THE DEVELOPMENT OF MOLD ON COLD STORAGE EGGS AND METHODS OF CONTROL

Thesis

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by
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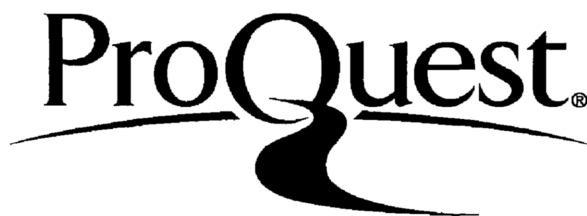

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PART I. ISOLATION AND IDENTIFICATION

Introduction

The need of producing a high quality storage egg is evidenced by the fact that approximately 10,000,000 cases of eggs were placed in cold storage during the year 1926 (6). This volume represented approximately 13 per cent of the entire crop. Unfortunately the preservation of eggs by cold as practiced today is still not the ideal to which the industry aspires, particularly for long periods of storage. The process of holding foods at temperatures of approximately 0° F. is a means of delayed decomposition rather than a process of total or complete inhibition of microbiological and enzymatic spoilage. If the humidity of the storage room is sufficiently high to form a microscopic film of moisture on the surfaces of the foods and their containers, then molds slowly but surely develop. If, on the other hand, the humidity of the storage room is kept at a point where the surfaces

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In the identification of the various fungi found by the writer in eggs and egg materials, assistance was received from Dr. E. A. Bessey, Department of Botany, under whose supervision the mycological portions of this investigation were carried on.

The remainder of the work, including the laboratory tests on mycostatic agents and the cold storage investigations, was carried out under the supervision of Dr. W. L. Mallmann of the Department of Bacteriology.

The main field tests were conducted at the plant of the United States Cold Storage and Ice Company, in Chicago, with the able assistance of Mr. Eugene A. Toop.

of the foods and containers do not carry a microscopic film of moisture, then molds are prevented from growing but the food gives up moisture to the air, and shrinking, with ultimate loss in quality of the food, results. In the operation of a cold storage plant, the humidities are, at present, kept at a point just below that which allows the development of microorganisms. This means that during storage a gradual loss of moisture from the foods results. In eggs there is not only loss in weight but the appearance of large air sacs, if the egg is held too long in storage.

The losses due to moldiness vary from season to season, and from one plant to another. Many factors enter to determine the extent and degree of spoilage that occurs. Most of the factors under present operating conditions are uncontrollable; at least there is no practical means of controlling contaminations of eggs and egg cases. Moisture content of the storage rooms and storage temperatures cannot be varied sufficiently to be of any value in controlling the growth of the molds without greater injury to the product itself.

Some method of mold control which will function regardless of the humidity and the initial contamination is necessary.

Mold has been reported as developing on a number of products in cold storage. The development of mold on meat in cold storage has been frequently observed. Monvoisin in 1922 (9)

found *Cladosporium herbarum*, *Thamnidium elegans*, *Mucor mucedo*, *Rhizopus nigricans*, and *Penicillium glaucum*. Bidault in 1922 (2) found *Chaetostylum fresenii*, *Thamnidium elegans*, *Penicillium crustaceum*, *Hormodendron cladosporioides*, *Cladosporium herbarum*, *Stysanus stemonites*, *Botrytis micheli*, and some varieties of Eubotrytis. Wright in 1923 (17) found that "black spot" of meat in cold storage could be produced by a number of molds, including *Cladosporium herbarum*, *Mucor mucedo*, and *Penicillium glaucum*. In 1923 Yesair (18) found that *Penicillium expansum* was one of the most common molds on the walls and equipment of meat packing houses. In 1927 Weston and Holman (15) demonstrated that a mold belonging to the genus Cladosporium was responsible for black discoloration of eggs. This discoloration, "black spot," was reproduced in eggs by treating clean eggs with spore suspensions of *Cladosporium herbarum*. They also found that a species of Penicillium could produce a similar condition. In 1929 James and Swenson (8), in a similar study, found two molds, a Penicillium and a Cladosporium.

In order to study the methods and procedures for mold control in the cold storage room, from a scientific attack, it is first essential to know exactly the species of molds encountered and the extent of their activity. Inasmuch as the literature does not reveal any such studies it was deemed necessary to make a careful and extensive survey on the incidence and identity of the molds occurring on eggs, fillers, flats, and cases in the cold storage plant.

Isolation and Identification of Molds

In order that a wide survey of the various species of molds be made representative of the whole industry, eggs were obtained over a period of two seasons from plants in Chicago, Fort Wayne, Detroit, Lansing, Omaha, and Topeka. Fresh eggs from the College poultry plant and moldy eggs from cold storage were cultured. In addition to eggs moldy fillers, flats, and crates from the packing plants, and new fillers, flats, and crates from a manufacturer of the same were cultured for molds.

The isolations of molds were made by using scrapings from the surfaces of affected areas on the various materials examined. These scrapings were smeared on the surface of dextrose agar or beanpod agar and incubated at room temperature until fruiting bodies of the molds appeared. The resulting mixed growths of molds were then purified by the dilution plate method. After the purity of each culture was definitely established, the cultures were transferred to the proper media upon which identifications are based.

Except where indicated, the Penicillia in this study were identified from Thom's "The Penicillia" (13). The genus Penicillium consists of a great many species which intergrade and which are exceedingly variable under different conditions of temperature, substratum, and manner of culture. The students of this

genus, of whom Dr. Charles Thom of the United States Department of Agriculture is foremost, recognize many strains within each species. A few species were identified by Dr. Thom. Where the description of an organism fell widely outside of any standard description in Thom's key, it is designated by its culture number and the description is presented in detail.

Organisms belonging to the order Mucorales were identified from the descriptions of N. A. Naumov (11). Several forms of Mucor racemosus were isolated which fit this species most closely but are aberrant in some particular.

The identification of the Aspergilli isolated correspond to the descriptions of Thom and Church (14).

A number of cultures were identified only as to genus. These belong to the Fungi Imperfecti and correspond to the description of the genus as found in standard works.

The descriptions of the aberrant species follow.

Penicillium No. 30

Colony after four days on Czapek's agar light green with slight sterile border; reverse tan; drops absent. Colony after two weeks bright green with white margin about 2000 u broad; colony 4 cm. broad and about 1000 u deep; distinctly zonate; reverse cream to rose; agar faint rose color. Colony after 24 days 5.5 cm., bright green, velvety, deeply zonate at margin; reverse yellow-rose; agar rose-tan. Colony after six weeks dark green,

deeply zonate at margin, with wide submerged margin; drops yellow; reverse dark red; agar dark red-brown; conidiophores slightly rough; metulae not divaricate, 9.44 - (11.0) - 11.8 u. Penicilli showing sterigmata and metulae in a compact brush or broom; sterigmata 5.9 - (8.0) - 10.6 u; conidia globose, smooth, 2.77 - (3.0) - 3.3 u; gelatin liquefied

Penicillium No. 36

Colony after four days on Czapek's agar pale green with white margin; drops absent; reverse light green. Colony after six weeks 8.5 cm. broad, velvety, thin felt, brown to gray-green, zonate at margin with wide submerged margin; white over-growth in center; reverse white to purple-black; conidiophores rough; metulae not divaricate, but penicilli subtended by one or more distant secondary divergent branches bearing secondary penicilli often mono-verticillate; metulae 12.5 - (13.0) - 15.0 u; sterigmata 7.5 - (12.0) - 15.0 u; conidia smooth, globose 3.1 - (3.58) - 4.1 u; gelatin not liquefied.

Penicillium No. 65

Colony after four days on Czapek's agar green with sterile white margin; drops absent; reverse tan-gray. Colony after twelve days 12.5 cm. x 5.5 cm., velvety, bright green with white margin 1000 u broad, zonate, about 900 u deep near edge; center about 2000 u deep; reverse orange yellow, zonate; agar colored red-brown;

conidiophores erect, rough, asymmetrical, over 300 u long; metulae not divaricate 9.6 - (9.65) - 10.0 u; sterigmata 9.6 - (9.65) - 10.0 u; conidia globose, smooth 3.0 - (3.7) - 3.84 u; gelatin liquefied.

Penicillium No. 84

Colony after four days on Czapek's agar green, white margin; reverse in shades of green; drops colorless. Colony after 26 days thin felt 3.5 cm. broad, floccose, olive-green, azonate; reverse in shades of green; agar uncolored; conidiophores rough; metulae not divaricate 10.0 - (11.5) - 15 u; sterigmata 7.5 - (10.5) - 12.5 u; conidia smooth, globose 3.86 - (4.0) - 4.16 u; gelatin liquefied.

Penicillium No. 102

Colony after four days on Czapek's agar light blue; drops colorless; reverse yellow-tan-rose. Colony after six weeks 8.5 cm., floccose, gray-green, faintly zonate at edge; wide submerged margin; drops colorless; reverse zones of rose red to cream; agar rose colored; conidiophores rough; metulae not divaricate 10 - (11.5) - 12.5 u; sterigmata 7.5 - (9.0) - 10.0 u; conidia globose, smooth, 3.3 - (3.48) - 3.6 u; gelatin liquefied.

Penicillium No. 123

Colony after four days on Czapek's agar floccose, green with white margin; drops colorless; reverse light yellow. Colony

after six weeks velvety, 4.5 cm. broad, dark green thin felt; wide submerged margin; slightly zonate at outer edge; drops colorless; reverse zones of pink to rose-tan; conidiophores rough; metulae not divaricate 12.5 - (16.5) - 20 u; sterigmata 12.5 - (13.0) - 15.0 u; conidia globose, smooth, 3.75 - (3.83) - 4.1 u; gelatin not liquefied.

Penicillium No. 131.1*

Colony after two months on Czapek's agar 4 cm. long by 1 - 1.5 cm. broad, lanate, dark green with narrow white margin; much wrinkled; zonate in age; reverse mouse gray-violet, wrinkled, zonate; conidiophores short, smooth, 48 - (60) - 72 u, asymmetrical; metulae divaricate 8.4 - (9.6) - 10.8 u; sterigmata 4.8 - (6.8) - 8.4 u; 3-4-5 at end of metulae; conidia globose, smooth, 2.3 - (3.1)-3.8 u; spore chains 36 u to 60 u.

Penicillium No. 166

Colony after four days on Czapek's agar blue; margin very fuzzy; drops colorless; reverse light green. Colony after two weeks velvety, gray-blue with white margin 2000 u, azonate; colony 500 u deep, center umbonate; reverse cream; agar slightly cream-colored. Colony after six weeks dark blue-gray; zonate; reverse cream to rose

*Dr. Thom examined this culture and could not satisfy himself as to its specific identity; it seemed to conform to his 131.1 to which he had not assigned a specific name.

shades; conidiophores slightly rough; metulae not divaricate 10 - (11.5) - 15 u; sterigmata 10 - (11.0) - 12.5 u; conidia globose, smooth, 2.77 - (3.0) - 3.5 u; gelatin liquefied.

Penicillium No. 208

Colony after four days on Czapek's agar pale blue, very fuzzy with white margin; drops colorless; reverse white. Colony after twenty-four days floccose, blue-green; zonate at margin, reverse green-white; agar uncolored; metulae not divaricate 10 - (10) - 10 u; sterigmata 7.5 - (9.5) - 10 u; conidia globose, smooth, 3.1 - (3.3) - 3.57 u.

Mucor racemosus Fresenius

The three forms of this species that were most frequently isolated were the following. They do not seem to fit other described species but are quite distinct from one another.

Form No. 1.

Colony on dextrose agar after ten days broadly spreading, mouse-gray; reverse light tan; colony 3.4 mm. high, very little branched; slender sporangiophores 1-3 mm. long; no stolons or rhizoids; sporangia 25-40 u, some sporangia encrusted; columella free, mostly oval, few conical, 19.2 - (25.4) - 33.6 u x 12.0 - (20.7) - 31.2 u; no chlamydospores; spores very slightly punctate, very slightly rough 4.0 - (6.7) - 11.5 x 3.6 - (5.4) - 9.8 u. This is a small form close to *Mucor racemosus*, but lacking chlamydospores, not cutinized, and not forming such tall sporangia as may occur in that species.

Form No. 2.

Colony on dextrose agar after ten days broadly spreading, gray; reverse light tan; colony 1 cm. high; sporangiophores about 2-6 mm.; branched irregularly; sporangium membrane rough but not hairy, breaks into pieces, does not deliquesce; sporangia 21.6 - (34.5) - 55.2 u; columella slightly darkened; small collar around base of columella; collars vary in size; columella absent in lateral branches; columella shape pyramidal to ovoid, 20-30 x 14-20 u; chlamydospores numerous; spores elliptical, very slightly rough, variable in size 3.3 - (6.6) - 9.6 x 2.4 (4.9) - 7.4 u.

Form No. 3.

Colonies after three weeks tan; sporangia 33.6 - (48) - 84 u; sporangium membrane rough all over, breaks up, does not deliquesce; columella mainly panduriform, 19.2 - (25.8) - 32.4 x 12 - (18.4) - 19.2 u; pale brown; chlamydospores numerous. The columella frequently contains one, rarely 2 chlamydospores. The sporangia are in 2 layers near the base of the tall sporangiospores. There are many branches with numerous sporangia of various sizes. These small sporangia have a columella but it is smaller, almost spherical, about 7.2 x 7.2 u. The chlamydospores are quite numerous near the base of the sporangia. The lower mycelium is much roughened. Spores hyaline, light brown enmass; spores smooth, variable in size and shape, 3.6 - (5.5) - 12.0 x 3.6 - (5.3) - 9.6 u.

The occurrence and incidence of molds on the various materials examined are presented in the following lists. The incidence of the molds does not represent the extent of the mold contamination, except to measure to a degree the predominance of the species on the particular material examined.

Various cold storage plants kindly submitted moldy eggs that were found at the time eggs were removed from storage. These eggs showed very marked evidence of mold contamination on the surfaces. Some of the eggs were completely covered with mycelial growth and fruiting bodies. Samples were made by using scrapings from the shell surface. The following molds were isolated:

	No. of isolations
Penicillium citreo-roseum Dierckx	12
Penicillium No. 102	2
Penicillium No. 36	5
Penicillium asperulum Bainier	10
Penicillium chloro-leucon Biourge	16
Penicillium ochro-chloron Biourge	4
Penicillium oxalicum Currie and Thom	10
Penicillium puberulum Bainier	36
Penicillium citrinum Thom	19
Penicillium janthinellum Biourge	16
Penicillium casei Staub	14
Penicillium crustosum Thom	16
Penicillium No. 131.1	17
Penicillium flavo-glaucum Biourge	10
Penicillium viridicatum Westling	6
Penicillium verrucosum Dierekx	12
Penicillium roqueforti Thom	9
Aspergillus flavus Link	15
Aspergillus niger van Tieghem	35
Fusarium sp.	5
Mucor racemosus Fresenius	50
Mucor lausannensis Lendner	30
Chaetomium globosum Kunze	24
Cephalosporium sp.	15
Protabsidia Blakesleana Lendner	12
Actinomyces sp.	Not counted

In all, 400 isolations were made. Of this total 214 strains were Penicillia, 50 strains were Aspergilli, and 80 strains were Mucors.

After examining the mold flora of the exterior of the eggs, the eggs were opened aseptically and cultures were made from the mold growths in the air sac. The molds found growing inside of the eggs were in all cases found on the surface of the membrane adjacent to the inner surface of the egg shell, in the air sac, and on the inner surface of the shell. In no case did the mold seem to penetrate into the egg contents.

The following organisms were obtained from eggs that showed evidence of having mold growth on the inside of the shell:

	No. of isolations
Penicillium chloro-leucon Biourge	34
Penicillium oxalicum Currie and Thom	6
Penicillium citreo-roseum Dierckx	12
Penicillium No. 30	8
Penicillium roqueforti Thom	10
Penicillium No. 102	6
Penicillium No. 36	12
Penicillium asperulum Bainier	6
Penicillium Kapuscinskii Zaleski	3
Penicillium No. 65	4
Penicillium No. 84	2
Penicillium puberulum Bainier	10
Penicillium citrinum Thom	6
Penicillium janthinellum Biourge	6
Penicillium casei Staub	2
Penicillium No. 123	3
Penicillium ochro-chloron Biourge	6
Penicillium No. 166	1
Penicillium No. 208	2
Penicillium viridicatum Westling	4
Penicillium verrucosum Dierckx	3
Penicillium crustosum Thom	4
Penicillium No. 131.1	6
Penicillium flavo-glaucum Biourge	8
Hormodendrum sp.	4

Of the total of 170 isolations, 166 strains were Penicillia. Only one other genus, Hormodendrum, was isolated. This is exceedingly interesting as it would appear that the only organisms capable of penetrating the egg shell to a marked degree were the Penicillia.

Moldy cases obtained from several cold storage houses were examined for molds. These cases showed visible evidence of mold development. The boxes were badly discolored by black and green mold spots. Cultures were taken from these areas. The following molds were isolated:

	No. of isolations
Penicillium No. 102	2
Penicillium citreo-roseum Dierckx	5
Penicillium Kapuscinskii Zaleski	4
Penicillium asperulum Bainier	4
Penicillium puberulum Bainier	5
Penicillium oxalicum Currie and Thom	3
Penicillium citrinum Thom	3
Penicillium janthinellum Biourge	8
Chaetomium globosum Kunze	4

Thirty-eight isolations were made. Thirty-four of the thirty-eight cultures were Penicillia. Only one other organism, Chaetomium globosum, was isolated.

In a similar manner, isolations were made from moldy fillers and flats obtained from cold storage houses. These fillers and flats like the cases showed marked evidence of mold formation. They were spotted with mold growth, generally black or green. In some instances the entire surface of the filler or flat was completely covered with mycelial growth and fruiting bodies. The fol-

lowing cultures were isolated:

	No. of isolations
Penicillium corylophilum Dierckx	16
Penicillium chloro-leucon Biourge	25
Penicillium No. 102	10
Penicillium No. 36	13
Penicillium asperulum Bainier	19
Penicillium ochro-chloron Biourge	19
Penicillium roqueforti Thom	9
Penicillium citreo-roseum Dierckx	12
Penicillium No. 208	1
Penicillium puberulum Bainier	18
Penicillium casei Staub	4
Penicillium viridicatum Westling	7
Penicillium verrucosum Dierckx	9
Penicillium crustosum Thom	11
Penicillium flavo-glaucum Biourge	14
Penicillium oxalicum Currie and Thom	10
Penicillium citrinum Thom	10
Penicillium janthinellum Biourge	16
Mucor racemosus Fresenius	27
Chaetomium globosum Kunze	4
Cephalosporium sp.	7
Fusarium sp.	3
Aspergillus niger van Tieghem	5

Of the total 269 cultures, 233 strains were Penicillia.

Assuming that the molds occurring on the eggs, both inside and outside, fillers, and flats are the molds that are significant in the spoilage of eggs; a compilation of the molds isolated from these sources was made to determine the relative relationship of the molds from each source. The data follow.

A total of 843 isolations were made. It is interesting to note that 607, or 70.8 per cent, of all organisms isolated were Penicillia. The most common species of Penicillia were _Penicillium chloro-leucon_ and _Penicillium puberulum_. The data do not show that

any particular Penicillium is significant. The data do show that the Penicillia appear to be the most significant as far as egg spoilage is concerned. Most species appear on the eggs, fillers, and flats.

To determine the significance of new cases as a source of molds, a package of case boards was obtained direct from the manufacturer. To be sure that the molds represented initial contamination at the source, cultures were taken from the boards in the interior of the package. These boards without exception showed no visible growth of molds. Slivers of wood were cut from the board using aseptic precautions. These slivers were then cultured by embedding in dextrose agar. From five packages examined only two molds were isolated, namely, Mucor racemosus and Chaetomium globosum. All of the boards showed a heavy incidence of both of these molds. In the light of studies on eggs, the absence of Penicillia and Aspergillus shows that the new case material certainly is not a source of objectionable molds.

In a similar manner new fillers and flats were examined. These were also packaged by the manufacturer. Samples for culture were taken from the center of each package. The organisms isolated are as follows:

Chaetomium globosum Kunze
Hormodendrum sp.
Aspergillus niger van Tieghem
Mucor racemosus Fresenius
Fusarium sp.
Penicillium asperulum Bainier
Penicillium oxalicum Currie and Thom
Penicillium Kapuseinskii Zaleski

These data show that the molds on the new fillers and flats, although the incidence was low, are genera that may cause mold spoilage of eggs. The use of new fillers and flats, although the incidence of molds would be low, does not eliminate, as far as this source of mold is concerned, the contamination of the egg.

The occurrence of molds on fresh eggs was also determined. Fresh eggs from the College poultry plant were obtained. Attempts to isolate the molds by plating some water used to wash the eggs was unsuccessful, showing that incidence of mold spores on the surface of the egg was low. That molds were on these eggs was demonstrated by placing the egg aseptically into a sterile deep culture dish and pouring dextrose agar over the egg and incubating at room temperature. By this means the mold spores present were able to germinate and grow. The molds isolated were identical with those found on moldy storage eggs.

Market eggs shipped to the cold storage plant for storage were also examined. These eggs showed a much higher incidence of molds than the fresh eggs cited above.

These data show that the eggs entering the cold storage plant carry on their surfaces the molds that may ultimately cause spoilage.

To show the ability of Penicillium to penetrate the shells, eggs showing no evidence of mold growth were washed with mercuric chloride and sterile water and inoculated with the spores by placing

them in the air sac without contamination of the outer shell surfaces. This was done by injecting the spores directly into the air sac with a hypodermic needle. After the infection was made, the hole made by the needle was covered with paraffin. After 14 months there was no evidence of mold growth on the outside of the eggshells. Upon opening the eggs, the mold was found to be alive. The mold had grown extensively between and on both the outer surface of the membrane and the inner surface of the shell. The molds had not penetrated either the shell or the membrane. The mold was also placed on the unbroken outer surface of the shells of eggs sterilized as above and kept in a moist atmosphere for this length of time. The molds were found to have penetrated the shells and were also found to be growing in the air sac of the eggs.

Discussion

All the fungi obtained from eggs and egg materials in this study belong to the large group of organisms which live primarily as saprophytes in the soil and on decaying **vegetative** material in the soil. They are, however, ubiquitous. They are present in the nest and on the feathers of the hen. They pass unharmed through the intestinal tract of chickens. Therefore, it is evident that eggs are laid in an environment heavily seeded with these spores.

That the packing materials also contain these organisms is demonstrated in this study. They are present not only on used

materials but on new cases, fillers, and flats. These organisms also inhabit the packing and storage houses, particularly when the humidity is high enough to support their growth.

Although the environment and the shells of both fresh and moldy eggs contains a large variety of molds, not all of them penetrate into the interior of the egg under cold storage conditions. Only two genera were included in those isolated from the interior of the shell. These consisted of twenty-five species of Penicillium and four strains of Hormodendrum.

Although the genus Penicillium was isolated most frequently in these experiments and was probably responsible for the contamination, no one species can be incriminated as a causative agent for the mold spoilage.

All the evidence of this study shows definitely that the mold found on moldy eggs comes from contamination from such sources as the handling and packing both before and after reaching the cold storage plant.

With conditions conducive to the growth of molds in the cold storage rooms, it would appear impossible to prevent mold formation irrespective of the sanitary precautions exercised in the handling and packing of the eggs. This statement should not be interpreted to the effect that sanitation need not be exercised, because the extent and degree of contamination can be materially reduced by sanitary precautions.

PART II. LABORATORY TESTS ON MYCOSTATIC AGENTS

The only attempts to control the development of mold on eggs in cold storage at the present time have been by the exposure of the eggs to either carbon dioxide or ozone during their storage period. Ewell 1936 (4) and 1938 (5) reports that 1.5 p.p.m. of ozone in the aisles of cold storage rooms provides ample protection against molds at a humidity of close to 90 per cent. However, the literature reveals some controversy as to the value of ozone when used as a mold preventive (1), although one man has stated that after 48 hours in an atmosphere containing five parts per million of ozone, molds on moldy eggs will "actually disappear as though they had evaporated." Another plant manager says "it cannot be done."

Probably one of the biggest drawbacks against the use of ozone in this connection is the cost of installing and maintaining the ducts. Most plants have trouble with the corrosive action of the ozone on this equipment (1). In many cold storage houses eggs are stored in rooms with other products. Another difficulty would arise here because of the destructive action of ozone on many food products that might be stored.

Moran 1938 (10) found that carbon dioxide would retard mold growth and permit a higher relative humidity. He found that at a relative humidity of 85 per cent, air containing two and one-

half per cent carbon dioxide was necessary. At 32° F. 60 per cent carbon dioxide was necessary to suppress mold growth if the atmosphere was saturated with water vapor. He also reports that at 60 per cent carbon dioxide storage the white as a whole was very watery.

Dr. Mary Pennington, in a discussion on carbon dioxide, states that it is difficult to maintain definite concentrations of a gas in rooms not adapted to gas storage (12), and that the average cold storage room is not designed for gas storage.

Therefore, it was deemed necessary in the search for a mycostatic agent to find a compound that was in a solid or liquid state rather than a gas, inasmuch as gas storage does not seem to be practical under present conditions in cold storage plants.

In the studies to find a suitable mycostatic agent of this kind, it was first necessary to find a suitable laboratory procedure that would produce results at least somewhat comparable to field conditions.

After a number of procedures had been tested it was found that the most suitable method was as follows: The mycostatic agent was mixed in varying concentrations with a suitable sterile nutrient medium, such as beanpod or dextrose agar. The media containing the mycostatic agents were poured into sterile petri dishes and the agar was allowed to harden. Spores of the various molds were placed on the surface of the medium in a circumscribed area. Growth on the plates was recorded quantitatively by the extent and degree of development.

Two methods of controlling mold growth appeared to be worth testing; first, an agent with marked mycostatic properties with low or negative vapor tension which acts by contact with the mold spore or mycelium; and, second, an agent with marked mycostatic properties with a vapor pressure sufficient to carry the inhibiting action to the mold without direct contact with the mold spore or mycelium. The first type of compound could at least theoretically be incorporated into the fillers and flats to hold the mold growth to the individual egg in each cell or it might be brought into direct contact with the eggs by dipping or spraying at the time they were placed in the case. The second type of compound could be incorporated into the fillers and flats or sprayed on the eggs in like manner, but not only could these agents inhibit the growth of molds on the fillers and flats, but they might also maintain sufficient vapor tension around the egg to prevent mold formation on the individual egg.

The first step in conducting these laboratory studies was to find suitable members of each type of compound.

In order to limit the amount of work, only three organisms were used in the early studies. These were picked largely at random except that in the case of the Penicillium one of the most frequently occurring species was selected. The three organisms were *Penicillium puberulum*, *Aspergillus niger*, and a species of Alternaria. Later studies were conducted with additional strains and the final

tests were made with mixtures containing 14 different commonly occurring species of molds.

Although a number of contact agents was tested, only a few will be presented, inasmuch as most of the compounds had little or no mycostatic action even in strong concentrations. Among these were several copper salts. Although copper is not permissible in foods, still it was hoped that dilute solutions might show sufficient mycostatic action to allow their use in fillers and flats. This would in no way affect the eggs.

Copper salts were selected because they are widely used in the control of fungus diseases of plants. The largest group of fungicides in present use contains as its active agent some form of copper. The following copper salts were tested: cupric nitrate, cupric oxide, cupric sulphate, cupric carbonate, cupric ammonium sulphate, cupric ammonium chloride, cupric acetate, and cupric chloride. These compounds were tested against *Penicillium puberulum*, *Aspergillus niger*, and a species of Alternaria. The data are presented in Tables I, II, and III.

The toxicity of the copper salts varies both with the specific nature of the salt as well as with the resistance of the test organism. Cupric oxide and cupric carbonate had no mycostatic action on all three organisms in concentrations up to 1.0 per cent, the strongest concentration tested. The remaining salts all inhibited the development of *Penicillium puberulum* and *Alternaria sp.* in concen-

trations of 0.5 per cent. Only cupric sulfate and cupric acetate inhibited *Aspergillus niger* in concentrations of 0.6 and 0.5 per cent, respectively. Doran 1923 (3) states that Clark found Aspergillus to be more resistant to copper than Penicillium, which agrees with the above data.

It is commonly believed that many factors enter into the amount of resistance of spores to toxic elements. Whetzel and McCallan 1930 (16) found that older spores were more sensitive to copper than young spores. Doran 1923 (3) found that dilutions of fungicides that were toxic near the maximum or minimum temperature for germination of spores were not toxic at the optimum temperature for germination. He also found that *Rhizopus nigricans* was eight times as susceptible to cupric sulphate as representatives of the Uredinales.

The data show that first the concentration necessary to inhibit mold growth is far too strong for practical use, and, second, that the marked variability in resistance of the three molds would make the use of such preparations vary unreliable in practical use.

The second series of compounds consisted of compounds having low toxicity which would not be objectionable in the presence of foods. Of this group, three are presented, namely, sodium borate, boric acid, and sodium benzoate. The results are presented in Tables IV, V, and VI. The same test organisms were used as those used in the tests on the copper salts.

In Table IV are presented the data for these three preparations using concentrations varying from one to 0.1 per cent. Sodium benzoate had no inhibitive effect even in concentrations of one per cent and boric acid showed only slight inhibiting action on *Alternaria sp.* and *Aspergillus niger*. Sodium borate, however, showed marked inhibiting action even in the weakest concentration on both *Aspergillus niger* and *Penicillium puberulum*. Alternaria grew in all dilutions except one per cent.

To determine whether or not there might be a difference in species resistance, 14 strains of Penicillia were tested in various dilutions of these three compounds. The test organisms were seven-day old cultures of *Penicillium puberulum* (4 strains), *Penicillium roqueforti*, *Penicillium verrucosum*, *Penicillium casei*, *Penicillium citrinum*, *Penicillium crustosum*, *Penicillium oxalicum*, *Penicillium 131.1*, *Penicillium flavo-glaucum*, *Penicillium janthinellum*, and *Penicillium viridicatum*.

All 14 cultures grew in all dilutions of sodium benzoate, showing that all of the strains tested were equally resistant to this compound.

The results with sodium borate are presented in Table VI. It will be observed that not only does the resistance of different species vary at the same concentration of sodium borate, but that 4 different strains of *Penicillium puberulum* show a varied resistance. Strain 1 is inhibited by a concentration of 0.2 per cent, strain 2

at 0.6 per cent, strain 3 by a concentration of 0.5 per cent, and strain 4 by a concentration of 0.7 per cent.

Of the three compounds reported, only sodium borate showed any promise of value and as indicated in Table IX, such marked variability occurred in the various strains of Penicillia coupled with the lack of inhibition of Alternaria sp. demonstrated that the use of this compound appeared to have little value.

Two commercial contact agents, "Moldex" and "Prevento," were submitted for examination. The "Moldex" was recommended as a mold preventative on eggs and "Prevento" for mold control on fruits. Both were recommended as contact agents. These preparations were tested in the same manner as the previous experiments and with the same three genera as before. From the data presented in Table VII, it is clear that the preparation "Moldex" has slight mycostatic powers, but the preparation "Prevento" has none in the strongest concentration tested.

As representatives of the second type of compounds with high vapor tension a group of phenol preparations were tested by the agar plate method. These included sodium-ortho-phenol phenate, sodium 2, 4, 5 trichlorophenate, sodium 2 chloro-ortho-phenyl phenate, sodium 2, 4, 5, 6 tetrachlorophenate, and sodium pentachlorophenate.

When sodium-ortho-phenyl phenate and sodium 2 chloro-ortho-phenyl phenate were tested against the 14 above strains of

Penicillia their mycostatic ability was found to be considerable and their effect against the different species quite constant. These data are shown in Tables VIII and IX.

The mycostatic value of these compounds, i.e., sodium-ortho-phenol phenate, sodium 2, 4, 5 trichlorophenate, sodium 2 chloro-ortho-phenyl phenate, sodium 2, 4, 5, 6 tetrachlorophenate, and sodium pentachlorophenate, was tested on 4 genera of molds, 3 of which have not been presented above. These data are presented in Table X. These three organisms, namely, *Aspergillus niger*, *Chaetomium globosum*, and *Mucor racemosus* were commonly isolated from eggs and egg-case material.

The mycostatic effect on these four molds showed *Mucor racemosus* the most resistant with *Penicillium puberulum* next. Inasmuch as the latter organism represents the most common egg contamination, the inhibition of *Mucor racemosus* represents an effective destruction or inhibition of all other molds.

Although the mycostatic effect of these phenol preparations was not constant against the four molds, they showed marked mycostatic ability against all of these organisms. Compounds sodium 2, 4, 5 trichlorophenate, sodium 2, 4, 5, 6 tetrachlorophenate, and sodium pentachlorophenate appear to be the most efficient mycostatic agents in this group of compounds.

To test the value of the phenol derivatives as mycostatic agents when applied to cases, fillers, and flats, two-inch squares

of cases, fillers, and flats were prepared. These were soaked for five minutes in the various dilutions of the phenol preparations and then placed individually in deep culture dishes. Subsequently they were seeded with a spore mixture of four different genera of molds, namely, *Aspergillus niger*, *Penicillium puberulum*, *Chaetomium globosum*, and *Mucor racemosus*. A piece of moist cotton was placed beneath each square in the deep culture dish during the incubation period. This cotton was kept saturated with water to insure proper moisture conditions for the growth of the molds. This method of application of the mycostatic agents did not insure that the egg-packing materials would soak up the same concentration of the compound as was present in the solution; but, as can be observed in Table XI, the egg-packing materials can be made to resist mold growth by being impregnated with effective concentrations of these phenol preparations.

A preliminary experiment was performed in which various methods of applying the chemical agents were used in an effort to determine which was most effective.

There were several methods that might be effective for this purpose, such as introducing the compound into the humidifiers, placing a solution of the compound in the storage room, using the compound in the dry form in the room, or simply impregnating the fillers and flats with the compound. Two-quart, wide-mouth Mason jars were used as containers. Four eggs were placed

on a wire screen raised above the bottom of the fruit jar by a 2 1/2 inch block of wood. The eggs were enclosed within a miniature "egg case" made from egg-case filler and flat material cut to fit the mar. Water was placed in the bottom of the jar to keep the atmosphere saturated with moisture. All cases, fillers, flats, and eggs were sprayed with a heavy suspension of molds before being packed. Sodium 2 chloro-ortho-phenyl phenate and sodium 2, 4, 5 trichlorophenate were used in three jars each, being applied as a dry powder, as a solution, and by impregnating the egg-case materials. The data obtained after two months at room temperature show (Table XII) that the impregnation of the packing materials is the most effective method of applying mycostatic agents to the eggs.

In order to determine which of the phenol preparations was the most effective, paper prepared by the Dow Chemical Co. and containing various amounts of the phenol derivatives was tested for its mycostatic efficacy. Three types of impregnated paper were used, containing 0.81 per cent sodium 2, 4, 5 trichlorophenate, 0.92 per cent sodium 2, 4, 5, 6 tetrachlorophenate, and sodium pentachlorophenate 0.86 per cent dry weight, respectively.

A second set of experimental jars was prepared using this impregnated paper for fillers and flats. The jars were set up as in the previous experiment and the humidity maintained in the same manner as before. After three months the eggs in the four control jars showed a solid mass of white and green mold and the fillers

were covered with mold. In the jars containing paper impregnated with sodium 2, 4, 5, 6 tetrachlorophenate all of the eggs were slightly moldy. The flats and fillers were free from mold. The paper containing 0.86 per cent sodium pentachlorophenate inhibited mold growth on the flats and fillers completely, but the eggs showed a slight mold development at the points of contact with the fillers. The paper impregnated with sodium 2, 4, 5 trichlorophenate completely inhibited all mold growth both on the eggs and on the flats and fillers.

Of the phenol preparations tested, the most satisfactory from the standpoint of inhibiting mold growth were sodium 2, 4, 5 trichlorophenate, sodium 2, 4, 5, 6 tetrachlorophenate, and sodium pentachlorophenate.

As judged from the laboratory tests, these phenol preparations are better mycostatic agents for use in preventing mold growth on eggs and egg-packing materials, not only because they are more effective mycostatic agents than the copper compounds or any of the other chemical agents tested, but also because they have a vapor pressure sufficient to exert a mycostatic effect on the shell when the compound is impregnated in the packing material.

PART III. COLD STORAGE UNDER COMMERCIAL CONDITIONS WITH HIGH AND LOW HUMIDITIES

With these laboratory tests completed, a field test was made using five half-cases of eggs. Four of these half-cases of eggs were sprayed heavily with a heavy suspension of the 18 species of molds previously described. In each instance the molds were applied as each layer of eggs was placed in the cases. The fifth half-case was kept as a control. Three of the wooden cases were sponged with a 0.1 per cent solution of a mycostat, three phenol preparations being used. As each layer of eggs was placed in the case, 0.1 per cent solution of the mycostat was sprayed lightly over the eggs. One of the cases seeded with mold spores was treated in the same manner with water without the addition of the mycostat. The fifth half-case, the control, was treated with water but no molds were applied. The cases of eggs were placed in cold storage for five months when the eggs were removed and examined carefully for mold formation. The data are presented in Table XIII. The data show that when sodium 2 chloro-ortho-phenyl phenate was applied, only three eggs showed signs of mold formation and all flats and fillers were mold free. Where sodium 2, 4, 5 trichlorophenate was used only 29.1 per cent of the eggs showed mold as compared to 94.1 per cent in the untreated case. Although the mycostatic agents were applied to the egg and egg-packing

materials as a light spray, and the spore load of the eggs was heavy, the phenol preparations used were able to decrease the development of mold on the eggs materially.

These eggs were stored under the conditions of a low relative humidity, about 85 per cent, but the cases, fillers, flats, and eggs were wet at the time of storage. Because the humidity was not accurately controlled, and the amount of moisture that the eggs were exposed to was uncertain, better storage conditions were sought for the succeeding experiments.

Humidity is such a primary factor in the development of mold in cold storage houses that it was thought necessary to test a number of mycostatic compounds under conditions of controlled humidity much higher than is ever used in cold storage rooms where eggs are stored.

Commercially packed eggs were stored in an experimental room* at relative humidities from 92 to 100 per cent and a temperature of 32° F. For comparative purposes, eggs were also stored at relative humidities of 88 per cent. Solutions of various mycostatic agents were sprayed over the eggs or containers or both immediately prior to storage. At the end of three and six months storage, the eggs were examined for evidence of mold growth. At

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*These tests were made possible through the cooperation of the Central Fiber Products Co., Dow Chemical Co., United States Cold Storage and Ice Co., American Institute of Poultry Industries, and Swift and Company. The main field tests were conducted at the United States Cold Storage and Ice Company plant at Chicago.

the end of six months the eggs were removed from storage, candled for appearance, and examined for off-taste. Observations from these experiments follow.

I. Influence of high relative humidity on growth of molds on eggs and egg packages as compared to low relative humidity.

Both oil-treated and regular-pack eggs were included in the experiments. In Table XIV is presented a comparison of relative humidities of 88 and 98 per cent on regular pack eggs. In this table are presented data obtained on eggs treated with water or dilutions of the mycostats, which were so dilute as to have little or no mycostatic properties. It will be observed that at a relative humidity of 88 per cent, with the exception of one batch of eggs, no mold was evident at the end of six months storage. With a relative humidity of 98 per cent, on the other hand, the incidence of mold was extremely high on eggs, fillers, flats, and cases. The drastic conditions of the experiments at 98 per cent relative humidity were clearly demonstrated by the exterior appearance of untreated cases, which presented a solid mass of mold mycelial growth and spores. In the case of eggs treated with water and dilute solutions of mycostatic agents, the cover of the case sometimes adhered to the top fillers by the attachment of the mycelial filaments to both surfaces. In many cases, the egg in each filler-cell was completely covered with white filaments of Mucors.

The effect of humidity on mold development on oil-treated eggs is presented in Table XI. It will be observed that no mold was present on eggs, fillers, flats, and cases at a relative humidity of 88; whereas at a relative humidity of 98 per cent, fillers, flats, eggs, and cases showed the presence of mold. The extent of contamination appeared to be less but the type of mold was materially different. The oil-treated eggs were covered with spots due to the growth of Penicillia on the shells; whereas the regular-pack eggs showed very little development of Penicillia on the shells but showed an abundant development of Mucors. This seemingly specific development of mold was probably due to different initial contamination of the eggs.

The above data show quite conclusively that high relative humidities (98 per cent) are more conducive to abundant mold growth than low humidities (88 per cent).

II. The effect of wetting eggs, fillers, flats, and cases on the keeping quality of eggs stored at various humidities.

In Table XVI are presented data showing the keeping quality of eggs which were wet or stored in wet fillers and flats and then stored at a relative humidity of 88 per cent. The eggs which were stored in dry fillers and flats at the end of six months failed to show any spoiled eggs, while of the batches of wet eggs or eggs stored in wet fillers and flats, four batches showed no spoilage and five batches showed 3, 6, 11, 31, and 95 per cent, respectively, of spoiled eggs.

The data for the eggs stored at a relative humidity of 98 per cent are presented in Table XVII. In contrast with the dry eggs stored in dry containers, which showed no spoilage at a relative humidity of 88 per cent, the eggs prepared similarly and stored at a relative humidity of 98 per cent showed 0.5, 6, and 9 per cent spoilage at the end of three months storage in the three batches of eggs tested. The wet eggs or eggs stored in wet fillers and flats at a relative humidity of 98 per cent showed, after three months storage, spoilage from 3 to 77 per cent for the 9 cases listed in the table with the general average well over 50 per cent. The data show that wetting the eggs or containers has marked effect on the ultimate spoilage of the egg, particularly if the humidity of the storage room is high. This spoilage was largely due to bacteria rather than fungi. It would appear that the presence of a water film on the surface of the egg destroys the protective film on the egg surface or that it offers a favorable medium for the growth of bacteria that ultimately penetrate the egg shell and thus invade to the egg contents. High humidities seem to be predisposing factors toward egg spoilage. It is interesting to note, however, that the presence of a mycostatic agent on the egg shell or on the fillers and flats seems to lessen this bacterial spoilage materially.

III. The investigation of wood used in containers.

The cases used in these experiments were manufactured from several kinds of wood. At the completion of three months storage, it was observed that certain containers, which were untreated by mycostatic agents, failed to show any mold formation, whereas other containers also untreated were completely covered with a heavy growth of mold. The cases which were resistant to mold growth were made of Sitka spruce heartwood.* The cases showing marked mold growth were made of Tupelo gum, swamp black gum, or cottonwood. Gum and cottonwood cases treated with sodium 2, 4, 5 trichlorophenate and sodium pentachlorophenate were resistant to mold development. These data are in agreement with the findings of Hubert 1938 (7) who demonstrated that pentachlorophenol, O-phenyl-phenol, 2 chloro-o-phenylphenol, and tetrachlorophenol were effective in the control of fungi in millwork products that were to be exposed to excessive moisture.

IV. The investigation of fillers for mold growth.

Three types of fillers were used in these experimental studies, namely, brown, gray, and white. In each case of eggs, brown and white or brown and gray fillers were used. It

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*Samples of each type of case were submitted to Dr. A. Koehler of the Forest Products Laboratory at Madison, Wisconsin, and Dr. A. J. Panshin of Michigan State College for identification.

was found that the amount of mold growth was much greater with brown fillers than with either gray or white fillers. In the case of the gray or white fillers, mold was generally present only at the point of contact with the egg; whereas, with the brown fillers, mold growth occurred over the entire surface of the filler. The development of Mucors was much more pronounced on the brown fillers.

All fillers treated with 0.5 per cent sodium 2, 4, 5 trichlorophenate or sodium pentachlorophenate were resistant to mold growth.

V. Investigation on the treatment of eggs by mycostats.

In the studies cited, experiments were set up in two ways, (1) fillers, flats, and cases treated but eggs untreated, and (2) fillers, flats, cases, and eggs treated. An examination of the dats shows that the condition of the eggs was similar to that of the fillers and flats used in the same case. The treated eggs and the untreated eggs appeared about the same where the fillers and flats were treated in a similar manner. There appeared to be little value in the treatment of the eggs themselves. This is, of course, exceedingly fortunate because the treatment of eggs would be difficult in commercial practice.

VI. Investigations on mycostat treated fillers and flats.

A practical solution to the problem of applying the mycostatic agents to the fillers and flats without wetting the eggs was thought to be by the chemical impregnation of these packing materials at the time of manufacture. This would eliminate the bacterial decomposition and lowered eating quality of the eggs due to excess wetting before storage. Therefore, the next cold storage experiment consisted of storing eggs, without any previous treatment, in the experimental cold storage room at the United States Cold Storage Plant at Chicago.

The fillers and flats used in packing these eggs had been impregnated with various concentrations of the three phenol preparations found to be the most effective in preventing mold development in the previous experiments,* namely, 0.81% sodium 2, 4, 5 trichlorophenate, 0.92% sodium 2, 4, 5, 6 tetrachlorophenate, and 0.86% dry weight sodium pentachlorophenate.

After 79 days in storage the control eggs stored in regular fillers and flats showed mold formation at the points of contact with the fillers, whereas the eggs in the treated fillers and flats showed none. After five months these eggs were still mold-free.

In these experiments in which compounds with high vapor tension were used, Azochloramide 100 p.p.m. available chlorine was included as an example of the contact type of compound to deter-

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*This paper was prepared by the Dow Chemical Co., Midland, Michigan.

mine whether or not the use of sterile fillers and flats would prevent the molding of the eggs stored under such conditions. This compound had been suggested as a possible solution for the control of mold on cold storage eggs. This did not prove to be the case as mold development did occur on the eggs stored in fillers and flats rendered sterile by the use of Azochloramide.

VII. The influence of the mycostatic agent on the quality of the eggs.

The phenol derivatives that are presented in this thesis are characterized, as stated previously, by having vapor pressures such that the vapor given off by the chemical is in sufficient strength to inhibit the growth of molds on the egg surfaces, although the compound is impregnated in the fillers and flats. These vapors are gradually but continuously given off by the compounds. Further, these vapors having taste and odor might affect the flavor and odor of the egg. It was deemed advisable to place eggs in storage in fillers and flats impregnated with a high content of the chemicals to see whether or not the quality would be affected.

Eggs were placed in storage using these treated fillers and flats at the United States Cold Storage plant at Chicago, October 28, 1938. The following concentrations of the mycostatic agents were used: 0.81% sodium 2, 4, 5 trichlorophenate; 0.92% sodium 2, 4, 5, 6 tetrachlorophenate; and 0.86% dry weight sodium pentachlorophenate. These concentrations were purposely made much higher

than will be used to inhibit mold growth under commercial conditions. At the same time eggs were stored under identical conditions in ordinary fillers and flats.

Eggs were removed and examined for odor and taste on January 12, 1939, and February 10, 1939. These examinations were made by Mrs. Kathryn Niles of the American Institute of Poultry Industries and Miss R. Griswold and Miss D. Grantham, of the Division of Home Economics, Michigan State College.

The results of these examinations showed that the eggs stored in flats and fillers treated with these phenol preparations were no different in odor and taste than the eggs stored under identical conditions in ordinary flats and fillers.

Discussion

The above results prove quite conclusively that the most effective method of controlling the development of mold on cold storage eggs is by the impregnating of the packing materials at the time of manufacture, by some chemical substance. The ideal substance indicated would be one (a) with maximum toxicity toward the fungi peculiar to eggs, (b) available at low cost, (c) having no odor, and (d) showing some but relatively low vapor pressure. The compound should possess this latter characteristic because egg-packing materials are used for more than one season, and also because a compound with a relatively low vapor pressure would insure

mycostatic protection during the life of the packing materials so treated.

None of the phenol compounds tested possessed all of the above characteristics, at the concentration of maximum efficiency. Sodium 2, 3, 4 trichlorophenol has the advantage of being a very effective fungicidal agent but its cost is relatively high for commercial use and it has a high vapor pressure. Sodium pentachlorophenol does not have as high a toxicity toward the fungi tested as sodium 2, 4, 5 trichlorophenol under the conditions of these experiments. But it will be remembered that the conditions set up were far more severe than would ever be experienced commercially. The experiments were purposely made more severe to test the mycostatic properties of the compounds to the fullest extent. Under conditions of ordinary commercial storage sodium pentachlorophenate was found to be the most satisfactory mycostatic agent. Sodium pentachlorophenate is also more suited to commercial use because of its mild odor, lower vapor pressure, and low cost.

SUMMARY

1. Representatives of ten genera were repeatedly isolated from eggs and egg packing material. The majority isolated belong to the genus Penicillium, but no one species predominated.

2. Not all of the molds penetrated into the interior of the egg under cold storage conditions. Only two genera were isolated from the interior of the shell. These consisted of twenty-five species of Penicillium and four strains of Hormodendrum.

3. Two groups of compounds were tested for their value as mycostatic agents to be used to control mold development on egg in cold storage. The first group were compounds having little or no vapor tension which act by contact with the mold spore or mycelium. The second group were compounds with a vapor pressure sufficient to carry the inhibiting action to the mold without direct contact with the mold spore or mycelium.

The compounds with relatively high vapor pressure proved to be the best mycostatic agents both in the laboratory tests and under commercial cold storage conditions.

4. Of all the compounds tested, the following proved to be the most effective in controlling the development of mold on eggs in cold storage: sodium 2, 4, 5 trichlorophenate, sodium 2, 4, 5, 6 tetrachlorophenate, and sodium pentachlorophenate.

Although sodium 2, 4, 5 trichlorophenate was the best mycostatic agent under the drastic experimental conditions used,

sodium pentachlorophenate was found to be the most satisfactory mycostatic agent under ordinary commercial conditions. It was also better from the standpoint of low cost, slight odor, and a relatively low vapor pressure.

5. Eggs stored in flats and fillers treated with these phenol preparations were no different in odor and taste than the eggs stored under identical conditions in ordinary flats and fillers.

6. The most effective method of applying the fungi-~~cide~~stat was by impregnating the fillers, flats, and cases at the time of manufacture. Treatment of the eggs themselves seems to be of little value.

Table I. Mycostatic properties of eight copper compounds on _Penicillium puberulum_

Mycostat	Per cent concentration of copper compounds						
	1.0	.9	.8	.7	.6	.5	.1	Control
Cupric nitrate	-	-	-	-	-	-	2+	3+
Cupric oxide	2+	2+	2+	2+	2+	2+	2+	3+
Cupric sulfate	-	-	-	-	-	-	2+	3+
Cupric carbonate	2+	2+	2+	2+	2+	2+	2+	3+
Cupric ammonium sulfate	-	-	-	-	-	-	2+	3+
Cupric ammonium chloride	-	-	-	-	-	-	2+	3+
Cupric acetate	-	-	-	-	-	-	2+	3+
Cupric chloride	-	-	-	-	-	-	-	3+

3+ excellent growth; 2+ good growth; - no growth

Table II. Mycostatic properties of eight copper compounds on *Aspergillus niger*.

Mycostat	Per cent concentration of copper compounds							
	1.0	.9	.8	.7	.6	.5	.1	Control
Cupric nitrate	2+	2+	2+	2+	2+	2+	2+	3+
Cupric oxide	2+	2+	2+	2+	2+	2+	2+	3+
Cupric sulfate	-	?	?	?	?	2+	2+	3+
Cupric carbonate	2+	2+	2+	2+	2+	2+	2+	3+
Cupric ammonium sulfate	2+	2+	2+	2+	2+	2+	2+	3+
Cupric ammonium chloride	2+	2+	2+	2+	2+	2+	2+	3+
Cupric acetate	-	-	-	-	-	-	2+	3+
Cupric chloride	2+	2+	2+	2+	2+	2+	2+	3+

3+ excellent growth; 2+ good growth; - no growth

Table III. Mycostatic properties of eight copper compounds on a species of Alternaria.

Mycostat	Per cent concentration of copper compounds							
	1.0	.9	.8	.7	.6	.5	.1	Control
Cupric nitrate	-	-	-	-	-	-	2+	3+
Cupric oxide	2+	2+	2+	2+	2+	2+	2+	3+
Cupric sulfate	-	-	-	+	+	+	2+	3+
Cupric carbonate	2+	2+	2+	2+	2+	2+	2+	3+
Cupric ammonium sulfate	-	-	-	-	-	-	2+	3+
Cupric ammonium chloride	-	-	-	-	-	-	2+	3+
Cupric acetate	-	-	-	-	-	-	2+	3+
Cupric chloride	-	-	-	-	-	-	+	3+

3+ excellent growth; 2+ good growth; + fair growth; - no growth

Table IV. The mycostatic properties of three compounds commonly used in food preservation.

Mycostat and test organism	Per cent concentration of mycostat										
	1.0	.9	.8	.7	.6	.5	.4	.3	.2	.1	Control
Boric acid:											
Alternaria sp.	-	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Aspergillus niger	-	-	2+	2+	2+	2+	2+	2+	2+	2+	3+
Penicillium puberulum	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Sodium borate:											
Alternaria sp.	-	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	3+
Penicillium puberulum	-	-	-	-	-	-	-	-	-	2+	3+
Sodium benzoate:											
Alternaria sp.	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Aspergillus niger	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+
Penicillium puberulum	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+

3+ excellent growth; 2+ good growth; - no growth

Table V. The resistance of fourteen cultures of Penicillia to sodium benzoate

Organism	Per cent concentration of sodium benzoate .2	.1	.02	.002	.0002	Control
P. puberulum strain 1	3+	3+	3+	3+	3+	3+
P. puberulum strain 2	3+	3+	3+	3+	3+	3+
P. puberulum strain 3	3+	3+	3+	3+	3+	3+
P. puberulum strain 4	3+	3+	3+	3+	3+	3+
P. roqueforti	3+	3+	3+	3+	3+	3+
P. verrucosum	3+	3+	3+	3+	3+	3+
P. casei	3+	3+	3+	3+	3+	3+
P. citrinum	3+	3+	3+	3+	3+	3+
P. crustosum	3+	3+	3+	3+	3+	3+
P. oxalicum	3+	3+	3+	3+	3+	3+
P. 131.1	3+	3+	3+	3+	3+	3+
P. flavo-glaucum	3+	3+	3+	3+	3+	3+
P. janthinellum	3+	3+	3+	3+	3+	3+
P. viridicatum	3+	3+	3+	3+	3+	3+

3+ excellent growth.

Table VI. The resistance of fourteen cultures of Penicillia to sodium borate.

Organism	Per cent concentration of sodium borate									
	.8	.7	.6	.5	.4	.3	.2	.1	.02	Control
P. puberulum strain 1	-	-	-	-	-	-	-	+	+	3+
P. puberulum strain 2	-	-	-	+	+	+	+	+	+	3+
P. puberulum strain 3	-	-	-	-	+	+	+	+	+	3+
P. puberulum strain 4	-	-	+	+	+	+	+	+	+	3+
P. roqueforti	-	-	-	-	-	-	+	+	+	3+
P. verrucosum	-	-	-	-	+	+	+	+	+	3+
P. casei	-	-	-	+	+	+	+	+	+	3+
P. citrinum	-	-	-	+	+	+	+	+	+	3+
P. crustosum	-	-	-	-	+	+	+	+	+	3+
P. oxalicum	-	-	-	-	+	+	+	+	+	3+
P. 131.1	-	-	-	-	+	+	+	+	+	3+
P. flavo-glaucum	-	-	-	-	-	-	-	+	+	3+
P. janthinellum	-	-	-	+	+	+	+	+	+	3+
P. viridicatum	-	-	-	-	+	+	+	+	+	3+

3+ excellent growth; + fair growth; - no growth

Table VII. The mycostatic powers of compounds on market sold for mold prevention.

Mycostat	Test organism	Per cent concentration of mycostat							
		.4	.3	.2	.04	.03	.02	.009	Control
Moldex	Aspergillus niger	-	-	-	3+	3+	3+	3+	3+
	Penicillium puberulum	-	-	-	3+	3+	3+	3+	3+
	Chaetomium globosum	-	-	-	3+	3+	3+	3+	3+
	Mucor racemosus	-	-	-	3+	3+	3+	3+	3+
Prevento	Aspergillus niger	3+	3+	3+	3+	3+	3+	3+	3+
	Penicillium puberulum	3+	3+	3+	3+	3+	3+	3+	3+
	Chaetomium globosum	3+	3+	3+	3+	3+	3+	3+	3+
	Mucor racemosus	3+	3+	3+	3+	3+	3+	3+	3+

3+ excellent growth; - no growth.

Table VIII. The resistance of fourteen cultures of Penicillia to sodium ortho-phenyl phenate.

	Per cent concentration of sodium ortho-phenyl phenate					
Organism	.2	.1	.02	.002	.0002	Control
P. puberulum strain 1	-	-	∓	+	+	3+
P. puberulum strain 2	-	-	∓	+	+	3+
P. puberulum strain 3	-	-	∓	+	+	3+
P. puberulum strain 4	-	-	∓	+	+	3+
P. roqueforti	-	-	∓	+	+	3+
P. verrucosum	-	-	∓	+	+	3+
P. casei	-	-	∓	+	+	3+
P. citrinum	-	-	∓	+	+	3+
P. crustosum	-	-	∓	+	+	3+
P. oxalicum	-	-	∓	+	+	3+
P. 131.1	-	-	∓	+	+	3+
P. flavo-glaucum	-	-	∓	+	+	3+
P. janthinellum	-	-	∓	+	+	3+
P. viridicatum	-	-	∓	+	+	3+

\+ fair growth; ∓ scanty growth; - no growth.

Table IX. The resistance of fourteen cultures of Penicillia to sodium 2 chloro-ortho-phenyl phenate.

Organism	Per cent concentration of sodium 2 chloro-ortho-phenyl phenate					
	.2	.1	.02	.002	.0002	Control
P. puberulum strain 1	-	-	-	+	+	3+
P. puberulum strain 2	-	-	-	+	+	3+
P. puberulum strain 3	-	-	-	+	+	3+
P. puberulum strain 4	-	-	-	+	+	3+
P. roqueforti	-	-	-	+	+	3+
P. verrucosum	-	-	-	+	+	3+
P. casei	-	-	-	+	+	3+
P. citrinum	-	-	-	+	+	3+
P. crustosum	-	-	-	+	+	3+
P. oxalicum	-	-	-	+	+	3+
P. 131.1	-	-	-	+	+	3+
P. flavo-glaucum	-	-	-	+	+	3+
P. janthinellum	-	-	-	+	+	3+
P. viridicatum	-	-	-	+	+	3+

3+ excellent growth; 2+ good growth; + fair growth; - no growth

Table X. The mycostatic powers of five phenol derivatives on four genera of molds.

Mycostat	Test organism	Per cent concentration of mycostat													Control
		.2	.04	.03	.02	.009	.008	.007	.006	.005	.004	.003	.002	.001	
Sodium ortho-phenyl phenate	Aspergillus niger	-	-	-	-	?	?	?	?	3+	3+	3+	3+	3+	3+
	Penicillium puberulum	-	-	-	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Chaetomium globosum	-	-	-	-	-	-	+	+	+	+	2+	3+	3+	3+
	Mucor racemosus	-	-	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Sodium B. 2, 4, 5 trichloro-phenate	Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	-	3+
	Penicillium puberulum	-	-	-	-	-	-	-	-	-	-	-	-	3+	3+
	Chaetomium globosum	-	-	-	-	-	-	-	-	-	-	-	-	-	3+
	Mucor racemosus	-	-	-	-	-	-	-	-	-	-	3+	3+	3+	3+
Sodium C. 2 chlor-ortho-phenyl phenate	Aspergillus niger	-	-	-	-	-	-	-	-	?	3+	3+	3+	3+	3+
	Penicillium puberulum	-	-	-	-	-	-	-	-	-	3+	3+	3+	3+	3+
	Chaetomium globosum	-	-	-	-	-	-	-	-	-	+	2+	2+	3+	3+
	Mucor racemosus	-	-	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

Table X (continued).

Mycostat	Test organism	Per cent concentration of mycostat													Control
		.2	.04	.03	.02	.009	.008	.007	.006	.005	.004	.003	.002	.001	
Sodium F. 2, 4, 5, 6 tetra-chloro-phenate	Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	3+	3+
	Penicillium puberulum	-	-	-	-	-	-	-	-	-	-	3+	3+	3+	3+
	Chaetomium globosum	-	-	-	-	-	-	-	-	3+	3+	3+	3+	3+	3+
	Mucor racemosus	-	-	-	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+
G. Sodium penta-chloro-phenate	Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	3+	3+	3+
	Penicillium puberulum	-	-	-	-	-	-	-	-	-	2+	3+	3+	3+	3+
	Chaetomium globosum	-	-	-	-	-	-	-	-	-	-	3+	3+	3+	3+
	Mucor racemosus	-	-	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

3+ excellent growth; 2+ fair growth; + slight growth; - no growth

Table XI. The mycostatic powers of egg-case materials impregnated with phenol derivatives.*

Mycostat	Medium	Per cent concentration of mycostat 2	1	0.1	.05	.03	.025	.02	.016	.014	.012	Control
A. Sodium ortho-phenyl phenate	Wood	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Flat	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+
	Filler	-	-	3+	3+	3+	3+	3+	3+	3+	3+	3+
B. Sodium 2, 4, 5 trichloro-phenate	Wood	-	-	-	-	-	-	-	-	-	+	3+
	Flat	-	-	-	+	+	+	+	2+	2+	2+	3+
	Filler	-	-	-	+	+	+	+	2+	2+	2+	2+
C. Sodium 2 chlor-ortho-phenyl phenate	Wood	-	-	-	-	-	-	-	+	+	+	3+
	Flat	-	-	-	+	+	+	+	2+	2+	2+	3+
	Filler	-	-	-	+	+	+	+	2+	2+	2+	3+
F. Sodium 2, 4, 5, 6 tetra-chloro-phenate	Wood	-	-	-	-	-	2+	2+	2+	2+	2+	3+
	Flat	-	-	+	2+	2+	2+	2+	2+	2+	2+	3+
	Filler	-	-	+	2+	2+	2+	2+	2+	2+	2+	3+
G. Sodium penta-chloro-phenol	Wood	-	-	+	+	+	3+	3+	3+	3+	3+	3+
	Flat	-	+	+	+	+	3+	3+	3+	3+	3+	3+
	Filler	-	+	+	+	+	3+	3+	3+	3+	3+	3+

*The materials were soaked 5 minutes in the mycostatic solutions.
3+ excellent growth; 2+ fair growth; + slight growth; - no growth.

Table XII. Results of applying phenol derivatives by different methods.

Mycostatic agent	Method of application	Amount of mold growth on eggs and packing materials
Sodium 2 chlor-ortho-phenyl phenate	Dry powder	Heavy growth of mold
	1-500 solution	Heavy growth of mold
	Packing materials impregnated	Slight amount of mold
Sodium 2, 4, 5 trichloro-phenate	Dry powder	Moderate growth of mold
	1-500 solution	Moderate growth of mold
	Packing materials impregnated	No mold growth

Table XIII. The mycostatic powers of phenol preparations applied to eggs in cold storage.

Mycostat	Treated with molds	Moldy eggs		Number moldy fillers	Number moldy flats
		Number	Per cent		
Water	-	32	26.6	2	0
Water	+	113	94.1	4	2
Sodium 2, 4, 5 trichloro-phenate	+	35	29.1	2	0
Sodium 2 chloro-phenyl phenate	+	3	2.5	0	0
Sodium penta-chloro-phenate	+	46	38.3	1	1

Table XIV. Influence of humidity on growth of molds on eggs and egg packages on fresh market eggs not oil-treated.

	Solution used	Treatment applied: Cases	Treatment applied: Fillers and flats	Treatment applied: Eggs	Number eggs tested	Appearance of mold: Cases	Appearance of mold: Fillers and flats	Appearance of mold: eggs
				Humidity -- 88 per cent				
106	none	none	none	none	176	none	none	none
107	none	none	none	none	36	none	none	none
112	1.0% Dow A	none	yes	none	36	none	none	none
113	0.05% Dow A	none	yes	none	36	none	8*	8
108	0.5% Dow B	none	yes	none	36	none	none	none
114	0.1% Dow B	none	yes	none	36	none	none	none
110	0.5% Dow C	none	yes	none	36	none	none	none
116	0.1% Dow C	none	yes	none	36	none	none	none
111	N.D.A.	none	yes	none	36	none	none	none
				Humidity - 98 per cent				
21B	Water	yes	yes	none	162	10	10	10
6	0.05% Dow A	none	yes	none	172	6	10	10
18	N.D.A.	yes	yes	none	172	8	8	8
16	0.1% Dow C	yes	yes	none	180	10	6	6
13	0.5% Dow C	yes	yes	yes	177	10	10	10

*Heavy mold growth scored - 10.

Table XV. Influence of humidity on growth of mold on eggs and egg packages on oil-treated eggs.

Serial Numbers	Solution used	Treatment applied: Cases	Treatment applied: Fillers and flats	Treatment applied: Eggs	Number eggs tested	Appearance of mold: Cases	Appearance of mold: Fillers and flats	Appearance of mold: Eggs
			Humidity - 88 per cent					
107	none	none	none	none	36	none	none	none
115	0.1% Dow B	none	yes	none	36	none	none	none
			Humidity - 98 per cent					
12a	none	none	none	none	177	none*	none	3+
12b	water	yes	yes	none	171	none*	2	4
3	0.1% Dow A	none	yes	none	180	8	1	2
4	0.1% Dow A	yes	yes	none	179	1	1	1
11	0.1% Dow B	yes	yes	none	324	none*	3	3
20	1% Dow G	yes	yes	none	164	-	3	8

*Sitka spruce case. + Heavy mold growth scored - 10.

Table XVI. Effect of wetting eggs and wet fillers and flats on keeping quality of eggs stored at 88 per cent humidity.

Serial number	Treatment	Treatment applied: Cases	Fillers and flats	Eggs	Number eggs tested	Length of storage in months	Per cent bad eggs
106	none	none	none	none	36	6	0
107	none	none	none	none	36	6	0
112	water	none	yes	none	36	6	31
113	water	none	yes	none	36	6	95
108	water	none	yes	none	36	6	6
114	water	none	yes	none	36	6	11
115	water	none	yes	none	36	6	3
110	water	none	yes	none	36	6	0
116	water	none	yes	none	36	6	0
111	water	none	yes	none	36	6	0
109	water	none	yes	none	36	6	0

Table XVII. Effect of wetting eggs and wet fillers and flats on keeping quality of eggs stored at 98 per cent humidity.

Serial number	Treatment	Treatment applied: Cases	Fillers and flats	Eggs	Number eggs tested	Length of storage in months	Per cent bad eggs
21A	none	none	none	none	179	3	0.5
12A	none	none	none	none	177	3	9.0
Spare	none	none	none	none	123	3	6.0
21B	water	yes	yes	none	162	3	61.0
12B	water	yes	yes	none	171	3	64.0
5	0.1% Dow A	yes	yes	yes	177	3	70.0
6	0.05% Dow A	none	yes	none	172	3	77.0
13	0.5% Dow C	yes	yes	yes	177	3	28.0
14	0.5% Dow C	yes	yes	none	179	3	31.0
15	0.2% Dow C	yes	yes	none	139	3	75.0
16	0.1% Dow C	yes	yes	none	180	3	20.0
17	0.1% Dow C	yes	yes	none	177	3	3.0

BIBLIOGRAPHY

1. Anonymous.
1936. Control of odors and molds by ozone and ionized oxygen. Food Industries 8: 307.

2. Bidault.
1922. (Molds on cold storage meat.) Bul. Soc. Sci. Hyg. Aliment. 10: 12-25. (Abstract) Experiment Station Record 48: 258.

3. Doran, W. L.
1923. Toxicity studies with some copper fungicides. Phytopath. 13:532-542. 9 tables.

4. Ewell, Arthur W.
1936. The use of ozone in cold storage plants. Ice and Refrigeration 91: 295-6.

5. Ewell, Arthur W.
1938. Present use and future prospects of ozone in food storage. Food Research 3: 101-108.

6. Heitz, Thomas W.
1929. The cold storage of eggs and poultry. United States Department of Agriculture Circular 73: 1-54. 25 figs., tables 1-10.

7. Hubert, Ernest E.
1938. The preservative treatment of millwork. Industrial and Engineering Chemistry 30: 1241-50. 4 figs., tables 1-5.

8. James, L. H., and T. L. Swenson.
1929. A preliminary study of pin-spot molding of shell eggs in cold storage. U. S. Egg and Poultry Magazine 25 (12): 47-49. 3 figs., 1 table.

9. Monvoisin, A.
1922. (Mold formation in meat kept in cold storage.) Recueil de Medecine Veterinaire 98: 149-161. 3 figs.

10. Moran, T.
1938. Gas storage of eggs. The U. S. Egg and Poultry Magazine 44: 304.

11. Naumov, N.
1935. Determination of Mucorales. Second revised edition, Botanical Institute of Academy of Sciences, U.S.S.R. 136 pp., 49 figs. Moscow and Leningrad (Russian). Unpublished translation by E. A. Bessey.

12. Pennington, Mary E.
1938. Roving thoughts on cold storage of eggs. U. S. Poultry and Egg Magazine 44: 242.

13. Thom, Charles.
1930. The Penicillia. xiii + 644 pp. 98 figs. Williams and Wilkins Co., Baltimore, Md.

14. Thom, Charles, and Margaret B. Church.
1926. The Aspergilli. ix + 272 pp., 4 plates, 14 figs. Williams and Wilkins Co., Baltimore, Md.

15. Weston, W. A. R. D., and E. T. Hallman.
1927. Black spot of eggs. Poultry Science 6: 251-258.

16. Whetzel, H. H., and S. E. A. McCallan.
1930. Studies on fungicides. I. Concepts and terminology. Cornell Agric. Exp. Sta. Memoir 128: 1-7.

17. Wright, A. M.
1923. Molds on frozen meat. Journal of the Society of Chemical Industry 42.

18. Yesair, John.
1928. The action of disinfectants on molds. Thesis, University of Chicago, Chicago, Ill. (Unpublished)