FUNGUS GROWTH IN SHELLED CORN AS AFFECTED BY MOISTURE[1]

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INTRODUCTION

Infection of corn caused by ear-rot fungi begins soon after pollination and may continue until stopped either by lack of moisture in the grain or by low temperature. Sometimes these ear-rot fungi cause considerable damage after the corn is cribbed or shelled and placed in storage. The study here reported was made to determine at what moisture limits growth of ear-rot fungi may take place at a temperature suitable for their development. To facilitate study, all the grain used in the experiment was first shelled from the cobs. A number of aspergilli and penicillia became conspicuous in the corn stored under controlled conditions and these were included in the study. These forms have often been recognized as important storage-rot fungi by other workers. They have not been observed by the writer to cause significant losses to corn ears prior to harvest except that they often produce moldy areas where the ears have been injured by birds or insects. Short progress reports of this work have been published (*9*, *12*).[2]

METHODS

STORAGE CHAMBERS

Two styles of storage chambers were used. When no disinfectant was applied because pure culture work was not desired, the shelled corn (*zea mays* L.) was placed in cylindrical wire baskets holding 100 g and hung over salt solutions in wide-mouthed half-gallon glass bottles. In most of the experiments the bottles were supplied with glass tubes 15 cm long with a 3-mm bore which were inserted through the rubber stoppers that closed the bottles. This supplied aeration sufficient to prevent the sour alcoholic odor which developed on corn above a certain moisture content in chambers entirely sealed, and at the same time checked appreciable evaporation. In fact, under sufficiently limited aeration the production of metabolic water by respiration seems to compensate fully for that lost by diffusion.

For pure culture work with a single fungus the apparatus shown in figure 1 was used. This method, adapted from Shippy (*15*, *pp. 370–372*) and Hatfield (*7*), provided a constant humidity and fresh sterile air. Before the surface-sterilized shelled corn, was introduced the entire apparatus was sterilized under steam pressure. Some of the rubber tubes were disconnected during the autoclaving process to prevent the solution from being forced from one flask into another. An additional flask of salt solution at first used in the chain was found

[1] Received for publication July 19, 1937; issued April 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 306.

unnecessary. A battery of 12 of these sets was operated at one time in a constant-temperature chamber. After the first few days of more thorough aeration, the air flow was adjusted to one bubble in 2 or 3 seconds.

HUMIDITY CONTROL

While partial saturations of sulphuric acid are commonly used for humidity control where the relative humidity must be known (*17*, *22*), in this case results were recorded in terms of moisture content of corn, and therefore a series of partial concentrations of any one of a number of salts would answer, and calcium chloride was chosen for use in the half-gallon bottles. A series of concentrations from 24 to 0 percent produced atmospheric humidities which caused the corn moistures to range from 14 to 29 percent.

In the sets where air was bubbled through the solutions, saturated solutions of a variety of chemical compounds were used. The chemicals used are given in table 1. They are arranged in ascending order

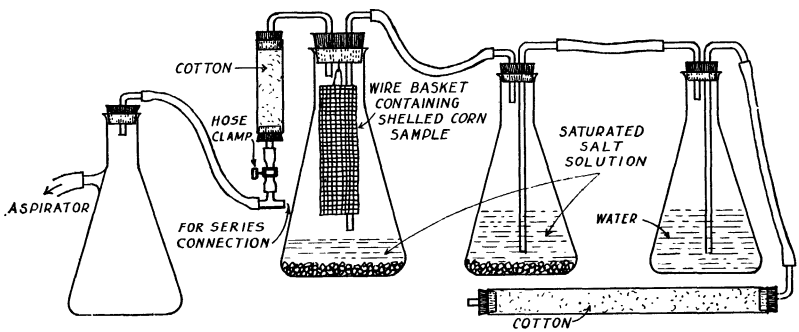

FIGURE 1.—Apparatus used for storing corn under constant-humidity conditions with a slow uniform change of air, and for protecting it from contamination with micro-organisms.

with respect to corn moisture, and therefore should also be in ascending order with respect to relative humidity. The values for relative humidity as compiled by Spencer (*16*) (table 1) are not in full agreement with this. Loss of water from a saturated solution does not affect the relative humidity over the solution. In actual use it was found that after a 3-month run, only a little water need be replenished in the end flask and there was no appreciable change in volume in the flasks containing chemical solutions.

Over some salt solutions, within certain limits, relative humidity is affected very little by temperature, while over others it is affected considerably. Furthermore, in either case a sudden change in temperature would temporarily throw the humidity out of balance, and at high humidities the dew point is easily reached, not only on the inner surface of the flask but also within the corn sample. Therefore, storage was carried out under constant temperature arbitrarily chosen at 70° F.

CORN USED

Well-developed, mature Illinois station Reid Yellow Dent ears free from blemishes were selected and 10 kernels from each were tested on a tray type germinator. This gave an approximation of the kind of internal infection, if any, the ears carried. Disease-free ears and

ears having grain infected with only one fungus, *Diplodia zeae* (Schw.) Lév., *Fusarium moniliforme* Sheldon, *Gibberella zeae* (Schw.) Petch, *Nigrospora sphaerica* (Sacc.) Mason (=*Basisporium gallarum* Moll.), or *Cephalosporium acremonium* Corda were selected. The ears were further selected after surface sterilizing 20 representative kernels from each ear and plating on potato-dextrose agar. Air-dry corn ranging from 10 to 13 percent in moisture and with the desired amount of water subsequently added was usually used, but some checks were made by selecting ears fresh from the field which contained a higher percentage of natural moisture. Whether or not the corn had previously been dried apparently made no difference in the results. This is in agreement with the finding of Swanson (*18*) in work with stored wheat.

TABLE 1.—*Moisture content of unsterilized shelled corn after storing over saturated solutions of different chemicals in closed containers* [1] *for 12 weeks at 70° F.*

Name of chemical	Relative humidity, at 20° C.[2]	Tests made	Moisture content of corn	
			Range	Mean
	Percent	*Number*	*Percent*	*Percent*
Ammonium sulphate	81	4	15.2–16.2	15.7
Potassium bromide	84	6	15.3–17.6	16.5
Potassium hydrogen sulphate	86	13	17.1–19.7	18.2
Zinc sulphate	90	12	17.0–20.7	18.6
Sodium sulphate	95	11	17.9–21.3	18.8
Sodium sulphite	93	15	18.5–22.4	19.9
Barium chloride	88	13	19.0–21.8	20.2
Potassium nitrate	93	12	20.7–22.6	21.6
Sodium tartrate	91	4	21.0–22.2	21.7
Ammonium dihydrogen phosphate	93	9	21.0–23.4	22.2
Sodium bromate	92	16	21.5–23.5	22.3
Dibasic sodium phosphate	95	14	23.2–25.7	24.6
Oxalic acid	96	4	25.1–27.5	26.0
Potassium sulphate	97	7	25.3–27.2	26.2
Gypsum	98	5	27.2–30.1	28.7

[1] As shown in fig. 1.

[2] As reported by Spencer (*16*).

The corn moistures were determined by placing about 50 g of the grain in an open vessel 65 mm in diameter and drying at 100° C. for 4 days in an electric oven without vacuum. Percentages are based on weight of corn before drying.

SURFACE STERILIZATION

Where pure culture work was desired, the grain was sterilized on the surface with the filtrate from a fresh chlorinated lime solution. The time of soaking ranged from 15 minutes to 1 hour, depending on how much water it was desired to take up. A solution of 80 g of chlorinated lime per liter of water was used for the short-time soaks, with decreasing strengths for the longer soaks. The grain was treated in an open beaker with frequent stirring and then was transferred to a sterile wire basket and placed in the sterilized storage apparatus. Thorough aeration with sterile air to remove chlorine fumes and thus allow the fungi to develop was provided by the apparatus shown in figure 1.

SOME FACTORS AFFECTING THE AMOUNT OF HYGROSCOPIC MOISTURE TAKEN UP BY GRAIN

VARIABILITY IN CORN STRAINS

Corn from different ears placed over the same solutions did not always come to equilibrium at the same grain moisture content (table 1). An experiment conducted with several distinctly different types of dent corn revealed significant differences in the extent to which water was taken up by the different types from two different atmospheres (table 2). Two cubic centimeters of formalin was added to 198 cc of water in the bottom of the jars to prevent mold growth on the kernels, and the corn used was selected for freedom from internal infection. The formalin solution proved unstable; it gradually lost its disinfecting power and, therefore, was replaced once a month. The largest quantity of moisture was taken up by the old type Reid Yellow Dent corn, which is a moderately rough "starchy" type. The Illinois high- and low-oil and high- and low-protein strains (*10*) differ markedly with respect to oil and protein content. All the hybrids used were of a distinctly horny dent type and the moistures at the close of the test were definitely lower than in either one of the two kinds of Reid Yellow Dent. It was shown by Alberts (*1*) that starchy corn takes up hygroscopic water more rapidly than horny corn. Bailey (*3*) found differences in three varieties of corn with respect to their capacity for taking up hygroscopic water.

TABLE 2.—*Moisture in 10 strains of dent corn when the shelled grain was stored free from mold in two constant atmospheric humidities in sealed half-gallon bottles for 105 days at 70° F.*

Kind of corn	Over 10-percent $CaCl_2$ solution containing 1 percent formalin					Over pure water containing 1 percent formalin				
	Moisture of grain in each of 4 replicates				Average moisture	Moisture of grain in each of 4 replicates				Average moisture
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Station strain Reid Yellow Dent	20.5	20.8	20.0	20.3	20.4	29.0	29.1	28.9	29.1	29.0
Old type Reid Yellow Dent	21.5	21.5	21.0	21.4	21.4	29.9	30.2	30.1	29.7	30.0
Illinois high protein	19.3	19.2	19.3	19.7	19.4	27.8	28.0	27.9	27.7	27.9
Illinois low protein	20.7	20.7	19.7	20.1	20.3	28.9	29.6	27.8	28.5	28.7
Illinois high oil	19.3	19.2	19.0	19.4	19.2	26.8	27.4	27.0	27.3	27.1
Illinois low oil	19.8	20.2	19.6	20.0	19.9	29.4	29.3	29.2	29.5	29.4
Hybrid A5 × Hy [1]	19.9	20.0	19.2	19.4	19.6	27.5	28.0	27.0	27.2	27.4
Hybrid A × K [1]	19.3	19.3	19.3	19.1	19.3	26.5	26.9	26.4	26.4	26.6
Hybrid R126 × JL [1]	19.9	19.9	19.4	19.8	19.8	27.0	27.3	26.5	26.6	26.9
Hybrid (A×L) (Hy×R4) [1]	19.2	19.0	19.7	19.4	19.3	26.7	26.9	26.1	26.8	26.6

[1] Supplied by J. R. Holbert, formerly with U. S. Department of Agriculture.

CONDITION OF GRAIN

The amount of moisture taken up also is influenced considerably by whether the grain is moldy or free from mold (fig. 2). The range or variability in corn moisture over each of the solutions given in table 1 is a combined effect of differences in corn used and differences in moldiness and kind of mold used in the different tests. Alberts (*2*) found that removal of the seed coat from the crown of corn kernels affected the speed with which hygroscopic water was taken up

or lost, but experiments by the writer indicated that this had no effect on the ultimate moisture content of the grain after equilibrium with the surrounding atmosphere was reached, nor did previous killing of the grain by low temperature affect this relationship.

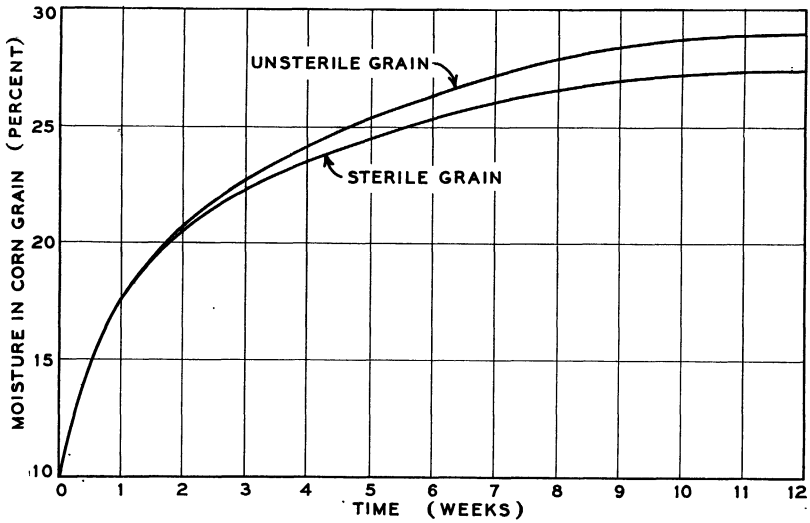

FIGURE 2.—Rise of hygroscopic moisture in shelled corn stored in a saturated atmosphere in closed containers for 12 weeks at 70° F. Grain from the same ears was divided into two lots; one was placed over plain water and molds developed; the other was placed over water containing 1 percent of formalin and remained sterile.

TIME AND TEMPERATURE OF STORAGE

At constant humidity and temperature, corn grain comes to equilibrium with the surrounding atmosphere very slowly, coming close to it in 12 weeks' time at 70° F., as shown in figure 2. To shorten this lag, sufficient water was added to the corn to bring it close to the desired moisture before placing it in the constant-humidity chambers, and a storage period of 12 weeks was chosen. Further fungus growth at low moistures might have been found if the storage period had been longer, for fungus growth is exceedingly slow when near the critical moisture (*18*, *23*). From a practical standpoint, on the other hand, it must be considered that the moisture of corn in storage is seldom static, and when it is above the critical point for mold growth there usually is a tendency for the moisture to decrease slowly.

The temperature chosen, 70° F., also has an important bearing on the results obtained. Swanson (*18*), for instance, found that wheat stored for 13 weeks at 95° F. was safe from mold growth only below 14 percent moisture content, but at 60° it was safe for the same length of time at 17.4 percent.

FUNGUS GROWTH IN RELATION TO MOISTURE OF GRAIN

ASPERGILLUS SPECIES

Members of the *Aspergillus glaucus* group [3] grow at a lower moisture content of grain than any other kind of fungi observed in these

[3] Some isolations of the *A. glaucus* group and *A. versicolar* were identified by Charles Thom, of the Bureau of Plant Industry, U. S. Department of Agriculture.

experiments. Some members of the group produced primarily conidia on the grain as well as on culture media while others produced primarily peritheeia, as shown in figure 3, *A* and *B*. The green conidial heads were observed with the naked eye at moistures as low as 14.3

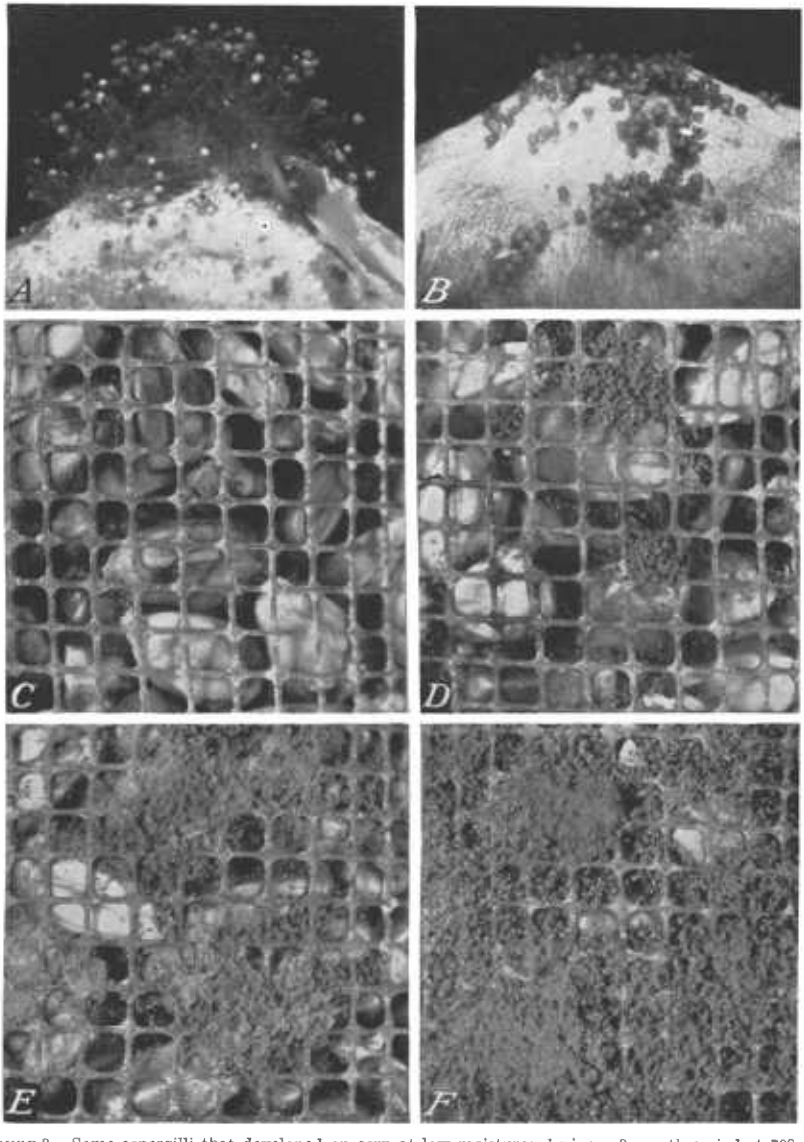

FIGURE 3.—Some aspergilli that developed on corn at low moistures during a 3-month period at 70° F.: *A*, Greenish conidial heads of *Aspergillus glaucus* growing on the tip end of grain with 15.5 percent moisture. *B*, Another form of *A. glaucus* producing bright-yellow perithecia on grain at 16 percent moisture. Both × 15. *A. glaucus* occurred at moistures as low as 14.3 percent. *C*, *D*, *E*, and *F*, *A. wentii* growing at moistures of 15.9, 16.8, 18.0, and 19.2 percent, respectively. × 2.

percent after 12 weeks' storage, as shown in table 3, and bright-yellow peritheeia appeared at a moisture percentage only slightly higher. Thom and Le Fevre (*20*) found members of the *A. glaucus* group to

be the first molds active in corn meal as the moisture rose above 12.5 or 13 percent. This was a somewhat lower moisture than that found in the present experiments with whole grain.

TABLE 3.—*Critical moisture content of shelled grain of Reid Yellow Dent corn at which certain fungi were able to grow*

Name of fungus	Source of fungus	Tests made	Highest moisture at which no fungus growth was observed	Lowest moisture at which some fungus growth was observed
GRAIN NOT SURFACE-STERILIZED, COMPETITION BY OTHER ORGANISMS				
		Number	*Percent*	*Percent*
Aspergillus glaucus group	Natural infection	12	14.8	14.4
A. wentii	do	2	15.2	15.1
Penicillium spp.	do	9	17.3	16.3
A. flavus	do	3	18.5	18.3
A. niger	do	12	20.8	18.3
Fusarium moniliforme	do	14	21.6	18.8
Diplodia zeae	do	9	22.4	21.2
Gibberella zeae	do	3	22.9	22.2
Nigrospora sphaerica	do	7	24.8	23.6
GRAIN SURFACE-STERILIZED, PURE CULTURE OF FUNGUS PRESENT				
A. glaucus group	Inoculation	2	14.0	14.3
A. versicolor	do	2	14.2	15.0
A. wentii	do	1	14.5	15.4
A. ochraceus	do	2	14.5	15.6
P. notatum	do	3	15.0	15.6
P. viridicatum	do	2	16.8	17.6
P. palitans	do	3	17.6	18.0
A. flavus	do	2	18.0	18.3
F. moniliforme	Natural infection	8	20.7	18.4
A. tamarii	Inoculation	1	18.7	19.8
A. niger	do	2	19.1	20.1
P. oxalicum	do	2	19.0	20.8
P. expansum	do	1	20.1	20.8
D. zeae	do	2	21.0	21.9
G. zeae	do	3	21.2	22.3
N. sphaerica	do	3	21.1	22.5
Cephalosporium acremonium	Natural infection	1	22.1	23.4

Aspergillus versicolor, *A. wentii*, and *A. ochraceus* developed at moistures only slightly higher than *A. glaucus* (table 3). *A. flavus* was not observed until a moisture content of 18.3 was reached, although it has been reported (*20*) as occurring in corn meal at a moisture as low as 16 percent. *A. niger* was found on grain with moistures of 18.3 percent and upward, while *A. tamarii*, observed in one test only, occurred at 19.8 percent. All of these species were observed growing naturally on unsterilized corn used in the experiments, but the lower limit for growth could not always be determined in that way. Some of the fungi that were observed were, therefore, isolated in pure culture and inoculations were made on surface-sterilized corn. No effort was made to determine the number and identification of all the fungi occurring naturally at the different moistures. In small grains *A. niger*, *A. flavus*, and *A. fumigatus* have been reported growing at 18 percent moisture (*6*). In another report (*14*) *A. glaucus* and *A. albus* were mentioned, the latter developing somewhat later and apparently requiring more moisture than *A. glaucus*.

Fungus growth at the lowest moistures occurred at the tip ends of the kernels where they had been attached to the cobs and at places where the seed coat was broken. At slightly higher moistures by close inspection a sparse amount of mycelium could be seen trailing through the spaces between the kernels, as shown in figure 3, *C*, and the formation of conidial heads was not limited to the places just mentioned. Different species showed some differences in growth habits.

PENICILLIUM SPECIES

Penicillium was found growing on the tips of corn kernels at moistures as low as 16.3 percent. By inoculating with some known cultures, the arrangement of species with respect to moisture requirement in ascending order was *P. notatum*,[4] *P. viridicatum* (in one of its forms),[4] *P. palitans*,[4] *P. oxalicum*,[5] and *P. expansum*.[6] The first three named were isolated from "blue eye" corn about which more is given below. *P. oxalicum* occurs commonly on corn ears before harvest when kernels have been mechanically injured by corn earworms or birds.

Penicillium expansum was included because McHargue (*14*) reported it as making more prolific growth than any of the molds observed in his corn-storage experiments. However, the culture of *P. expansum* used by the writer made the least vigorous growth on corn of any of the penicillia used. This difference in results may very likely be explained by differences in physiologic behavior of different strains of *P. expansum*. There is the question also whether *P. expansum* is considered in the broad sense in which some forms may be difficult to distinguish from some members of the *P. viridicatum* series, or whether the species is limited to that organism which, in addition to answering the morphological description, causes rot of apple.

A special study was made of the condition known to the grain trade as "blue eye" (fig. 4). It is caused by the growth of blue penicillia between the germ and the seed coat. Through the courtesy of W. B. Combs and H. P. English, of the Bureau of Agricultural Economics United States Department of Agriculture, samples of blue eye were obtained from the markets at Chicago, Milwaukee, Minneapolis, Cedar Rapids, Peoria, Toledo, and Nashville. Representative kernels but with unbroken seed coats were selected from each lot. They were surface-sterilized with a chlorinated lime solution, and the seed coat covering the germ was then opened and a transfer of the fungus spores made to sterile water. This was followed by the pouring of agar dilution plates. Sometimes the colonies obtained in the plates from a transfer from a single kernel appeared to be all alike; sometimes two different kinds of colonies developed in abundance, and occasionally there were three different kinds. All the isolations were penicillia. To make more sure of their purity, a needle transfer was made from the one or from each of the several kinds of colonies isolated from each kernel and a second set of dilution plates was poured.

After spending a whole winter season isolating, purifying, culturing and classifying these penicillia, and studying the keys and descriptions (*19*), the writer felt that he could not definitely name any of them

[4] Identified by Thom.

[5] Received from Helen Johann, of the Bureau of Plant Industry, U. S. Department of Agriculture, who had it identified by Thom.

[6] Received from H. W. Anderson, University of Illinois, who isolated it from a rotting apple.

unless he were to make a long-time study of the whole *Penicillium* group and secure an abundance of authentic specimens. As this was not the main purpose of the investigation, such a study was not attempted. Many of the isolations could be classified into three large groups within each of which there was a great deal of similarity. A fourth group was composed of various types which did not classify into the first three. No further work was done with the last group. Representative samples of the first three groups sent to Dr. Charles Thom in the spring of 1935 and again in 1936 were identified, while others were said to be mixtures. From the results of these identifications it appeared that for the most part one group represented members of the *P. chrysogenum* series, one group was *P. palitans*, a

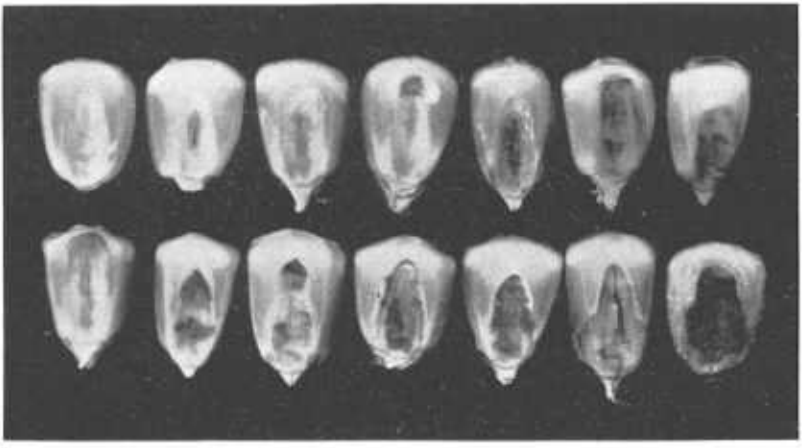

FIGURE 4.—Blue eye of corn caused by a growth of *Penicillium* over the germ and beneath the seed coat on grain with moistures of 18.5 to 24.0 percent. These specimens were received from Federal grain inspectors. Very little blue eye has been observed above the moistures given, for at higher moistures other molds usually predominated over the penicillia.

member of the *P. viridicatum* series, and the other group was composed of members of the "Fasciculata," ranging, according to Thom, "from *P. cyclopium* through the *viridicatum* series."

The lower moisture limits for the development of blue eye was determined by (1) observing its natural development from surface-borne spores, and (2) by inoculating surface-sterilized corn with *Penicillium notatum*, *P. palitans*, and with three different cultures belonging to the third group mentioned above. Corn not inoculated did not always develop blue eye even though moisture was adequate, but at times abundant blue eye developed. In the inoculated series *P. notatum* produced blue eye in 2 percent of the kernels at 16.7 percent moisture, in 10 percent of the kernels at 17.7 percent moisture, and in about 60 percent of the kernels at 19.0 percent moisture. *P. palitans* produced 10 percent of blue eye kernels at 19.5 percent moisture and about 50 percent at 21.2 percent moisture. The general appearance of the blue eye from the two inoculations was practically alike, being blue in color. The three unnamed species caused blue eye at about the same moistures as *P. palitans*, and one of them produced blue eye identical in appearance with it. Of the other two, one produced a grayish-blue color of the germ area, and the other produced a some-

what reddish or purple color. When kernels of the latter kind were placed in an alkaline solution the color became a bright blue. *P. oxalicum* and *P. expansum*, although growing and fruiting over the surfaces of the kernels, did not cause the typical blue eye condition.

At the lowest moistures, blue eye appeared mostly as a narrow stripe over the germ, as illustrated in figure 4, top row, second kernel from the left. This condition, called "hair line blue eye" by grain inspectors, in some forms is easily confused with the genetic condition known as purple plumule (*8*). When a kernel is cut crosswise through the plumule, however, the genetic purpling is seen to be in the tissue of the plumule, while the fruiting heads of penicillia causing the color of blue eye are located between the germ and the seed coat. With somewhat higher moistures blue eye becomes more general over the surface of the germ. At moistures above 23 to 26 percent, depending no doubt largely on what kind of fungi the grain is carrying, blue eye is likely to become obliterated by the growth of other organisms. Sometimes it is covered over by various aspergilli even at moistures below 23 percent. In natural, unsterilized stored shelled corn with moistures of 17 to 23 percent, sometimes the penicillia, sometimes the aspergilli, predominate.

FUSARIUM MONILIFORME

That there are wide differences in culture characters of *Fusarium moniliforme* is well known, and marked differences in pathogenicity to corn have been reported (*13*, *21*). Differences were also found with respect to moisture requirements. Because of the variability in the results obtained, more tests were made with this fungus than with any other. Only naturally infected seed was used. Infection caused by *F. moniliforme* is the commonest of all internal seed infections in corn, and a great many kernels of each lot used in these particular tests carried this fungus. The term "pure culture" as applied to *F. moniliforme* in table 3 means only that no other kinds of fungi were present; most certainly there were a considerable number of strains of *F. moniliforme* in each lot of corn used and the growth at the lower moisture limit was determined by the particular strain that had the lowest moisture requirement. The 22 different experiments were conducted with 18 different lots of corn over a period of 8 years. Corn internally infected with other organisms of course was avoided in these tests.

In surface-sterilized grain growth of *Fusarium moniliforme* was seen at 18.4 percent moisture in one lot, while in another lot no growth occurred at 20.7 but growth did occur at 22.1 percent. In grain not surface-sterilized the end point for growth was more difficult to determine because some aspergilli and penicillia grow luxuriantly at moistures where growth of *F. moniliforme* is very feeble. However, by using a microscope and pouring dilution plates from needle-point transfers from suspected places, the presence of *F. moniliforme* could be definitely verified. In some of these tests the fungus was found to grow at moistures as low as 18.8 percent, while in others the grain was free from growth up to or above 21.6 percent. There seems to be no doubt that the variability of different strains of *F. moniliforme* was the principal cause of some of these differences. Furthermore, the strains used or observed probably do not represent the whole range,

for, as already pointed out, measurements were concerned entirely with those strains in each lot of corn that had the lowest moisture requirement.

Above 21 to 24 percent moisture *Fusarium moniliforme* grew vigorously, completely enveloping the grain with a powdery pink mass and with mycelium crisscrossing the spaces between the kernels. Frequently many of the germs became rotted and turned a deep reddish-purple color. In mixed cultures it sometimes was difficult to distinguish this discoloration from that produced by certain penicillia, although the usual type of blue eye produced by penicillia is decidedly bluish in color.

Commercial lots of corn examined over a period of years have always shown 10 percent or more infection from *Fusarium moniliforme*. Many infected kernels look normal and healthy. Two methods of testing have been used. (1) a tray germinator in which the corn is tested as received, and (2) plating the surface-sterilized grain on potato-dextrose agar. The percentage of infection has always been much higher by the first method than by the second, indicating that much of the infection was superficial enough to be killed by the disinfectant used. It is evident that most if not all lots of corn from the Corn Belt contain enough *F. moniliforme* infection to cause serious rot above 23 percent moisture if the temperature and oxygen supply are suitable. At this moisture *F. moniforme* will usually compete well and frequently will predominate over the aspergilli and penicillia, but if *Diplodiazeae* is present in sufficient quantity it, in turn, will predominate over *F. moniliforme*.

DIPLODIA ZEAE AND OTHER FUNGI

The moisture limits for growth of *Diplodia zeae*, *Gibberella zeae*, and *Nigrospora sphaerica* are very close together, as shown in table 3, and the general appearance of the three is very much the same (fig. 5). At 23.8 percent moisture *Diplodia* caused a dull dark discoloration of the germs and at slightly higher moistures all the kernels became tightly bound together by the white *Diplodia* mycelium. It grew aggressively and usually predominated over all other organisms.

Gibberella zeae had a pure white mycelium like *Diplodia* when grown at the lowest moisture limit, but at somewhat higher moistures the mycelium was pink in some areas and yellow and white in others, all colors occurring in the same basket of corn. At this moisture many of the kernels developed deep-red discolorations of the germs and other limited parts of the kernels. When the corn was not surface-sterilized, *G. zeae* did not usually predominate over aspergilli, penicillia, and *Fusarium moniliforme* until the moisture reached 26 percent and over. Above this moisture it became aggressive.

Nigrospora sphaerica grew as well as the two fungi just discussed when stored at corresponding moistures in pure culture (fig. 5). The mycelium was usually white but sometimes had a grayish appearance caused by the presence of spores. Some strains of *N. sphaerica* produced spores under the conditions of these experiments, while others produced none. The white parts of affected corn kernels turned a yellowish color at moistures allowing abundant growth of *Nigrospora*. When forced to compete with other fungi, this fungus grew poorly in stored corn containing less than 30 percent moisture. In three tests

with naturally infected grain, not surface-sterilized, and with moistures ranging up to 25 percent, *Nigrospora* could not be detected, whereas several other fungi were present in great abundance. In other similar tests some growth of *Nigrospora* could be detected by its characteristic spores at a moisture of 23.6 percent. The results obtained with *Nigrospora* infection in nonsterile seed are in agreement with some results reported by Durrell (*4*). Apparently this fungus is not ordinarily of importance as a cause of storage rot in corn even though it is present and there is sufficient moisture for its growth.

Cephalosporium acremonium infection in corn grain, while not ordinarily as frequent as that caused by *Fusarium moniliforme*, is never-

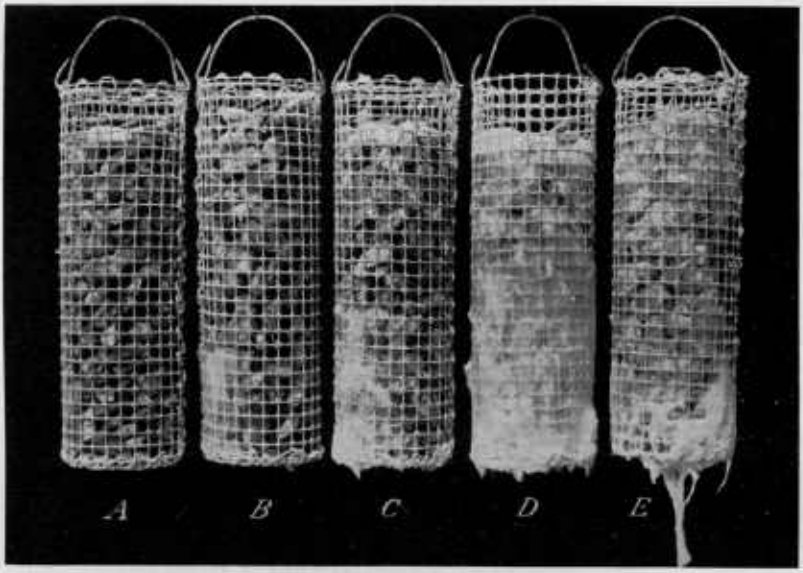

FIGURE 5.—*Nigrospora sphaerica* growing on corn in four baskets, *B* to *E*. No growth occurred in *A*. The storage period was 3 months at 70° F. with constant moisture of the grain as follows: *A*, 20.7 percent; *B*, 22.6; *C*, 23.4; *D*, 26.1; *E*, 29.2. At the same moisture content *Gibberella zeae* and *Diplodia zeae* present a similar appearance except that kernels infected by *G. zeae* turn a reddish color and portions of the mycelium may show yellowish to reddish hues, and those infected by *D. zeae*, which is a white mold, turn a brownish color and the mold usually is denser than that shown.

theless of very common occurrence. It is rarely found when plating surface-sterilized rot-damaged grain but is often observed when plating apparently sound grain. The explanation seems to be that *Cephalosporium* is not ordinarily a rot-producing organism. This belief was further strengthened by the storage experiments here reported. As is true with *F. moniliforme*, much of the *C. acremonium* infection is superficial enough so that thorough surface disinfection frees half or more of the kernels carrying this fungus. Nevertheless, by careful selection of corn ears, grain composites were prepared carrying 30 or more percent of *C. acremonium*, and no other fungus, after surface disinfection.

When corn grain was placed in the storage equipment without surface sterilization, other fungi developed in profusion at the higher moistures and *Cephalosporium* could not be detected by microscopic examination up to 27 percent moisture of the grain, which was the highest used. With surface-sterilized grain a pure growth of *Cephalo-*

sporium developed at suitable moistures. At 23.4 percent typical spore heads developed on sparse mycelium at the tip ends of the kernels and some of the kernels developed narrow longitudinal white streaks which were caused by the mycelium lifting the colorless seed coat away from the yellow endosperm. With higher moistures *Cephalosporium* growth became more abundant, but even at 27 percent moisture the growth was limited to the region at the tip ends of the kernels and there were no prominent indications of rotting after 3 months' storage.

DISCUSSION

A considerable number of tests were made with natural shelled corn, that is, unsterilized and uninoculated. Members of the *Aspergillus glaucus* group never failed to appear at 14.5 to 15.5 percent moisture in the grain and sometimes without apparent competition from other organisms. Some other aspergilli appeared at slightly higher percentages of moisture and some penicillia regularly appeared near 17 to 18 percent. They could be readily identified as penicillia among the aspergilli by the use of a microscope. A vertical illuminator which throws the light down on the object through condensers built around the objectives was found especially useful for distinguishing the various kinds of fungi. *A. niger*, a very easily recognized fungus, appeared in only about two-thirds of the tests at moistures suitable for its growth. Either this fungus was not universally present or slight amounts of it might have been inhibited by the growth of other organisms. This fungus, unlike some others, is so conspicuous that it would hardly be overlooked if present. *A. flavus* appeared in less than one-third of the tests.

In interpreting the data it must be borne in mind that in each test only a limited number of moisture constants were used with intervals of ½ to 2 percent of grain moisture ranging between them. Thus the exact moisture limit for fungus growth could not always be observed in any one experiment. Furthermore, experimental results did not always check closely. For instance, *Aspergillus wentii* was observed growing naturally on corn in two different tests made in two different seasons. In one case it was found at 15.1 percent but not at 14.3; in the other it was found at 16.2 but not at 15.2. Therefore, the data in table 3 show it as not present at 15.2 in one test, but as present at 15.1 in another. This fungus was not observed in 19 other tests with unsterilized seed, but this does not necessarily mean that it was absent, for it is not particularly conspicuous and can easily be overlooked when other fungi are abundant.

The variations in the results obtained in different tests can probably be accounted for by the fact that the corn used at different times came from different lots grown under different conditions. Moreover, different strains of the fungus species were present at different times. The results obtained with *Fusarium moniliforme* were especially variable, and this was charged primarily to the fungus itself as explained earlier under the discussion of that fungus.

Except in seed lots that had been carefully selected for freedom from *Fusarium moniliforme*, this fungus predominated above a moisture content of 23 percent provided *Diplodia zeae* was not present and provided the desired moisture was added at one time or the corn had not previously been dried. If dry corn was hung over solutions and the mois-

ture was taken up from the atmosphere the rise in moisture was slow (fig. 2) and aspergilli made heavy growth before *F. moniliforme* could get started. A fungus already in possession of the field may block the growth of another fungus which might have predominated if it had had an equal opportunity.

At a moisture suitable for vigorous growth of several fungi, the relative amount of inoculum of each kind present often determines which one will predominate. When different lots of unsterilized corn were dusted with several pure cultures of aspergilli and penicillia, respectively, the result in a number of cases was a growth of an almost pure culture of the fungus used for inoculation, almost as pure as though pure culture methods had been used.

When a *Penicillium* or *Aspergillus* species was found growing on grain at its lowest moisture limit it not only made weak growth but it also grew only on a minority of the kernels. At a slightly higher moisture the growth was not only more vigorous but it also occurred on a higher percentage of kernels. This appeared to be a combined effect of chance inoculation and actual difference in moisture content of individual kernels. It is also probable that some kernels possess resistance of a chemical nature to some extent (*11*). When kernels were thoroughly dusted with spores of one of these fungi before the experiment started, the fungus occurred on a much higher percentage of kernels even though it made weak growth for lack of water; still some kernels remained free from fungus growth. It has already been shown (table 2) that different kinds of corn may take up moisture at different rates or may come to equilibrium with their surrounding atmosphere with different percentages of moisture in the grain. This being true, individual kernels, especially of an open-pollinated variety, will no doubt behave differently in this respect also.

Actual damage to grain from rot was never observed at the lowest moisture limit for fungus growth. Damaged kernels of the kind classified as "commercial damage" by grain inspectors usually occurred to some extent with the various fungi tested at a moisture of about 1½ to 2 percent higher than the minimum moisture for growth. If the storage period had been longer, the critical moisture for development of damaged kernels might have been slightly lower. With temperatures higher than 70° F. the minimum moisture requirement and the margin for commercial damage both appear to be lowered slightly. When temperatures become sufficiently high heat damage takes place in addition to rot damage.

The oxygen requirements of the corn-rot fungi studied were very moderate. When 100 g of corn was suspended over a salt solution in a sealed bottle containing nearly 2 liters of air, growth with the various molds usually proceeded nearly as well as in an aerated bottle. A sour alcoholic odor developed in sealed bottles in which the moisture of the corn was over 16 percent. This odor was not observed in bottles aerated with only a fine caliber glass tube as mentioned in the discussion of methods. Rapid aeration with sterile air was used to carry off the chlorine when the corn had been surface-sterilized with chlorinated lime. If the sealed bottles had been filled full with grain, lack of aeration might have greatly retarded mold growth as Swanson found with wheat (*18*). In deep storage bins aeration also is exceedingly limited. Duvel (*5*) made observations in a 65-foot elevator bin filled with shelled corn at 17.0 to 18.8 percent moisture.

Storage was carried out during the late winter and spring months, the temperature being 36° to 39° F. when the experiment was started. In 5 weeks' time the mustiness had extended down to 7 feet below the surface but no lower. In 7 weeks no mustiness was observed at a 12-foot depth although there was an abundance of molds at the surface. When the bin was emptied after 9 weeks' storage the lower half still appeared to be free from significant mold growth.

SUMMARY

Shelled yellow dent corn was stored in atmospheres maintained at constant humidities of various degrees by placing it in wire baskets over salt solutions in closed containers. Storage was for a period of 3 months at a temperature of 70° F. Three methods of aeration were tried. The one supplying the least oxygen appeared adequate for growth of the fungi studied, but more liberal aeration was supplied for most of the tests.

Growth limits of a number of fungi as determined by moisture in grain was studied in two ways, in competition with surface-borne fungi, and in pure culture after surface-sterilizing the grain. The fungus to be studied was supplied by either selecting corn carrying the fungus as an internal infection or by inoculating the grain.

Different strains of corn showed distinct differences in the percentage of moisture in the grain when in equilibrium with a saturated atmosphere. There was good evidence also for believing that individual kernels showed variations in moisture content in experiments with open-pollinated corn. Thus one would not be able to predict accurately the moisture content of grain stored in an atmosphere with a known constant humidity.

Aspergillus glaucus grew at 14.3 percent moisture of grain, which was lower than could be utilized by any other fungus, but several other aspergilli appeared at only slightly higher moistures. *A. flavus* and *A. niger*, however, were not found below a moisture content of 18.3 percent.

Five species of penicillia were found to vary in their minimum moisture requirement from 15.6 to 20.8 percent. The blue color of the germ known to grain inspectors as "blue eye" was found to be caused by the growth of certain kinds of penicillia between the germ and the seed coat. *P. notatum* caused blue eye at a minimum moisture of 16.7 percent, while *P. palitans* required 19.5 percent, in these experiments. In each case somewhat more moisture was required for the extensive development of the blue-eye condition.

The minimum moisture requirement for *Fusarium moniliforme* ranged from 18.4 to 21.2 percent in tests in which competition from other organisms was excluded. A similar range was observed where competition was allowed. This was a wider variation than was observed with any other fungus species and demonstrated a significant variation in the moisture requirement of different strains of *F. moniliforme*.

Various aspergilli and penicillia grew well in mixed combinations in natural uninoculated seed. When the spores of a single species of these same fungi were dusted on the grain before a storage period was started, that particular fungus predominated more or less to the exclusion of all others provided moisture conditions were suitable.

Above 23 percent moisture *Fusarium moniliforme* competed well and often predominated over all other fungi except when *Diplodia zeae* was present. While in many cases one fungus tended to dominate another under growing conditions suitable for both, the relative abundance of inoculum of each one present was often an important factor in determining dominance.

Diplodia zeae, Gibberella zeae, and *Nigrospora sphaerica* all grew well on corn above 21.5 to 23 percent moisture content in the absence of competition from other organisms. When forced to compete, *D. zeae* was the most aggressive and *G. zeae* next. *N. sphaerica* was very weak.

For the production of commercial damage to the grain from rot by fungus growth an increase of 1½ to 2 percent in moisture over the minimum moisture requirement for growth was usually needed under the conditions of these experiments.

While *Cephalosporium acremonium* grew well on naturally infected corn under pure culture conditions, at moistures of 23.4 to 27 percent, it was not observed to cause commercial damage.

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