HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 12 OCTOBER, 1938 NUMBER 1

CONTENTS

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 12 OCTOBER, 1938 No. 1

THE TOXIC EFFECT OF CERTAIN CHEMICAL SOLUTIONS ON SPORES OF PENICILLIUM ITALICUM AND P. DIGITATUM[1, 2]

LIANG HWANG[3] AND L. J. KLOTZ[4]

INTRODUCTION

THE BLUE AND THE GREEN molds (*Penicillium italicum* Wehmer and *P. digitatum* Sacc.) are the most common fungi causing soft decay in citrus fruits. They are world-wide in distribution, affecting fruits in orchards, in packing-houses, during transportation, and on the markets. In 1908, Powell (*27*)[5] reported that the losses from blue-mold decay in oranges during transportation from California were from $750,000 to $1,500,000 annually.

According to Sawada's (*34*) report in 1922, the two molds caused decay of oranges in Italy, the United States, Japan, and Formosa. Tindale (*38*) stated that in Victoria blue and green molds are the greatest enemies of oranges in cold storage and elsewhere. He (*39*) also reported that after two months' cold storage blue mold developed extensively. In 1928, Barker (*7*) stated that green mold causes serious losses in oranges from Spain, Palestine, Brazil, and the Argentine; less extensive damage is caused to oranges from South Africa, Australia, and California, and to grapefruit from Florida, Puerto Rico, and South Africa. In the same year Reichert and Littauer (*32*) reported that blue and green molds developed on picked fruit in Palestine. Bates (*8*) has shown that ship-

[1] Received for publication February 2, 1938.

[2] Paper No. 367, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[3] Research Fellow of China Foundation for the Promotion of Education and Culture.

[4] Associate Plant Pathologist in the Experiment Station.

[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

ment of early varieties in South Africa without precooling was attended by a very marked increase in mold wastage on discharge overseas. Yu (*43*) has reported that blue and green molds are found on all kinds of citrus fruits on the markets in China. Recently, Nattrass (*24*) reported that wastage of Cyprus oranges arriving in Europe was caused by blue and green molds.

In 1925, H. S. Fawcett (*13*) reported that of the decay of citrus fruits on arrival at eastern markets, 1.4 per cent was due to blue mold and 0.8 per cent was due to green mold. He also mentioned that the principal and almost the only kinds of decay found in California were the green mold and blue mold. In 1928, Barger (*4*) reported that Fawcett, after inspecting 500 field boxes of navel oranges in 8 Tulare County packing-houses in California in 1927, found that among the 1 per cent of rot in these oranges, 52 per cent of it was due to green mold, 32 per cent to a mixture of blue and green molds, 11 per cent to blue mold, and 5 per cent to other types of decay. According to Hopkins' report (*20*) for 1929, it was shown that the greatest losses during storage were due to *Penicillium italicum* and other fungi. Takeuchi (*37*) reported in 1929 that the rotting of satsuma oranges in storage or transit in Japan was caused by *P. italicum, P. digitatum,* and two other species of *Penicillium.* It was estimated that 89 per cent of the decayed fruits had *P. italicum* and 73 per cent had *P. digitatum.*

The prevention of any form of wounds is the most important means of reducing decay, but the use of solutions of certain substances has been tried out as a supplementary means to prevent the rot. A solution of borax was first tested and described by Fulton and Bowman (*17*) in 1924. Further suggestions and confirmations for its use have been reported by the following: Fulton and Winston (*18*), Barger and Hawkins (*6*), Barger (*4*), the Brogdex Company (*10*), Powell (*28*), Young and Read (*42*), Benton (*9*), Bates (*8*), Nattrass (*24*), Putterill (*30*), and by Winston (*41*). The solutions of soap and of borax used in citrus-fruit packing houses for washing and disinfecting have been reported by Fawcett (*14*) and by Shiver (*35*). Hodgson (*19*) claimed that the use of borax was rapidly going out of favor in the California citrus industry. Some workers (*8, 29*) have emphasized that borax treatment can be considered only as an adjunct to careful handling.

"Metbor," a new material said to equal borax as a decay preventive, was reported recently by Stewart (*36*) to have marked advantages over borax in regard to cold water solubility and other properties.

According to the Charter Oak House tests (*11*), the sodium hypochlorite process will prevent blue and green molds from developing in fruit

while it is in transit. Recently a stabilized sodium hypochlorite concentrate (*2*) has been manufactured; this contained 6 per cent sodium hypochlorite and when diluted according to directions was effective in controlling blue mold on apples and pears. Baker and Heald (*3*) found that rinsing apples for one minute with a sodium hypochlorite solution containing 0.4 per cent available chlorine was very effective in reducing the number of viable spores of *Penicillium expansum* on the surface and in the lenticels of apples and in decreasing losses from decay by this fungus.

Sodium bicarbonate and sodium carbonate are used in countries where borax treatment is prohibited by law. In 1928, Barger (*5*) first used sodium bicarbonate in controlling molds. The results were confirmed by Young and Read (*42*), by the Australian Citrus Preservation Committee (*1*), by Benton (*9*), by Bates (*8*), by Putterill and Davies (*31*), and by Putterill (*30*). Sodium carbonate was shown by Doidge (*12*) to be best for the control of *Penicillium* molds.

Tomkins and Trout (*40*) stated that storage of oranges in a humid atmosphere with ammonium carbonate or with crystals of ammonium carbonate reduces green-mold decay.

The study reported here was made for the purpose of securing more definite and effective means of controlling the blue and green molds by the use of chemical solutions.

MATERIALS USED

The original cultures of *Penicillium italicum* (No. 1746) and *P. digitatum* (No. 1438) were obtained from the stock cultures of the Division of Plant Pathology, Citrus Experiment Station, Riverside, California. The former was isolated by L. J. Klotz in 1930 from a decayed Valencia orange, and the latter by G. Savastano in 1927 from a decayed lemon. The medium used for these two fungi was 2 per cent glucose potato agar in the form of test-tube slants.

All cultures used throughout the experiments were incubated at 77° F (25° C), which was near the optimum temperature for growth on culture media as well as on orange fruits, as shown by Fawcett and Barger (*15*). The rate of sporulation of *Penicillium digitatum* is much slower than that of *P. italicum*. For the purpose of getting fair sporulations of these two fungi, *P. digitatum* was transferred to the slant 8 days earlier than *P. italicum*. For all treatments throughout the experiments, the age of *P. italicum* cultures used was 6 days and that of *P. digitatum* was 14 days.

The substances described below were used in solution form for treating both kinds of spores in the experiments:

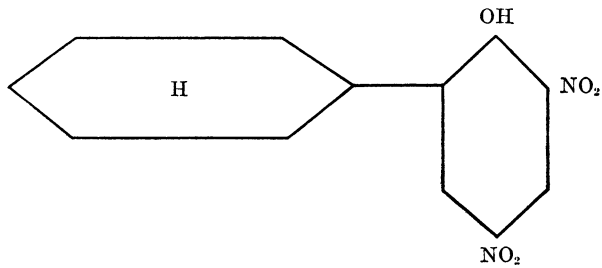
1. It was thought that a neutral soap of high purity might be used to facilitate wetting and to prevent clumping of spores. Accordingly a good grade of Castile soap was selected and tested in various concentrations.

2. Various concentrations of borax (sodium tetraborate decahydrate), $Na_2B_4O_7 \cdot 10H_2O$, were used. This preparation is also called sodium biborate or sodium pyroborate. It is a colorless monoclinic crystal or white powder and is slightly soluble in water and insoluble in alcohol.

3. A 6 per cent solution of a mixture of 2 parts borax and 1 part boric acid, H_3BO_3, was employed.

4. Metbor, which consists of 95 to 97 per cent sodium metaborate ($Na_2B_2O_4 \cdot H_2O$) and 3 to 5 per cent borax, was used in various concentrations.

5. Dinitro-o-cyclohexylphenol, is a yellow powder, having the empirical formula $C_{12}H_{14}N_2O_5$, and the structural formula:



It is only slightly soluble in distilled water, dissolving to the extent of 6.2 milligrams per liter of water. It was used in concentrations representing saturation, half saturation, and one-fourth saturation.

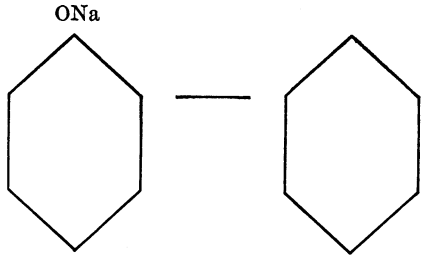
6. A 6 per cent sodium hypochlorite, NaOCl, was used as stock solution and then diluted to three concentrations of 1.0, 0.6, and 0.4 per cent.

7. Various concentrations of sodium bicarbonate, $NaHCO_3$, were used. This is also called acid sodium carbonate and baking soda. It is a white opaque powder or colorless crystals, soluble in water.

8. Sodium carbonate, Na_2CO_3 (anhydrous), was used in various concentrations. It is a white powder which is soluble in water. The form commonly used commercially is called soda ash.

9. A 0.4 per cent solution of chloramine-T or chlorazene, $CH_3C_6H_4SO_2Na:NCl \cdot 3H_2O$ (sodium p-toluene-sulfo-chloramine), was also tested. It is also called mianine, actirin, tochlorine, chloroamine, tolamine, and Dakin's antiseptic. It takes the form of colorless crystals which are soluble in water.

10. Sodium o-phenylphenate was used as a 0.15 per cent solution. The chemical is represented by the formula:



It is a white powder which is difficultly soluble in water.

11. A 1 per cent solution of a commercial washing powder containing mostly soda ash (anhydrous sodium carbonate) with some caustic and a trace of pine oil, was also used.

METHODS

The methods described here have been used throughout the experiments as the standard procedure, unless otherwise stated. The more specific methods will be described under separate headings.

As soon as preparations for a certain experiment were completed the spore suspensions were made up in 0.25 per cent soap solution from agar slant cultures of *Penicillium italicum* and *P. digitatum*. About 10 minutes after shaking these suspensions, 5 cc of each of the suspensions was transferred with sterile pipettes to sterile centrifuge tubes. In order to precipitate the spores from suspension, these tubes were centrifuged for 3 minutes. Immediately after that the supernatant solution was decanted and into each tube was poured 10 cc of a desired concentration of the designated chemical. The tube was then shaken thoroughly. About 3 minutes before the desired treatment time had expired, the tubes were put in the centrifuge in order to precipitate the treated spores. At the end of 3 minutes, the treating solution was decanted and the spores were washed with sterile distilled water.

When experiments on the effect of various temperatures were conducted, treating solutions at the desired temperature were poured on the spores and the tubes immersed immediately in water baths at definite, controlled temperatures for a period of about 3 minutes less than the desired period of exposure. Then these tubes were put in the centrifuge which was fixed in an electric oven adjusted to the same temperature as the water bath. After the tubes were centrifuged for 3 minutes, the treat-

ing solution was decanted and the spores washed with sterile distilled water as before. The check tubes were secured after the spore suspensions in soap were centrifuged and decanted, by using 10 cc of sterile distilled water instead of the treating solution. At the end of the treatments, the number of spores per cubic centimeter was estimated by means of a Howard counting chamber. Generally, two counts for each fungus were made, one of the treated suspension and one of the untreated suspension. In order to determine the viability of the treated and untreated spores, germination and dilution plate tests in triplicate were conducted as follows.

Germination.—For the sake of obtaining accurate results in spore-germination tests, several factors mentioned by McCallan and Wilcoxon (*23*) were considered. They stated that the most important factors are: cleanliness of glassware, source and age of spores, density of spore suspension, germination medium, concentration of toxic agent, temperature, and time. For the germinations two Van Tieghem cells were sealed to each glass slide with vaseline. A drop of sterile distilled water was placed in each cell and a small amount of vaseline on the upper edge of each ring. A very small drop of fresh sweet-orange juice and one loopful (4 mm) of spore suspension were placed on a sterile cover glass which was then inverted over a cell. Then these cells were placed in the incubator at 77° F (25° C). After 24 hours of incubation, a drop of chloroform was introduced into one cell of a slide to stop the growth during the period of measurement; this was repeated in the other cell after 48 hours.

Dilution Method.—As soon as the hanging-drop germination tests were completed, dilutions of 1:10,000, 1:100,000, and 1:1,000,000 were made by means of sterile pipettes, and 9 cc and 99 cc water blanks. Each of the dilutions was transferred with a pipette to a sterile petri dish. Melted glucose potato agar was poured into these petri dishes, and was mixed and incubated at 77° F. After periods of 2 days and 3 days, the number of colonies in the dishes was counted.

All results shown in table 2 were recorded as an average of three tests for each experiment, unless otherwise noted. The average number of colonies per cubic centimeter in the dilution-plate counts was calculated by dividing the total number of colonies of the three dilutions by the decimal 0.000111, since dilutions of 1:10,000 (0.0001), 1:100,000 (0.00001), and 1:1,000,000 (0.000001) were used. The viability index was calculated by dividing the average number of colonies per cubic centimeter of dilution-plate counts by the average number of spores per cubic centimeter of the microscopic counts. In the last column of table 2 the viability of each fungus after each treatment is calculated on the

basis of an assumed value of 100 for the viability in the water check. This permits comparison of the two fungi in any given solution and gives at a glance the relative efficiency of the several treatments.

MEANS OF WETTING SPORES

Before starting the experiments on the effect of various chemical solutions, three different experiments were made with soap, the object being to determine the most suitable concentration for wetting spores and preventing clumping.

The Effect of Various Concentrations of Soap Solution.—Spore suspensions were made in five different concentrations of Castile soap, 10

TABLE 1

CONCENTRATION OF SOAP SOLUTION IN RELATION TO THE NUMBER OF SPORE CLUMPS AT ROOM TEMPERATURE

Fungus	Treatment		Average number of clumps per field*	Number of spores per cubic centimeter
	Per cent soap	Time, in minutes		
Penicillium italicum	0.10	10	4.8	3,827,157
	0.25	10	6.7	
	0.50	10	8.9	
	1.00	10	6.1	
	2.00	10	6.5	
	Check	..	9.6	
Penicillium digitatum	0.10	10	7.4	2,592,590
	0.25	10	3.1	
	0.50	10	4.8	
	1.00	10	6.8	
	2.00	10	3.4	
	Check	..	3.8	

* Average of 20 fields under the low power of the microscope.

cc of each being placed in sterile centrifuge tubes. These were centrifuged at room temperature for about 3 minutes, the total length of exposure being 5 minutes. Immediately after centrifuging, all the soap solutions were decanted and the tubes filled with 10 cc of sterile distilled water. The germination tests and plate method were then carried out as described above. The results are shown in table 2, entries 1 to 12.

Although none of these concentrations showed much inhibition of spore germination, the results (table 2) indicated that 0.25 per cent soap solution had the least effect on both spore germination and growth in petri dishes in the case of *Penicillium digitatum.*

TABLE 2

TOXICITY OF VARIOUS CHEMICAL SOLUTIONS TO THE CONIDIA AT SEVERAL TEMPERATURES AND PERIODS OF EXPOSURE

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination		Average number of colonies per cc by dilution plate counts*	Average number of spores per cc by microscopic counts	Viability index†	Relative viability (water at 69° F = 100)
							After 24 hrs.	After 48 hrs.	*A*	*B*	*A* ÷ *B*	
1	*P. italicum*....	Castile soap.....	0.10	9.73	69	5	98.9	100.0	2,801,801	3,950,614	0.709	77.3
2			0.25	9.90	69	5	99.5	100.0	3,108,108	3,950,614	0.787	85.8
3			0.50	9.88	69	5	99.1	100.0	3,576,576	3,950,614	0.905	98.6
4			1.00	9.97	69	5	98.6	100.0	4,279,279‡	3,950,614	1.083	118.1
5			2.00	10.05	69	5	99.5	100.0	5,189,189‡	3,950,614	1.313	143.1
6		Distilled water.....	Check	4.98	69	5	99.5	100.0	4,189,189	4,567,898	0.917	100.0
7	*P. digitatum*...	Castile soap.....	0.10	9.73	69	5	68.8	92.5	1,522,522	2,304,525	0.661	90.3
8			0.25	9.90	69	5	76.9	98.7	1,531,531	2,304,525	0.664	90.7
9			0.50	9.88	69	5	70.8	84.0	1,423,423	2,304,525	0.618	84.4
10			1.00	9.97	69	5	72.8	100.0	1,270,270	2,304,525	0.551	75.2
11			2.00	10.05	69	5	71.5	82.5	1,324,324	2,304,525	0.575	78.5
12		Distilled water.....	Check	4.98	69	5	76.6	92.5	1,837,837	2,510,289	0.732	100.0
13	*P. italicum*....	Castile soap; borax.....	0.10; 6¶	9.30	69	10; 5¶	52.2	93.4	2,909,909	3,497,939	0.832	71.5
14			0.25; 6	9.30	69	10; 5	46.6	99.2	2,765,765	3,497,939	0.791	68.0
15			0.50; 6	9.30	69	10; 5	29.1	91.9	3,090,090	3,497,939	0.883	75.9
16			1.00; 6	9.30	69	10; 5	32.3	75.8	4,189,189‡	3,497,939	1.198	103.0
17			2.00; 6	9.30	69	10; 5	31.8	96.4	4,171,171‡	3,497,939	1.192	102.5
18			0.00; 6	9.30	69	.. 5	41.4	98.7	3,090,090	3,497,939	0.883	75.9
19		Distilled water.....	Check	4.98	69	5 ..	96.2	100.0	5,981,981‡	5,144,022	1.163	100.0
20	*P. digitatum*...	Castile soap; borax.....	0.10; 6	9.30	69	10; 5	18.8	28.1	1,000,000	2,716,047	0.368	62.6
21			0.25; 6	9.30	69	10; 5	13.8	54.3	846,846	2,716,047	0.312	53.1
22			0.50; 6	9.30	69	10; 5	4.1	36.5	936,936	2,716,047	0.345	58.7
23			1.00; 6	9.30	69	10; 5	22.7	75.5	684,684	2,716,047	0.252	42.9
24			2.00; 6	9.30	69	10; 5	43.6	74.9	990,990	2,716,047	0.365	62.1
25			0.00; 6	9.30	69	.. 5	59.2	93.6	1,045,045	2,716,047	0.385	65.5
26		Distilled water.....	Check	4.98	69	5 ..	97 7	100.0	1,765,765	3,004,113	0.588	100.0

27			....	4.98	69	5	99.6	100.0	5,108,828	5,226.333	0.977	100.0
28	*P. italicum*....	Distilled water.....	....	5.04	100	5	97.2	100.0	1,846,846	4,444,107	0.415	42.5
29			....	5.06	110	5	58.6	98.4	747.747	4,444,107	0.168	17.2
30			....	5.30	120	5	0.8	11.5	9,009	4,444,107	0.002	0 2
31			....	4.98	69	5	99.2	100.0	1,504,504	3,168,721	0.475	100.0
32	*P. digitatum*...	Distilled water.....	....	5.04	100	5	95.8	99.6	792,792	2,921,808	0.271	57.1
33			....	5 06	110	5	59.2	99.6	509,509	2,921,808	0.174	36.6
34			....	5.30	120	5	0.4	8.2	9,009	2,921,808	0.003	0.6
35			6	8.98	110	2	65.5	100.0	3,666,666	8,768,759	0.418	88.4
36			6	8.98	110	4	80.0	100.0	2,882,882	8,768,759	0.329	69.6
37			6	8.98	110	6	13.6	81.6	2,774,774	8,768,759	0.316	66.8
38		Borax.....	6	8.98	110	8	13.1	57.0	1,549,549	8,768,759	0.177	37.4
39			6	8.98	110	10	1.2	24.4	531,531	8,768,759	0.061	12.9
40	*P. italicum*....		6	8.98	110	12	2.4	39.9	792,792	8,768,759	0.090	19.0
41			6	8.98	110	14	0.1	24.3	441,441	8,768,759	0.050	10.6
42			6	8.98	110	16	0.1	22.1	230,630	8,768,759	0.026	5.5
43		Distilled water.....	Check	4.98	69	5	100.0	100.0	4,441,441	9,386,042	0.473	100.0
44			6	8.98	110	2	68.1	99.1	1,171,171	3,580,177	0.327	60.8
45			6	8.98	110	4	82.6	99.4	837,837	3,580,177	0.234	43.5
46			6	8.98	110	6	32.1	64.2	864,864	3,580,177	0.247	45.9
47		Borax.....	6	8.98	110	8	3.6	12.4	504,504	3,580,177	0.141	26.2
48			6	8.98	110	10	3.9	33.4	301,801	3,580,177	0.084	15.6
49	*P. digitatum*...		6	8.98	110	12	3.6	27.9	141,141	3,580,177	0.039	7.2
50			6	8.98	110	14	1.0	6.5	193,693	3,580,177	0.054	10.0
51			6	8.98	110	16	0.9	4.9	128,828	3,580,177	0.036	6.7
52		Distilled water.....	Check	4.98	69	5	99.8	100.0	2,306,306	4,283,165	0.538	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

¶ In this column in entries 13 to 26, the first number refers to castile soap and the number following the semicolon refers to borax.

TABLE 2—(*Continued*)

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination		Average number of colonies per cc by dilution plate counts*	Average number of spores per cc by microscopic counts	Viability index†	Relative viability (water at 69° F=100)
							After 24 hrs.	After 48 hrs.	*A*	*B*	*A*÷*B*	
53			6	9.30	69	5	49.0	78.6	3,162,162	4,691,354	0.674	62.1
54			6	9.14	80	5	40.9	73.2	4,108,108	4,691,354	0.876	80.7
55		Borax	6	9.00	100	5	36.5	40.9	3,261,261	4,691,354	0.695	64.0
56	*P. italicum*		6	8.96	110	5	12.9	40.8	1,567,567	4,691,354	0.334	30 8
57			6	8.93	120	5	0.1	2.3	0	4,691,354	0.000	0.0
58		Distilled water	Check	4.98	69	5	100.0	100.0	5,720,720‡	5,267,419	1.086	100.0
59			6	9.30	69	5	51.5	77.1	1,207,207	3,786,005	0.319	56.8
60			6	9.14	80	5	55.7	62.3	1,297,297	3,786,005	0.343	61.0
61		Borax	6	9.00	100	5	39.7	58.2	1,027,027	3,786,005	0.271	48.2
62	*P. digitatum*		6	8.96	110	5	5.2	53.5	585,585	3,786,005	0.155	27.6
63			6	8.93	120	5	1.0	1.3	2,702	3,786,005	0.001	0.1
64		Distilled water	Check	4.98	69	5	99.0	100.0	2,450,450	4,362,136	0.562	100.0
65			4	8.95	110	5	13.7	85.9	1,729,729	5,843,620	0.296	31.1
66			6	8.98	110	5	6.0	41.6	1,324,324	5,843,620	0.227	23.9
67		Borax	8	9.03	110	5	0.8	23.7	1,387,387	5,843,620	0.237	24.9
68	*P. italicum*		10	9.08	110	5	0.6	7.9	1,144,144	5,843,620	0.196	20.6
69			12	9.11	110	5	0.0	1.7	936,936	5,843,620	0.160	16.8
70		Distilled water	Check	4.98	69	5	100.0	100.0	6,621,621	6,962,546	0.951	100.0
71			4	8.95	110	5	59.4	89.2	1,297,297	4,444,441	0.292	76.2
72			6	8.98	110	5	5.7	51.0	1,045,045	4,444,441	0.235	61.4
73		Borax	8	9.03	110	5	4.1	39.4	909,909	4,444,441	0.205	53.5
74	*P. digitatum*		10	9.08	110	5	2.2	31.9	711,711	4,444,441	0.160	41.8
75			12	9.11	110	5	0.0	1.3	401,801	4,444,441	0.090	23.5
76		Distilled water	Check	4.98	69	5	100.0	100.0	1,891,891	4,938,268	0.383	100.0

77			6	8.18	100	5	59.6	99.6	2,342,342	4,067,771	0.576	56.7
78		Borax-boric acid	6	8.15	110	5	0.0	40.8	743,243	4,067,771	0.183	18.0
79	*P. italicum*		6	8.14	120	5	0.0	11.9	7,207	4,067,771	0.002	0.2
80		Distilled water	Check	4.98	69	5	100.0	100.0	5,225,225‡	5,144,029	1.016	100.0
81			6	8.18	100	5	90.8	100.0	1,153,153	2,757,199	0.418	94.1
82		Borax-boric acid	6	8.15	110	5	4.6	59.9	486,486	2,757,199	0.176	39.6
83	*P. digitatum*		6	8.14	120	5	0.0	0.0	2,702	2,757,199	0.001	0.2
84		Distilled water	Check	4.98	69	5	99.9	100.0	1,369,369	3,086,084	0.444	100.0
85			6	10.18	69	2	99.2	100.0	2,261,261	3,621,396	0.624	61.7
86		Metbor	6	10.18	69	5	91.8	100.0	2,216,216	3,621,396	0.612	60.5
87	*P. italicum*		6	10.18	69	10	87.7	99.5	1,882,882	3,621,396	0.520	51.4
88			6	10.18	69	15	65.8	91.7	1,756,756	3,621,396	0.485	48.0
89		Distilled water	Check	4.98	....	5	99.6	100.0	4,036,036‡	3,991,432	1.011	100.0
90			6	10.18	69	2	97.4	100.0	1,261,261	3,004,112	0.420	89.9
91		Metbor	6	10.18	69	5	96.9	100.0	1,099,099	3,004,112	0.366	78.4
92	*P. digitatum*		6	10.18	69	10	77.6	98.4	981,981	3,004,112	0.327	70.0
93			6	10.18	69	15	49.6	79.2	900,900	3,004,112	0.300	64.2
94		Distilled water	Check	4.98	69	5	99.8	100.0	1,576,576	3,374,482	0.467	100.0
95			6	10.18	69	5	91.8	100.0	2,216,216	3,621,396	0.612	62.6
96		Metbor	6	9.95	100	5	87.1	99.8	2,198,198	4,444,107	0.495	50.7
97	*P. italicum*		6	9.86	110	5	78.1	99.2	1,981,981	4,444,107	0.446	45.6
98			6	9.79	120	5	0.0	0.6	23,423	4,444,107	0.005	0.5
99		Distilled water	Check	4.98	69	5	99.6	100.0	5,108,108	5,226,333	0.977	100.0
100			6	10.18	69	5	96.9	100.0	1,000,000	3,004,112	0.333	70.1
101		Metbor	6	9.95	100	5	59.5	98.0	639,639	2,921,808	0.219	46.1
102	*P. digitatum*		6	9.86	110	5	36.3	91.0	630,630	2,921,808	0.216	45.5
103			6	9.79	120	5	0.0	0.4	2,702	2,921,808	0.001	0.2
104		Distilled water	Check	4.98	69	5	99.2	100.0	1,504,504	3,168,721	0.475	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

TABLE 2—(*Continued*)

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination		Average number of colonies per cc by dilution plate counts*	Average number of spores per cc by microscopic counts	Viability index†	Relative viability (water at 69° F=100)
							After 24 hrs.	After 48 hrs.	*A*	*B*	*A*÷*B*	
105			4	10.07	69	5	78.0	94.5	2,171,171	3,621,396	0.599	59.2
106			6	10.18	69	5	91.8	100.0	2,216,216	3,621,396	0.612	60.5
107		Metbor	8	10.22	69	5	73.2	91.2	1,909,909	3,621,396	0.527	52.1
108	*P. italicum*		10	10.27	69	5	44.3	76.8	1,900,900	3,621,396	0.525	51.9
109			12	10.31	69	5	31.9	60.2	1,864,864	3,621,396	0.515	50.9
110		Distilled water	Check	4.98	69	5	99.6	100.0	4,036,036‡	3,991,432	1.011	100.0
111			4	10.07	69	5	97.8	99.0	1,198,198	3,004,112	0.399	85.4
112			6	10.18	69	5	96.9	100.0	1,099,099	3,004,112	0.366	78.4
113		Metbor	8	10.22	69	5	78.2	92.5	918,918	3,004,112	0.306	65.5
114	*P. digitatum*		10	10.27	69	5	56.5	83.8	576,576	3,004,112	0.192	41.1
115			12	10.31	69	5	35.4	65.4	441,441	3,004,112	0.147	31.5
116		Distilled water	Check	4.98	69	5	99.8	100.0	1,576,576	3,374,482	0.467	100.0
117		Dinitro-o-cyclohexylphenol	0.001	5.35	69	2	92.2	99.6	1,189,189	2,551,438	0.466	47.6
118	*P. italicum*		0.0005	5.16	69	2	91.5	100.0	1,162,162	2,551,438	0.455	46.5
119			0.00025	4.96	69	2	91.7	100.0	954,954	2,551,438	0.374	38.2
120		Distilled water	Check	4.98	69	5	99.5	100.0	5,117,117	5,226,333	0.979	100.0
121		Dinitro-o-cyclohexylphenol	0.001	5.35	69	2	88.0	100.0	864,864	2,181,068	0.396	88.2
122	*P. digitatum*		0.0005	5.16	69	2	94.4	100.0	1,018,018	2,181,068	0.466	103.8
123			0.00025	4.96	69	2	95.0	100.0	1,063,063	2,181,068	0.487	108.5
124		Distilled water	Check	4.98	69	5	99.7	100.0	1,459,459	3,251,026	0.449	100.0
125			0.4	11.10	69	2	0.0	0.0	0	7,736,619	0.000	0.0
126		NaOCl	0.6	11.14	69	2	0.8	2.0	0	7,736,619	0.000	0.0
127	*P. italicum*		1.0	11.14	69	2	0.0	0.0	0	7,736,619	0.000	0.0
128		Distilled water	Check	4.98	69	5	99.4	100.0	6,369,369	11,275,711	0.565	100.0

129			0.4	11.10	69	2	0.0	0.0	0	2,716,047	0.000	0.0
130		NaOCl	0.6	11.14	69	2	0.0	0.0	0	2,716,047	0.000	0.0
131	*P. digitatum*		1.0	11.14	69	2	0.0	0.0	0	2,716,047	0.000	0.0
132		Distilled water	Check	4.98	69	5	99.0	100.0	1,387,387	3,415,635	0.406	100.0
133			6	7.96	86	2	99.8	100.0	4,801,801	5,802,465	0.827	73.3
134		$NaHCO_3$	6	7.96	86	5	99.7	100.0	4,657,657	5,802,465	0.803	71.2
135	*P. italicum*		6	7.96	86	10	99.6	100.0	4,459,459	5,802,465	0.768	68.1
136		Distilled water	Check	4.98	69	5	99.7	100.0	7,522,522‡	6,666,661	1.128	100.0
137			6	7.96	86	2	99.7	100.0	1,171,171	3,086,417	0.379	62.9
138		$NaHCO_3$	6	7.96	86	5	99.6	100.0	1,261,261	3,086,417	0.409	67.8
139	*P. digitatum*		6	7.96	86	10	97.4	100.0	1,072,072	3,086,417	0.347	57.5
140		Distilled water	Check	4.98	69	5	99.8	100.0	2,009,009	3,333,330	0.603	100.0
141			6	7.96	86	5	99.7	100.0	4,657,657	5,802,465	0.803	69.2
142		$NaHCO_3$	6	7.85	100	5	99.2	100.0	2,540,540	4,444,437	0.572	49.3
143			6	7.85	110	5	96.9	100.0	2,162,162	4,444,437	0.486	41.9
144			6	7.82	120	5	0.0	25.2	18,018	4,444,437	0.004	0.3
145	*P. italicum*		6	10.16	86	5	48.3	98.1	747,747	4,067,771	0.184	15.8
146		Na_2CO_3	6	9.83	100	5	39.4	93.9	365,765	4,444,437	0.082	7.1
147			6	9.74	110	5	1.4	33.3	28,828	4,444,437	0.006	0.5
148			6	9.64	120	5	0.0	0.0	0	4,444,437	0.000	0.0
149		Distilled water	Check	4.98	69	5	100.0	100.0	5,540,540‡	4,773,659	1.161	100.0
150			6	7.96	86	5	99.8	100.0	1,261,261	3,086,417	0.409	78.4
151		$NaHCO_3$	6	7.85	100	5	99.2	100.0	1,351,351	2,921,808	0.462	88.5
152			6	7.85	110	5	98.5	100.0	1,153,153	2,921,808	0.395	75.7
153			6	7.82	120	5	0.5	11.8	23,423	2,921,808	0.008	1.5
154	*P. digitatum*		6	10.16	86	5	61.5	91.8	459,459	2,757,199	0.166	31.8
155		Na_2CO_3	6	9.83	100	5	44.7	78.0	450,450	2,921,808	0.154	29.5
156			6	9.74	110	5	0.4	6.0	28,828	2,921,808	0.010	1.9
157			6	9.64	120	5	0.0	0.0	0	2,921,808	0.000	0.0
158		Distilled water	Check	4.98	69	5	99.6	100.0	1,738,738	3,333,330	0.522	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

TABLE 2—(*Concluded*)

Entry No.	Fungus	Fungicide	Concentration of fungicide, per cent	pH of fungicide	Temperature during treatment, °F	Exposure, minutes	Average per cent germination		Average number of colonies per cc by dilution plate counts*	Average number of spores per cc by microscopic counts	Viability index†	Relative viability (water at 69° F=100)
							After 24 hrs.	After 48 hrs.	*A*	*B*	*A*÷*B*	
159			2	8.30	86	5	99.6	100.0	5,243,243	5,802,465	0.904	92.4
160		$NaHCO_3$	6	8.13	86	5	99.7	100.0	4,657,657	5,802,465	0.803	82.1
161	*P. italicum*		10	8.00	86	5	100.0	100.0	4,657,657	5,802,465	0.803	82.1
162		Distilled water	Check	4.98	69	5	99.7	100.0	6,522,522	6,666,661	0.978	100.0
163			2	8.30	86	5	99.1	100.0	1,729,729	3,086,417	0.560	92.9
164		$NaHCO_3$	6	8.13	86	5	99.8	100.0	1,261,261	3,086,417	0.409	67.8
165	*P. digitatum*		10	8.00	86	5	85.1	100.0	1,189,189	3,086,417	0.385	63.8
166		Distilled water	Check	4.98	69	5	99.8	100.0	2,009,009	3,333,330	0.603	100.0
167			6	10.16	86	2	94.6	100.0	810,810	4,067,771	0.199	19.6
168		Na_2CO_3	6	10.16	86	5	48.3	98.1	747,747	4,067,771	0.184	18.1
169	*P. italicum*		6	10.16	86	10	32.4	83.6	558,558	4,067,771	0.137	13.5
170		Distilled water	Check	4.98	69	5	100.0	100.0	5,225,225‡	5,144,029	1.016	100.0
171			6	10.16	86	2	70.7	100.0	657,657	2,757,199	0.238	53.6
172		Na_2CO_3	6	10.16	86	5	61.5	91.8	459,459	2,757,199	0.167	37.6
173	*P. digitatum*		6	10.16	86	10	30.9	63.4	324,324	2,757,199	0.118	26.6
174		Distilled water	Check	4.98	69	5	99.9	100.0	1,369,369	3,086,084	0.444	100.0
175			2	10.20	86	5	99.0	100.0	2,837,837	4,067,771	0.698	68.7
176		Na_2CO_3	6	10.15	86	5	48.3	98.1	747,747	4,067,771	0.184	18.1
177	*P. italicum*		10	9.90	86	5	39.4	72.9	209,903	4,067,771	0.052	5.1
178		Distilled water	Check	4.98	69	5	100.0	100.0	5,225,225‡	5,144,029	1.016	100.0
179			2	10.20	86	5	99.4	100.0	1,036,036	2,757,199	0.376	84.7
180		Na_2CO_3	6	10.15	86	5	61.5	91.8	459,459	2,757,199	0.167	37.6
181	*P. digitatum*		10	9.90	86	5	28.3	64.9	243,243	2,757,199	0.088	19.8
182		Distilled water	Check	4.98	69	5	68.9	100.0	1,369,369	3,086,084	0.444	100.0

183			0.4	6.52	69	5	87.1	98.5	873,873	4,444,107	0.197	24.5
184		Chloramine-T	0.4	6.47	100	5	94.4	100.0	711,711	2,592,590	0.274	34.1
185	*P. italicum*		0.4	6.45	110	5	0.0	78.5	9,009	2,592,590	0.003	0.4
186			0.4	6.55	120	5	0.0	0.0	0	2,592,590	0.000	0.0
187		Distilled water	Check	4.98	69	5	99.2	100.0	2,774,774	3,455,787	0.803	100.0
188			0.4	6.52	69	5	97.9	100.0	1,108,108	2,921,808	0.379	92.0
189		Chloramine-T	0.4	6.47	100	5	97.6	100.0	1,045,045	2,716,041	0.385	93.4
190	*P. digitatum*		0.4	6.45	110	5	20.8	97.1	234,234	2,716,041	0.086	20.9
191			0.4	6.55	120	5	0.0	0.0	0	2,716,041	0.000	0.0
192		Distilled water	Check	4.98	69	5	99.2	100.0	1,270,270	3,086,417	0.412	100.0
193		Sod. o-phenylphenate	0.15	9.67	69	5	42.4	98.6	1,468,468	4,444,107	0.330	33.8
194	*P. italicum*	Chloramine-T	0.40	6.52	69	5	87.1	98.5	873,873	4,444,107	0.197	20.2
195		Washing powder	1.00	9.90	69	5	98.1	100.0	2,648,648	4,444,107	0.596	61.0
196		Distilled water	Check	4.98	69	5	99.6	100.0	5,108,108	5,226,333	0.977	100.0
197		Sod. o-phenylphenate	0.15	9.67	69	5	5.2	63.6	128,828	2,921,808	0.044	9.3
198	*P. digitatum*	Chloramine-T	0.40	6.52	69	5	97.9	100.0	1,108,108	2,921,808	0.379	79.8
199		Washing powder	1.00	9.90	69	5	98.5	100.0	1,153,153	2,921,808	0.395	83.2
200		Distilled water	Check	4.98	69	5	99.2	100.0	1,504,504	3,168,721	0.475	100.0

* Total number of colonies in three dilution plates *divided by* 0.000111.

† Average number of colonies per cubic centimeter determined by dilution plate count *divided by* the average number of spores per cubic centimeter determined by microscopic count *equals* the viability index.

‡ Theoretically, this figure should not have exceeded the microscopic count.

The Concentrations of Soap Solution in Relation to Number of Spore Clumps.—Five concentrations of Castile soap solution were used to determine the best concentration for dispersing spores of the two molds. Spore suspensions were made with sterile, distilled water; each of 6 centrifuge tubes was then filled with 5 cc of the suspension. After centrifuging for 3 minutes, the supernatant water was decanted and the tubes were filled, respectively, with 10 cc of the above five concentrations of soap solution; the check tubes were filled with sterile, distilled water. Ten minutes after the solutions had been added, the spore clumps were counted by means of a Howard counting chamber under the low power of the microscope. The results are shown in table 1 (p. 7).

Table 1 shows that 0.25 per cent soap solution is the best concentration for dispersing spores of *Penicillium digitatum,* that is, fewer clumps appeared when this concentration was used. This is in harmony with the results on the effect of various concentrations of soap solution on spore germination and on the growth in petri dishes. In the case of *P. italicum* the fewest clumps appeared in the 0.10 per cent solution. Because of the indications of these experiments and because the several soap concentrations were all relatively nontoxic, a concentration of 0.25 per cent was chosen for use with both fungi in all experiments subsequent to the third (entries 35 *et seq.* of table 2).

Killing Effect of Borax in Relation to Concentration of Soap Solution.—In comparing the effect of the concentration of soap solution on the killing effect of borax, the following procedures were followed: First, treatment was made with different concentrations of soap solution for 10 minutes. This was followed by immersion in a 6 per cent borax solution for 5 minutes at room temperature (66° to 72°, average 69° F). Secondly, treatment was made with 6 per cent borax solution but without any previous treatment with soap solution. Finally, for checks, the spores were not subjected to borax treatment. Results are given in table 2, entries 13 to 26.

According to the results, a 6 per cent borax solution is more effective in checking the spore germination and the plate growth of both fungi when used in conjunction with concentrations of 0.1 to 1.0 per cent soap solutions.

From the results of the three experiments discussed above, it is also seen that 0.25 per cent soap solution is suitable for wetting spores of these fungi. Hereafter, unless otherwise noted, the spores for all the experiments were first wetted with this concentration of soap before any further tests were made.

EFFECT OF TEMPERATURE ON SPORES IN DISTILLED WATER

Experiments were conducted to find how different temperatures affected the spores of the two molds. Winston (*41*) had reported that water at 110° F or above showed effective control of decay.

The general procedure for this experiment was the same as stated above, except that special attention was paid to obtaining and maintaining the desired temperatures. The more important steps for this experiment may be described briefly as follows: When the spore suspensions were centrifuged and the supernatant soap solution was poured off, distilled water which had been previously heated to the desired temperature was poured into the centrifuge tube and shaken thoroughly. Immediately after that, the tubes were put in the water bath for about 2 minutes and then into the centrifuge, which had been placed in an electric oven at the same temperature. After having been centrifuged for about 3 minutes, the tubes were removed and the warm water replaced with sterile distilled water at room temperature. Germination and plating tests were then made; the results are reported in table 2, entries 27 to 34.

The data show that the percentage of germination of the spores as measured by direct counts or plate cultures decreases as the temperatures increase. At 120° F, germination and plate growth are greatly repressed.

EFFECT OF BORAX

The preliminary results of the experiments of Fulton and Bowman (*17*) have shown that a commercial borax solution of 5 per cent or 10 per cent greatly reduces blue-mold rot of citrus fruits under experimental conditions. Further experiments by Fulton and Winston (*18*) suggest the use of the 5 per cent borax solution at 120° F for 5 minutes. This procedure was later supported and patented by the Brogdex Company (*10*). The experiments by Barger and Hawkins (*6*) at first indicated that 2.5 per cent boric acid at 120° F gave very promising results in controlling blue mold (*Penicillium italicum*). Later borax was tested and found to be as effective as boric acid and was much cheaper than the latter. From the results based on experimental data and data obtained from the commercial shipments, Barger (*4*) concluded that both blue- or green-mold decay can be controlled by 7 per cent borax solution at 110° F for 5 minutes. This has been confirmed by Reichert and Littauer (*33*). As a result of experiments in South Africa, Powell (*28*) suggests the use of a hot 2.5

per cent borax solution, or boric acid, or mixtures of both for the control of the green mold (*P. digitatum*). Benton (*9*) found that a 4-minute immersion in 8 per cent borax solution at 110° F was effective in preventing decay in oranges. By the results of tests, Putterill (*30*) supports the use of 8 per cent borax. He also mentions that 4 per cent borax and 4 per

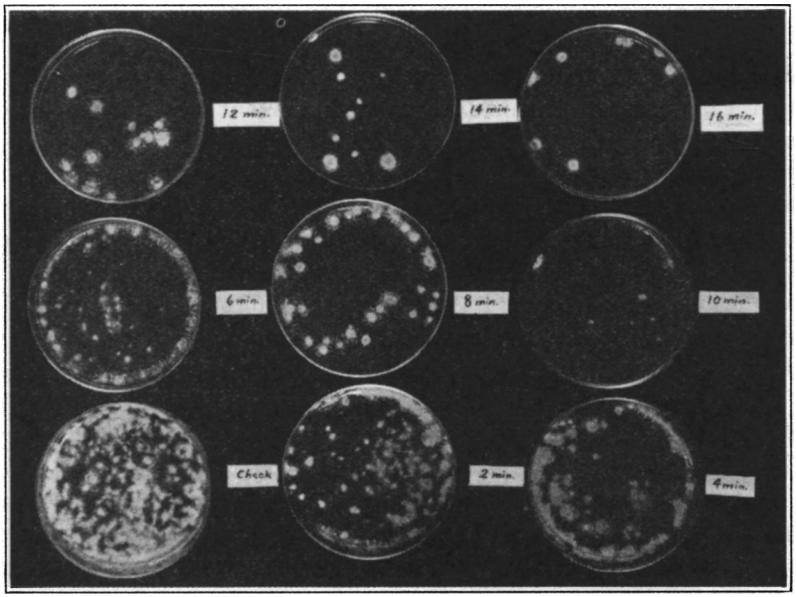


Fig. 1.—Effect on spores of *Penicillium italicum* exposed to 6 per cent borax solution at 110° F for various lengths of time. The plates represent a 1:100,000 dilution of the spore suspensions. The untreated suspension yielded numerous colonies, while treatments of 10 minutes or longer killed most of the spores.

cent sodium bicarbonate are of equal effectiveness at high temperatures, but the former is less effective at lower temperatures.

In order to determine the effect of the interrelation of concentrations of borax solution, with the length of treatment and various temperatures on the spores of both *Penicillium italicum* and *P. digitatum,* the following three experiments were conducted.

Time of Exposure.—In each series of 16 tests, 6 per cent borax solution at 110° F (43° C) was used for 2, 4, 6, 8, 10, 12, 14, and 16 minutes; as checks, 2 of the tests were made without chemical treatment except in 0.25 per cent soap solution. The results are given in table 2, entries 35 to 52, and shown in figure 1.

The results show that the percentage of spore germination of *Penicillium italicum* in 6 per cent borax at 110° F for 16 minutes is 0.1, but

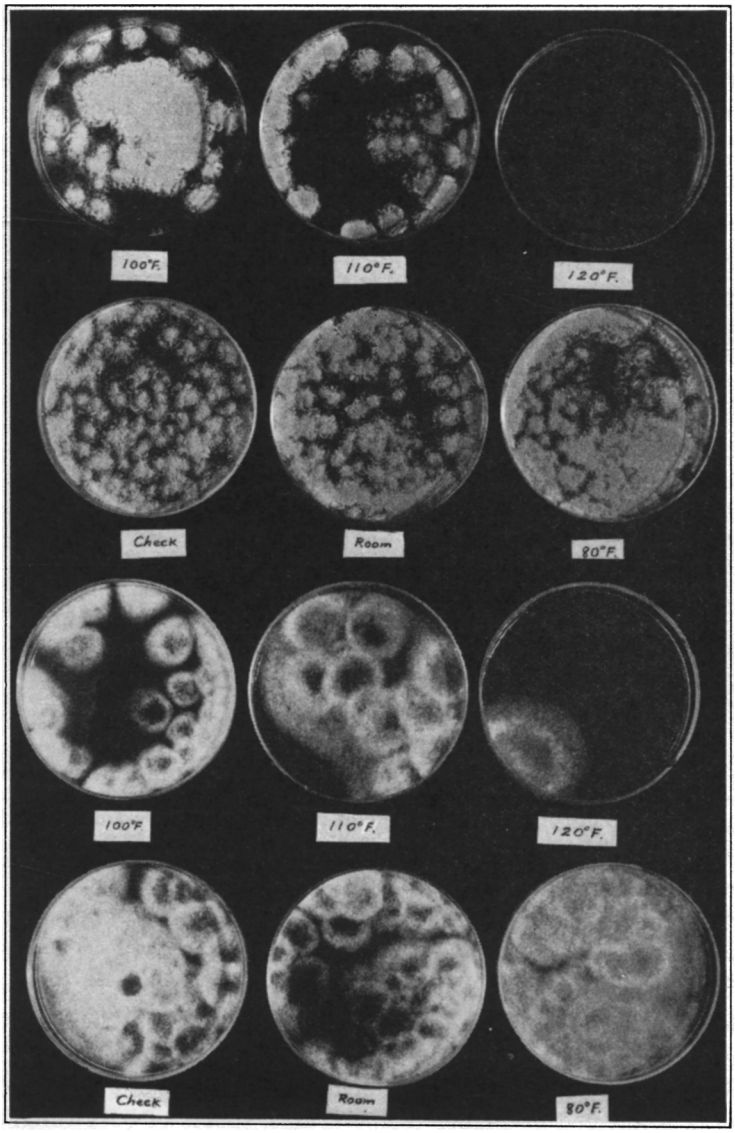


Fig. 2.—Effect of 6 per cent borax solution at various temperatures (from room temperature to 120° F), on spores of *Penicillium italicum* (upper 6 plates), and on *P. digitatum* (lower 6 plates); exposure time, 5 minutes. The plates represent a 1:100,000 dilution of the spore suspensions. The untreated spore suspensions of the two species at temperatures below 100° F yielded numerous colonies, while treatments at 120° F killed all the spores of *P. italicum* and nearly all those of *P. digitatum*.

at the same temperature for 10 minutes it is 1.2. Similar relations were found with *P. digitatum;* that is, 0.9 per cent of the conidia germinated after 110° F for 16 minutes, 3.9 per cent germinated after 110° F for 10 minutes. The results indicate that the longer the time of exposure to 6 per cent borax at the same temperature (110° F), the more effective

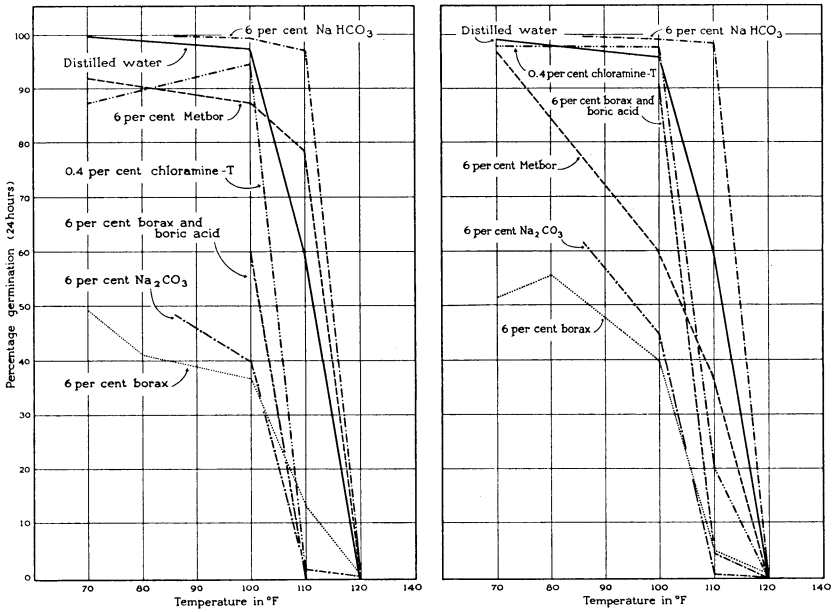


Fig. 3.—Left: Comparative effect of six solutions and distilled water at various temperatures, on the spore germination of *Penicillium italicum;* exposure time, 5 minutes. Six per cent borax-boric acid and 0.4 per cent chloramine-T at 110° F killed all the spores while 6 per cent sodium carbonate killed nearly all.

Right: Effect of the same solutions on the spore germination of *P. digitatum.* Six per cent solutions of sodium carbonate, borax-boric acid, and borax at 110° F killed nearly all the spores.

For convenience in plotting, the graphs are shown as starting at 70° F instead of 69° as shown in the tables; that temperature is still within the room-temperature range of 66°–72°, although not the exact average.

is the material in reducing both spore germination and the growth in plates of the two fungi.

Temperature Relations.—In order to determine whether the toxicity of borax is affected by higher temperatures, spores of both fungi were treated with 6 per cent borax solution for 5 minutes at the following temperatures: 66° to 72° (room temperature), 80°, 100°, 110°, and 120° F. The results are summarized in table 2, entries 53 to 64, and illustrated in figure 2.

The results show that, when the temperature increased from room tem-

perature to 120° F (49° C), the spore germination (24 hours later) decreased from 49.0 per cent to 0.1 per cent in the case of *Penicillium italicum*. In the case of *P. digitatum*, the same tendency is found, that is, germination decreased from 51.5 per cent to 1.0 per cent.

However, even in distilled water the higher temperatures are effective in decreasing germination (table 2, entries 27 to 34), though 6 per cent borax at these temperatures is seen to be even more effective, indicating

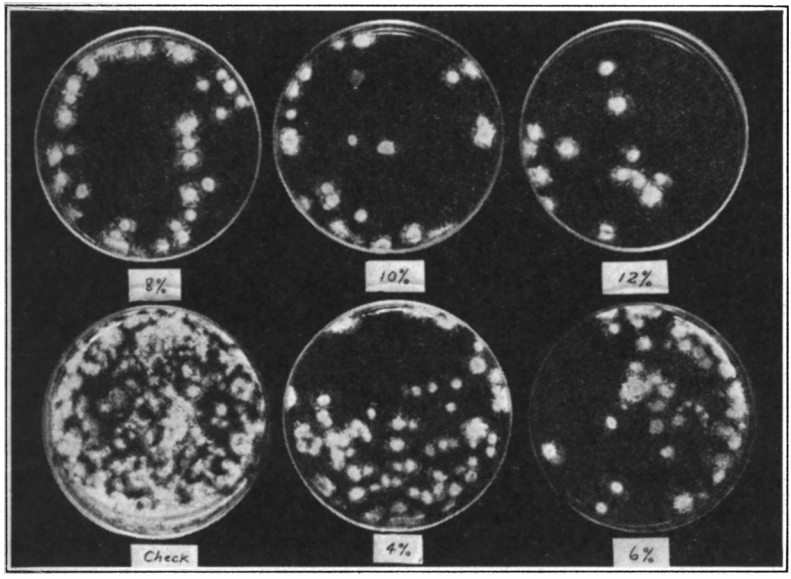


Fig. 4.—Effect of various concentrations of borax solution, at 110° F for 5 minutes, on spores of *Penicillium italicum*. The plates represent a 1:100,000 dilution of spore suspensions. The untreated suspension yielded numerous colonies, but treatments at 10 or 12 per cent killed large numbers of spores.

an intrinsic fungicidal action of the borax. Furthermore, in the case of *Penicillium digitatum* spores treated at 80° F, it seems that both germination and growth in plates are greater than at room temperature. This was true in Winston's (*41*) recent experiments. His conclusion is that increasing the temperature of borax was not ordinarily accompanied by a corresponding reduction in decay, except at 110° F. The comparative results are shown in figure 3.

Concentration.—Tests were made to determine the best concentration of borax solution. The concentration of the chemical ranged from 4 to 12 per cent in steps of 2 per cent, and the treatments were made at 110° F with a 5-minute period of exposure. The results are given in table 2, entries 65 to 76, and illustrated in figure 4.

The results show that as the concentration of borax solution increases the percentage viability of the treated spores decreases. Working on the control of decay in citrus fruit Barger (*4*) obtained a similar result. Winston (*41*) found that increasing the concentration of the borax solution up to 10 per cent, progressively decreased decay in the treated oranges; above 10 per cent, however, there was no marked increase in effectiveness of decay control.

INFLUENCE OF TEMPERATURES ON EFFECTIVENESS OF A MIXTURE OF BORAX AND BORIC ACID

From the result of experiments, Powell (*28*) suggested that the use of 2.5 to 5.0 per cent hot solutions of borax or boric acid, or a mixture of both gave complete control of green mold. This has been confirmed by Winston (*41*). He claimed, however, that boric acid, alone or in combination with borax, injured the rinds of oranges, grapefruit, and tangerines, although the effectiveness in decay control equaled that of borax.

Since borax and boric acid (2:1) solution is still commonly used in some packing-houses of California as an aid in washing as well as for better decay control, 8 tests including 2 checks were conducted at one time. For these treatments, the 6 per cent combined solution was adjusted to 100°, 110°, and 120° F and used for 5 minutes. The results are summarized in table 2, entries 77 to 84.

These results show that there is no appreciable reduction of germination at 100° F. A comparison with table 2, entries 27 to 34, shows, however, that the chemical solution is more effective than water at all three temperatures. As indicated by germination after 48 hours, the solution is more toxic to *Penicillium digitatum* than to *P. italicum*. At 110° F the combined chemical solution of borax-boric acid is slightly more toxic than is a 6 per cent borax solution; at 120° F the former is more toxic to *P. digitatum* but slightly less toxic to *P. italicum* than is the latter.

EFFECT OF METBOR

Metbor is a new material which has been mentioned by Stewart (*36*) as fully equal to borax in fungicidal efficiency, and as having very marked advantages over borax in regard to solubility in cold water and other properties. It is completely and quickly soluble in cold water in concentrations even greater than are necessary to obtain the equivalent of 8 per cent borax solution. In order to determine its effectiveness on spores of *Penicillium italicum* and *P. digitatum* as compared with borax, three separate experiments were conducted.

Time of Exposure.—This experiment consisted of 8 tests in which was used a 6 per cent solution of the chemical at room temperature (66° to 72° F) for 2, 5, 10, and 15 minutes respectively. The results are given in table 2, entries 85 to 94.

It is seen from the results that at the longest exposure time the germination after 24 hours is 65.8 per cent for *Penicillium italicum* and 49.6 per cent for *P. digitatum*. This shows much less reduction in germination than does borax under similar exposures.

Since the longest treatment was not effective, and since such treatments are impractical in the packing-house, 6 per cent Metbor has no usefulness at room temperature.

Temperature Relations.—Tests were made to determine temperature relations. Spores of both fungi were given 5-minute exposures to 6 per cent Metbor solution at temperatures of 66° to 72° F, 100°, 110°, and 120° F. The results are given in table 2, entries 95 to 104, and shown in figure 3.

In comparison with the results of the effect of temperature of distilled water (table 2, entries 27 to 34), 6 per cent Metbor is much more effective from the standpoint of spore germination, though the reduction in plate colonies is less significant.

Concentration.—Ten tests were made in which concentrations ranging from 4 to 12 per cent were used for 5 minutes at room temperature (66° to 72° F). The results are summarized in table 2, entries 105 to 116.

Although there is some reduction of germination and number of colonies at higher concentrations at 5-minute exposure, the results indicate that the chemical is not so effective in killing spores as borax.

EFFECT OF DINITRO-O-CYCLOHEXYLPHENOL

Since dinitro-o-cyclohexylphenol, used as a 0.01 per cent emulsion, has been shown to be effective in decreasing the number of brown-rot infections on lemon, caused by *Phytophthora citrophthora,* from 51.45 to 1.45 infections per fruit,[6] tests for its effectiveness on *Penicillium italicum* and *P. digitatum* were conducted, using three concentrations. The spores of the two fungi were treated separately at room temperature for 2 minutes. Results are tabulated in table 2, entries 117 to 124.

From the results it is evident that both spore germination and the growth on plates are but slightly affected by this substance under the conditions of the experiment although some reduction in number of colonies is noted as compared to the checks.

[6] Klotz, L. J., and L. L. Huillier. Dinitro-o-cyclohexylphenol as a treatment for brown rot of citrus. Unpublished data on file at Citrus Experiment Station, Riverside, California. 1936.

EFFECT OF SODIUM HYPOCHLORITE

Sodium hypochlorite (NaOCl) has been reported (*2, 3*) to be effective in controlling blue mold on apple and pear and in sterilizing packing rooms, etc. Klotz and Huillier[7] likewise found a 0.4 per cent solution completely effective in controlling brown rot of lemon.

To determine the toxicity of the chemical to blue and green molds of citrus, 6 tests were made with 0.4, 0.6, and 1.0 per cent solutions at room temperature for 2 minutes at each time; two checks were left without chemical treatment. The solutions were prepared by using a concentrated stock solution containing 6 per cent NaOCl. To prepare a solution containing 1.0 per cent available chlorine, the amount to be prepared is multiplied by 0.2. This gave the volume of stock solution to be used. The volume was made up with distilled water. The factor for 0.6 per cent is 0.111 and that for 0.4 per cent is 0.072.

The effectiveness of sodium hypochlorite is shown in table 2, entries 125 to 132. A low concentration (0.4 per cent) of the chemical in contact with the fungi for 2 minutes was completely lethal. Lack of success in some of the earlier experiments with this material was found to be due to an insufficient mixing and wetting of the mold spores.

Practically the hypochlorite has some disadvantages. Chlorine escapes, making it necessary to test and correct the treating solution frequently to maintain an effective concentration. The rate of loss of chlorine increases as the temperature of the treating solution is raised. This material is injurious to metal, cement, and wooden tanks. In the apple industry (*3*) these objections were in large measure overcome by use of a small, separate tank where the fruit was treated cold with hypochlorite solution stabilized by certain organic chemicals, and by maintaining the chlorine concentration by frequent colorimetric measurements with orthotolidine and the addition of concentrated sodium hypochlorite.

EFFECT OF SODIUM BICARBONATE

From the results of experiments in California, Barger (*5*) suggests the use of 3 to 5 per cent cold or hot solution of sodium bicarbonate for reducing mold. Negative results were obtained by Reichert and Littauer (*33*), who state that 3 and 5 per cent solutions of sodium bicarbonate for 5 and 15 minutes gave no control of wastage. Other results, however, have shown that 3 per cent and 5 per cent solutions of the chemical at 32° C

[7] Klotz, L. J., and L. L. Huillier. Sodium hypochlorite as a treatment for brown rot of citrus. Unpublished data on file at Citrus Experiment Station, Riverside, California. 1936.

(89.5° F) for 5 minutes gave some reduction of rot. Putterill and Davies (*31*) mentioned the beneficial use of 3 per cent sodium bicarbonate solution for controlling green mold. Recently Putterill (*30*) showed that 4 per cent sodium bicarbonate at high temperature was effective in controlling mold.

To determine the effect of sodium bicarbonate on spores of *Penicillium italicum* and *P. digitatum* for various periods of exposure and at several temperatures and concentrations of the chemical ($NaHCO_3$), 3 separate experiments were conducted. The effectiveness of sodium bicarbonate was also compared with that of sodium carbonate at several temperatures.

Time of Exposure.—Six tests were made with 6 per cent sodium bicarbonate at 86° F (30° C) for 2, 5, and 10 minutes respectively. The results are given in table 2, entries 133 to 140.

As indicated, a 6 per cent solution of sodium bicarbonate at 86° F and for a period of 10 minutes is practically inocuous to the spores of either mold. The plate tests show that the chemical slightly inhibited growth. These results are in contrast with those of Marloth (*22*), who found that a 6 per cent solution for 2 or 5 minutes was decidedly toxic to the spores of *P. digitatum*.

Temperature Relations.—With the aim of comparing more critically 6 per cent sodium bicarbonate solution with the same concentration of sodium carbonate (Na_2CO_3) these two substances were tested at the same time. The results are given in table 2, entries 141 to 158.

The lack of efficacy of sodium bicarbonate may be summed up by saying that the chemical at 120° F showed no advantage over water at that temperature. In fact the fungi after the bicarbonate treatment showed slightly greater viability than after the treatment in distilled water. Sodium carbonate, on the other hand, showed complete effectiveness at 120° F; and at 110° and 100° greatly reduced germination and growth.

It was also found that the temperatures of 6 per cent sodium bicarbonate solution below 120 F do not affect the viability of spores very much. This supports Barger's (*5*) conclusion that a treating temperature of 60° is as ineffective as one of 95° for this substance.

Concentration.—The effect of various concentrations of sodium bicarbonate solution was determined; the results are given in table 2, entries 159 to 166. The reduction in germination and growth of the two fungi was slight. With *Penicillium digitatum,* however, there is some reduction at 10 per cent but these tests show far less effect than is shown by the experiments of Marloth (*22*).

The results obtained by Barger (*4*) with experimentally injured and inoculated fruit show that 3 per cent sodium bicarbonate at 100° F re-

duces decay to 35.3 per cent and that 5 per cent sodium bicarbonate reduces it to 32.0 per cent. He concludes that a 3 per cent solution of sodium bicarbonate appears to be as effective in reducing mold on fruit as a 5 per cent solution.

EFFECT OF SODIUM CARBONATE

Sodium carbonate (Na_2CO_3) is used for controlling molds in many lemon packing-houses and in some orange houses in California. Doidge (*12*) has suggested the use of a 5 per cent solution of sodium carbonate for the control of *Penicillium* molds. As a result of comparative treatments of spores, Marloth (*22*) has concluded that the same concentration of carbonate is considerably more toxic than a similar concentration of bicarbonate. Recently Winston (*41*) has shown that a 3.5 per cent solution of sodium carbonate ($Na_2CO_3 \cdot 10H_2O$) at 80° F gave nearly the same result as that of the check without chemical treatment.

With the hope of obtaining more information in regard to the effectiveness of sodium carbonate in relation to time of treatment, temperature, and concentration, 3 separate experiments were conducted.

Time of Exposure.—Six tests with 6 per cent solution of sodium carbonate at 86° F were made, the spores being exposed for 2, 5, and 10 minutes.

The results shown in table 2, entries 167 to 174, indicate that the reduction of spore germination and growth in plates of both fungi become more significant as the time of exposure is extended. The results are more or less in harmony with those of Marloth (*22*).

Temperature Relations.—In this experiment, to determine temperature relations, the spores were treated with 6 per cent sodium carbonate at temperatures of 86°, 100°, 110°, and 120° F for 5 minutes. The comparative results are summarized in table 2, entries 144 to 149, and 154 to 158.

According to the results, it is shown that 6 per cent sodium carbonate at 120° F gave complete killing of spores of both fungi; that is, this treatment permitted neither germination nor the growth of a single colony.

In comparison with the results of the effect of temperature in distilled water, 6 per cent sodium carbonate at 120° F is much more effective in preventing germination than distilled water at the same temperature. This also shows that sodium carbonate apparently has an intrinsic fungicidal action on the two fungi not accounted for by the temperature of the treating solution.

It was also found that 6 per cent sodium carbonate solution at 110° F

was very toxic to both kinds of spores but this toxicity was slightly less than that at 120°.

Concentration.—In order to determine the best concentration of sodium carbonate for controlling blue and green molds, spores were treated with concentrations of 2, 6, and 10 per cent for 5 minutes at 86° F. The results are recorded in table 2, entries 175 to 182. They show that after 5-minute exposures the percentage of germination and the growth in plates of both fungi reduce significantly as the concentration increases. Under the conditions of this experiment, a 10 per cent solution of sodium carbonate was the most efficient concentration.

The results agreed with Marloth's (*22*) more in the case of *Penicillium italicum* than in that of *P. digitatum*.

A comparison of the results with those of sodium bicarbonate indicates that the same concentration of sodium carbonate was much more toxic than in the case of sodium bicarbonate.

RELATION OF TEMPERATURES TO EFFECTIVENESS OF CHLORAMINE-T

Klotz and Huillier[8] have shown that 0.4 per cent solution of chloramine-T reduces brown rot of inoculated lemons from 28.65 to 0.05 infections per lemon. To test the effect of this chemical in controlling blue and green molds, 0.4 per cent solution was used at room temperature, and at 100°, 110°, and 120° F, for 5 minutes. The effects of the various temperatures on the toxicity are given in table 2, entries 183 to 192. The reduction of spore germination and growth in plates of both fungi is very marked at higher temperatures, especially at 120° F. No germination and growth of either fungus were found at 120°. Chloramine-T is apparently more toxic to *Penicillium italicum* than to *P. digitatum* at the three higher temperatures used. At temperatures used below 110°, it showed but slight toxicity.

EFFECT OF OTHER SUBSTANCES

Sodium o-phenylphenate at 0.15 per cent has been used by Klotz and Huillier[9] in unreported experiments on the control of brown rot of lemons. They show that a 0.15 per cent solution reduces the rot of inoculated fruit from 28.65 to 0.55 infections per lemon. A commercial

[8] Klotz, L. J., and L. L. Huillier. Chloramine-T as a treatment for brown rot of citrus. Unpublished data on file at the Citrus Experiment Station, Riverside, California. 1936.

[9] Klotz, L. J., and L. L. Huillier. Sodium o-phenylphenate as a treatment for brown rot of citrus. Unpublished data on file at the Citrus Experiment Station, Riverside, California. 1936.

washing powder containing mostly soda ash with some caustic and a trace of pine oil, has also been commonly used in some of the packing-houses in California, and chloramine-T is reported to have been tried.

An experiment was set up to test the relative effectiveness of these three substances. The time of exposure was 5 minutes, the temperature, 66° to 72° F, and the concentrations as follows: sodium o-phenylphenate, 0.15 per cent; chloramine-T, 0.4 per cent; and washing powder, 1.0 per cent. The results are shown in table 2, entries 193 to 200.

Under the conditions used, sodium o-phenylphenate is the most effective substance of the three in reducing germination and the growth of *Penicillium digitatum;* it was also slightly more effective than chloramine-T and washing powder in reducing germination of *P. italicum.* The latter two substances are almost without effect on *P. digitatum* under the conditions mentioned above. With *P. italicum,* 0.4 per cent chloramine-T is more effective in reducing germination and the number of colonies than is 0.15 per cent sodium o-phenylphenate or the 1 per cent washing powder.

DISCUSSION

The inhibiting or the lethal effect of a given solution on the spores of a fungus is dependent upon a number of factors, including concentration of the fungicide and the spore suspension, duration of exposure, solvent for the fungicide, temperature, H- and OH-ion concentration, and the characteristically specific nature of the cations and anions. In the tests reported in this paper at the lower temperatures of 69°, 86°, and 100° F, and with a 5-minute treatment (2 minutes for sodium hypochlorite) the solutions in order of toxicity from highest to lowest are as shown in table 3, the numbers in the fourth column being the relative viability of the treated spores based on the combined mean of the percentage of germination after 24 hours and the viability on a culture medium.

The sodium hypochlorite solutions acting for only 2 minutes were fatal to the spores of both *Penicillium italicum* and *P. digitatum.* These results are similar to those of Baker and Heald (*3*) who found that rinsing apples for one minute with a sodium hypochlorite solution containing 0.4 per cent available chlorine was very effective to reducing the number of viable spores of *P. expansum* on the surface and in the lenticels, and in decreasing losses from decay by this organism.

Sodium carbonate occupies a relatively high position in the toxicity tables, and shows a greater effect on *Penicillium italicum* than on *P. digitatum.*

Borax in the cool solutions was more toxic to *P. digitatum* than to *P.*

TABLE 3

AVERAGE VIABILITY OF THE SPORES AFTER TREATMENT WITH VARIOUS FUNGICIDES AT VARIOUS TEMPERATURES

Penicillium italicum				*Penicillium digitatum*			
Order of toxicity of fungicide	Fungicide	Concentration of fungicide, per cent	Viability*	Order of toxicity of fungicide	Fungicide	Concentration of fungicide, per cent	Viability*
			Treatment at 69°, 86°, and 100° F				
1	Sodium hypochlorite†	1.0	0.0	1	Sodium hypochlorite†	1.0	0.0
2	Sodium hypochlorite†	0.6	0.0	2	Sodium hypochlorite†	0.6	0.0
3	Sodium hypochlorite†	0.4	0.0	3	Sodium hypochlorite†	0.4	0.0
4	Sodium carbonate	6.0	30.4	4	Sodium o-phenylphenate	0.15	7.3
5	Sodium o-phenylphenate	0.15	38.2	5	Castile soap for 10 minutes	0.25	
6	Castile soap for 10 minutes	0.25			followed by borax	6.0	33.8
	followed by borax	6.0	58.4	6	Sodium carbonate	6.0	48.3
7	Chloramine-T	0.4	58.0	7	Borax	6.0	56.5
8	Borax-boric acid (2:1)	6.0	58.15	8	Metbor	6.0	78.1
9	Borax	6.0	58.17	9	Sodium bicarbonate	6.0	87.7
10	Dinitro-o-cyclohexylphenol†	0.001	70.1	10	Dinitro-o-cyclohexylphenol†	0.001	88.2
11	Metbor	6.0	74.7	11	Washing powder	1.0	91.2
12	Washing powder	1.0	79.7	12	Borax-boric acid (2:1)	6.0	94.1
13	Sodium bicarbonate	6.0	83.9	13	Chloramine-T	0.4	93.5
			Treatment at 110° and 120° F				
1	Chloramine-T	0.4	0.1	1	Sodium carbonate	6.0	0.6
2	Sodium carbonate	6.0	0.5	2	Chloramine-T	0.4	10.4
3	Borax-boric acid	6.0	4.6	3	Borax-boric acid	6.0	11.1
4	Borax	6.0	19.3	4	Metbor	6.0	20.6
5	Metbor	6.0	31.1	5	Borax	6.0	22.4
6	Sodium bicarbonate	6.0	34.8	6	Sodium bicarbonate	6.0	44.1

* Calculations all based on percentage germination after 24 hours and relative viability, being related to germination and relative viability of water check (100).

† All treatments for 5 minutes except in the case of dinitro-o-cyclohexylphenol and sodium hypochlorite treatments of 2 minutes.

italicum, although the borax-boric acid mixture had a greater effect on the latter.

Sodium o-phenylphenate was effective on both fungi but more toxic to *Penicillium digitatum.*

Cold 6 per cent solutions of Metbor and of sodium bicarbonate were relatively ineffective on the two fungi. A 0.4 per cent solution of chloramine-T decreased the viability of *P. italicum* 42 per cent but *P. digitatum* only 7 per cent.

At the higher temperatures of 110° and 120° F, and with a 5-minute treatment (6 minutes at 110° F in one experiment with borax), the order of toxicity of the several solutions is shown in table 3.

One of the striking features of this record is the toxicity positions occupied by the warm chloramine-T and borax-boric acid solutions as compared with those of the solutions at the lower temperatures. Another surprising result is the consistently greater resistance of *Penicillium digitatum* to the growth inhibition of the several solutions. This would indicate that *P. digitatum* would be found more difficult to control with the solutions mentioned above than would *P. italicum.* But most of the results of experiments on decay prevention in citrus fruits show that borax or borax-boric acid is much more effective against the green than against the blue mold. As one possible explanation for this difference may be offered the suggestion that when the solution comes in contact with the oil of the rind, some chemical action takes place whereby the original specific toxicity of the treating solution to the particular spores is altered. Another explanation may be that some of the treating substances remaining on the rind surface or in injuries may react differently to germinated spores of one fungus than to those of the other. Marloth (*22*) found that germinated spores of both fungi were more readily killed by borax, sodium carbonate, and sodium bicarbonate than non-germinated spores.

The comparative results are shown in figure 3. Sodium bicarbonate again shows the least toxicity, while sodium carbonate averages higher for the two fungi. The importance of temperature is readily seen from the above and from a consideration of the results in table 2. The lower toxicity at the lower temperatures may be due in part to poor wetting of the spores, although that factor was decreased as much as possible by the pretreatment with soap solution. The greater toxicity of the chemical solutions at the higher temperatures should be largely related to the increased velocity and penetrating power of the toxic ions and molecules and to the kinetic energy of the particles of the solvent itself, since water at 120° F greatly inhibited subsequent germination. A 5-minute exposure

to sodium carbonate, borax-boric acid mixture, chloramine-T, and Metbor at 120° F was fatal to the spores of the two fungi, while water, sodium bicarbonate, and borax at the same temperature and exposure permitted survival of a small percentage. At 110° F nearly all the spores of *Penicillium italicum* were killed by chloramine-T and the borax-boric acid mixture. Not all the spores of *P. digitatum* were killed by any of the solutions tried at 110° F, although 6 per cent sodium carbonate permitted only 0.4 per cent germination after 24 hours and a relative viability of only 1.9.

As would be expected, the longer the period of exposure to any of the several chemicals used, the smaller the percentage of germination of either fungus; this is especially striking in the experiments with 6 per cent borax solution at 110° F. The comparative results are given in figure 5.

The results of the concentration experiments show that all the spores of both blue and green molds were killed at room temperature by the weakest solution (0.4 per cent) of sodium hypochlorite tried. After treatment with 12 per cent borax solution at 110° F, no spore germinations were observed after 24 hours. Conidia of *Penicillium italicum* treated with 8 per cent borax solution at 110° F were affected significantly, while those of *P. digitatum* were affected significantly in 10 per cent borax solution at the same temperature. A 10 per cent solution of Metbor at 66° to 72° began to reduce the germination of *P. italicum* markedly. In the case of *P. digitatum* spores were affected significantly in 12 per cent Metbor solution at 66° to 72°. From this point of view, it is also indicated that *P. digitatum* is more resistant to the toxicity of higher concentrations of borax and Metbor than *P. italicum*. Both spores began to be affected by a 10 per cent sodium carbonate solution at 86° F. Spores of *P. digitatum* and *P. italicum* were but slightly affected by a 10 per cent solution of sodium bicarbonate at 86° F, although the former were less inhibited than the latter. The differences among concentrations of dinitro-o-cyclohexylphenol as to their effect upon spores of both fungi were not sufficiently great to be of practical significance.

As far as the results of these experiments are concerned, it may be concluded that the most effective and economical solution for killing blue and green mold spores is the 0.4 per cent sodium hypochlorite used for 2 minutes at room temperature. The following solutions may also be effective when used at 120° F for 5 minutes: 0.4 per cent chloramine-T, 6 per cent mixture of borax and boric acid, 6 per cent sodium carbonate, and 6 per cent Metbor. Eight to 12 per cent solutions of borax are effective if used at 110° for 14 to 16 minutes.

As Fawcett (*14*) has pointed out, 120° F for 2 to 4 minutes is gener-

ally considered the danger point for the temperature of washing or treating solutions. With freshly picked fruit, especially with lemons, it is

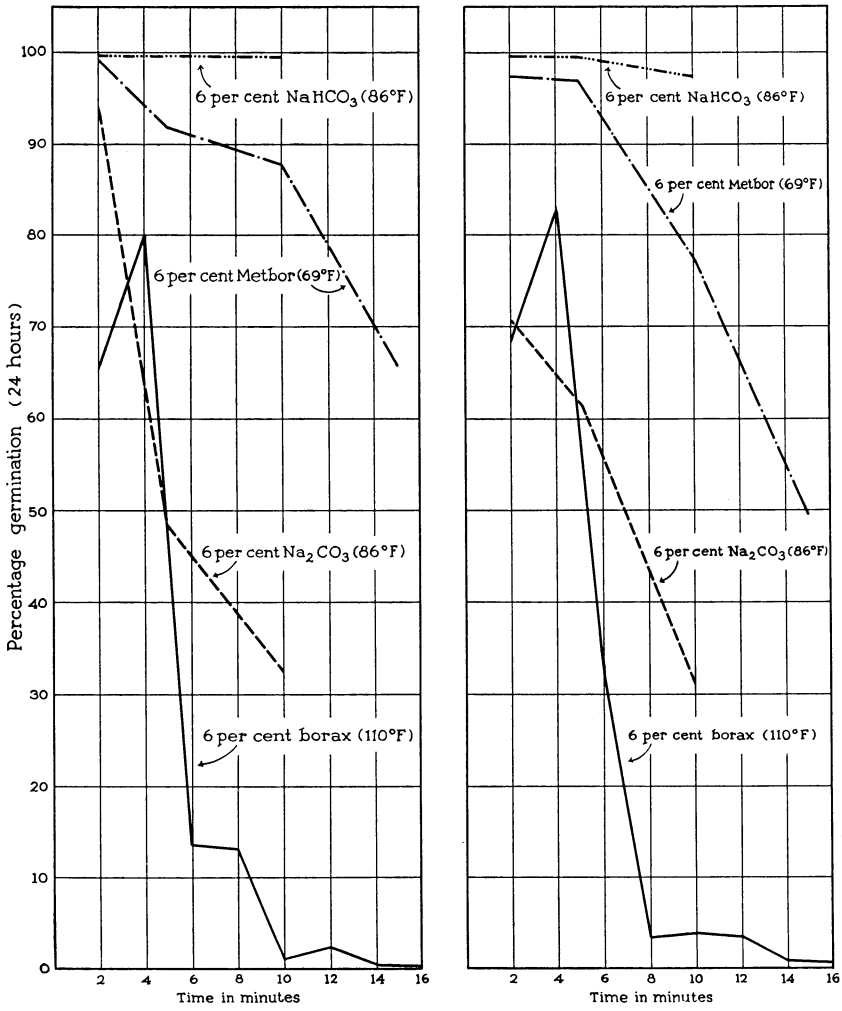


Fig. 5.—Left: Comparative effect on spore germination of *Penicillium italicum* of various exposures to four different solutions at recommended temperatures. Six per cent borax solution at 110° F for 10 minutes or longer killed nearly all the spores. Right: Effect of the same solutions on the spore germination of *Penicillium digitatum*. Six per cent borax solution at 110° F for 8 minutes or longer killed nearly all the spores.

necessary to use lower temperatures or to dry the fruit for several days before treatment, as pointed out by Fawcett and Klotz (*16*). To avoid injury to freshly picked fruit and at the same time secure partial control

of blue and green molds the four solutions mentioned above may be used at 110° to 115° F, providing the fruit is first allowed to dry 3 to 5 days.

The spores showed a great tolerance toward hydroxyl ions. A 5-minute treatment in 2 per cent Castile soap having a pH 10.05 had no effect on the subsequent viability of the spores. A 2-minute treatment in 6 per cent soda ash solution (pH 10.16) and a 5-minute immersion in a solution of 1 per cent washing powder (pH 9.90) had but slight effect on germination. However, a 2-minute exposure to the 0.4 per cent alkaline sodium hypochlorite solution (pH 11.1) was fatal. While the high OH-ion concentration was likely an important factor in the toxicity of the hypochlorite solution, the lethal effects may have been due in a large measure to the toxicity of free chlorine, OCl ions, and nascent oxygen. Klotz (*21*) has shown that clorine even in small concentration is lethal to *Penicillium italicum* and *P. digitatum*. Marloth (*22*) found that the spores of the two fungi showed abundant germination in a slightly buffered Duggar's solution to which orange extract had been added and which had been adjusted to the acidity-alkalinity range of pH 2.4 to pH 9.4. In the range pH 3.0 to pH 9.2 the germination in that medium was relatively indifferent to H-ion concentration. No germination of either organism was obtained in Sörensen's glycocoll buffer above pH 9.7 or in 2.6 and 10.0 per cent solutions of sodium carbonate, potassium carbonate, sodium bicarbonate, and potassium bicarbonate, or in 4 per cent sodium tetraborate solution. Since the estimations of H-ion concentration in those studies were made with the quinhydrone electrode they are reliable only up to approximately pH 7.5. For example, the pH of 10 per cent solutions of bicarbonate and carbonate which were reported as 8.6 and 11.4, respectively, were estimated by the glass electrode and reported in the present paper as pH 8.0 and pH 9.9.

The results seem to indicate that $\overset{+}{H}$ and $\overset{-}{OH}$ ions in the concentration range of pH 2.4 to pH 10.0 are in themselves relatively innocuous to the spores of *P. italicum* and *P. digitatum*. However, owing to their possible effect in altering the permeability of the fungus protoplasts these very mobile ions may affect the results with other toxic ions. Osterhout (*25, 26*) found that alkali increases permeability, and that acid at first decreases then rapidly increases permeability in the seaweed *Laminaria saccharinum*.

Unlike the technique of the former paper (*22*) in which the spores were germinated directly in media whose pH was adjusted, the procedure of the present paper exposed the spores for only a short period to the action of the $\overset{-}{OH}$ and other ions, then rinsed with water and mounted them in a medium favorable for germination. The short period of exposure to

the toxic solution followed by a rinse would correspond to that treatment usually given in a packing-house. Some packing-house procedures, however, as in the case of the water-wax method for lemons, allow the toxic solution, containing soda ash in this case, to dry on the fruit and thus maintain a protective coating. As suggested by Marloth (*22*) the toxic salt thus deposited would form a relatively concentrated solution in condensation water that might subsequently form on the surface of the fruit and would, by killing the tender swollen spores and germ tubes, repulse invasion of the fungi that might lodge in that water.

Some discrepancies are seen between the results of the germination tests and those of the dilution plate methods. These may be due to the clumps of spores forming individual colonies which would be recorded as arising from single spores.

SUMMARY

To obtain information on the toxicity of various chemical solutions, at several temperatures and concentrations, to *Penicillium italicum* and *P. digitatum* (the causal agents of blue and green mold of citrus fruits), the spores of the fungi were immersed for certain time periods, and their subsequent viability compared with that of untreated spores by means of germination and dilution-plate tests. The technique of the methods employed is described in detail.

It was shown that a 0.25 per cent solution of a nontoxic soap effectively wets and prepares the spores of *Penicillium italicum* and *P. digitatum* for the chemical treatment that follows. No decrease in germination followed the pretreatment with the soap.

Distilled water at 120° F for 5 minutes killed approximately 90 per cent of the spores.

Tests in which 6 per cent borax at 110° F was used for 2, 4, 6, 8, 10, 12, 14, and 16 minutes, and at room temperature (66° to 72°), 80°, 100°, 110°, and 120° F for 5 minutes, and at concentrations of 4, 6, 8, 10, and 12 per cent for 5 minutes at 110° F, showed, as would be expected, that the longer the exposure to, the higher the temperature of, and the greater the concentration of the chemical, the more effective was the solution in reducing viability. Similar relations were found with sodium carbonate and Metbor.

Under the conditions of the experiments toxicity of the several solutions to spores of *Penicillium italicum* and *P. digitatum* was more dependent on temperature than on concentration of the chemicals or the period of immersion. A 5-minute exposure at a temperature of 120° F

in a 6 per cent borax-boric acid mixture, or 6 per cent Metbor, or 0.4 per cent chloramine-T, or in 6 per cent sodium carbonate, was lethal to the spores of both fungi. Details of the effects of the several temperatures may be secured from tables 2 and 3.

A saturated solution of dinitro-o-cyclohexylphenol and a 1 per cent proprietary washing powder used at room temperature for 2 minutes and 5 minutes, respectively, showed only a slight inhibitory effect on spore germination.

A 5-minute exposure of the spores in 6 per cent sodium bicarbonate at 86°, 100°, 110°, and 120° F showed no advantage of the chemical over water. At 86° F, immersion in a 10 per cent solution of sodium bicarbonate for 5 minutes or in one of 6 per cent for 10 minutes, had but little effect on the spores.

Two-minute exposures to 0.4, 0.6, and 1.0 per cent solutions of sodium hypochlorite were fatal to the spores of both fungi.

Excluding the sodium hypochlorite solutions which killed all the spores of both fungi, the three most efficacious solutions, when used at 100° F and below for 5 minutes, were 6 per cent sodium carbonate, 0.15 per cent sodium o-phenylphenate and 6 per cent borax; at 110° and 120° F the 3 most toxic were 0.4 per cent chloramine-T, 6.0 per cent sodium carbonate, and the 6 per cent mixture (2:1) of borax-boric acid.

ACKNOWLEDGMENTS

The writers wish to express their deep appreciation to Dr. H. S. Fawcett for assistance in the direction of the investigation and review of the manuscript. Thanks are especially due to Dr. M. W. Gardner for his constructive criticism of the manuscript and for the method of reporting toxicity index.

LITERATURE CITED

1. AUSTRALIAN CITRUS PRESERVATION COMMITTEE.
1931. Export of citrus fruit. Jour. Australia Council Sci. and Indus. Research 4:96–99.

2. B & C SCIENTIFIC PRODUCTS, INC.
1935. The Staklor method of blue mold control. 12 p. B & C Scientific Products, Inc., Seattle, Wash. (Special folder.)

3. BAKER, K. F., and F. D. HEALD.
1934. Investigations on methods of control of the blue-mold decay of apples. Washington Agr. Exp. Sta. Bul. 304:1–32.

4. BARGER, W. R.
1925. Treating oranges with borax solution for control of blue and green mold. California Citrograph 10:149.

5. BARGER, W. R.
1928. Sodium bicarbonate as a citrus fruit disinfectant. California Citrograph 13:164, 172–74.

6. BARGER, W. R., and L. A. HAWKINS.
1925. Borax as a disinfectant for citrus fruit. Jour. Agr. Research 30:189–92.

7. BARKER, J.
1928. Wastage in fruit commerce. [Gt. Brit.] Dept. Sci. and Indus. Research. Food Invest. Bd. Rept. 1927:38–42.

8. BATES, G. R.
1933. II. Wastage during the 1932 export season. British South Africa Co. Mazoe Citrus Exp. Sta. Pub. 2c:155–76.

9. BENTON, R. J.
1931. Prevention of decay in oranges. Agr. Gaz. N. S. Wales 42:411–13.

10. BROGDEX COMPANY.
[1925.] The successful control of blue mold decay in marketing citrus fruits. The borax treatment and the patent situation. 49 p., Brogdex Co., Los Angeles and Winterhaven.

11. CHARTER OAK HOUSE.
1925. Charter Oak House tests sodium hypochlorite process. California Citrograph 10:417, 446–47.

12. DOIDGE, E. M.
1929. Some diseases of citrus prevalent in South Africa. So. African Jour. Sci. 26:320–25.

13. FAWCETT, H. S.
1925. The decay of citrus fruits on arrival and in storage at eastern markets. California Citrograph 10:79, 98, 103.

14. FAWCETT, H. S.
1936. Citrus diseases and their control. 2nd ed. 656 p. (See specifically p. 387–99.) McGraw-Hill Book Co. Inc., New York, N. Y.

15. FAWCETT, H. S., and W. R. BARGER.
1927. Relation of temperature to growth of *Penicillium italicum* and *P. digitatum* and to citrus-fruit decay produced by these fungi. Jour. Agr. Research 35:925–31.

16. FAWCETT, H. S., and L. J. KLOTZ.
1936. Protecting the fruit and foliage of citrus from brown rot. Univ. California Citrus Exp. Sta. 2 p. (Mimeo.)

17. FULTON, H. R., and J. J. BOWMAN.
1924. Preliminary results with the borax treatment of citrus fruits for the prevention of blue mold rot. Jour. Agr. Research 28:961–68.

18. FULTON, H. R., and J. R. WINSTON.
1924. Controlling blue mold rot of citrus fruits with borax solution. Florida Grower 30(18):7.

19. HODGSON, R. W.
1928. Borax treatment. A letter addressed to the Director of Agriculture and Forests, Jerusalem. Palestine Citrograph 1:3.

20. HOPKINS, J. C.
1930. Report of plant pathologists for year ending December 31, 1929. South. Rhodesia Dept. Agr. Rept. of Secretary 1929:84–86.

21. KLOTZ, L. J.
1936. Nitrogen trichloride and other gases as fungicides. Hilgardia 10(2):27–52.

22. MARLOTH, R. H.
1931. Influence of hydrogen-ion concentration and of sodium bicarbonate and related substances on *P. italicum* and *P. digitatum*. Phytopath. 21:169–98.

23. MCCALLAN, S. E. A., and F. WILCOXON.
1932. The precision of spore germination tests. Boyce Thompson Inst. Contrib. 4:233–43.

24. NATTRASS, R. M.
1935. Prevention of wastage of citrus fruit in transit. Cyprus Agr. Jour. 30: 84–87.

25. OSTERHOUT, W. J. V.
1914. The effect of alkali on permeability. Jour. Biol. Chem. 19:335–43.

26. OSTERHOUT, W. J. V.
1914. The effect of acid on permeability. Jour. Biol. Chem. 19:493–501.

27. POWELL, G. H., *et al.*
1908. The decay of oranges while in transit from California. U. S. Dept. Agr. Bur. Plant Indus. Bul. 123:1–79.

28. POWELL, H. C.
1926. The control of blue and green mold of oranges. So. Africa Fruit Grower 9(13):232.

29. PUTTERILL, V. A.
1930. The prevention of mould wastage in oranges. Union So. Africa Dept. Agr. Bul. 64:1–20.

30. PUTTERILL, V. A.
1935. Citrus wastage investigations progress report No. 3, 1934. Union So. Africa Dept. Agr. Bul. 149:5–27.

31. PUTTERILL, V. A., and R. DAVIES.
1934. Citrus wastage investigations carried out at Zebediela, Transvaal, during the seasons 1931 and 1932. Union So. Africa Dept. Agr. Bul. **128**:7–49.

32. REICHERT, I., and F. LITTAUER.
1928. The decay of citrus fruits in Palestine and its prevention. Palestine Citrograph **1**(8):4–7; **1**(9):5–8.

33. REICHERT, I., and F. LITTAUER.
1931. Preliminary disinfection experiments against mould wastage in oranges. Hadar **4**(3&4):3–18.

34. SAWADA, K.
1932. Blue mold of sweet orange (*Penicillium italicum*). Descriptive catalogue of Formosan fungi, part II. Formosa Dept. Agr. Gov. Research Inst. Rept. **2**:128–30.

35. SHIVER, H. E.
1925. Disinfecting and washing citrus fruit. Chem. and Metall. Engin. **32**:812.

36. STEWART, R. M.
1935. Interesting new uses of soluble borates in the packing houses. Florida State Hort. Soc. Proc. **48**:42–44.

37. TAKEUCHI, H.
1929. Penicillium rots of citrus fruits. Kjusu Imp. Univ. Bul. Sci. Fakultato Terkultura **3**:333–49.

38. TINDALE, G. B.
1927. Cool storage of Washington Navel oranges. Results of 1926 experiments. Jour. Dept. Agr. Victoria **25**:74–80.

39. TINDALE, G. B.
1927. Valencia late oranges. Cool storage experiments. Jour. Dept. Agr. Victoria **25**:276–79.

40. TOMKINS, R. G., and S. A. TROUT.
1931. The use of ammonia and ammonium salts for the prevention of green mold in citrus. Jour. Pomol. and Hort. Sci. **9**:257–64.

41. WINSTON, J. R.
1935. Reducing decay in citrus fruits with borax. U. S. Dept. Agr. Tech. Bul. **488**:1–32.

42. YOUNG, W. J., and F. M. READ.
1930. The preservation of citrus fruit. Progress report of the citrus preservation committee. Jour. Australia Council Sci. and Indus. Research **3**:69–76.

43. YU, T. F.
1934. Notes on the storage and market diseases of fruits. I. Jour. Agr. Assoc. China **123**:16–27.