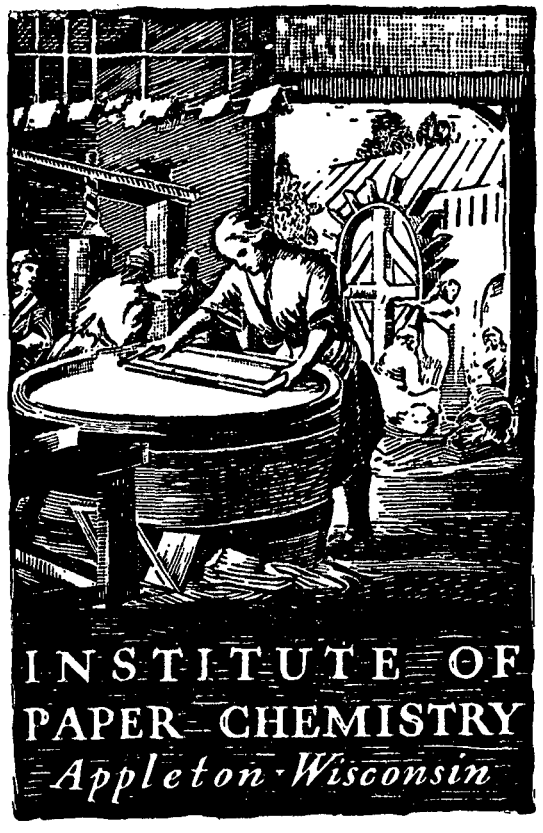

THE EFFECT OF VARIOUS CHEMICALS ON THE GROWTH OF FOUR CITRUS SPOILAGE ORGANISMS

Project 1108-7-D

Progress Report Two

to

FOURDRINIER KRAFT BOARD INSTITUTE, INC.

April 23, 1953

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

THE EFFECT OF VARIOUS CHEMICALS ON THE GROWTH OF FOUR CITRUS SPOILAGE ORGANISMS

Project 1108-7-D

Progress Report Two

to

FOURDRINIER KRAFT BOARD INSTITUTE, INC.

April 23, 1953

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

INTRODUCTION

The success which has been achieved in the use of biphenyl as a means of preventing the decay of oranges packed in fiberboard containers raises the question as to whether there may be other materials which will function with equal success but without some of the attendant disadvantages of biphenyl. As a preliminary approach in the search for such materials, two compounds known to have certain fungistatic properties have been investigated for their ability to inhibit the growth of four citrus spoilage organisms. The evaluation was so designed as to determine the comparative fungistatic effect of each material in the gaseous state on four different species of fungi. In addition, control tests and tests with biphenyl were run for comparative purposes.

MATERIALS USED

The three chemicals used as fungistatic agents were as follows:

1. Ortho captan, Technical (92% captan)
2. 8-Hydroxyquinoline
3. Biphenyl

The four species used as test organisms were:

1. Penicillium italicum
2. Penicillium digitatum
3. Phomopsis citri
4. Diplodia natelensis

These two species of penicillium are commonly associated with the blue mold of citrus fruit. The Diplodia natelensis and Phomopsis citri are generally associated with stem end rot.

PROCEDURE

Each of the three chemicals used in this study was evaluated as follows: 0.5 gram of the compound was placed in a sterile flat bottom glass cup (16 mm. in diameter and 10 mm. high) and the cup in turn was centrally located in a 100-mm. sterile Petri plate. The cup was surrounded with approximately 25 ml. of melted sterile 1-1/2% malt agar. The agar was allowed to harden thereby securing the cup containing the chemical to the center of the Petri plate. For each chemical twelve such units were prepared so that the test could be carried out in triplicate for each of the four fungi. Controls containing no chemicals were similarly prepared.

The malt agar was prepared by dissolving 15 grams plain malt extract and 20 grams agar in 1000 ml. of distilled boiling water. The medium thus prepared was distributed in Erlenmeyer flasks, plugged with cotton, and sterilized in an autoclave for 20 minutes at 15 p.s.i. steam pressure (121°C.). After sterilization the agar was allowed to cool to 45 to 50°C. before pouring into the Petri plates.

The inoculum for the Petri dishes was prepared by suspending the growth from a 7-day culture of test organism in 100 ml. of sterile

distilled water. One ml. of the resultant spore-mycilial suspension was used as the inoculum for each Petri dish. After inoculation each plate was sealed with masking tape and placed in a 28°C. incubator. The effectiveness of each chemical was judged by periodically inspecting the plates for the degree of mold growth.

DISCUSSION OF RESULTS

The results of the tests performed as described in the previous section of this report are summarized in Table I. It may be observed that the effectiveness of each chemical varies with the organism used. In other words, the chemicals exhibit a certain degree of selectivity in regard to their fungistatic inhibiting properties. In general, Ortho captan and 8-hydroxyquinoline were more effective, or equally as effective, as biphenyl against all four molds.

Because of the above mentioned selectivity, a second series of tests was set up using the same procedure and chemicals as was used in obtaining the above results. In addition, a mixture of equal portions (by weight) of Ortho captan and 8-hydroxyquinoline were used. The total amount of the mixture used was 0.5 gram. This series of tests was incubated for 14 days. The results obtained at the end of three-day incubation are given in Table II. It may be observed that the fungistatic effect obtained with the 50/50 mixture of Ortho captan and 8-hydroxyquinoline was equal to, or better than, that obtained with the biphenyl. The portions of each used in the mixture were purely arbitrary

TABLE I

EFFECT OF VARIOUS CHEMICALS ON MOLD GROWTH

	Relative Rate of Growth*															
	Penicillium italicum				Penicillium digitatum			Phomopsis citri			Diplodia natelensis			
Incubation, days	3	7	11	14	3	7	11	14	3	7	11	14	3	7	11	14
Chemical																
Ortho captan	+	+	+1	+1	0	0	0	+	0	+	+	+	0	+2	+4	+4
8-hydroxyquinoline	+	+	+	+	+2	+3	+3	+3	0	0	0	0	0	0	0	0
Biphenyl	+2	+3	+3	+4	+	+2	+2	+3	0	+1	+2	+2	0	+3	+3	+3
Control	+4	+4	+4	+4	+4	+4	+4	+4	+	+3	+3	+3	+4	+4	+4	+4

* 0 indicates no growth
\+ indicates very slight growth
+1 indicates slight growth
+2 indicates moderate growth
+3 indicates good growth
+4 indicates luxuriant growth

TABLE II

EFFECT OF VARIOUS CHEMICALS ON MOLD GROWTH
(Incubation Period = 14 days)

Chemical	Diplodia natelensis	Penicillium digitatum
Ortho captan	+4	+1
8-hydroxyquinoline	0	+3
Mixture 50/50*	+	+1
Biphenyl	+3	+1
Control	+4	+4

*50/50 by weight Ortho captan and 8-hydroxyquinoline.

0 indicates no growth

+ indicates very slight growth

+1 indicates slight growth

+3 indicates good growth

+4 indicates luxuriant growth

and it would appear from the above results that possibly even better results would be obtained if the amount of 8-hydroxyquinoline were cut slightly and the Ortho captan increased accordingly.

It should be emphasized that these results are in no way conclusive nor should they be interpreted as necessarily being more than indicative of the trend which might be obtained if these materials were used in the packaging of oranges in paperboard cases. However, they do appear sufficiently promising to warrant further investigation.