6/5/52	Imperial Chem.
23	Neomycin sulfate	6/3/52	Upjohn Co.
24	Netropsin sulfate	4/25/52	Pfizer & Co.
25	Netropsin disulfate	2/2/53	Nat.Res.Council, C.B.C.
26	Nystatin	9/18/52	Squibb Institute
27	Ortho	9/18/52	" "
28	Patulin	6/5/52	Imperial Chem.
29	Polypeptin	12/3/52	U.S. Publ. Health
30	Protoanemonin	3/27/53	Columbia Univ.
31	Rimocidin	4/7/52	Pfizer & Co.
32	Rimocidin, Cu salt	4/7/52	" "
33	Rimocidin SO_4	3/31/52	" "
34	Streptomycin SO_4	11/14/52	Heyden Chem.
35	Streptomycin SO_4, dihydro	11/14/52	" "
36	Streptothricin SO_4	11/14/52	" "
37	Thiolutin	4/7/52	Pfizer & Co.
38	Thiolutin	3/31/52	" "
39	Thiolutin, Crude	4/7/52	" "
40	Tomatine	12/15/52	U.S.D.A., E.R.R.L.
41	Trichothecin	1/7/53	Imperial Chem.
42	Ustilagic acid	6/9/52	U.S.D.A., N.R.R.L.

* Puromycin was formerly called Achromycin Hydrochloride.

Table 1.--continued.

No.	Name of Antibiotic	Date Received	Source
43	Ustilagic acid	10/6/52	U.S.I. Chem.
44	Viridin	6/5/52	Imperial Chem.
45	1501	4/16/53	Ben Venue Lab.
46	1523A	4/16/53	" " "
47	1537A	4/16/53	" " "
48	A7604-5	8/4/52	Bristol Labs.
49	BL-3	8/4/52	" "
50	BL-7	8/4/52	" "
51	BL-31	8/4/52	" "
52	BL-60	8/4/52	" "
53	BL-72	8/4/52	" "
54	BL-81	8/4/53	" "
55	BL-96	8/4/53	" "
56	BL-99	8/4/52	" "
57	BL-100	8/4/52	" "
58	BL-104	8/4/52	" "
59	BL-130	8/4/52	" "
60	BL-138	8/4/52	" "
61	BL-190	8/4/52	" "
62	BL-226	8/4/52	" "
63	BL-245	8/4/52	" "
64	BL-247	8/4/52	" "
65	BL-251	8/4/52	" "
66	BL-262	8/4/52	" "
67	BL-336	8/4/52	" "
68	BL-404	8/4/52	" "
69	BL-413	8/4/52	" "
70	BL-415	8/4/52	" "
71	BL-456	8/4/52	" "
72	BL-471	8/4/52	" "
73	BL-496	8/4/52	" "
74	9R7098	11/25/52	Merck & Co.
75	52R282	11/25/52	" "
76	52R3023	11/25/52	" "
77	52R3899	11/25/52	" "
78	52R4009	11/25/52	" "
79	52R5208	11/25/52	" "
80	52R5989	11/25/52	" "
81	52R6106	11/25/52	" "
82	52R6107	11/25/52	" "
83	Pfizer #1	6/25/52	Pfizer & Co.
84	Pfizer #3	6/25/52	" "

Table 1.--continued.

No.	Name of Antibiotic	Date Received	Source
85	Pfizer #4	6/25/52	Pfizer & Co.
86	Pfizer #5	6/25/52	" "
87	Pfizer #6	6/25/52	" "
88	Pfizer #7	6/25/52	" "
89	Pfizer #8	6/25/52	" "
90	Pfizer #9	6/25/52	" "
91	Pfizer #10	6/25/52	" "
92	Pfizer #11	6/25/52	" "
93	Pfizer #12	6/25/52	" "
94	Pfizer #13	6/25/52	" "
95	Pfizer #14	6/25/52	" "
96	Pfizer #16	6/25/52	" "
97	Pfizer #18	6/25/52	" "
98	Pfizer #20	6/25/52	" "
99	Pfizer #21	6/25/52	" "
100	Pfizer #22	6/25/52	" "
101	Pfizer #24	6/25/52	" "
102	Pfizer #27	6/25/52	" "
103	M4019	11/18/52	Squibb Institute
104	M4348	11/18/52	" "
105	M4489	11/18/52	" "
106	M4575	11/18/52	" "
107	MC2113, SEP_4-B-14	9/18/52	" "
108	MC3277, SEP_4-D	9/18/52	" "
109	MC3702	3/12/53	" "
110	MC3711	3/12/53	" "
111	MC7728	3/12/53	" "
112	MC7729	3/12/53	" "
113	MC7730	3/12/53	" "
114	MC7731	3/12/53	" "
115	MC7732	3/12/53	" "
116	MC7733	3/12/53	" "
117	MC7734	3/12/53	" "
118	MC7735	3/12/53	" "
119	MC7736	3/12/53	" "
120	MC7737	3/12/53	" "
121	See #13	10/5/53	Upjohn Co.
122	Antimycin A-35	10/13/53	Univ. of Wisconsin
123	Helixin B	10/13/53	Univ. of Wisconsin
124	See #77	10/14/53	Merck & Co.
125	Magnamycin	10/22/53	Pfizer & Co.
126	Bacillomycin "B"	12/11/53	Wyeth Inst.

Table 1.--continued.

No.	Name of Antibiotic	Date Received	Source
127	See #108	12/16/53	Squibb Institute
128	2-Chloroethyl mucochlorate 9R3122	12/22/53	Merck & Co.
129	Isoamyl mucochlorate 9R4528	12/22/53	Merck & Co.
130	See #107	1/8/54	Squibb Institute
131	CPI-H-2	3/12/54	Crop Protection Inst.
132	CPI-H-3	3/12/54	" " "
133	CPI-H-8	3/12/54	" " "
134	CPI-H-10	3/12/54	" " "
135	CPI-H-13	3/12/54	" " "
136	CPI-F-8	3/12/54	" " "
137	CPI-F-20	3/12/54	" " "
138	CPI-F-57	3/12/54	" " "
139	CPI-F-126	3/12/54	" " "
140	CPI-NF-1	3/12/54	" " "
141	CPI-NF-4	3/12/54	" " "
142	CPI-NF-6	3/12/54	" " "
143	CPI-NF-7	3/12/54	" " "
144	CPI-NF-16	3/12/54	" " "
145	CPI-NF-20	3/12/54	" " "
146	CPI-NF-24	3/12/54	" " "
147	CPI-NF-102	3/12/54	" " "

Table 2.--Chemical and Biological Properties Known, of Candidate Antibiotics.

Name of Antibiotic	Organism Producing	Formula	Reported toxicity to *A	B	F	P
Actidione	Streptomyces griseus	$C_{15}H_{23}NO_4$	x	x	x	x
Albidin	Penicillium albidum	$(C_5H_4O_2)n$		x	x	
Alternaric acid	Alternaria solani				x	x
Aspergillic acid	Aspergillus flavus	$C_{12}H_{20}N_2O_2$		x	x	
Bacitracin, Zn salt	Bacillus subtilis			x		
Candicidin A	----- -----				x	
Clavacin	see Patulin	$C_7H_6O_4$		x	x	x
Comirin	Bacterium sp.				x	
Endomycin	Streptomyces sp.			x	x	
Fradicin	Streptomyces fradiae		x		x	
Frequentin	Penicillium frequentans	$C_{14}H_{20}O_4$		x	x	
Gladiolic acid	Penicillium gladioli			x	x	x
Gliotoxin	Trichoderma viride Aspergillus fumigatus Penicillium terlikowskii Penicillium cinerascens	$C_{13}H_{14}N_2S_2O_4$	x	x	x	x
Griseofulvin	Penicillium spp.	$C_{17}H_{17}O_6Cl$				
Kojic acid	Aspergillus spp. Penicillium daleae	$C_6H_6O_4$				
Methyl gallate	Koelrenteria paniculata			x	x	
Mycophenolic acid	Penicillium sp.	$C_{17}H_{20}O_6$		x	x	
Neomycin SO_4	Streptomyces fradiae		x	x		
Netropsin SO_4	----- -----				x	
Nystatin	----- -----				x	
Patulin	Penicillium spp. Aspergillus spp. Gymnoascus sp.	$C_7H_6O_4$		x	x	x
Polypeptin	Bacillus circulans		x	x	x	
Protoanemonin	Anemone pulsatilla		x	x	x	
Rimocidin	Streptomyces spp.		x		x	
Rimocidin SO_4	Streptomyces rimosus		x			
Streptomycin SO_4	Streptomyces griseus		x	x		x
Thiolutin	Streptomyces albus	$C_{13}H_{14}N_3O_3S_3$	x	x	x	x
Tomatine	Lycopersicon esculentum			x	x	
Trichothecin	Trichothecium roseum	$C_{19}H_{24}O_5$			x	
Ustilagic acid	Ustilago zeae		x	x	x	
Viridin	Trichoderma viride	$C_{19}H_{16}O_6$			x	x
1501	Bacterium sp.				x	
1523A	Bacterium sp.				x	

*A-animals, B-bacteria, F-fungi, P-plants.

Table 2.--continued.

Name of Antibiotic	Organism Producing	Formula	Reported toxicity to *A	B	F	P
1537A	Bacterium sp.				×	
M4019	-----				×	
M4348	-----				×	
M4489	-----				×	
M4575	-----				×	
MC2113, SEP_4-B-14	synth.	C_5H_5NOS				
MC3277, SEP_4-D	synth.	C_5H_4ONSNa				
Antimycin A-35	Streptomyces sp.				×	
Helixin B	Streptomyces sp.				×	
Magnamycin	Soil fungus		×	×		
Bacillomycin "B"	Bacillus subtilis				×	

*A-animals, B-bacteria, F-fungi, P-plants.

Table 3.--Physical Properties of Candidate Antibiotics.

Name of Antibiotic	Color	State	M.P. °C	Solubility*
Puromycin	white	solid		s.w.
Actidione	white	crystal	115-116	s.w.;s.chl.
Albidin	red	crystal		sl.s.w.;s.h.al.;sl.s. bz.,al.
Alternaric acid	white	powder	134	sl.s.w.;s.chl.,al.,bz.
Aspergillic acid	pale yellow	powder	93	sl.s.w.;s.al.,act.
Aureothricin	dark brown	solid		
Bacitracin, Zn salt	grey	solid		sl.s.w.;sl.s.al.,act.
Candicidin A	buff	solid		
Candicidin B	light green	solid		
Clavacin	white	crystal	109	s.w.;s.et.,al.,act., i.bz.
Comirin	sandy	powder		sl.s.w.;sl.s.al.,act.
Endomycin	light brown	granular		[1]i.w.;s.al.;i.et.,chl., bz.
Fradicin	yellow	crystal		sl.s.w.;s.al.,chl.;sl. s.et.bz.,act.
Frequentin	light brown	powder	128	sl.s.w.;s.act.,chl.,al., bz.
Gladiolic acid	white	powder	160	s.h.w.;s.al.,act.
Gliotoxin	light brown	powder	185	sl.s.w.;s.chl.;al.s.al., bz.
Griseofulvin	white	powder	220	sl.s.w.;s.chl.;sl.s.bz., al.
Kojic acid	white	crystal	158	s.al.;sl.s.bz.
Methyl gallate	white	solid		
Musarin	sandy	solid		
Mycophenolic acid	yellow	crystal	140	sl.s.w.;s.et.,chl.
Neomycin SO_4	white	powder		s.w.
Netropsin SO_4	white	solid		
Netropsin disulfate	white	solid		
Nystatin	buff	solid		s.al.
Ortho	white	powder		
Patulin	colorless	powder	111	s.w.;s.et.,al.,act., i.bz.
Polypeptin	tan	crystal	235	sl.s.w.;s.al.

*s.-soluble, sl.s.-slightly soluble, i.-insoluble, w.-water, h.-hot, al.-alcohol, act.-acetone, bz.-benzene, chl.-chloroform, et.-ether.

[1]Acid form.

Table 3.--continued.

Name of Antibiotic	Color	State	M.P. °C	Solubility*
Protoanemonin	light yellow	liquid		
Rimocidin	white	solid		
Rimocidin, Cu salt	blue	powder		
Rimocidin SO_4	cream	crystal	151	s.al.
Streptomycin SO_4	white	solid		s.w.;i.al.
Streptomycin SO_4, dihydro	white	solid		s.w.;i.al.
Streptothricin SO_4	yellow-white	solid		s.w.;i.al.
Thiolutin	yellow	solid	about	s.w.;s.al.;sl.s.et.
Tomatine	white	crystal	270	s.w.;s.al.;sl.s.et.
Trichothecin	white	crystal	118	sl.s.w.;s.al.,act.
Ustilagic acid	white	powder		
Ustilagic acid	buff	solid		
Viridin	light brown	powder	208-217	sl.s.w.;sl.s.al.,bz.
Ben Venue 1501	cream-white	solid		s.w.
Ben Venue 1523A	yellow-brown	solid		s.w.
Ben Venue 1537A	brown	solid		s.w.
Bristol Lab. A7604-5	tan	solid		s.w.
Bristol Lab.-3	white	solid		s.w.
Bristol Lab.-7	white	solid		s.w.
Bristol Lab.-31	white	solid		s.w.
Bristol Lab.-60	white	solid		s.w.
Bristol Lab.-72	tan-white	solid		s.w.;s.al.
Bristol Lab.-87	white	crystal		s.w.
Bristol Lab.-96	white	solid		s.w.
Bristol Lab.-99	white	crystal		s.w.;s.al.,act.
Bristol Lab.-100	white	solid		s.w.;s.al.
Bristol Lab.-104	white	solid		s.w.
Bristol Lab.-130	buff	solid		s.w.
Bristol Lab.-138	white	solid		s.w.
Bristol Lab.-190	white	crystal		s.w.
Bristol Lab.-226	white	solid		s.w.
Bristol Lab.-245	white	solid		s.w.
Bristol Lab.-247	white	solid		s.w.
Bristol Lab.-251	white	solid		s.w.

*s.-soluble, sl.s.-slightly soluble, i.-insoluble, w.-water, h.-hot, al.-alcohol, act.-acetone, bz.-benzene, chl.-chloroform, et.-ether.

Table 3.--continued.

Name of Antibiotic	Color	State	M.P. °C	Solubility*
Bristol Lab.-262	white	crystal		s.w.;s.al.
Bristol Lab.-336	white	solid		s.w.
Bristol Lab.-404	white	solid		s.w.;s.al.,act.
Bristol Lab.-413	white	solid		s.w.;s.al.
Bristol Lab.-415	white	crystal		s.w.
Bristol Lab.-456	white	crystal		s.w.
Bristol Lab.-471	white	solid		s.w.;s.al.,act.
Bristol Lab.-496	white	crystal		s.w.
Merck 9R7098	white	solid		s.al.
Merck 52R282	dark brown	solid		sl.s.w.
Merck 52R3023	grey-white	solid		s.w.
52R3899 Benzyl muco-chlorate	colorless	liquid		sl.s.w.;s.al.
9R3122 2-Chloroethyl mucochlorate	colorless	liquid		
9R4528 Isoamyl mucochlorate	colorless	liquid		
Merck 52R4009	cream	solid		s.w.
Merck 52R5208	buff	solid		s.w.
Merck 52R5989	tan	solid		s.w.
Merck 52R6106	orange	solid		s.al.
Merck 52R6107	light tan	solid		sl.s.al.
Pfizer #1	buff	powder		s.w.
Pfizer #3	white	powder		s.w.
Pfizer #4	white	powder		s.w.
Pfizer #5	buff	solid		s.w.
Pfizer #6	buff	solid		s.w.
Pfizer #7	brown	solid		s.w.
Pfizer #8	yellow	solid		s.w.
Pfizer #9	brown	solid		s.w.
Pfizer #10	white	crystal		s.w.
Pfizer #11	white	solid		s.w.
Pfizer #12	brown	solid		s.w.
Pfizer #13	buff	powder		s.w.
Pfizer #14	yellow	crystal		s.w.
Pfizer #16	white	solid		s.w.

*s.-soluble, sl.s.-slightly soluble, i.-insoluble, w.-water, h.-hot, al.-alcohol, act.-acetone, bz.-benzene chl.-chloroform, et.-ether.

Table 3.--continued.

Name of Antibiotic	Color	State	M.P. °C	Solubility*
Pfizer #18	yellow-brown	powder		sl.s.act.
Pfizer #20	gold	crystal		s.w.
Pfizer #21	orange-yellow	solid		sl.s.act.
Pfizer #22	orange-yellow	solid		s.w.
Pfizer #24	buff	solid		s.act.
Pfizer #27	dark-brown	powder		sl.s.w.
Squibb M4019	brown	solid		
Squibb M4348	light brown	solid		s.w.
Squibb M4489	light brown	solid		s.w.
Squibb M4575	brown	solid		s.w.
Squibb MC2113, SEP_4-B-14	pale yellow	solid		sl.s.w.;s.al.
Squibb MC 3277, SEP_4-D	cream	powder		s.w.
Squibb MC 3702	light green	solid		i.w.
Squibb MC 3711	white	solid		i.w.
Squibb MC7728	light green	solid		i.w.
Squibb MC7729	black	solid		i.w.
Squibb MC7730	black	solid		i.w.
Squibb MC7731	white	solid		i.w.
Squibb MC7732	cream-white	solid		i.w.
Squibb MC7733	white	solid		i.w.
Squibb MC7734	light brown	solid		i.w.
Squibb MC7735	light yellow	solid		i.w.
Squibb MC7736	light yellow	solid		i.w.
Squibb MC7737	white	solid		s.w.
Antimycin A-35	white	crystal		sl.s.w.;s.al.
Helixin B	orange	solid		sl.s.w.
Magnamycin	white	powder		
Bacillomycin "B"	light tan	powder		
CPI-H-2	colorless	liquid		s.w.,act.,xyl.,cyclo.
CPI-H-3	tan-white	solid		sl.s.w.,act.,xyl., cyclo.

*s.-soluble, sl.s.-slightly soluble, i.-insoluble, w.-water, h.-hot, al.-alcohol, act.-acetone, bz.-benzene, chl.-chloroform, et.-ether.

Table 3.--continued.

Name of Antibiotic	Color	State	M.P. °C	Solubility*
CPI-H-8	white	crystal		sl.s.w.,xyl.,cyclo.; s.act.
CPI-H-10	white	crystal		s.w.,act.;sl.s.cyclo.; i.xyl.
CPI-H-13	white	crystal		sl.s.w.;act.,xyl., cyclo.
CPI-F-8	yellow-orange	liquid		s.w.,act.,xyl.,cyclo.
CPI-F-20	light tan	solid		i.w.;sl.s.act.,xyl.; s.cyclo.
CPI-F-57	cream-white	solid		i.w.;sl.s.act.,xyl., cyclo.
CPI-F-126	amber	semi-solid		i.w.;s.act.,xyl., cyclo.
CPI-NF-1	cream	solid		i.w.;s.act.,xyl., cyclo.
CPI-NF-4	cream	crystal		s.w. act.,cyclo.; sl.s.xyl.
CPI-NF-6	tan	crystal		sl.s.w.,xyl.;s.act., cyclo.
CPI-NF-7	yellow	solid		i.w.,xyl.;sl.s.act., cyclo.
CPI-NF-16	amber	semi-solid		s.w.,act.,xyl.,cyclo.
CPI-NF-20	yellow	solid		i.w.,s.act.,xyl. cyclo.
CPI-NF-24	cream	crystal		i.w.,s.act.,xyl., cyclo.
CPI-NF-102	yellow	solid		i.w.,s.act.,xyl., cyclo.

*s.-soluble, sl.s.-slightly soluble, i.-insoluble, w.-water, h.-hot, al.-alcohol, act.-acetone, bz.-benzene, chl.-chloroform, et.-ether, xyl.-xylol, cyclo.-cyclohexane.

METHODS

Four techniques were employed in the project for evaluating the antifungal action of compounds. The first may be termed the "petri plate method" in which the fungitoxic substance is incorporated in a nutrient agar and the ability of a test fungus to subsist on it is measured. The second has been called the "spore germination method" in which spores of a test fungus are exposed to a known concentration of toxicant in solution and the subsequent germination of spores recorded. The third has been termed the "pad plate method" in which one-half inch disk of filter paper is permitted to absorb a measured amount of toxicant prior to placement on a nutrient agar surface seeded with test fungi. The ability of the toxicant to inhibit the growth of the fungi is recorded. The fourth, and perhaps most meaningful technique, is the "thread impregnation method." Therein a uniformly treated cotton cord is exposed to a known concentration of the candidate mold-inhibiting agent in solution. After transfer to a nutrient agar surface inoculated with test fungi and incubation for 14 days, the residual breaking strength of the cord is determined. Adequate controls were run in all tests. Details of procedure for each technique are described below.

Agar Plate Method In the "Official Screening Test Method for Determining Fungicide Activity of Candidate Fungicides" each test compound was incorporated directly into melted nutrient agar medium and mixed vigorously in a sterile Waring Blendor jar so that the compound might be evenly dispersed throughout the medium. Dilution series were prepared from the initial nutrient-toxic-agar. Sterile petri plates were poured with the prepared nutrient-toxic-agar, and the latter allowed to harden. Twenty-five milliliters were allowed for each plate in the series. It was found that Myrothecium verrucaria produced better growth on nutrient agar at pH 7 with 1 percent yeast extract and 2 percent tryptone added; Chaetomium globosum seemed to require mascerated filter paper in the medium in addition. Thus these special media were used when M. verrucaria and C. globosum were grown.

Spore suspensions were prepared from 11-day old cultures of the desired organisms in 100 milliliters of water containing 0.05 percent of a non-toxic wetting agent, Gardinol WA. Aspergillus niger and Aspergillus terreus produced spores satisfactorily on potato dextrose agar; the addition of yeast extract to the potato dextrose agar favored satisfactory spore production by Chaetomium globosum and Myrothecium verrucaria.

One drop of the desired spore suspension was placed in the center of each hardened agar gel plate by using a nichrome loop of 1/8 inch inside diameter. The plates were incubated at 30°C ±2 in an upright position for not more than 48 hours and were then inverted to prevent seeding of new colonies.

At the end of 96 hours measurements were made of the extent of mycelial growth. The diameters of the colonies of the test organisms were measured in millimeters, and results were calculated as percent inhibition against the normal control:

$$\text{Percentage Inhibition} = 100 - \frac{\text{Radial growth in mm. test compound plate}}{\text{Radial growth in mm. untreated control plate}} \times 100$$

The concentrations used and the mean percentage inhibitions obtained are tabulated in this report (Tables 4 and 5) for all the compounds tested.

Forty-three compounds selected because of their inhibitory effects against the test fungi have been compared with the standard Copper 8-quinolinolate on the basis of inhibitory dose values (ID_{50} and ID_{95} or ID_{100}). These values were obtained by plotting the percent inhibition for each dosage in the series on log-probability graph paper and drawing the best possible straight line equidistant from the various points on the graph. The inhibiting dosage values in parts per million were then read directly from the graph. In the cases where curved lines were formed regularly from the plotted data, values were obtained from the steepest portion of the curve.

Inhibition of Spore Germination This method was outlined by a committee of the American Phytopathological Society and is used by plant pathologists to evaluate fungicides. Chemically clean glass slides were dipped in a cellulose-nitrate solution (.25 gm. cellulose nitrate in 50 ml. ethyl alcohol and 50 ml. ether) and dried. Each test chemical solution was prepared by making a stock solution of 100 ppm. of the candidate chemical in distilled water. A 10 mg. sample of the compound was weighed out on a chainomatic balance and placed in a 100 milliliter volumetric flask. If the compound was insoluble in water, the sample was first dissolved in a suitable solvent, using as small an amount as possible and never more than 5 milliliters. The solution was then brought up to a volume of 100 milliliters with distilled water. Dilution series were made from this stock solution. The initial screening test involved 100 ppm. and 10 ppm. concentrations. If the compound showed promise as a fungicide, a second dosage series range was determined from the initial assay.

The cellulose nitrate-treated slides were marked on the underneath side with a wax glass-marking pencil, four cross marks being placed equidistant in a staggered line. The slides were placed on a slide warmer with the upper surface exposed and marked with the chemical name and concentration near one edge. A chemically clean 5-milliliter pipette was used to place a drop of the desired concentration over each of the four cross marks on the slide, the highest dilution being used first and followed by the lower dilutions. When all the slides for a given chemical had been treated, the slide warmer, set for 75°F, was turned on, and the drops were allowed to dry.

A spore suspension of Alternaria solani, or other test fungus, was prepared by scraping an area of a spore-producing culture grown on potato dextrose agar. The scrapings were suspended in distilled water and filtered through four layers of cheese cloth in order to remove mycelial fragments. Each suspension was standardized to obtain a spore count of about 25 per microscope field (100X) by placing one

drop of the suspension from a 5-milliliter pipette on a slide and counting 45-50 spores in one path across the drop at 100X magnification. Dilutions were made with distilled water if there were more than this number in one path, or spores were added if there were less. The above method of standardization appears to have greater uniformity than the use of a haemacytometer (Horsfall, 1945).

One drop of the standardized spore suspension was placed over each cross mark from a 5-milliliter pipette, thereby exposing the spores to a known amount of the fungitoxic agent. Control slides were prepared in the same manner, but untreated with any antifungal material. Each slide was then placed in an individual petri dish. The bottom halves of the petri dishes were lined with a 9 cm. disk of No. 613 filter paper moistened with distilled water. The moist incubation chambers were placed in a constant temperature room held at approximately 74°F for about 16 hours. In determination of results control slides were read first. Twenty-five spores at 100X magnification in one microscopic field in each spore drop were counted for inhibition of spore germination. The total number of spores read for any one slide was one hundred, the number inhibited in each spore drop being recorded and the total being given. Slides treated with chemicals were read in the same manner. However, in some cases it was necessary to count more than 100 spores to compensate for variation in germination of the test fungus on the control slides. Thus, if in the first drop on the control slide 2 of the 25 spores read had not germinated, 27 spores were counted in the first drop of the chemically treated slides with the number of ungerminated spores being recorded. The spores should germinate 95 percent or more on the control slide for the test to be valid. In this way the mean percent inhibition of the candidate chemicals may be calculated. This was done by subtracting the total number of ungerminated spores on the control slide from the total number not germinating on the chemically-treated slide; the difference was the mean percent inhibition for the chemical at the particular concentration employed.

Lethal dose values (LD_{50} and LD_{84}) were also calculated for the compounds showing promise as fungicides. The mean percent inhibition for each dosage was plotted on log-probability graph paper. A straight line was drawn equidistant from the various points obtained. Then the concentrations required to inhibit spore germination 50 percent (LD_{50}) and spore germination 84 percent (LD_{84}) were read off directly. Results were obtained from the steepest portion of the curve in those cases where curved lines were formed regularly from plotted data.

Pad Plate Method Rapid evaluation of the relative fungistatic potency of candidate fungicides may be attained by the following method developed in this project.

Oblong pyrex plates (9 1/2 x 6 x 1 1/2 inches) were autoclaved and then 100 milliliters of melted nutrient agar inoculated with Aspergillus niger, Aspergillus terreus, Myrothecium verrucaria, or Chaetomium globosum were dispensed into each before covering the top with heavy aluminum foil.

Aspergillus niger and A. terreus produced good growth on standard nutrient agar, while M. verrucaria and C. globosum appear to require a supplemented medium. Consequently standard nutrient agar was prepared, adjusted to pH 7 and then 1 gm. of yeast extract plus 2 gms. of tryptone were added for each liter of medium. Inocula were prepared by flooding 11-day old agar slant cultures with 5 milliliters of sterile 0.05 percent Gardinol WA aqueous solution, agitating with a needle, and pouring the resulting spore suspensions into 95-milliliter portions of the 0.05 percent Gardinol WA solution in the case of A. niger and A. terreus. Then 0.1 milliliter of the spore suspension was incorporated into 100 milliliters of melted nutrient agar. M. verrucaria and C. globosum slants were flooded with 0.05 percent Gardinol solution and agitated with a needle; the resulting spore suspensions were incorporated directly into 100 milliliters of the special medium described above. To obtain optimum growth of C. globosum mascerated filter paper was added to the agar. The agar substrate in the plates was allowed to harden for at least three hours.

For tests stock solutions of the candidate antibiotics were prepared in distilled water. A 10 mg. sample of antibiotic was weighed out on a chainomatic balance and placed in a 100-milliliter volumetric flask. The antibiotic, if insoluble in water, was first dissolved in a suitable solvent using as small an amount as possible, but never more than 5 milliliters. The solution was brought up to the 100-milliliter volume with distilled water. Desired concentrations for a dilution series were made from the stock solutions in sterile test tubes with sterile distilled water to reduce the possibility of contamination. Initially the concentrations were 100, 40, 16, and 6.4; the dilution factor being 2.5.

The desired concentrations were applied to filter paper disks, one-half inch in diameter. The #613 filter paper manufactured by the Eaton-Dikeman Co. was used, since by test it was found to adsorb more antibiotic than other samples of filter paper. Each disk received 0.1 milliliter of the desired concentration.

The disks were allowed to dry in an oven at approximately 60°C. When dry, the disks were placed on the pyrex plates so that the distance between the centers of any two disks was at least 1 1/4 inches, with no more than 28 pads being placed on one plate. The pads were arranged so that the pad with the least dilution in one column was next to the pad with the greatest dilution in the next column. See diagram below:

100	6.4	100	6.4	100	6.4	100
40	16	40	16	40	16	40
16	40	16	40	16	40	16
6.4	100	6.4	100	6.4	100	6.4

The plates were incubated in a constant temperature room at 30°C for 48 hours. Results were determined by measuring the zones of inhibition in millimeters. Notes were also taken on the absence of or degree of mycelial growth on the cellulose pads aided by a 10X magnifying lens.

Thread Impregnation Tests Breaking strength retention was studied by exposing treated 4-cord cotton thread to cellulolytic fungi. The untreated thread had a breaking point of approximately 7 pounds; 15 to 20 pound strength would have been better. Non-fibrous materials such as starch and proteins were removed from the thread by using 5 percent Diastofor L at 158-160°F until a negative reaction was obtained with the iodine test for starch and Millon's reagent for protein; then the thread was extracted with distilled water to remove the enzyme solution.

Lots of 20 non-consecutive pieces of desized thread, 8 inches in length, were dried to constant weight and submerged in 50 milliliters of fresh solutions of test chemicals in such concentrations as available amounts of the samples allowed. One lot of 20 threads was soaked in the solvent, while another lot was subjected only to drying and weighing to serve as controls. The threads were soaked at room temperature for 60 minutes with intermittent agitation. Then the segments were removed from the respective solutions and allowed to dry for 30 minutes at room temperature in sterile, partially covered petri dishes. The lots were returned to the original weighing bottles, heated for 5 hours at 100°C, and weighed again in order to determine the weight of toxicant absorbed.

Spore suspensions of M. verrucaria and C. globosum were prepared using 100 milliliters of 0.05 percent Gardinol WA solution and 11-day old cultures of the organisms. Three milliliters of each suspension were incorporated into each 100 milliliters of standard nutrient agar minus dextrose with pH 6.5. The agar was poured into petri dishes or large pyrex plates; 25 milliliters being allowed for each dish or 100 milliliters for each large plate. A sufficient number of plates was poured to allow for five replications.

When the agar was almost gelled, a sterile one-inch square of cotton cloth and two lengths of thread were aseptically placed on each agar surface. The square of cloth served as a carbon source. In the case of the large pyrex plates, a 9 x 1 inch strip of heat sterilized cotton cloth was placed on the agar. Eight or ten threads were placed on the large pyrex plates, only two threads for each dilution being placed on one plate. Of the 20 threads in each lot, ten were exposed to the fungus and ten were held as controls. After a 14-day incubation or exposure period at approximately 26°C the thread lengths remaining intact were removed from the plates, sterilized by autoclaving, conditioned in desiccators for uniform humidity and tested for tensile strength. A wall-mounted Scott tester was used.

Heat and pH stability of antibiotics Culture plates of agar were poured as described for the "pad plate method" and allowed to harden. A 200 ppm. solution of each antibiotic was prepared for testing. A 20-milliliter aliquot of the stock solution was adjusted to pH 5.5 and another 20-milliliter aliquot to pH 8. A phosphate buffer solution of pH 7 was used as well as 1N HCl or 1N NaOH to adjust the H-ion concentration. The solutions were placed in an incubator at 30°C for 4-6 hours; the solutions were then brought back to pH 7. The volumes were made up to 40 milliliters. The desired concentrations for a dilution series were made from this new 100 ppm. "stock" and applied to the filter paper disks; 0.1 milliliter being

applied to each pad.

Another 20-milliliter portion was diluted to 40 milliliters, and desired concentrations for a dilution series were made. The series of concentrations were applied to at least three sets of filter paper pads, and the pads allowed to dry in the oven at approximately 60°C. One set of pads was subjected to flowing steam in the autoclave for one hour. Another set of pads was placed in the electric oven and heated at 200°C for one hour. The third set of pads served as a control.

The dried pads were arranged on the plates in the manner described for the "pad plate method." Pads treated with copper 8-quinolinolate as a standard were also placed on the plates.

After incubation in an upright position for 48 hours at 30°C the zones of inhibition were measured, and the absence or degree of growth recorded. A comparison was made with the control in regard to the stability of the compound being studied.

RESULTS OF FUNGITOXICITY TESTS

Effects of antibiotics in agar substrate. An initial screening test was made of all the compounds received during the two-year period of the project used at a concentration of 100 ppm. in agar against Aspergillus niger and A. terreus. The percentage inhibition of vegetative growth obtained at this initial concentration is shown in Table 4. Only those compounds exhibiting complete inhibition of growth by 100 ppm. in agar gel were further evaluated at lower concentrations against all four test fungi; A. niger, A. terreus, C. globosum, and M. verrucaria.

The percentage inhibition of the more antifungal compounds at various dosages is shown in Table 5. The results indicate that 43 compounds are fairly effective against cellulose-decomposing fungi. The inhibitory dose (ID50 and ID95 or ID100) values were calculated for the 43 compounds showing most promise as mildew-proofing agents. These values may be compared with the standard copper 8-quinolinolate in Tables 6, 7, and 8. The data show that comirin, endomycin, rimocidin, copper rimocidin, rimocidin sulfate, and benzyl mucochlorate compared rather favorably with the standard copper 8-quinolinolate and merited further trials. It is interesting to note, however, that of the four fungi tested, these antibiotics are least effective against Aspergillus terreus and do not compare favorably with Copper 8 in this respect, since Copper 8 seems to be most effective against this organism. Nystatin, netropsin sulfate, ortho, Pfizer #16 ,and Pfizer #21 are apparently somewhat effective against C. globosum and M. verrucaria, but to a lesser degree than the previously mentioned antibiotics and Copper 8.

Results indicate that thirteen of the Squibb pyridine-thiol compounds are very effective against all four test fungi. The copper salt is effective against A. terreus, C. globosum, and M. verrucaria, but relatively ineffective against A. niger and might be ruled out because of this. All, with the exception of the copper salt, compare favorably with Copper 8.

Although data show that five of the candidate Crop Protection Institute fungicides are fairly effective against cellulose-decomposing fungi, they do not have sufficient ID values to warrant further trials as mildew-proofing agents when compared to the standard.

Effect of antibiotics on spore germination. Ninety-nine of the candidate antifungal agents were tested according to the standard glass-slide technique for inhibition of spore germination, as described above. Results of an initial assay against Alternaria solani spores at 100 and 10 ppm. are found in Table 9. Forty-nine of the 80 tested against Alternaria showed sufficient fungitoxicity to justify testing at a second dosage-series range. Table 10 indicates that only Comirin equals Copper 8 on this basis. Table 11 illustrates the instability of ten of the antibiotics from Bristol Laboratories, which had shown some promise in initial assays.

Six compounds were tested for ability to stop germination of Helminthosporium spores to learn something of their specificity. Table 12 shows the results.

The Crop Protection Institute provided materials which were tested against Curvularia and the results are shown in Table 13. These products all contain the furfuran structure and some appear to merit further tests.

The lethal dose (LD_{50} and LD_{84}) values of the more potent antifungal antibiotics assayed against A. solani spores are presented in Table 14. From the data it is evident that none of the antibiotics tested is better in fungicidal activity than Copper 8. However, comirin seems to be the leading antibiotic, while actidione, albidin, gliotoxin, thiolutin (R. I. #37), trichothecin, and Pfizer #11 may also be considered potential Class A fungicides against plant pathogens.

Compounds CPI-F-20 and CPI-NF-102 are also worthy of consideration as fungicides according to the tests against Curvularia spores. The LD_{50} for F-20 equals 6.2 ppm. and the LD_{95} is 26 ppm.; the LD_{50} for NF-102 is 3.1 ppm. and the LD_{95}, 5.2 ppm.

It must be kept in mind that the chemical and physical nature of the spore surface differs from that of mycelium and consequently differences in the toxicity of chemical substances occur.

Effects of antibiotics absorbed in cellulose. Forty compounds were evaluated by the "pad plate method," as shown in Table 15. The effects of four concentrations of each antibiotic against each of the four test fungi are recorded. This permits direct comparison and estimation of inhibiting dosages of the candidate toxicants. The ability or inability of an antibiotic to prevent growth in the zone of agar surrounding the cellulose disk (Column A) and on the cellulose paper pad (Column B) is illustrated.

The rather complex data indicate that some antibiotics stimulate rather than inhibit growth of particular fungi. Alternaric acid and gladiolic acid so affect Chaetomium globosum and similarly endomycin affects Myrothecium verrucaria, as examples. According to the results obtained, copper rimocidin permits growth of Aspergillus niger on the cellulose pad, but rimocidin alone permits little or no growth. This may be explained on the basis of ion antagonism.

Squibb MC 2113, Squibb MC 3277, and benzyl mucochlorate appear to be outstanding antifungal substances by this technique. The Squibb compounds were capable of protecting the cellulose disks, as well as sizable areas beyond the edges of the pads. Benzyl mucochlorate was shown to be a good preventative of mold growth on the cellulose, but because of its insolubility in water it could not check growth of the fungi much beyond the edges of the cellulose disks.

The stability of substances MC 2113 and MC 3277 after storage in aqueous solution up to 63 days is presented in Table 16. The data indicate that in general there is no loss in fungitoxicity with storage, except at extremely low concentrations.

A comparison of the storage life of Copper 8 with MC 2113 and MC 3277 is recorded in Table 17. Copper 8 seemed to permit growth of the test organisms on the disks, allowed bacteria to grow, and even appeared to stimulate the mycelium of M. verrucaria. The Squibb compounds thus seem to be superior antifungal agents on this basis.

Nine "promising" antibiotics were tested for pH and heat stability by the "pad plate method". Examination of Table 18 reveals that some candidate fungitoxic compounds are unaffected by pH while others are altered. Temperature appears to be the more critical factor. Wet or dry heat affects some compounds such as comirin, benzyl mucochlorate, rimocidin, copper rimocidin, and rimocidin sulfate. Others appear to be inactivated by dry heat but not by moist heat, e.g. Squibb MC 2113 and MC 3277.

Table 4.--Inhibition of mycelial growth by antibiotics when tested by the "Official Screening Test Method for Determining Fungicidal Activity of Candidate Fungicides".

Antibiotic	Aspergillus niger	Aspergillus terreus
Puromycin	*8	8
Actidione	90	56
Albidin	100	24
Alternaric acid	38	80
Alternaric acid	40	66
Aspergillic acid	0	4
Aureothricin	8	17
Bacitracin, Zn salt	19	6
Candicidin A	56	9
Candicidin B	58	37
Clavacin	51	77
Comirin	100	96
Endomycin	100	100
Fradicin	28	13
Frequentin	100	100
Gladiolic acid	100	100
Gliotoxin	100	90
Griseofulvin	12	19
Kojic acid	0	0
Methyl gallate	9	3
Musarin	1	2
Mycophenolic acid	22	50
Neomycin sulfate	0	2
Netropsin sulfate	100	76
Netropsin disulfate	34	37
Nystatin	100	78
Ortho	70	60
Patulin	51	84
Polypeptin	18	10
Protoanemonin	100	100
Rimocidin	100	100
Rimocidin, Cu salt	100	79
Rimocidin SO_4	100	100
Streptomycin SO_4	9	8
Streptomycin SO_4 dihydro	12	12
Streptothricin SO_4	15	11
Thiolutin	100	80

*Results expressed as percent inhibition of mycelial growth at 100 ppm. concentration after 96 hours at 30° C ± 2.

Table 4.--continued

Antibiotic	Aspergillus niger	Aspergillus terreus
Thiolutin	*100	42
Thiolutin, Crude	100	39
Tomatine	26	17
Trichothecin	100	100
Ustilagic acid	100	100
Ustilagic acid	100	100
Viridin	38	19
Ben Venue 1501	0	0
Ben Venue 1523A	82	20
Ben Venue 1537A	0	0
Bristol Lab. A7604-5	28	30
Bristol Lab.-3	10	10
Bristol Lab.-7	1	0
Bristol Lab.-31	0	0
Bristol Lab.-60	17	8
Bristol Lab.-72	2	2
Bristol Lab.-81	11	5
Bristol Lab.-96	7	0
Bristol Lab.-99	0	0
Bristol Lab.-100	11	5
Bristol Lab.-104	16	0
Bristol Lab.-130	59	20
Bristol Lab.-138	0	0
Bristol Lab.-190	4	0
Bristol Lab.-226	18	10
Bristol Lab.-245	13	18
Bristol Lab.-247	22	4
Bristol Lab.-251	4	0
Bristol Lab.-262	6	0
Bristol Lab.-336	9	3
Bristol Lab.-404	31	17
Bristol Lab.-413	32	2
Bristol Lab.-415	8	0
Bristol Lab.-456	0	0
Bristol Lab.-471	100	78
Bristol Lab.-496	1	0
Merck 9R7098	Tested only by "Pad Plate Method"	
Merck 52R282	0	35
Merck 52R3023	12	0

*Results expressed as percent inhibition of mycelial growth at 100 ppm. concentration after 96 hours at 30°C ± 2.

Table 4.--continued.

Antibiotic	Aspergillus niger	Aspergillus terreus
Benzyl mucochlorate	*100	100
2-Chloroethyl mucochlorate	100	100
Isoamyl mucochlorate	100	100
Merck 52R4009	15	2
Merck 52R5208	11	8
Merck 52R5989	Tested only by "Pad Plate Method"	
Merck 52R6106	Tested only by "Pad Plate Method"	
Merck 52R6107	Tested only by "Pad Plate Method"	
Pfizer #1	100	0
Pfizer #3	0	0
Pfizer #4	2	0
Pfizer #5	2	0
Pfizer #6	0	0
Pfizer #7	0	0
Pfizer #8	5	0
Pfizer #9	7	0
Pfizer #10	18	0
Pfizer #11	60	26
Pfizer #12	2	2
Pfizer #13	0	0
Pfizer #14	11	4
Pfizer #16	100	59
Pfizer #18	7	6
Pfizer #20	11	17
Pfizer #21	100	73
Pfizer #22	8	21
Pfizer #24	0	0
Pfizer #27	8	2
Squibb M4019	**	
Squibb M4348	**	
Squibb M4489	**	
Squibb M4575	**	
Squibb MC2113	100	100
Squibb MC3277	100	100
Squibb MC3702	37	100
Squibb MC3711	100	100
Squibb MC7728	100	100

*Results expressed as percent inhibition of mycelial growth at 100 ppm. concentration after 96 hours at 30°C ± 2.

**100% inhibition shown at a concentration of 10,000 ppm.

Table 4.--continued.

Antibiotics	Aspergillus niger	Aspergillus terreus
Squibb MC7729	*100	100
Squibb MC7730	100	100
Squibb MC7731	100	100
Squibb MC7732	100	100
Squibb MC7733	100	100
Squibb MC7734	100	100
Squibb MC7735	100	100
Squibb MC7736	100	100
Squibb MC7737	100	100
Antimycin A-35	53	30
Helixin B	69	57
Magnamycin	24	8
Bacillomycin "B"	44	47
CPI-H-2	46	
CPI-H-3	100	
CPI-H-8	52	
CPI-H-10	22	
CPI-H-13	42	
CPI-F-8	89	
CPI-F-20	49	
CPI-F-57	36	
CPI-F-126	75	
CPI-NF-1	71	
CPI-NF-4	20	
CPI-NF-6	100	
CPI-NF-7	44	
CPI-NF-16	100	
CPI-NF-20	100	
CPI-NF-24	100	
CPI-NF-102	100	

*Results expressed as percent inhibition of mycelial growth at 100 ppm. concentration after 96 hours at 30°C ± 2.

Table 5.--Fungitoxic Dosage Response of Selected Candidate Antibiotics.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Actidione	100	*95	54	100	90
	50	84	39	80	75
	25	44	22	69	40
	10	26	15	46	28
	5	-	-	32	5
Albidin	100	100	39	38	22
	50	74	32	0	28
	25	48	22	0	16
	10	36	19	0	22
	5	-	-	0	10
Alternaric acid (R. I. #4)	100	28	68		
	50	24	72		
	25	14	57		
	10	7	44		
Alternaric acid (R. I. #5)	100	32	74		
	50	24	72		
	25	14	56		
	10	2	41		
Candicidin A	100	56	9		
	50	44	8		
	25	34	4		
	10	29	13		
Candicidin B	100	58	37		
	50	56	30		
	25	52	26		
	10	50	30		
Clavacin	100	58	61		
	50	56	48		
	25	45	26		
	10	35	13		

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C ± 2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Comirin	100	*100	85	100	100
	50	100	70	-	-
	25	100	56	-	-
	10	100	40	100	100
	5	-	-	100	100
	3.3	100	28	-	-
	2.5	-	-	76	100
	1	76	0	70	100
Endomycin	100	100	100	100	100
	50	100	100	-	-
	25	100	90	100	-
	10	100	49	100	100
	5	-	-	100	100
	3.3	50	37	-	-
	2.5	-	-	100	29
	1	17	14	90	26
Frequentin	100	100	100	100	
	50	100	88	82	34
	25	86	57	55	24
	10	51	25	6	28
	5	-	-	7	16
	2.5	-	-	0	10
Gladiolic acid	100	68	100		
	50	42	64		
	25	37	45		
	10	27	18		
Gliotoxin	100	100	52	100	100
	50	100	52	100	-
	25	54	28	66	-
	10	18	15	57	43
	5	-	-	46	25
	2.5	-	-	43	26
	1	-	-	12	25

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

Table 5.--continued

Antibiotic	Concentration in ppm	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Netropsin sulfate	100	*100	41	100	100
	50	100	29	100	100
	25	18	11	100	100
	10	8	8	40	93
Netropsin disulfate	100	34	37		
	50	18	22		
	25	0	4		
	10	0	2		
Nystatin	100	100	76	100	100
	50	100	24	-	-
	25	100	18	-	-
	10	84	11	54	17
	5	-	-	36	6
	2.5	-	-	92	0
	1	-	-	52	0
Ostho	100	100	100	100	100
	50	100	91	-	-
	25	68	46	100	100
	10	47	26	100	13
	5	-	-	19	11
	2.5	-	-	0	10
	1	-	-	0	0
Patulin	100	55	59		
	50	54	48		
	25	46	33		
	10	33	13		
Protoanemonin	100	100	100	100	100
	50	100	18	100	100
	25	100	2	50	100
	10	34	6	38	8
	5	30	1	0	2

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C ± 2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Rimocidin	100	*100	100	100	100
	50	100	100	-	-
	25	100	81	-	-
	10	100	42	100	100
	5	-	-	100	100
	3.3	46	27	-	-
	2.5	-	-	87	100
	1	32	25	15	14
Rimocidin, Cu salt	100	100	100	100	100
	50	100	68	-	-
	25	100	50	-	-
	10	88	28	100	100
	5	-	-	100	100
	2.5	-	-	39	18
	1	-	-	0	16
Rimocidin SO_4	100	100	100	100	100
	50	100	88	-	-
	25	100	96	-	-
	10	100	38	100	100
	5	-	-	100	100
	3.3	50	25	-	-
	2.5	-	-	56	34
	1	32	23	0	20
Thiolutin (R. I. #37)	100	100	80	100	100
	50	100	64	-	-
	25	100	50	-	-
	10	70	10	80	42
	5	-	-	60	30
	2.5	-	-	50	22
	1	-	-	20	22

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C ± 2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Thiolutin (R. I. #38)	100	*100	42	100	100
	50	100	39	-	-
	25	100	26	-	-
	10	18	0	63	15
	5	-	-	90	18
	2.5	-	-	19	5
	1	-	-	0	0
Thiolutin, crude	100	100	70	100	44
	50	100	43	-	-
	25	100	26	-	-
	10	28	11	91	36
	5	-	-	100	1
	2.5	-	-	59	24
	1	-	-	53	16
Trichothecin	100	100	94	21	35
	50	90	74	50	28
	25	79	52	21	0
	10	44	33	0	0
	5	-	-	0	5
	3.3	24	20	-	-
	1	2	6	-	-
Ustilagic acid (R. I. #42)	100	100	100	100	100
	50	100	100	-	93
	25	100	100	8	20
	10	68	44	0	8
	5	-	-	0	12
	2.5	-	-	0	-
Ustilagic acid (R. I. #43)	100	100	100	80	0
	50	100	100	19	0
	25	74	50	0	0
	10	60	33	0	0
	5	-	-	0	0

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C ± 2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Bristol Lab.-130	100	*45	6		
	50	50	15		
	25	50	6		
	10	36	9		
Bristol Lab.-471**	100	100	22	-	100
	50	44	13	-	100
	25	17	13	-	100
	10	9	10	-	45
Benzyl mucochlorate	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	62	100	90
	5	-	-	17	20
	3.3	57	25	-	-
	2.5	-	-	21	15
	1	37	10	17	0
2-Chloroethyl mucochlorate	100	100	100	92	100
	10	56	42	42	16
	5	44	22	26	4
	2.5	26	18	15	4
	1	10	8	2	2
Isoamyl mucochlorate	100	100	100	100	100
	10	82	35	59	30
	5	71	22	33	18
	2.5	58	18	31	12
	1	48	15	17	4
Pfizer #1	100	26	2		
	50	0	7		
	25	1	12		
	10	1	7		

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

**A later test showed 31 percent inhibition of A. niger and 11 percent inhibition of A. terreus at a 100 ppm. concentration.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Pfizer #11	100	*6	7		
	50	6	8		
	25	5	10		
	10	0	4		
Pfizer #16	100	100	100	100	100
	50	100	76	100	100
	25	16	18	74	100
	10	21	1	74	100
	5	-	-	46	90
Pfizer #21	100	100	89	100	100
	50	100	55	-	-
	25	86	44	100	100
	10	29	24	70	29
	5	-	-	57	7
	2.5	-	-	-	22
Squibb MC 2113	100	*100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	100	100	-	-
	2.5	-	-	100	100
	1	98	82	100	80
Squibb MC3277	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	84	98	-	-
	2.5	-	-	100	100
	1	30	72	100	92

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Squibb MC3702	100	*35	100	100	100
	50	57	100	-	-
	25	48	62	-	-
	10	35	100	100	100
	5	-	-	100	100
	3.3	36	91	-	-
	2.5	-	-	100	100
	1	10	100	100	100
Squibb MC3711	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	90	100	-	-
	2.5	-	-	100	100
	1	27	44	91	100
Squibb MC7728	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	100	96	-	-
	2.5	-	-	100	100
	1	49	52	100	100
Squibb MC7729	100	100	100	100	100
	50	100	100	-	-
	25	100	93	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	96	100	-	-
	2.5	-	-	100	100
	1	19	65	100	100

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Squibb MC7730	100	*100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	95	97	100	100
	5	-	-	100	100
	3.3	95	95	-	-
	2.5	-	-	100	100
	1	20	46	100	100
Squibb MC7731	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	100	100	-	-
	2.5	-	-	100	100
	1	56	84	83	96
Squibb MC7732	100	100	100	100	100
	50	100	100	-	-
	25	100	96	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	100	100	-	-
	2.5	-	-	100	100
	1	24	58	100	90
Squibb MC7733	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	100	100	-	-
	2.5	-	-	100	100
	1	50	76	100	100

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
Squibb MC7734	100	*100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	84	89	-	-
	2.5	-	-	100	100
	1	29	52	100	92
Squibb MC7735	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	52	100	-	-
	2.5	-	-	100	100
	1	32	20	100	57
Squibb MC7736	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	100	100	100	100
	5	-	-	100	100
	3.3	36	100	-	-
	2.5	-	-	100	96
	1	34	26	100	6
Squibb MC7737	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	98	100	100	100
	5	-	-	100	100
	3.3	80	100	-	-
	2.5	-	-	100	100
	1	46	92	100	47

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
CPI-H-3	100	*30	23		
	50	44	23		
	25	25	19		
	10	0	11		
	5	0	21		
CPI-F-8	100	31	17	-	11
	50	22	13	-	6
	25	17	13	-	0
	10	19	7	-	0
	5	17	2	-	0
CPI-NF-6	100	100	100	-	100
	50	100	100	-	100
	25	94	66	-	100
	10	52	34	-	20
	5	41	26	-	18
CPI-NF-16	100	100	100	-	100
	50	100	61	-	100
	25	58	41	-	22
	10	42	22	-	17
	5	24	13	-	11
CPI-NF-20	100	100	100	-	100
	50	100	100	-	100
	25	75	46	-	100
	10	44	15	-	19
	5	29	2	-	11
CPI-NF-24	100	100	100	-	100
	50	100	100	-	100
	25	100	51	-	100
	10	44	15	-	33
	5	36	28	-	4

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±2.

Table 5.--continued.

Antibiotic	Conc. in ppm.	Aspergillus niger	Aspergillus terreus	Chaetomium globosum	Myrothecium verrucaria
CPI-NF-102	100	*100	100	-	100
	50	100	100	-	100
	25	91	57	-	100
	10	53	28	-	9
	5	47	34	-	7
Copper 8-quinolinolate	100	100	100	100	100
	50	100	100	-	-
	25	100	100	-	-
	10	53	100	100	100
	5	-	-	100	46
	3.3	34	100	-	-
	2.5	-	-	94	27
	1	2	100	35	6

*Results expressed as percent inhibition of mycelial growth after 96 hours incubation at 30°C±?

Table 6.--Antifungal antibiotics compared on the basis of inhibitory dose (ID_{50} and ID_{100}) values as determined by the Defense Agencies "Official Screening Test Method" on petri plates.

Antibiotic	A. niger		A. terreus		C. globosum		M. verrucaria	
	ID_{50} ppm.	ID_{100} ppm.	ID_{50} ppm.	ID_{100} ppm.	ID_{50} ppm.	ID_{100} ppm.	ID_{50} ppm.	ID_{100} ppm.
Actidione	20	>100	90	>100	40	100	29	>100
Albidin	42	100	>100	>100	>100	>100	>100	>100
Comirin	.7	3.3	20	>100	2.1	5	-	<1
Endomycin	3.0	10	20	50	.6	2.5	2.8	5
Frequentin	12	50	36	100	25	100	>50	>50
Gliotoxin	18	50	48	>100	21	100	11	100
Netropsin SO_4	28	50	>100	>100	10.5	25	8	25
Nystatin	7.2	25	68	>100	7.6	100	14	100
Ortho 406	15	50	21	>100	7	10	12.7	25
Protoanemonin	11	25	58	100	17	50	13	20
Rimocidin	3.5	10	20	50	1.6	5	1.2	2.5
Rimocidin, Cu salt	6.6	25	25	100	2.6	5	2.9	5
Rimocidin SO_4	3.3	10	12	100	2.4	5	2.7	5
Thiolutin	8.6	25	25	>100	3.2	100	12	100
Thiolutin, crude	11.3	25	54	>100	4.8	10	>100	>100
Thiolutin	12	25	>100	>100	6.8	100	10	100
Trichothecin	8	100	15	>100	>100	>100	>100	>100
Ustilagic acid	8.6	25	11	25	37	100	32	100
Ustilagic acid	22	50	25	50	73	>100	>100	>100
Benzyl mucochlorate	2.2	10	9	25	5.7	10	7.8	100
2-Chloroethyl mucochlorate	9.2	94	11	95	13	>100	16	95
Isoamyl mucochlorate	2.5	95	12	95	8.8	95	13	98
Pfizer #16	21	50	28	100	21	50	3.5	10
Pfizer #21	14	50	33	100	8.4	25	9.4	25
Copper 8	3.7	25	<1	<1	1.2	5	5.2	10

__-Underscore indicates figure obtained by using steepest portion of a curve. > =greater than; < =less than

Table 7.--Compounds supplied by Squibb & Co. compared on the basis of inhibitory dose (ID50 and ID100) values as determined by the Defense Agencies "Official Screening Test Method" on petri plates.

Chemical	Squibb Code	A. niger		A. terreus		C. globosum		M. verrucaria	
		ID50 ppm.	ID100 ppm.	ID50 ppm.	ID100 ppm.	ID50 ppm.	ID100 ppm.	ID50 ppm.	ID100 ppm.
Copper salt of N-hydroxy-2-pyridine-thione	MC3702	>* 100	>100	-	<1	-	<1	-	<1
Zinc salt of N-hydroxy-2-pyridine-thione	MC3711	1.5	10	1.1	3.3	.6	2.5	-	<1
Manganese salt of 2-mercapto-pyridine-1-oxide	MC7728	1.0	3.3	1.1	10	-	<1	-	<1
Iron (ferrous) salt of 2-mercapto-pyridine-1-oxide	MC7729	1.5	10	.3	3.3	-	<1	-	<1
Iron (ferric) salt of 2-mercapto-pyridine-1-oxide	MC7730	1.5	10	1.2	10	-	<1	-	<1
Mercuric salt of 2-mercapto-pyridine-1-oxide	MC7731	1.0	3.3	.7	3.3	.8	2.5	.5	2.5
Silver salt of 2-mercapto-pyridine-1-oxide	MC7732	1.2	3.3	1.0	3.3	-	<1	.6	2.5
Antimony salt of 2-mercapto-pyridine-1-oxide	MC7733	1	3.3	.8	3.3	-	<1	-	<1
Cobalt salt of 2-mercapto-pyridine-1-oxide	MC7734	1.6	10	1.2	10	-	<1	.6	2.5
Lead salt of 2-mercapto-pyridine-1-oxide	MC7735	2	10	1.3	3.3	-	<1	1.0	2.5
Bismuth salt of 2-mercapto-pyridine-1-oxide	MC7736	3.7	10	1.2	3.3	-	<1	1.6	5
Barium salt of 2-mercapto-pyridine-1-oxide	MC7737	1.7	25	.5	3.3	-	<1	1.1	2.5
Sodium salt of 2-pyridinethiol 1-oxide	MC3377	1.3	10	.9	3.3	-	<1	.6	2.5
2-Pyridinethiol 1-oxide	MC2113	.2	3.3	.8	3.3	-	<1	.8	2.5

*>greater than; <less than

Table 8.--Crop Protection Institute Chemicals compared on the basis of inhibitory dose (ID_{50} and ID_{95}) values as determined by the "Official Screening Test Method."

Chemical	Value	Aspergillus niger	Aspergillus terreus	Myrothecium verrucaria
Cu 8	ID_{50}	3.7 ppm.	< 1 ppm.	5.2 ppm.
	ID_{100}	25 ppm.	< 1 ppm.	10 ppm.
CPI-NF-6	ID_{50}	*15 ppm.	23 ppm.	12 ppm.
	ID_{95}	26 ppm.	32 ppm.	16 ppm.
CPI-NF-16	ID_{50}	24 ppm.	48 ppm.	29 ppm.
	ID_{95}	33 ppm.	66 ppm.	37 ppm.
CPI-NF-20	ID_{50}	21 ppm.	26 ppm.	12 ppm.
	ID_{95}	31 ppm.	34 ppm.	16.5 ppm.
CPI-NF-24	ID_{50}	10 ppm.	24 ppm.	11 ppm.
	ID_{95}	14 ppm.	34 ppm.	16 ppm.
CPI-NF-102	ID_{50}	18 ppm.	24 ppm.	13 ppm.
	ID_{95}	27 ppm.	33 ppm.	17 ppm.

*All results calculated from the steepest portion of plotted curves.

Table 9.--Inhibition of germination of Alternaria solani spores by 100 ppm. and 10 ppm. of antibiotics tested by the standard glass-slide technique.

Antibiotic	Concentration in parts per million	
	100	10
Puromycin	*39	0
Actidione	90	76
Albidin	100	100
Alternaric acid (R. I. #4)	1	0
Aspergillic acid	17	8
Aureothricin	0	0
Bacitracin, zinc	91	7
Clavacin	71	32
Comirin	97	94
Endomycin	82	0
Fradicin	90	1
Frequentin	100	-
Gladiolic acid	100	26
Gliotoxin	99	94
Griseofulvin	30	46
Kojic acid	40	0
Methyl gallate	0	0
Musarin	0	0
Mycophenolic acid	18	1
Neomycin sulfate	94	0
Netropsin	100	3
Nystatin	85	0
Ortho	91	47
Patulin	66	5
Rimocidin	100	54
Rimocidin sulfate	100	1
Thiolutin (R. I. #37)	100	100
Trichothecin **	95	80
Ustilagic acid (R. I. #42)	63	38
Ustilagic acid (R. I. #43)	0	0
Viridin	100	50
Bristol Lab. A7604-5	100	0
BL-3	100	44
BL-7	100	0
BL-31	96	60
BL-60	99	62

*Results expressed as mean percent inhibition of spore germination.
**One-day old solution tested.

Table 9.--continued.

Antibiotic	Concentration in parts per million	
	100	10
BL-72	*0	3
BL-81	22	0
BL-96	99	0
BL-99	25	21
BL-100	100	0
BL-104	0	16
BL-130	99	0
BL-138	100	36
BL-190	94	17
BL-226	22	0
BL-245	43	0
BL-247	99	39
BL-251	98	0
BL-262	0	0
BL-336	94	42
BL-404	100	40
BL-413	11	0
BL-415	0	10
BL-456	100	33
BL-471	93	11
BL-496	61	0
Benzyl mucochlorate	100	89
Pfizer #1	100	47
Pfizer #3	100	3
Pfizer #4	0	0
Pfizer #5	15	0
Pfizer #6	16	1
Pfizer #7	0	0
Pfizer #8	4	0
Pfizer #9	0	0
Pfizer #10	95	68
Pfizer #11	96	98
Pfizer #12	0	0
Pfizer #13	0	0
Pfizer #14	98	0
Pfizer #16	99	1
Pfizer #18	0	0

*Results expressed as mean percent inhibition of spore germination.

Table 9.--continued.

Antibiotics	Concentration in parts per million	
	100	10
Pfizer #20	*13	3
Pfizer #21	99	0
Pfizer #22	15	7
Pfizer #24	2	0
Pfizer #27	9	0
Squibb MC2113	92	38
Squibb MC3277	75	49

*Results expressed as mean percent inhibition of spore germination.

Table 10.-- Inhibition of germination of Alternaria solani spores by various dosages of selected antibiotics tested by the standard glass-slide techniques.

Antibiotic	Concentration of antibiotic in parts per million						
	100	50	25	10	5	2.5	1
Actidione **	*99	-	-	95	58	9	3
Albidin **	100	-	-	72	52	22	8
Bacitracin, zinc**	96	65	32	1	-	-	-
Clavacin **	65	-	-	0		-	-
Comirin	100	-	-	-	72	41	13
Endomycin	100	99	74	1	-	-	-
" **	99	99	93	0	-	-	-
Fradicin **	100	95	53	0	-	-	-
Frequentin **	100	-	-	0	-	-	-
" ***	100	100	99	0	-	-	-
Gladiolic acid **	100	80	12	0	-	-	-
Gliotoxin **	100	-	-	94	7	0	-
Neomycin sulfate**	97	98	97	-			
" **	-	-	21	1	-	-	-
Netropsin sulfate	100	100	31	3	0	-	-
Nystatin **	89	12	0	0	-	-	-
Ortho *** o	84	63	44	12	67	-	-
Rimocidin **	100	-	-	0	0	0	-
" ***	100	99	63	0	-	-	-
Rimocidin sulfate	100	99	12	0	-	-	-
" **	99	99	10	0	-	-	-
Thiolutin (R. I. #37)	100	-	-	99	0	0	0
Trichothecin **	95	96	89	80	-	-	-
Ustilagic acid (R. I. #42)	10	-	-	0			
Viridin **	100	-	-	55	22	3	-
Bristol Lab. A7604-5	100	100	100	0	-	-	-
" " **		100	100	0	-	-	-
BL-7	100	99	94	0	-	-	-
" **	-	90	9	0	-	-	-
" ***	-	94	79	0	-	-	-
"	97	93	57	3	-	-	-
BL-96 **	99	94	58	8	-	-	-
"	99	93	0	0	-	-	-
BL-99	98	0	0	0	-	-	-

*Results expressed as mean percent inhibition of spore germination.
**One-day old solution used.
***Two-day old solution used.
oVariable results may be due to the mineral oil used as the solvent.

Table 10.--continued.

Antibiotic	Concentration of antibiotic in parts per million						
	100	50	25	10	5	2.5	1
BL-100	*100	97	45	-	-	-	-
BL-104	100	97	88	18	-	-	-
BL-130	99	93	9	0			
BL-138**	100	97	5	0			
BL-336**	89	15	8	0	1	-	-
"	82	23	31	-	-	-	-
Benzyl mucochlorate**	0	-	-	0	0	-	0
Pfizer #1	100	100	57	47	-	-	-
" " ***	98	99	95	47	5	-	-
Pfizer #3	100	66	36	3	-	-	-
" " ***	100	96	34	2	0	-	-
Pfizer #10**	98	-	-	63	4	0	2
Pfizer #11	95	-	-	98	46	0	0
Pfizer #14	100	99	42	0	-	-	-
" **	100	85	3	0	-	-	-
Pfizer #16 **	98	98	26	2	-	-	-
Pfizer #21	99	39	0	0	-	-	-
" **	100	0	0	0	-	-	-
" ***	100	0	-	-	-	-	-
Squibb MC2113 **	93	86	48	38	-	-	-
Squibb MC3277 **	83	81	78	58	-	-	-
" " ***	78	-	-	47	36	15	-
Copper 8	100	-	-	97	81	41	14

*Results expressed as mean percent inhibition of spore germination.
**One-day old solution used.
***Two-day old solution used.

Table 11.--Instability of some antibiotics from Bristol Laboratories as shown by the glass slide, dosage-response method against Alternaria solani.

Code no.	Date	Conc. in ppm.				
		100	50	25	10	5
BL-3	8/12/52	*100	100	37	44	
	9/24/52	100	8	9	6	
	2/3/53	97	32	0	0	
BL-31	8/19/52	96	98	62	60	
	**8/20/52	49	16	13	8	
	***8/21/52	6	2	2	0	
	9/18/52	0	0	0	0	
	2/3/53	14	0	0	0	
BL-60	8/26/52	99	98	64	62	
	**8/27/52	-	4	10	10	0
	9/24/52	96	88	89	90	
	2/3/53	17	3	0	0	
BL-190	8/19/52	94	25	19	17	
	9/16/52	0	0	0	0	
	2/3/53	2	0	0	0	
BL-247	8/26/52	99	100	71	39	
	**8/27/52	-	99	26	2	5
	9/24/52	100	94	85	10	
	2/3/53	97	32	1	0	
BL-251	8/26/52	98	8	0	0	
	**8/27/52	40	28	7	0	
	9/16/52	0	0	0	0	
	2/3/53	-	4	0	0	
BL-404	8/19/52	100	98	99	40	
	**8/20/52	-	96	52	18	2
	2/3/53	94	0	0	0	
	9/16/52	51	0	1	0	
BL-456	8/19/52	100	93	11	33	
	**8/20/52	-	-	4	6	7
	9/18/52	1	0	0	0	
	2/3/53	5	1	0	0	

*Percent inhibition of spore germination.
**One-day old solution.
***Two-day old solution.

Table 11.--continued.

Code no.	Date	Conc. in ppm.				
		100	50	25	10	5
BL-471	8/26/52	*93	12	11	11	
	**8/27/52	35	1	0	0	
	9/16/52	0	0	0	0	
	2/3/53	0	0	0	0	
BL-496	8/19/52	61	36	9	0	
	9/16/52	1	0	0	0	
	2/3/53	1	1	0	0	

*Percent inhibition of spore germination.
**One-day old solution.

Table 12.-- Inhibition of germination of Helminthosporium sp. spores by antibiotics tested by the standard glass-slide technique.

Antibiotic	Conc. in ppm.			
	100	50	25	10
Candicidin A	*0	0	0	0
Candicidin B	4	0	0	0
Netropsin, disulfate	95	24	4	2
Bristol Lab.-99	0	0	2	0
BL-190	0	0	0	0
BL-251	0	0	0	0

*Results expressed as mean percent inhibition.

Table 13.--Inhibition of germination of Curvularia sp. spores by dosages of Crop Protection Institute compounds according to the standard glass-slide technique.

Compound	Conc. in ppm.						
	100	50	25	10	5	2.5	1
CPI-H-2	*0			**55			
CPI-H-3	3			0			
CPI-H-8	1			0			
CPI-H-10	7			4			
CPI-H-13	0			2			
CPI-F-8	54			53			
CPI-F-20	100			63			
	***100	77	78	100	69		
	100	98	-	86	60	0	4
CPI-F-57	22			42o			
CPI-F-126	43			47			
CPI-NF-1	***17			33oo			
CPI-NF-4	***10			9			
CPI-NF-6	***69			6			
CPI-NF-7	***11			8			
CPI-NF-16	46			3			
CPI-NF-20	14			26ooo			
CPI-NF-24	***9			2			
CPI-NF-102	***100			77			
	100	99	100	100	100		
	100	-	100	100	94	78	51

*Results expressed as mean percent inhibition.
**Scratched slide may account for inconsistent results.
***Solution one-day old.
oDrops on slide dried out at time of reading.
ooOne of four drops dried out at time of reading.
oooOne of four drops showed high inhibition.

Table 14.-- Antifungal antibiotics compared on the basis of lethal dose (LD_{50} and LD_{84}) values determined by the inhibition of germination of Alternaria solani spores, standard A. P. S. method.

Antibiotic	LD_{50} in ppm.	LD_{84} in ppm.
Actidione	4.4	8
Albidin	4.4	10
Bacitracin, zinc	37	68
Comirin	2.8	7.4
Endomycin	22	30
Fradicin	24	38
Frequentin	18	20
Gladiolic acid	37	49
Gliotoxin	7	9
Neomycin sulfate	16	19
Netropsin sulfate	26	38
Nystatin	70	92
Rimocidin	25	33
Rimocidin sulfate	30	37
Thiolutin (R. I. #37)	8	8.8
Trichothecin	4	16
Viridin	8.8	16
Bristol Labs. A7604-5	17	19
BL-7	24	38
BL-96	42	47
BL-99	82	92
BL-100	33	47
BL-104	17	27
BL-130	36	49
BL-138	33	43
BL-336	59	88
Pfizer #1	12	21
Pfizer #3	29	40
Pfizer #10	8.6	14
Pfizer #11	6	7.6
Pfizer #14	40	50
Pfizer #16	27	40
Pfizer #21	70	76
Squibb MC2113	26	56
Squibb MC3277	7.4	66
Copper 8	3	5.8

Table 15.--Evaluation of the fungistatic potency of different antibiotics by the "Pad Plate Method". (Legend at end of table).

Antibiotic	Conc. ppm.	A. niger		A. terreus		M. verrucaria		C. globosum	
		A*	B**	A	B	A	B	A	B
Copper 8	100	-	±	20	-	25	-	32	-
	40	-	±	17	-	19	-	24	-
	16	-	+++	-	±	tr	-	(tr)	±
	6.4	-	+++	-	+	-	-	stm	+
G_4	100	-	-	-	-	13	-	18	-
	40	-	+	-	+	-	-	tr	±
	16	-	++	-	+	-	-	-	+
	6.4	-	++	-	++	-	-	-	++
Actidione	100	tr	-	-	±	tr	-	tr	+
	40	-	±	-	±	-	-	-	++
	16	-	+	-	++	-	-	-	+++
	6.4	-	++	-	++	-	-	-	+++
Alternaric acid	100	-	++	24	±	-	-	stm	+++
	40	-	++	-	±	-	-	stm	+++
	16	-	+++	-	+	-	-	stm	+++
	6.4	-	+++	-	+	-	-	stm	+++
Comirin	100	20	-	-	+	15	-	18	-
	40	18	-	-	++	tr	-	tr	-
	16	tr	±	-	++	tr	-	-	±
	6.4	tr	-	-	++	tr	-	-	+
Endomycin	100	16	-	tr	-	stm	-	22	-
	40	-	±	-	-	stm	-	16	-
	16	-	+++	-	±	stm	-	tr	±
	6.4	-	+++	-	+	stm	±	-	++
Frequentin	100	-	±	tr	-				
	40	-	+	tr	-				
	16	-	++	-	+				
	6.4	-	+++	-	++				
Gladiolic acid	100	-	±	22	±	-	-	stm	+++
	40	-	+++	tr	±	-	-	stm	+++
	16	-	+++	-	±	-	-	stm	+++
	6.4	-	+++	-	±	-	-	stm	+++

Table 15.--continued.

Antibiotic	Conc. ppm.	A. niger		A. terreus		M. verrucaria		C. globosum	
		A*	B**	A	B	A	B	A	B
Gliotoxin	100	tr	±	tr	±	21	-	20	±
	40	-	+	-	+	16	-	stm	+++
	16	stm	++	-	+	16	-	stm	+++
	6.4	stm	+++	-	+	tr	-	stm	+++
Netropsin sulfate	100	-	+++	-	+++	ctn	ctn	ctn	ctn
	40	-	+++	-	++	ctn	ctn	ctn	ctn
	16	-	+++	-	+++	ctn	ctn	ctn	ctn
	6.4	-	+++	-	+++	ctn	ctn	ctn	ctn
Streptomycin sulfate	100	-	+++	-	++	(tr)	-	ctn	ctn
	40	-	+++	-	++	stm	±	ctn	ctn
	16	-	+++	-	++	-	±	ctn	ctn
	6.4	-	+++	-	++	-	±	ctn	ctn
Thiolutin	100	-	-	tr	-	stm	-	tr	±
	40	-	±	tr	±	stm	-	tr	±
	16	-	±	-	±	stm	-	-	±
	6.4	-	±	-	+	stm	-	-	++
Tomatine	100	-	+++	-	++	-	-	stm	+++
	40	-	+++	-	±	-	-	stm	+++
	16	-	+++	-	++	-	-	stm	+++
	6.4	-	+++	-	++	-	-	stm	+++
Tomatine + NH_3 vapor	100	-	+++	-	++	-	+++	-	+++
	40	-	+++	-	++	-	+++	-	+++
	16	-	+++	-	++	-	+++	-	+++
	6.4	-	+++	-	++	-	+++	-	+++
Trichothecin	100	18	-	18	-	22	-	ctn	ctn
	40	16	±	tr	-	20	-	ctn	ctn
	16	-	±	-	++	tr	-	ctn	ctn
	6.4	-	+++	-	+++	-	-	ctn	ctn
Ustilagicacid	100	tr	-	-	±	-	-	-	++
	40	-	+	-	+++	-	-	-	++
	16	-	++	-	+++	-	-	-	++
	6.4	-	+++	-	+++	-	±	-	++

Table 15.--continued.

Antibiotic	Conc. ppm.	A. niger		A. terreus		M. verrucaria		C. globosum	
		A*	B**	A	B	A	B	A	B
BL-247	100	-	++	stm	++	stm	-	-	±
	40	-	++	stm	++	stm	-	stm	+
	16	-	++	stm	++	stm	-	stm	++
	6.4	-	++	stm	++	stm	±	stm	++
Squibb M4019	100	-	++	19	-	-	-	-	+++
	40	-	++	18	±	-	-	-	+++
	16	-	+++	tr	±	-	-	-	+++
	6.4	-	+++	-	±	-	±	-	+++
Squibb M4348	100	-	+++	-	+++	-	-	-	
	40	-	+++	-	+++	-	-	-	
	16	-	+++	-	+++	-	-	-	
	6.4	-	+++	-	+++	-	-	-	
Squibb M4489	100	-	+++	-	+++	31	-	32	
	40	-	+++	-	+++	25	-	24	
	16	-	+++	-	+++	20	-	14	
	6.4	-	+++	-	+++	14	-	-	
Ortho	100	20	-	tr	±	14	-	tr	+
	40	17	±	tr	+	tr	-	-	+
	16	tr	+++	-	++	-	-	-	+++
	6.4	-	+++	-	++	-	-	-	+++
Pfizer #12	100	-	++	stm	++	stm	-	stm	+
	40	-	++	stm	++	stm	-	stm	++
	16	-	+++	stm	++	-	-	stm	++
	6.4	-	+++	stm	++	-	±	stm	+++
Pfizer #16	100	-	+++	-	+++	40	-	34	-
	40	-	+++	-	+++	34	-	30	-
	16	-	+++	-	+++	32	-	28	-
	6.4	-	+++	-	++	22	-	20	-
Pfizer #21	100	-	++	stm	+	-	-	stm	++
	40	-	++	stm	++	-	-	stm	++
	16	-	+++	stm	+++	-	-	stm	++
	6.4	-	+++	stm	+++	-	-	stm	++

Table 15.--continued.

Antibiotic	Conc. ppm.	A. niger		A. terreus		M. verrucaria		C. globosum	
		A*	B**	A	B	A	B	A	B
Polypeptin	100	-	+	-	+	-	++	-	±
	40	tr	+	-	+	-	+	-	±
	16	-	++	-	++	-	+	-	±
	6.4	-	++	-	++	-	+	-	±
Rimocidin	100	-	±	-	+	-	-	-	+
	40	-	±	-	+	-	-	-	+
	16	-	±	-	++	-	-	-	+
	6.4	tr	-	-	+	-	-	-	+
Rimocidin, copper	100	-	+++	-	++	ctn	ctn	ctn	ctn
	40	-	+++	-	+++	ctn	ctn	ctn	ctn
	16	-	+++	-	++	ctn	ctn	ctn	ctn
	6.4	-	+++	-	+++	ctn	ctn	ctn	ctn
Rimocidin, sulfate	100	-	±	-	+++	ctn	ctn	ctn	ctn
	40	-	±	-	+++	ctn	ctn	ctn	ctn
	16	-	+++	-	+++	ctn	ctn	ctn	ctn
	6.4	-	+++	-	+++	ctn	ctn	ctn	ctn
Streptomycin	100	-	+++	-	++	-	-	ctn	ctn
	40	-	+++	-	++	-	-	ctn	ctn
	16	-	+++	-	++	-	-	ctn	ctn
	6.4	-	+++	-	++	-	±	ctn	ctn
Streptomycin, dihydro	100	-	+++	-	++	-	-	ctn	ctn
	40	-	+++	-	++	-	-	ctn	ctn
	16	-	+++	-	++	-	-	ctn	ctn
	6.4	-	+++	-	++	-	±	ctn	ctn
Squibb M4575	100	17	-	-	-	-	-	-	-
	40	14	-	-	-	-	-	-	-
	16	-	±	-	++	-	-	-	-
	6.4	-	+++	-	++	-	-	-	-
Squibb MC2113	100	30	-	40	-	47	-	40	-
	40	28	-	34	-	38	-	28	-
	16	28	-	26	-	31	-	26	-
	6.4	tr	-	20	-	23	-	14	-

Table 15.--continued.

Antibiotic	Conc. ppm.	A. niger		A. terreus		M. verrucaria		C. globosum	
		A*	B**	A	B	A	B	A	B
Squibb	100	40	-	60	-	42	50	ctn	ctn
MC3277	40	36	-	48	-	32	44	ctn	ctn
	16	36	-	36	-	29	40	ctn	ctn
	6.4	26	-	24	-	22	28	ctn	ctn
Merck	100	-	±	-	+	-	±	-	-
9R7098	40	-	±	-	++	-	±	-	±
	16	-	+	-	+++	-	±	-	±
	6.4	-	+++	-	+++	-	±	-	++
Merck	100	-	+++	-	++	-	-	-	
52R282	40	-	+++	-	++	-	-	-	
	16	-	+++	-	++	-	±	-	
	6.4	-	+++	-	++	-	-	-	+++
Merck	100	-	+++	-	++	-	-	-	+++
52R3023	40	-	+++	-	++	-	-	-	+++
	16	-	+++	-	++	-	±	-	+++
	6.4	-	+++	-	++	-	±	-	+++
Benzyl	100	25	-	20	-	19	-	-	+
mucochlorate	40	tr	±	tr	-	-	-	-	-
	16	-	+	-	++	-	-	-	+
	6.4	-	+	-	++	-	-	-	++
Merck	100	-	+++	-	++	-	-	-	+++
52R4009	40	-	+++	-	++	-	-	-	+++
	16	-	+++	-	++	-	±	-	+++
	6.4	-	+++	-	++	-	±	-	+++
Merck	100	-	+++	-	+++	-	-	-	+++
52R5208	40	-	+++	-	+++	-	-	-	+++
	16	-	+++	-	+++	-	-	-	+++
	6.4	-	+++	-	+++	-	-	-	+++
Merck	100	-	+	-	++	-	±	-	±
52R5989	40	-	+	-	+++	-	+	-	±
	16	-	++	-	+++	-	+	-	±
	6.4	-	+++	-	+++	-	++	-	-

Table 15.--continued.

Antibiotic	Conc. ppm.	A. niger		A. terreus		M. verrucaria		C. globosum	
		A*	B**	A	B	A	B	A	B
Merck 52R6106	100	-	+	-	+	17	-	-	±
	40	-	+++	-	+++	16	+	-	+
	16	-	+++	-	++	-	+	-	+
	6.4	-	+++	-	+++	-	+	-	+
Merck 52R6107	100	-	+	-	+++	tr	±	-	-
	40	-	+	-	+++	-	±	-	±
	16	-	++	-	+++	-	±	-	±
	6.4	-	+++	-	+++	-	+	-	+
Comirin	50	20	-	-	+	tr	-	tr	-
	40	19	-	-	++	tr	-	tr	-
	30	14	±	-	+++	stm	±	tr	±
	20	15	±	-	+++	stm	-	tr	±
Endomycin	50	tr	±	tr	-	tr	-	22	-
	40	tr	±	-	-	stm	-	16	-
	30	-	+	-	±	stm	-	16	±
	20	-	+++	-	±	stm	-	22	-
Endomycin and Comirin 50-50	50	18	-	tr	+	(15)	-	15	-
	40	18	-	-	+	(14)	-	14	-
	30	17	-	-	++	tr	-	tr	±
	20	16	-	-	++	stm	-	tr	-

*A - Zone of inhibition in millimeters, including 12.5 mm. diameter of cellulose paper pad.

tr - Trace of inhibition.

__ - Underline indicates superficial aerial mycelial growth over a zone of inhibition in agar.

( )- Parenthesis indicates "stimulation" of fungus growth around zone of inhibition.

stm - Stimulates rather than inhibits fungus growth.

**B - Growth of test fungus on cellulose paper pads.

± - Growth confined to margin of cellulose disk.

+ - Slight growth of test fungus on cellulose disk.

++ - Moderate growth on cellulose disk.

+++ - Heavy growth on cellulose disk.

ctn - Contamination by bacteria of agar in test zone.

- - Hyphen under column A indicates the absence of a zone of inhibition; hyphen under column B indicates the absence of growth of test fungus on cellulose paper pad.

Table 16.-- Effect of duration of storage of cellulose pads impregnated with a dosage series of antibiotics (Squibb MC3277 and MC2113) on their subsequent fungistatic action.

Fungus	Days stored	Conc. in ppm. 100	40	16	6.4	Days stored	Conc. in ppm. 5	2.5	1.25	1
MC3277										
A. niger	0	40*	36	36	26	0	25**	19	tr	-
	14	48	42	34	32	30	18	15	-	-
	56	36	28	22	tr	63	22	tr	-	-
A. terreus	0	60	48	36	24	0	34	24	tr	tr
	14	54	44	38	32	30	20	17	-	-
	56	44	42	26	18	63	22	tr	tr	tr
M. verrucaria	0	42	32	29	22	0	25	18	16	15
	14	48	40	34	28	30	34	25	17	16
	56	50+	50+	30	28	63	34	30	22	18
C. globosum	0	50	44	40	28	0	24	16	tr	tr
	14	ctn	ctn	ctn	ctn	30	26	18	tr	tr
	56	50+	50+	40	24	63	ctn	ctn	ctn	ctn
MC2113										
A. niger	0	36	28	28	?	0	30	21	19	21
	14	43	40	31	25	30	23	17	18	16
	56	46	34	24	22	63	30	22	22	tr
A. terreus	0	46	34	26	20	0	38	27	19	15
	14	54	44	38	28	30	33	22	17	17
	56	52	42	32	22	63	34	23	tr	tr
M. verrucaria	0	47	38	31	23	0	38	22	22	20
	14	47	39	31	30	30	36	34	28	17
	56	50+	52	36	32	63	48	30	tr	tr
C. globosum	0	40	28	26	14	0	30	22	17	15
	14	ctn	ctn	ctn	ctn	30	25	ctn	ctn	ctn
	56	50	50	38	32	63	ctn	ctn	ctn	ctn

*Zone of inhibition in millimeters including 12.5 mm. diameter of cellulose paper pad.
**Underline indicates superficial aerial mycelial growth over a zone of inhibition in agar.
ctn-Contamination by bacteria of agar in test zone.
tr-Trace of inhibition.

Table 17.-- Effect of storage duration and concentration of fungistatic agent impregnated in cellulose pad on the inhibition of fungus growth assessed by the "Pad Plate Method".

	Days stored	Conc. ppm.	A. niger A*	A. niger B**	A. terreus A	A. terreus B	M. verrucaria A	M. verrucaria B	C. globosum A	C. globosum B
Copper 8	14	100	-	±	28	-	(16)	-	ctn	ctn
		40	-	+	23	-	(tr)	-	ctn	ctn
		16	-	++	tr	-	stm	-	ctn	ctn
		6.4	-	++	-	+	stm	-	ctn	ctn
	30	100	-	±	22	-	21	-	ctn	ctn
		40	-	±	19	-	16	-	ctn	ctn
		16	stm	+	-	±	tr	-	ctn	ctn
		6.4	stm	+++	-	++	-	±	ctn	ctn
	56	100	-	±	26	-	24	-	30	-
		40	-	++	17	-	17	-	24	-
		16	-	+++	-	±	-	++	-	±
		6.4	-	+++	-	+++	-	+++	-	+
	63	100	tr	-	25	-	36	-	ctn	ctn
		40	-	±	19	-	22	-	ctn	ctn
		16	-	+	-	±	tr	-	ctn	ctn
		6.4	-	+++	-	+++	-	-	ctn	ctn
Squibb MC2113	14	100	43	-	54	-	47	-	ctn	ctn
		40	40	-	44	-	39	-	ctn	ctn
		16	31	-	38	-	31	-	ctn	ctn
		6.4	25	-	28	-	30	-	ctn	ctn
	56	100	46	-	52	-	50+	-	50	ctn
		40	34	-	42	-	52	-	50	ctn
		16	24	-	32	-	36	-	38	ctn
		6.4	22	-	22	-	32	-	32	ctn
Squibb MC3277	14	100	48	-	54	-	48	-	ctn	ctn
		40	42	-	44	-	40	-	ctn	ctn
		16	34	-	38	-	34	-	ctn	ctn
		6.4	32	-	32	-	28	-	ctn	ctn
	56	100	36	-	44	-	50+	-	50+	-
		40	28	-	42	-	50+	-	50+	-
		16	22	-	26	-	30	-	40	-
		6.4	tr	±	18	-	28	-	24	-

Legend on next page.

Table 17.--continued.

Legend

A* - Zone of inhibition in millimeters including 12.5 mm. diameter of cellulose paper pad.
( ) - Parenthesis indicates "stimulation" of fungus growth around zone of inhibition.
tr - Trace of inhibition.
__ - Underline indicates superficial aerial mycelial growth over zone of inhibition in agar.
stm - Stimulates rather than inhibits fungus growth.
B** - Growth of test fungus on cellulose paper pads.
± - Growth confined to margin of cellulose disk
+ - Slight growth of test fungus on cellulose disk.
++ - Moderate growth on cellulose disk.
+++ - Heavy growth on cellulose disk.
ctn - Contamination by bacteria of agar in test zone.
-- Hyphen under column A indicates the absence of a zone of inhibition; hyphen under column B indicates absence of growth of test fungus on cellulose paper pad.

Table 18.-- The effect of pH and temperature on the stability of different antibiotics as determined by the "Pad Plate Method". (Legend at end of table.)

Antibiotic	Conc. in ppm.	Pad pH or temp.	A. niger A*	A. niger B**	A. terreus A	A. terreus B
Comirin	100	5.5	16	-	-	+
	100	8.0	13	±	-	++
	100	100°C	tr	±	-	++
	100	200° C	-	++	-	+++
	100	C'	17	-	-	++
	40	5.5	-	+	-	++
	40	8.0	-	+	-	+++
	40	100°C	-	+++	-	+++
	40	200°C	-	++	-	++
	40	C'	-	+	-	+++
Endomycin	100	5.5	14	±	tr	±
	100	8.0	-	±	tr	±
	100	100°C	-	+	tr	++
	100	200°C	-	+	-	++
	100	C'	tr	±	tr	±
	40	5.5	-	+++	tr	+
	40	8.0	-	+++	tr	±
	40	100°C	-	++	-	++
	40	200°C	-	+++	-	++
	40	C'	-	+++	tr	++
Squibb MC2113	10	5.5	36	-	32	-
	10	8.0	37	-	22	-
	10	100°C	28	-	21	-
	10	200°C	-	+++	-	++
	10	C'	35	-	35	-
	4	5.5	30	-	tr	-
	4	8.0	21	-	21	-
	4	100°C	24	-	tr	±
	4	200°C	-	+++	-	++
	4	C'	32	-	19	-
Squibb MC3277	10	5.5	36	-	20	-
	10	8.0	33	-	22	-
	10	100°C	32	-	24	-
	10	200°C	-	+++	-	++
	10	C'	31	-	26	-

Table 18.--continued.

Antibiotic	Conc. in ppm.	Pad pH or temp.	A. niger A*	A. niger B**	A. terreus A	A. terreus B
Squibb MC3277 continued	4	5.5	24	±	tr	+
	4	8.0	30	-	tr	+
	4	100°C	28	-	T9	+
	4	200°C	-	+++	-	++
	4	C'	25	-	tr	±
Rimocidin	100	5.5	20	-	stm	±
	100	8.0	20	-	stm	+
	100	100°C	-	+++	-	+
	100	200°C	-	+++	-	++
	100	C'	15	-	stm	±
	40	5.5	17	-	stm	+
	40	8.0	tr	±	stm	++
	40	100°C	-	+++	-	±
	40	200°C	-	+++	-	+
	40	C'	-	±	stm	++
Rimocidin, copper	100	5.5	21	-	-	±
	100	8.0	-	±	-	+
	100	100°C	-	++	-	±
	100	200°C	-	++	-	±
	100	C'	tr	±	-	++
	40	5.5	tr	±	-	++
	40	8.0	-	+++	-	+
	40	100°C	-	+++	-	±
	40	200°C	-	++	-	+
	40	C'	-	+	-	++
Rimocidin sulfate	100	5.5	15	-	-	++
	100	8.0	tr	±	-	++
	100	100°C	-	++	-	±
	100	200°C	-	++	-	+
	100	C'	-	±	-	+
	40	5.5	-	±	-	++
	40	8.0	-	+	-	++
	40	100°C	-	++	-	±
	40	200°C	-	++	-	+
	40	C'	-	+	-	++

Table 18.--continued.

Antibiotic	Conc. in ppm.	Pad pH or temp.	A. niger A*	A. niger B**	A. terreus A	A. terreus B
Thiolutin	100	5.5	-	±	-	+
	100	8.0	-	±	tr	±
	100	100°C	tr	-	tr	±
	100	200°C	-	++	-	++
	100	C'	-	±	-	±
	40	5.5	-	±	-	++
	40	8.0	-	±	-	+
	40	100°C	-	±	-	±
	40	200°C	-	+++	-	++
	40	C'	-	±	-	++
Benzyl mucochlorate	100	5.5	27	-	tr	-
	100	8.0	24	-	tr	-
	100	100°C	-	+++	-	+++
	100	200°C	-	+++	-	+
	100	C'	31	-	tr	-
	40	5.5	19	±	tr	±
	40	8.0	tr	±	tr	±
	40	100°C	-	+++	-	+++
	40	200°C	-	+++	-	±
	40	C'	19	-	tr	+
Copper 8	50	5.5	-	±	24	-
	50	8.0	-	±	28	-
	50	100°C	-	±	23	-
	50	200°C	-	+++	-	±
	50	C'	-	±	20	-
	10	5.5	-	++	-	±
	10	8.0	-	++	-	±
	10	100°C	-	++	-	+
	10	200°C	-	+++	-	±
	10	C'	-	+++	-	±

A*-Zone of inhibition in millimeters, including 12.5 mm. diameter of cellulose paper pads.

tr-Trace of inhibition.

-Underline indicates "stimulation" of fungus growth around zone of inhibition.

stm-Stimulates rather than inhibits fungus growth.

B**-Growth of test fungus on cellulose paper pads.

±-Growth confined to margin of cellulose disk. (Legend continued on next page.)

Table 18.--continued.

(Legend continued.)

+-Slight growth of test fungus on cellulose disk.
++-Moderate growth on cellulose disk.
+++-Heavy growth on cellulose disk.
C'-Pad prepared from unadjusted solution (100 ppm.) of antibiotic in distilled water.
-- Hyphen under column A indicates the absence of a zone of inhibition; hyphen under column B indicates absence of growth of test fungus on cellulose paper pad.

Effect of Antibiotics on Tensile Strength Retention of Cotton Thread

Twenty-seven compounds were deemed worthy of testing by this method, as depicted in Table 19. The occasional reduction in tensile strength without evidence of growth of the cellulose-decomposing fungi on treated threads necessitates a descriptive account for each of the candidate fungicides tested. Also no loss in strength occurred in some instances where no weighable residue of protective toxicant was present.

Comirin

Although not completely soluble in ethanol, comirin was used at concentrations of 1000, 500, and 250 mg./liter. The dried thread lengths contained from 0.40 to 1.28 percent of the chemical. There was partial growth on three of the threads at 1000 mg./liter, on four at 500 mg./liter, and on five at 250 mg./liter nearest the one-inch untreated cotton squares. It was possible to pick up all the thread lengths intact.

Benzyl mucochlorate (Test of 1 September 1953).

This compound is soluble in ethanol and was prepared at concentrations of 1000, 500, and 250 mg./liter. The percentage uptake was 0.65, 0.63, and 0.66 respectively. At 1000 mg./liter there was evidence of growth on six strings; all the cotton squares were covered with growth. At the 500 mg./liter concentration there was growth on eight thread lengths, and two could not be removed from the plates intact. Six strings could not be removed intact at the 250 ppm. concentration, with evidence of growth on all the treated thread lengths.

Benzyl mucochlorate (Test of 22 October 1953).

The candidate chemical was prepared at concentrations of 4000, 2000, and 1000 mg./liter. The percentage uptake of toxicant ranged from 1.38 percent at the lowest concentration to 2.91 percent at the highest. There was no evidence of growth on the treated thread lengths after the 14-day exposure period at the rate of 4000 mg./liter; all the untreated one-inch squares were covered with growth. At 2000 mg./liter and at the 1000 mg./liter there was slight growth on seven thread lengths. Only eight of the treated lengths at the latter concentration could be removed from the plates intact.

Endomycin (Test of 3 July 1953).

Ethanol suspensions of endomycin were employed at 4000, 2000, and 1000 mg./liter. The antibiotic content of treated thread was from 1.62 to 2.59 percent of the weight of the thread. There was no evidence of growth after the 14-day exposure period, and a zone of inhibition was present around the strings. All the one-inch cotton squares were covered with fungus growth.

Endomycin (Test of 25 September 1953).

This compound, not completely soluble in ethanol at a concentration of 2000 mg./liter, was prepared at concentrations of 2000, 1000, 500, and 250 mg./liter. At 2000 mg./liter with 2.07 percent endomycin in the thread very slight growth of the test fungi was found at the tip of one thread length after the exposure period. When 1.84 percent of the antibiotic was absorbed into the treated thread lengths from a concentration of 1000 mg./liter, there was slight sporulating growth on ten strings; nine could be removed from the plates intact. At 500 mg./liter with 1.29 percent endomycin in the thread all the strings evidenced some sporulating growth, and only three thread lengths could be removed intact. At 250 mg./liter and with 1.2 percent of the antibiotic in the threads, the latter were so decomposed by the fungi that they could not be removed from the plates.

Endomycin (Test of 29 October 1953).

A new sample of endomycin was prepared at concentrations of 4000, 2000, 1000, and 500 mg./liter. The compound was almost entirely in solution in ethanol, the remaining particles forming a suspension. The percentages of compound in the thread lengths were respectively; 1.72, 1.75, 1.38, and 1.06. There was no evidence of growth at the rate of 4000 mg./liter. At 2000 mg./liter there was growth on four threads, but only nine of the ten threads could be removed intact. The thread lengths were so decomposed at 500 and 1000 mg./liter that they could not be removed from the plates.

Netropsin sulfate

Although not completely soluble in ethanol, this chemical was used at 3000, 2000, and 1000 mg./liter concentrations. The percentage uptake of antibiotic was 1.49, 1.02, and 1.38 respectively of the dry weight of the thread. After a 14-day incubation period there was no evidence of growth on the treated cords. All one-inch squares of untreated cotton were covered with fungus growth.

Nystatin

Nystatin is soluble in methanol and was prepared at concentrations of 1000, 500, and 250 mg./liter. The compound was unable to protect thread lengths containing 0.40, 0.20, and 0.14 percent of the toxicant against the test fungi for 14 days. All threads were decomposed by the fungi, and none could be removed from the plates intact.

Ortho

Ortho is soluble in acetone at 1000 mg./liter, and gave little protection to treated thread lengths. At 250 mg./liter with 0.18 percent of the compound only five strings could be removed intact, whereas at 500 mg./liter, with 0.27 percent of the compound seven were removed intact. Although the strings at 1000 mg./liter containing 0.44 percent were partially covered with growth, all could still be removed from the plates.

Protoanemonin

A protoanemonin solution in water was prepared at concentrations of 1000, 500, and 250 mg./liter. The percentage uptake at the respective concentrations was 0.27, 0.02, and 0.05 percent of the weight of the thread. The antibiotic was unable to protect the thread against the cellulolytic fungi for a 14-day period. None of the threads could be removed from the plates intact.

Pfizer #16

This compound is soluble in water and was employed at concentrations of 1000, 500, and 250 mg./liter. The percentage uptake of the antibiotic at the respective concentrations was 0.96, 0.64, and 0.51. After a 14-day exposure period there was no visible growth on the thread lengths at the rate of 1000 mg./liter. At 500 mg./liter there was slight mycelial growth on two of the threads; at 250 mg./liter there was slight mycelial growth on four of the thread lengths nearest the one-inch cotton squares.

Pfizer #21

Pfizer #21 is only slightly soluble in water and was prepared at concentrations of 1000, 500, and 250 mg./liter. The percentage uptake of the antibiotic was respectively; 0.38, 0.25, and 0.18. The compound was unable to protect the treated thread at these concentrations for the 14-day period. All the threads were decomposed by the fungi, and none could be removed from the plates intact.

Rimocidin

This compound is not completely soluble in ethanol and was employed at concentrations of 4000, 2000, and 1000 mg./liter. After a 14-day incubation period partial growth was noted nearest the one-inch cotton squares on the strings treated with 4000 mg./liter, on five strings with 2000 mg./liter, and on eight threads with 1000 mg./liter. The compound content of the treated lengths ranged from 0.79 to 2.55 percent of the weight of the string. It was possible to pick up all the treated threads intact.

Rimocidin sulfate

Because of the small size of the sample, concentrations of 500 and 250 mg./liter only were used. The percentage uptake was 0.77 and 0.80 respectively. The antibiotic was not completely soluble in ethanol. Within 14 days C. globosum and M. verrucaria invaded and decomposed the strings so that they could not be removed from the plates.

Trichothecin

Trichothecin is soluble in ethanol and was employed at concentrations of 300 and 150 mg./liter because of the small size of the sample. The antibiotic content of the thread was 0.38 and 0.40 percent of the weight of the thread respectively. Trichothecin was unable to protect the thread for a 14-day period; all were decomposed and could not be removed from the plates intact.

Ustilagic acid (R. I. #43).

Ustilagic acid did not entirely dissolve in ethanol and when used at the rate of 2000, 1000 and 500 mg./liter was unable to protect the thread. The percentage uptake of compound at the respective concentrations was 1.03, 1.09, and 1.02 percent of the dry weight of the thread. Within 14 days the threads were overgrown by the test organisms and badly decomposed.

Squibb MC2113 (Test of 30 June 1953).

The thread lengths were treated at 4000, 2000, and 1000 mg./liter. Ethanol was the solvent used. In 14 days there was no growth on the treated strings; the percentage uptake of the compound ranged from 0.54 to 1.35 on a dry weight basis.

Squibb MC2113 (Test of 1 September 1953).

Concentrated ammonium hydroxide (3 drops) with water was the solvent employed. The compound was prepared at concentrations of 100, 500, and 250 mg./liter. The percentage uptake by the thread lengths was respectively: 0.41, 0.00, 0.37. No growth was observed on any of the treated thread. At 1000 and 500 mg./liter the one-inch untreated cotton squares were partially sterile, while the squares were covered in growth at 250 mg./liter. This indicates diffusion of the toxicant.

Squibb MC2113 (Test of 25 September 1953).

With the aid of ten drops of concentrated ammonium hydroxide, the compound dissolved in water at a concentration of 2000 mg./liter. After a 14-day exposure period to cellulolytic fungi only one thread showed slight mold growth on one end and this had been soaked in the solution containing 250 mg./liter. The threads exposed to 2000, 1000, and 500 mg./liter concentrations exhibited no hyphal growth. In the five petri plates each containing two threads exposed to a 2000 mg./liter dosage, three of the one-inch cotton squares were sterile and two partially so; one square was sterile and four partially sterile at 1000 mg./liter, while at 500 mg./liter two squares were partially sterile and three were covered with growth. All of the five one-inch untreated squares were covered at the lowest concentration. The percentage of compound absorbed in the thread at each concentration was found to be 0.00, or an insufficient amount to be determined by weight measurements.

Squibb MC3277

Thread lengths were treated with water solutions of this compound at 4000, 2000, and 1000 mg./liter. The toxicant content of the treated thread was respectively: 0.76, 0.34, and 0.06 percent of the dry weight. With the exception of slight growth at the lowest concentration tested, there was no growth on the treated strings and all could be removed from the plates intact.

Squibb MC3702

Although not completely soluble in acetone, this copper salt was prepared at concentrations of 1000, 500, and 250 mg./liter. The percentage uptake at the respective concentrations was 0.81, 0.06, and 0.03. There was slight growth on all

treated strings at the points of contact with the 9" x 1" strip of cotton material used as the carbon source. However, all the threads could still be removed from the plates intact.

Squibb MC3711

This zinc salt, although not completely soluble in acetone, was prepared for 1000, 500, and 250 mg./liter treatment. The percentage uptake ranged from 0.71 to 0.05 of the dry weight of the thread. There was slight growth on the treated thread at the points of contact with the 9" x 1" strips of untreated cotton material. All the threads were removed intact, however, after 14 days of exposure.

Squibb MC7728

This manganese salt was not completely soluble in acetone, but concentrations of 1000, 500, and 250 mg./liter were prepared. When thread lengths contained as much as 0.79 percent of the compound or as little as 0.18 percent, there was no visible mold growth on the strings. At 1000 mg./liter the one-inch cotton squares were partially sterile, and at 500 mg./liter two of the squares were partially so. There was slight growth on the ends of the three strings nearest the one-inch squares at the lowest concentration tested where the percentage uptake was 0.10; all five untreated squares were covered with growth.

Squibb MC7729

This ferrous salt was found to be fairly soluble in acetone, but not completely so at the rate of 1000 mg./liter. At that concentration with 0.99 percent of the compound by weight absorbed in the thread, there was very slight growth on one string nearest the one-inch square. At 500 mg./liter with 0.37 percent of the compound in the thread there was growth on the ends of the five strings nearest the squares, whereas at 250 mg./liter with 0.36 percent toxicant in the thread there was growth on the portions of four strings nearest the squares. MC7729 is black in color and imparted a grey color to the thread. All the thread could be removed intact from the incubation plates.

Squibb MC7730

The ferric salt is not completely soluble in acetone. Concentrations of 1000, 500, and 250 mg./liter were prepared. The 1000 mg./liter concentration imparted a grey tinge to the strings, the compound being black in color. The percentage uptake at the respective concentrations was 0.86, 0.22, and 0.00. At the highest concentration there was no growth on the thread lengths and two one-inch squares were sterile, whereas at 500 mg./liter there was slight growth on two strings nearest the one-inch squares. Although the percentage uptake at the lowest concentration tested was 0.00, five strings had no visible growth. Even though the five lengths nearest the one-inch cotton squares permitted growth, all could be removed intact for tensile strength determinations.

Squibb MC7731

This mercuric salt was very slightly soluble in acetone, but was prepared at the rate of 1000, 500, and 250 mg./liter. The percentage uptake ranged from 0.12 at the lowest to 0.61 at the highest concentration. There was no growth on the thread at the two highest concentrations and the untreated squares were partially sterile. At 250 mg./liter there was slight growth on one thread length nearest the one-inch square.

Squibb MC7732

Very little of the silver compound went into solution in acetone at 1000 mg./liter. The percentage uptake was 0.42 and there was no growth on the thread at this concentration. At 500 mg./liter there was growth on five strings nearest the one-inch squares but there was moderate growth on eight thread lengths at the 250 mg./liter concentration. At both of these latter concentrations the percentage uptake of compound was calculated as 0.00. All threads could be removed intact for breaking tests.

Squibb MC7733

Although very little of the antimony salt was soluble in acetone at 1000 mg./liter, thread lengths treated at 1000, 500, and 250 mg./liter concentrations were fairly well protected against the cellulose-decomposing fungi. The percentage uptake at the respective concentrations was 0.51, 0.39, and 0.00. At the highest rate the five squares were partially sterile, and there was no evidence of growth on the thread. At the two lower concentrations there was slight growth on two strings nearest the one-inch squares.

Squibb MC7734

This cobalt salt, although not completely soluble in acetone, was prepared at concentrations of 1000, 500, and 250 mg./liter. It imparted a brown-grey tinge to the threads treated at 1000 mg./liter. At the highest concentration with 1.17 percent uptake there was no growth on the thread, and five one-inch squares were partially sterile. At 500 mg./liter with 0.09 percent uptake there was mycelial growth on one string, whereas at the lowest concentration with 0.18 percent toxicant uptake there was no evidence of growth.

Squibb MC7735

This lead salt was prepared at concentrations of 1000, 500, and 250 mg./liter by using acetone as the solvent. The percentage uptake was 1.82, 0.38, and 0.06 at the respective concentrations. The threads treated at 1000 mg./liter and exposed to the fungi had a greyish color. There was no growth on any of the treated lengths. At the highest concentration the one-inch squares were partially sterile.

Squibb MC7736

Threads were treated with the bismuth salt, which is not completely soluble in

acetone; at concentrations of 1000, 500, and 250 mg./liter the percentage uptake was 0.28, 0.04, and 0.00 respectively. At 1000 mg./liter there was no growth on the strings. At the two lower concentrations there was growth on five strings nearest the one-inch squares; however, all could be removed from the plates intact.

Squibb MC7737

The unusual water-soluble barium salt was dissolved at the rates of 1000, 500, and 250 mg./liter. The percentage uptake of the compound at the latter two concentrations was 0.00. At 1000 mg./liter with 0.91 percent by weight of the compound absorbed in the thread, the strings showed no growth and the untreated squares were partially sterile. The 500 mg./liter concentration exhibited slight mycelial growth on three strings nearest the one-inch squares, and the squares were partially sterile. At the lowest concentration there was growth on five strings nearest the one-inch squares, but all could be removed from the plates.

Copper 8 (Test of 5 June 1953).

Although the compound was not completely soluble in ethanol, the thread lengths were treated in solutions of 4000, 2000, and 1000 mg./liter. The percentage uptake at the respective concentrations was 4.27, 3.37, and 2.53. In 14 days all the untreated cotton strips were covered with spore-producing mycelia, and there was evidence of growth on the treated lengths of string. However, it was still possible to pick up the thread lengths intact.

Copper 8 (Test of 1 September 1953).

The compound, although not completely soluble in ethanol, was prepared at concentrations of 4000, 2000, 1500, 1000, and 500 mg./liter. The percentage of compound in the thread lengths at the various concentrations was 3.58, 2.74, 1.97, 1.67, and 1.66 respectively. At the highest concentration there was no growth on the thread. Slight mycelial growth was evident on five thread lengths nearest the one-inch untreated squares at each of the remaining concentrations.

Copper 8 (Test of 22 October 1953).

Copper 8 solutions were prepared at the rate of 4000, 2000, 1000, and 500 mg./liter. Thread lengths treated in the solutions took up the following percentages of compound: 2.87, 1.91, 1.55, and 1.30 respectively. At the rate of 4000 mg./liter there was no evidence of growth after a 14-day exposure period. At the 2000 and 1000 mg./liter concentrations there was very slight growth on five thread lengths nearest the one-inch squares. There was also slight growth on five thread lengths at the lowest concentration tested. All threads, however, could be removed from the plates intact.

Cunilate #2174 (Test of 1 October 1953).

This compound, almost completely soluble in xylol, was prepared at concentrations of 2000, 1000, 500, and 250 mg./liter after bringing the percentage of solubilized Copper 8 up to 100. The calculated percentages of compound in the thread lengths showed great variation; 3.10, 1.48, 5.57, and 5.92 at the respective concentrations. At the highest concentration slight mold growth occurred on two strings nearest the one-inch squares. At 1000 mg./liter there was slight growth in evidence on 5 thread lengths nearest the squares, and there was slight growth evident on six strings at 500 mg./liter. Five threads exhibited slight growth at the lowest concentration tested.

Cunilate #2174 (Test of 29 October 1953).

Cunilate #2174 was prepared in xylol at concentrations of 4000, 2000, 1000, and 500 mg./liter after bringing the percentage of solubilized Copper 8 up to 100. As in the previous test, the percentage uptake was erratic; 6.04, 3.04, 6.07, and 4.67 respectively. At the rate of 4000 mg./liter there was no evidence of growth on the threads, the edges of the one-inch squares being sterile. At 2000 mg./liter there was also no growth on the treated thread, but the cotton squares were covered. There was slight mycelial growth on three threads at the 1000 mg./liter concentration, but one untreated cotton square was partially sterile. At the lowest concentration tested there was growth on eight of the thread lengths; however, all could be removed from the plates intact.

Cunilate #2174 (Test of 9 December 1953).

The compound was prepared at 2000, 1000, 500, and 250 mg./liter concentrations after bringing the percentage of solubilized Copper 8 up to 100. The uptake was 2.98, 1.40, 0.50, and 0.45 percent of the dry weight of the thread lengths respectively. At the highest concentration there was no evidence of growth on the treated thread, while at 1000 mg./liter there was slight growth on one string. Slight growth developed on the ends of two thread lengths at 500 mg./liter, while at the lowest concentration there was growth on three threads and only nine could be removed from the plates intact.

Cunilate #2174-WP (Test of 9 October 1953).

This compound, almost completely soluble in xylol, was prepared at concentrations of 2000, 1000, 500, and 250 mg./liter after the percentage of solubilized Copper 8 was adjusted to 100. At the highest concentration with 4.32 percent of the compound absorbed in the thread, there was no evidence of growth. At the rate of 1000 mg./liter with 2.60 percent in the thread, only three lengths could be removed intact. With 1.71 percent of the compound in the thread at 500 mg./liter, the threads supported mold growth and again only three lengths could be removed intact. At the lowest concentrations there was slight non-sporulating growth on all ten treated strings containing 2.95 percent toxicant, but all could not be removed intact.

Cunilate #2174-WP (Test of 9 December 1953).

After adjusting the percentage of solubilized Copper 8 to 100, the compound was prepared at the rate of 2000, 1000, 500, and 250 mg./liter. The uptake of compound at the respective concentrations was 5.46, 2.82, 1.47, and 0.77 percent. At the two highest concentrations there was no evidence of growth after the 14-day exposure period to the test fungi. At 500 mg./liter there was growth on one string, while at the rate of 250 mg./liter there was growth on four thread lengths. All could be removed from the plates intact.

Cunilate #2419 (Test of 9 October 1953).

An emulsion of Cunilate #2419 in water was prepared at concentrations of 2000, 1000, 500, and 250 mg./liter, after adjusting the percentage of solubilized Copper 8 to 100. The compound in the thread at the respective concentrations was 3.70, 0.64, 0.46, and 0.00 percent. Although all untreated one-inch squares were covered with growth, there was no evidence of growth on any of the treated thread lengths.

Cunilate #2419 (Test of 16 December 1953).

Concentrations were prepared as in the above test. The uptake of compound was found to be 5.21, 2.19, 0.91, and 0.30 percent of the weight of the thread at the respective concentrations. There was no evidence of growth on any of the treated thread lengths after the 14-day exposure period.

The foregoing observations and the tabulated data (Table 19) indicate that comirin, benzyl mucochlorate, endomycin, netropsin sulfate, and rimocidin are protective agents at the higher concentrations used. Lack of new samples of rimocidin sulfate and trichothecin made testing of these promising antifungal antibiotics at the higher concentrations impossible. Thirteen of the 14 Squibb pyridinethiol salts tested were shown to be effective mildew-proofing agents. In comparison to Copper 8 and Cunilates #2174, 2174-WP, and 2419 these compounds may even be slightly superior, since a smaller percentage of compound in the thread produces equivalent antifungal effects. Some of these compounds are white or light in color.

All the pyridinethiol salts, with the exception of the copper salt, proved to be effective against the cellulose-decomposing fungi on the treated threads. In the agar plate method, however, the copper salt was also effective against the cellulolytic fungi.

Large scale tests will have to be made on new lots of cotton cord at the same and higher concentrations, before definite evaluation of the selected candidate fungicides can be determined.

Table 19.-- Efficiency of candidate fungicides impregnated in 4-cord cotton thread on the retention of tensile strength when exposed to a combination of Chaetomium globosum and Myrothecium verrucaria for 14 days.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
			Comirin			
1000	1.28	4.82	6.48	1.66	-25.6	74.4
500	0.47	4.06	7.57	3.51	-46.4	53.6
250	0.40	3.80	6.11	2.31	-37.8	62.2
(control) 0	0.00	0.00	7.49	7.49	-100.0	0.0
(alcohol) 0	0.00	0.00	4.50	4.50	-100.0	0.0
			Benzyl mucochlorate (9/1/53)			
1000	0.65	2.83	5.88	3.05	-51.9	48.1
500	0.63	1.12	5.69	4.57	-80.3	19.7
250	0.66	0.00	4.64	4.64	-100.0	0.0
(control) 0 *	0.00	0.00	2.06	2.06	-100.0	0.0
(alcohol) 0 *	0.00	0.00	2.26	2.26	-100.0	0.0
			Benzyl mucochlorate (10/22/53)			
4000	2.91	6.67	7.29	0.62	-8.5	91.5
2000	1.90	6.60	7.67	1.07	-14.0	86.0
1000	1.38	5.65	7.89	2.24	-28.4	71.6
(control) 0	0.00	0.00	7.19	7.19	-100.0	0.0
(alcohol) 0	0.00	0.00	6.01	6.01	-100.0	0.0

*Unusually low results from unexposed control thread.

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
			Endomycin (7/3/53)			
4000	2.59	3.83	5.20	1.37	-26.3	73.7
2000	1.93	4.01	6.02	2.01	-33.4	66.6
1000	1.62	4.23	4.58	0.35	-7.6	92.4
(control) 0	0.00	0.00	6.58	6.58	-100.0	0.0
(alcohol) 0	0.00	0.00	5.28	5.28	-100.0	0.0
			Endomycin (9/25/53)			
2000	2.07	5.55	7.60	2.05	-27.0	73.0
1000	1.84	1.43	6.67	5.24	-78.6	21.4
500	1.29	0.00	7.29	7.29	-100.0	0.0
250	1.20	Decomposed	Not tested	-	-100.0	0.0
(control) 0	0.00	0.00	6.60	6.60	-100.0	0.0
(alcohol) 0	0.00	0.00	7.05	7.05	-100.0	0.0
			Endomycin (10/29/53)			
4000	1.72	5.21	6.45	1.24	-19.2	80.8
2000	1.75	4.05	6.35	2.30	-36.2	63.8
1000	1.38	Decomposed	Not	-	-100.0	0.0
500	1.06	by fungi	tested	-	-100.0	0.0
(control) 0	0.00	0.00	5.43	5.43	-100.0	0.0
(alcohol) 0	0.00	0.00	7.03	7.03	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposure	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
			Netropsin sulfate			
3000	1.49	5.20	5.59	0.39	-7.0	93.0
2000	1.02	4.54	5.07	0.53	-10.4	89.6
1000	1.38	5.38	6.41	1.03	-16.1	83.9
(control) 0	0.00	0.00	7.25	7.25	-100.0	0.0
(alcohol) 0	0.00	0.00	5.33	5.33	-100.0	0.0
			Nystatin			
1000	0.40	Decomposed	Not		-100.0	0.0
500	0.20	by fungi	tested		-100.0	0.0
250	0.14		because of		-100.0	0.0
(control) 0	0.00		failure of		-100.0	0.0
(methanol) 0	0.00		compound		-100.0	0.0
			Ortho			
1000	0.44	0.39	4.10	3.71	-90.5	9.5
500	0.27	0.27	4.21	3.94	-93.6	6.4
250	0.18	0.00	5.54	5.54	-100.0	0.0
(control) 0	0.00	0.00	7.12	7.12	-100.0	0.0
(acetone) 0	0.00	0.00	5.11	5.11	-100.0	0.0
			Protoanemonin			
1000	0.27	Decomposed	Not		-100.0	0.0
500	0.02	by fungi	tested		-100.0	0.0
250	0.05		because of		-100.0	0.0
(control) 0	0.00		failure of		-100.0	0.0
(water) 0	0.00		compound		-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposure	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
			Pfizer #16			
1000	0.96	4.59	6.94	2.35	-33.9	66.1
500	0.64	5.29	6.58	1.29	-19.6	80.4
250	0.51	4.95	6.49	1.54	-23.7	76.3
(control) 0 *	0.00	0.00	2.06	2.06	-100.0	0.0
(water) 0 *	0.00	0.00	4.14	4.14	-100.0	0.0
			Pfizer #21			
1000	0.38	Decomposed	Not		-100.0	0.0
500	0.25	by fungi	tested		-100.0	0.0
250	0.18		because of		-100.0	0.0
(control) 0	0.00		failure of		-100.0	0.0
(water) 0	0.00		compound		-100.0	0.0
			Rimocidin			
4000	2.55	6.24	6.56	0.32	-4.9	95.1
2000	1.29	2.10	5.00	2.90	-58.0	42.0
1000	0.79	3.53	5.50	1.97	-35.8	64.2
(control) 0	0.00	0.00	7.25	7.25	-100.0	0.0
(alcohol) 0	0.00	0.00	5.33	5.33	-100.0	0.0
			Rimocidin sulfate			
500	0.77	Decomposed	Not		-100.0	0.0
250	0.80		tested		-100.0	0.0
(control) 0	0.00	0.00	4.50	4.50	-100.0	0.0
(alcohol) 0	0.00	0.00	7.49	7.49	-100.0	0.0

*Unusually low results from unexposed control thread.

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
			Trichothecin			
300	0.38	Decomposed	Not		-100.0	0.0
150	0.40	by fungi	tested		-100.0	0.0
(control) 0	0.00		because of		-100.0	0.0
(ethanol) 0	0.00		failure of compound		-100.0	0.0
			Ustilagic acid (R. I. #43)			
2000	1.03	Decomposed	Not		-100.0	0.0
1000	1.09	by fungi	tested		-100.0	0.0
500	1.02		because of		-100.0	0.0
(control) 0	0.00		failure of		-100.0	0.0
(ethanol) 0	0.00		compound		-100.0	0.0
			Squibb MC2113 (6/30/53)			
4000	1.35	7.06	6.88	0.18	+2.6	102.6
2000	0.73	6.22	5.93	0.29	+4.9	104.9
1000	0.54	6.44	6.67	0.23	-3.4	96.6
(control) 0	0.00	0.00	6.95	6.95	-100.0	0.0
(ethanol) 0	0.00	0.00	6.61	6.61	-100.0	0.0
			Squibb MC2113 (9/1/53)			
1000	0.41	3.36	6.36	3.00	-47.2	52.8
500	0.00	1.65	4.71	3.06	-65.0	35.0
250	0.37	3.21	6.04	2.83	-46.8	53.2
(control) 0 *	0.00	0.00	2.06	2.06	-100.0	0.0
(ammonia + water) 0*	0.00	0.00	1.78	1.78	-100.0	0.0

*Unusually low results from unexposed control thread.

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
			Squibb MC2113 (9/25/53)			
2000	0.00	3.92	4.88	0.96	-19.7	80.3
1000	0.00	7.03	6.52	0.51	+7.8	107.8
500	0.00	6.97	6.71	0.26	+3.9	103.9
250	0.00	6.50	7.20	0.70	-9.7	90.3
(control) 0	0.00	0.00	6.60	6.60	-100.0	0.0
(ammonia + water) 0	0.00	0.00	7.26	7.26	-100.0	0.0
			Squibb MC3277			
4000	0.76	4.94	4.68	0.26	+5.5	105.5
2000	0.34	6.30	6.80	0.50	-7.3	92.7
1000	0.06	5.61	6.14	0.53	-8.6	91.4
(control) 0	0.00	0.00	6.95	6.95	-100.0	0.0
(water) 0	0.00	0.00	6.19	6.19	-100.0	0.0
			Copper salt of n - hydroxy -2- pyridine - thione MC3702			
1000	0.81	0.00	5.39	5.39	-100.0	0.0
500	0.06	0.00	5.54	5.54	-100.0	0.0
250	0.03	0.00	6.89	6.89	-100.0	0.0
(control) 0	0.00	0.00	6.63	6.63	-100.0	0.0
(acetone) 0	0.00	0.00	5.42	5.42	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Zinc salt of N - hydroxy -2- pyridine - thione MC3711				
1000	0.71	6.01	6.94	0.93	-13.4	86.6
500	0.12	0.52	6.68	6.16	-92.2	7.8
250	0.05	0.07	6.02	5.95	-98.8	1.2
(control) 0	0.00	0.00	6.63	6.63	-100.0	0.0
(acetone) 0	0.00	0.00	5.42	5.42	-100.0	0.0
		Manganese salt of 2 - mercapto - pyridine -1- oxide MC7728				
1000	0.79	4.40	4.91	0.51	-10.4	89.6
500	0.18	6.26	7.89	1.63	-20.6	79.4
250	0.10	4.30	5.28	0.98	-18.6	81.4
(control) 0	0.00	0.00	6.63	6.63	-100.0	0.0
(acetone) 0	0.00	0.00	5.42	5.42	-100.0	0.0
		Iron (ferrous) salt of 2-mercapto- pyridine-1-oxide MC7729				
1000	0.99	6.78	6.45	0.33	+5.1	105.1
500	0.37	4.18	4.87	0.69	-14.2	85.8
250	0.36	3.55	4.71	1.16	-24.6	75.4
(control) 0	0.00	0.00	6.63	6.63	-100.0	0.0
(acetone) 0	0.00	0.00	5.42	5.42	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Iron (ferric salt) of 2-mercapto-pyridine-1-oxide MC7730				
1000	0.86	6.61	7.02	0.41	-5.8	94.2
500	0.22	5.53	7.89	2.36	-29.9	70.1
250	0.00	4.60	6.89	2.29	-33.2	66.8
(control) 0	0.00	0.00	7.90	7.90	-100.0	0.0
(acetone) 0	0.00	0.00	6.11	6.11	-100.0	0.0
		Mercuric salt of 2-mercapto-pyridine-1-oxide MC7731				
1000	0.61	5.78	5.60	0.18	+3.2	103.2
500	0.22	6.19	6.44	0.25	-3.9	96.1
250	0.12	6.80	6.69	0.11	+1.6	101.6
(control) 0	0.00	0.00	7.90	7.90	-100.0	0.0
(acetone) 0	0.00	0.00	6.11	6.11	-100.0	0.0
		Silver salt of 2-mercapto-pyridine-1-oxide MC7732				
1000	0.42	6.34	7.16	0.82	-11.4	88.6
500	0.00	3.86	7.00	3.14	-44.8	55.2
250	0.00	4.04	7.16	3.12	-43.6	56.4
(control) 0	0.00	0.00	7.90	7.90	-100.0	0.0
(acetone) 0	0.00	0.00	6.11	6.11	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Antimony salt of 2-mercapto-pyridine -1-oxide MC7733				
1000	0.51	7.17	7.62	0.45	-5.9	94.1
500	0.39	3.76	3.50	0.26	+7.4	107.4
250	0.00	7.01	7.05	0.04	-0.6	99.4
(control) 0	0.00	0.00	7.90	7.90	-100.0	0.0
(acetone) 0	0.00	0.00	6.11	6.11	-100.0	0.0
		Cobalt salt of 2-mercapto-pyridine-1-oxide MC7734				
1000	1.17	5.95	6.38	0.43	-6.7	93.3
500	0.09	6.54	6.83	0.29	-4.2	95.8
250	0.18	5.81	6.15	0.34	-5.5	94.5
(control) 0	0.00	0.00	6.39	6.39	-100.0	0.0
(acetone) 0	0.00	0.00	6.19	6.19	-100.0	0.0
		Lead salt of 2-mercapto-pyridine-1-oxide MC7735				
1000	1.82	5.71	6.94	1.23	-17.7	82.3
500	0.38	5.34	6.32	0.98	-15.5	84.5
250	0.06	6.15	6.54	0.39	-6.0	94.0
(control) 0	0.00	0.00	6.39	6.39	-100.0	0.0
(acetone) 0	0.00	0.00	6.19	6.19	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Bismuth salt of 2-mercapto-pyridine-1-oxide MC7736				
1000	0.28	5.74	6.90	1.16	-16.8	83.2
500	0.04	4.60	7.81	3.21	-41.1	58.9
250	0.00	3.72	5.03	1.31	-26.0	74.0
(control) 0	0.00	0.00	6.39	6.39	-100.0	0.0
(acetone) 0	0.00	0.00	6.19	6.19	-100.0	0.0
		Barium salt of 2-mercapto-pyridine-1-oxide MC7737				
1000	0.91	5.72	5.86	0.14	-2.4	97.6
500	0.00	3.96	3.26	0.70	+21.5	121.5
250	0.00	4.83	5.26	0.43	-8.2	91.8
(control) 0	0.00	0.00	6.39	6.39	-100.0	0.0
(water) 0	0.00	0.00	6.57	6.57	-100.0	0.0
		Copper 8 - quinolinolate (6/5/53)				
4000	4.27	3.86	6.01	2.15	-35.8	64.2
2000	3.37	3.44	5.30	1.86	-35.1	64.9
1000	2.53	1.49	5.57	4.08	-73.2	26.8
(control) 0	0.00	0.00	5.98	5.98	-100.0	0.0
(alcohol) 0	0.00	0.00	6.10	6.10	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Copper 8 - quinolinolate (10/1/53)				
4000	3.58	3.16	2.71	0.45	+16.6	116.6
2000	2.74	4.22	5.07	0.85	-16.8	83.2
1500	1.97	5.04	6.60	1.56	-23.6	76.4
1000	1.67	6.48	6.89	0.41	-6.0	94.0
500	1.66	4.87	7.28	2.41	-33.1	66.9
(control) 0	0.00	0.00	7.01	7.01	-100.0	0.0
(alcohol) 0	0.00	0.00	7.01	7.01	-100.0	0.0
		Copper 8 - quinolinolate (10/22/53)				
4000	2.87	8.02	7.00	1.02	+14.6	114.6
2000	1.91	7.50	7.35	0.15	+2.0	102.0
1000	1.55	7.55	8.49	0.94	-11.1	88.9
500	1.30	7.02	8.13	1.11	-13.6	86.4
(control) 0	0.00	0.00	7.19	7.19	-100.0	0.0
(alcohol) 0	0.00	0.00	6.01	6.01	-100.0	0.0
		Cunilate #2174 (10/1/53)				
2000	3.10	3.44	4.91	1.47	-29.9	70.1
1000	1.48	4.41	6.04	1.63	-27.0	73.0
500	5.57	4.38	6.55	2.17	-33.1	66.9
250	5.92	4.26	7.28	3.02	-41.5	58.5
(control) 0	0.00	0.00	7.01	7.01	-100.0	0.0
(xylol) 0	0.00	0.00	5.46	5.46	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Cunilate #2174 (12/9/53)				
2000	2.98	5.91	6.34	0.43	-6.8	93.2
1000	1.40	4.93	4.99	0.06	-1.2	98.8
500	0.50	7.00	6.77	0.23	+3.4	103.4
250	0.45	4.55	6.07	1.52	-25.0	75.0
(control) 0	0.00	0.00	6.73	6.73	-100.0	0.0
(xylol) 0	0.00	0.00	6.23	6.23	-100.0	0.0
		Cunilate #2174 - WP (10/9/53)				
2000	4.32	5.67	6.02	0.35	-5.8	94.2
1000	2.60	0.63	7.11	6.48	-91.1	8.9
500	1.71	0.93	5.98	5.05	-84.4	15.6
250	2.95	3.44	6.58	3.14	-47.7	52.3
(control) 0	0.00	0.00	5.99	5.99	-100.0	0.0
(xylol) 0	0.00	0.00	6.43	6.43	-100.0	0.0
		Cunilate #2174 - WP (12/9/53)				
2000	5.46	5.85	6.64	0.79	-11.9	88.1
1000	2.82	5.14	6.02	0.88	-14.6	85.4
500	1.47	5.33	6.05	0.72	-11.9	88.1
250	0.77	5.18	6.16	0.98	-15.9	84.1
(control) 0	0.00	0.00	6.73	6.73	-100.0	0.0
(xylol) 0	0.00	0.00	6.23	6.23	-100.0	0.0

Table 19.--continued.

Conc. of solution mg./liter	% of compound in thread	Thread treated & exposed	Thread treated not exposed (lbs.)	Strength difference (lbs.)	% loss or gain	% remaining strength
		Cunilate #2419 (10/9/53)				
2000	3.70	4.82	5.39	0.57	-10.6	89.4
1000	0.64	4.34	5.62	1.28	-22.8	77.2
500	0.46	4.67	5.67	1.00	-17.6	82.4
250	0.00	5.97	6.39	0.42	-6.6	93.4
(control) 0	0.00	0.00	5.99	5.99	-100.0	0.0
(water) 0	0.00	0.00	6.94	6.94	-100.0	0.0
		Cunilate #2419 (12/16/53)				
2000	5.21	5.65	5.84	0.19	-3.2	96.8
1000	2.19	5.09	5.83	0.74	-12.7	87.3
500	0.91	5.50	6.28	0.78	-12.4	87.6
250	0.30	5.55	5.73	0.18	-3.1	96.9
(control) 0	0.00	0.00	6.65	6.65	-100.0	0.0
(water) 0	0.00	0.00	5.67	5.67	-100.0	0.0

DISCUSSION OF RESULTS

The chief aim of the study was to determine the antifungal activity of obtainable antibiotics and particularly the effective range of the most potent substances as inhibitors of cellulose-decomposing fungi. Antibiotics were requested of governmental agencies and commercial producers for this investigation, but many of the candidate agents supplied were synthetic in nature. The composition and purity of these latter were known and hence an accurate judgement of them could be obtained. The natural antibiotics, however, were not all submitted in a pure state, i. e. the active ingredient was unknown. Hence, the full potential of their activity may not have been exhibited, since all were subjected to tests in the form received. Since the chemical and physical form of a material affects its antifungal action, considerable further study will be required before full inherent fungitoxicity can be known and utilized.

The test fungi employed are generally known to range from most to least resistant to toxic compounds in the following order: Aspergillus niger, Aspergillus terreus, Myrothecium verrucaria, and Chaetomium globosum. In the destruction of cotton cloth fibers, Chaetomium globosum is the least visible but perhaps the most important; but Myrothecium verrucaria is a very active decomposer and stainer of cellulose. Aspergillus niger is least important, although it does cause discoloration and/or spotting. The agar plate test results are fairly well in agreement with the above generality. However, there is evidence of specifity of toxic action to the various fungi. The organisms, in these instances, do not follow the general pattern of resistance. Undoubtedly, a major factor is penetration of the spore or hyphal wall which would exclude or permit ingress to the site of action in the protoplasm. Also the enzyme systems operative in the fungous metabolism play a part, as the case of copper sensitivity of A. niger.

Although the simplified screening procedure suggests the use of only one organism, Aspergillus niger, in the initial assay, Aspergillus terreus was also tested. The copper salt of the pyridinethiones would have been eliminated in the initial assay, since it did not yield 100 percent inhibition of A. niger; but did completely inhibit A. terreus at 100 ppm. Therefore, it was tested against the other organisms; against all of which it was effective. Thus the use of the second organism to eliminate somewhat the factor of specificity appears to be justified. Many years of personal experience with the method of incorporating a chemical in an agar gel, as a measure of its fungitoxicity, has resulted in questioning of its validity. For example, if thiram and mercury chloride are incorporated in agar gels and inoculated with a test fungus, growth is likely on the substrate poisoned with mercury but not with thiram. Yet, if a solution of each toxicant is allowed to stand in a culture for 5 minutes and then segments of the thallus cut, washed and implanted on fresh nutrient agar, the thiram-exposed hyphae will grow while the mercury-treated hyphae are killed. Thus too great a reliance should not be placed on the agar plate method alone.

The standard glass-slide technique was employed, since it was thought a correlation might exist between this method and the agar plate test. Also most investigators developing chemicals for the control of fungi causing plant disease find that the method of inhibiting spore germination on glass slides with a series of dosages of the candidate toxicant is a sensitive, quantitative measure of fungitoxicity. Comirin, however, was the only antibiotic found to be a leading fungicide by both methods; with this exception the relative potency of compounds tested by each method differed. Specificity again may be a possible explanation, since essentially different types of organisms are represented by the two tests. Another explanation is the diffusability and volatility of some chemicals as influenced by inclusion in aqueous agar gels.

The "pad plate method" is a means of evaluating a large number of candidate chemicals in a short period of time. However, results can only be scored on a qualitative basis, and results on a quantitative, numerical basis cannot be determined. For this reason, the method was employed only to a limited extent. However, experience with the method does permit the observer to judge whether the toxicant diffuses out of or is retained by the cellulose, and to assess the concentration that will inhibit the mold from entering and growing on the pad.

Thread impregnation tests are considered to be an accurate index of effectiveness, but results were often inconsistent. The percentage uptake of compound at the various concentrations in which the threads were immersed did not follow a predictable order in many cases, a greater amount often being taken up at a lower concentration than at the higher concentrations. Although the thread supplied by the Air Force was presumed to have a breaking strength of approximately 14 lbs., the average for the untreated, desized thread was about seven pounds according to the Scott tester used throughout the project. Rather large variations were sometimes found in the tensile strength in each lot of cords tested and results were often not reproducible. Variations in twist of individual threads and imperfections in the large spools from which the thread lengths were cut may be possible explanations. Also the process of desizing may have weakened the fibers to a certain degree. In spite of the uptake of compounds being inconsistent and results not always reproducible, the general trend of the candidate compounds' fungicidal activity is evident from the data.

The ability of Squibb MC 2113 (2-pyridinethione-1-oxide, sodium salt) to protect the threads against the cellulolytic fungi, when the percentage of compound in the thread was calculated to be 0, is of great interest. Whether atmospheric conditions affected the weighings after the treatment, or whether trace amounts are capable of protecting the threads, is unknown. The latter is indicated by the data, however. Noteworthy is the fact that although some materials were not considered soluble in water but only in such organic solvents as acetone and ethanol, yet they diffused sufficiently through the agar gel to stop fungal growth on the untreated cotton squares. The weakness of this technique is that the weight of 10 lengths of cotton 4-thread cord is so little that the chainomatic balance is not sufficiently sensitive (0.1 milligram) to indicate the relatively minute residue absorbed from the treating bath when brought to constant weight by drying.

Interpretation of the term "antibiotic" varies. Some would restrict its use to organic substances synthesized by fungi which have the capability to retard or stop the growth of microorganisms. Bacteria, lichens and seed-bearing plants also produce growth-inhibiting substances, which has necessitated enlargement of the concept. Then the research chemist successfully synthesized the active compound (e.g. chloromycetin) which was produced naturally by a fungus. Thus, from the standpoint of use, the literal meaning "against living substance" is of greater interest than the origin. The chemist with control over conditions can be looked to for "synthetic" antibiotics of the desired potency and characteristics for use.

Certain of the antibiotics (e.g. comirin, netropsin sulfate, rimocidin, and endomycin) appear to have sufficient merit to justify obtaining one or more in requisite quantities to run a pilot test with material for which copper 8-quinolinolate is unsatisfactory. Likewise, trial runs with the salts of 2-pyridinethiol, 1-oxide impregnated in cotton cloth should be made and exposure tests carried out. The versatility of this group as to color and solubility, in addition to its potent fungitoxicity, should make it adaptable to many applications. Details for use were given in the "Invention Report" of 27 November 1953. The material could be made in unlimited quantities at a reasonable price. Although the muchochlorates are of interest, the furans should be given particular consideration in the future because of their high potency as fungicides, bactericides and even nematocides.

SUMMARY

The premise that antibiotics equal in antifungal action the currently used copper 8-quinolinolate has been verified by this study. Comirin, netropsin sulfate, rimocidin, and endomycin were superior to copper 8-quinolinolate in retention of tensile strength of 4-thread cotton cord exposed to the cellulose-destroying fungi, Chaetomium globosum and Myrothecium verrucaria. In addition, some pyridinethiols have evidenced sufficient fungitoxicity to make them competitive with present mildew-proofing agents. Salts can be prepared to permit precipitation in cloth and thus confer "permanent" mold protection. Another active antifungal group brought to light is the benzyl and the p-chlorobenzyl mucochlorates. In medical practice a group of furan derivatives have proved to have a wide spectrum of toxicity to resistant microorganisms. A few of these have also exhibited very high antifungal action.

Several antibiotics that have been reported effective for plant disease control were inferior against the test fungi employed in this study. No one test method can be completely relied upon to select or to serve as the basis for the recommendation for a particular application. Temperature appears to be a more critical factor than hydrogen-ion concentration in affecting the stability of toxicants.

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