DETERIORATION OF STORED GRAINS BY FUNGI [1]

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Although deterioration in stored grain typical of that caused by fungi was described more than 40 years ago (17, 45), only recently has the problem become recognized as one of some importance. It is supposed that combine-harvesting of wheat and other small grains has led to increased amounts of seed being stored with a high moisture content. The greater quantities of grain now commonly stored in bulk for two to five years have certainly increased all of the hazards that accompany storage, including those caused by fungi. Realization that fungi are involved in many of the deteriorative processes in bulk stored grains also is relatively new. Often deterioration is subtle in onset and accompanied by no indications of trouble that are apparent to the practical men in the grain trade, and hence losses that we now know to be caused mainly or solely by invasion of the stored seeds by fungi, have been attributed by them to mysterious causes.

NATURE OF LOSSES

Deterioration in stored grains is manifested by: *a*) decrease in germination, important in barley to be used for malting, and in seed to be used for planting; *b*) decrease in processing quality, as in wheat and corn for milling; corn and other seeds used for starch; and flax, soybeans, cottonseed and other seeds used for oils; *c*) sick or germ-damaged grain, a trade and grading term used to designate brown color of the germs, especially in wheat and corn; *d*) heating; and *e*) mustiness. Factors other than fungi may at times be involved in some of these types of deterioration, but fungi can and do cause all of these deleterious changes in stored seeds.

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FUNGI INVOLVED

Somewhat more than 50 species of fungi and a considerable number of species of bacteria have been isolated from agricultural seeds (42). Bacteria do not normally appear to be involved in the deterioration of stored seeds because, so far as known, they require free water to grow, and seeds seldom are stored under conditions where free water is available. There are, of course, some exceptions to this, but to date they appear to be minor in extent and significance.

Fungi in seeds may be rather arbitrarily divided into two groups, designated field fungi and storage fungi. The field fungi are those that invade the developing or mature seed while it is still on the plant. The major genera are *Alternaria, Helminthosporium* and *Fusarium,* in non-weathered seed, plus *Cladosporium, Diplodia, Chaetomium, Rhizopus, Absidia* and a number of others in weathered seed. With the possible exception of *Fusarium,* even heavy invasions of seed by these fungi do not seem to result in decreased storability. We have encountered, however, a few cases in which wheat, with the germs moderately infected by *Fusarium,* developed sick or germ-damaged seeds in storage at moisture contents below those where *Fusarium* originally present could continue to grow. This at least suggests that *Fusarium* spp. should be investigated more thoroughly as possible contributors to one of the common types of storage deterioration in wheat.

The storage fungi are those which develop on and within seeds at moisture contents often encountered in storage, principally *Aspergillus* and *Penicillium.*

Aspergillus glaucus is one of the major fungi that invade stored seeds. This group species is divided into nine " series ", each of which contains a number of subspecies (49). Four of these series —*A. amstelodami, A. ruber, A. repens, A. restrictus*—have been found associated with many cases of deterioration in stored wheat, and appear to constitute the major organisms that invade wheat whose moisture content is 13.2 to 15 per cent, wet weight basis (7, 8, 52). With increasing moisture content above 15 per cent, *A. candidus, A. ochraceus, A. flavus, A. versicolor, A. tamarii* and perhaps a few other species of *Aspergillus,* and *Penicillium* appear. Essentially the same moisture limits prevail for the growth of these fungi in corn (29) as in wheat. These various species and subspecies of *Aspergillus* differ from one another not only in taxonomic characters but also in such aspects as the moisture content and temperature at which they grow, the speed with which they invade, kill and discolor the germs of the seeds, and the biochemical changes they produce in the seed. Recent unpublished evidence from work in our own laboratory shows that within a given species, strains or varieties differing in these characters may also occur. Thus many of the problems associated with deterioration of stored seeds involve not only a moderate number of species of the ubiquitous *Aspergillus* group but also strains within the species. Some of these species and strains can be isolated from seeds in which they are present only by the use of rather special technics and media.

DETERMINING NUMBER AND KINDS OF STORAGE FUNGI IN SEEDS

It is almost axiomatic in microbiological work that any culture medium used to isolate microorganisms from materials in which they are present is to a certain extent selective, but this very obvious principle has at times been disregarded in work with fungi related to deterioration of stored grains. We have repeatedly found, for example, that acid potato dextrose agar, so widely used in plant pathology laboratories for the isolation of fungi from plant materials, is totally unsuited to detecting the presence of some of the common subspecies of *A. glaucus* that are most prevalent in stored seeds. Also Czapek-Dox agar, a standard medium for some kinds of mycological work and essential for the identification of many species of *Aspergillus,* is of limited value in determining the presence of some of these species of *Aspergillus* in seeds. After tests of 11 different media it was stated (4): " It is obvious —that the standard Czapek-Dox medium—is not suitable for determining the mold count of these samples. It not only gave one of the lowest total mold counts, but—*Aspergillus glaucus* did not appear at all, whereas the other media indicated that this fungus was present in considerable numbers ".

No one medium or technic is sufficient to disclose all of the organisms that might be present in a given lot of seeds, and at times it has even been difficult to isolate *Aspergillus restrictus* on agar media, on which this fungus grows fairly well, and from seeds in which the fungus could be detected by microscopic examination (7). Recently we encountered what appears to be a new species of *Aspergillus* in the germs of nearly 100 per cent of seeds that had been stored for some time at a moisture content of 13.5 per cent; when the seeds were cultured, this fungus grew and sporulated only on the germs, not on the agar; when transferred it grew very slowly on agar media containing 15–20 per cent sodium chloride or 40–80 per cent sucrose, but often did not grow at all on media of lower osmotic pressure. It seems unlikely that even the technics which have been developed to isolate these very xerophytic fungi are adequate to detect them in all cases where they are present in seeds. This is especially true when more rapid growing fungi also are present. In work of this type, as in much other research, the validity of results and of conclusions derived from them depends very greatly upon the technics used and upon the worker's understanding of the limitations and qualifications that attach to the technics. With the use of inadequate technics or of media on which the fungi concerned grow poorly or not at all, it is very easy to conclude that a given sample of seed contains no fungi of significance in storage deterioration, when actually these fungi may be present in large amounts.

While the media and technics of preparation of the seed before culturing are varied considerably in our laboratories, depending on the information desired, several more or less standard media and technics have been developed to determine the number and kinds of storage fungi present on and in seeds. These will be described briefly.

The principal culture medium used is malt-salt agar, consisting of 1–2 per cent malt extract, 2 per cent agar, and 7.5 to 20 per cent sodium chloride. This serves to isolate a larger number of fungi significant in storage deterioration, especially in the *A. glaucus* and *A. candidus* groups, than any other of the media tested (7, 52). For isolation of members of the *A. restrictus* series of the *A. glaucus* group, a medium high in salt or sugar seems to be essential, and even on such media most isolates of most subspecies of the *A. restrictus* series grow very slowly (52). On some media commonly used in microbiological work they do not grow at all. Malt agar, containing 7.5 to 20 per cent sodium chloride, and Czapek-Dox, containing 40–80 per cent sucrose, have proved fairly satisfactory for isolation of this fungus.

Before culturing, seeds usually are surface disinfected for one half to one minute in one per cent sodium hypochlorite, then rinsed twice in a sterile 7.5 per cent solution of sodium chloride. Implausible as it sounds, even such a light surface disinfection sometimes appears to prevent some of the species of the *A. restrictus* series from growing out of the germs of wheat seed. Sometimes seeds are scattered on the agar without any pre-treatment, or are washed, before culturing, in a jet stream of water, with addition of soap powder or detergent, to remove external dirt and debris. The latter technics have been used especially in determining the fungus flora of supposedly fungus-free seed that was to be used in special tests.

The plates usually are incubated at room temperature, although incubation temperatures of from 10 to 50° C. have been used for special cases. Incubation at 30° C. results in a more rapid appearance of most of the common species of the *A. glaucus, A. candidus* and *A. flavus* groups than incubation at 25° C. It is not unusual to encounter parcels of seeds from commercial storage which contain *A. repens, A. amstelodami, A. chevalieri, A. ruber, A. restrictus* and *A. candidus,* and sometimes most of these may appear from each seed; in those cases the subspecies of *A. glaucus* frequently grow out first, and *A. candidus* appears only after a week or so. If a mixture of *A. restrictus* with other subspecies of the *A. glaucus* group is present, as not infrequently happens, especially in parcels containing some sick seeds, it usually is preferable to culture the seeds on agar media containing 15–20 per cent sodium chloride, as well as on a medium containing 7.5 per cent. In practice we often culture 50 to 100 seeds (25 seeds per plate) on each of several different media; experience has shown that only in this way can we be moderately certain of determining the range of fungi present in the seeds.

To determine the number of viable spores of storage fungi present, five grams of seed are weighed to the nearest seed, on a torsion balance, put in 500 ml. of a sterile solution of 0.2 per cent agar and 7.5 per cent sodium chloride in a Waring blendor, and comminuted for one and one half minutes. Five-ml. amounts are removed from this suspension with a pipette whose end has been cut and fused to give a larger opening, calibrated to deliver 5 ml. of this rather viscous liquid, and this is added to 45 ml. of the suspension medium of the same composition as that noted above. This is shaken vigorously to distribute the suspended material, then one-ml. aliquots are removed, using either a one-ml. syringe or a one-ml. pipette calibrated to deliver one ml. of this suspension, and placed in each of two or more replicate dishes. Malt-salt agar, melted and cooled to 50° C., is added; the agar is swirled to distribute the suspended material and allowed to harden; the dishes are then incubated.

Agar is added to the suspension medium to give a uniform suspension of spores and seed particles. In extensive tests to determine the sources of variability in numbers of colonies obtained in replicate plates of blendorized seeds, it was found that, when water was used as the suspending medium, the major source of variability was in successive aliquots taken from any given suspension. Adding 0.2 per cent agar to the medium reduced the variability greatly, and with the present technic colony counts on replicate plates commonly vary only plus or minus 5–10 per cent, and frequently less. Salt is added to the suspension medium because in numerous comparative trials it was found that this resulted in higher counts of some of the *A. glaucus* group, particularly *A. restrictus.*

The plates are incubated at room temperature or at 30° C. for two days, then inverted, without removing the cover, on the stage of a stereoscopic microscope, and with the aid of transmitted light the young colonies are counted and their location marked. This is, at times, an unnecessary refinement, but at other times it is desirable because it is not unusual, where even as few as 50 colonies are present per culture plate, for a number of them to arise near each other. Where *A. restrictus* and some of the faster growing subspecies of the *A. glaucus* group, such as *A. repens, A. amstelodami* and *A. ruber,* are all present in considerable numbers, the faster growing colonies may obscure the slower growing colonies of *A. restrictus* if the colonies are not counted in this way. The plates are similarly examined after three days, then left until the colonies can be identified. Frequently it is necessary to transfer colonies to Czapek-Dox agar containing 20 to 40 per cent sucrose to identify them positively.

Essentially the same method is used for determining number and kinds of molds in flour, although with that material no comminution in a blender is required; it has been accepted as standard by the American Association of Cereal Chemists and is included in the latest edition (VI) of Cereal Laboratory Methods (1).

WHERE AND IN WHAT FORM FUNGI OCCUR WITHIN SEEDS

Mycelium is common under the pericarps of wheat seeds (6, 27) and under the hulls and pericarps of barley (53) and rice (16). At the time of harvest, most of this mycelium appears to be that of *Alternaria* or other field fungi and relatively little of storage fungi (6). However, light and superficial infections by storage fungi appear to be so general in cereal seeds that it is difficult to obtain even small amounts of cereal seed that are totally free of storage fungi, or that can be freed of them by surface disinfection or other technics that do not injure the seed, even when the seed is produced in the dryland or irrigated regions of northwestern U. S. One of the problems in separating the effects of storage fungi from the effects of processes inherent in the seeds themselves has been that of obtaining seed that could be stored free of storage fungi for periods of several months to a year. In some cases, to be cited later, we have been able to do this.

WHEN STORAGE FUNGI INVADE SEEDS

Rather extensive tests with wheat and barley over a period of some years indicate that there is no significant invasion of the seeds by storage fungi before harvest (52, 53). This has been so even when the harvest season was moist or when the grain was left in the field, either standing, lodged or windrowed, for as long as two weeks during rainy weather after ripening. Some of these tests have involved wheat grown in various portions of the hard and soft winter wheat regions, from Texas to Oklahoma to New York and Virginia, as well as more extensive tests of grain grown in Minnesota. In no single case have we ever found an appreciable amount of infection of the seed by storage molds prior to harvest.

The reason for this is not certainly known, but there is evidence that so long as the seeds remain attached to the rachis and surrounded by glumes, they are much less susceptible to invasion by the fungi that cause deterioration in storage than seed that is threshed out (52). As stated above, light and apparently superficial infections by storage fungi are common and in our experience almost universal in wheat and barley; but infections by these fungi heavy enough to account for later deterioration, without more extensive growth by these fungi during the storage period, have not been encountered. Rather extensive invasion may, however, develop within a week or two after harvest if the seed is stored under conditions that permit or promote rapid growth of the fungi concerned.

CONDITIONS THAT INFLUENCE INVASION OF STORED GRAINS BY FUNGI WHICH CAUSE DETERIORATION

A complex of conditions influences the invasion of stored grains by fungi, and in practice these operate together. The major ones are moisture content, temperature, amount of previous infection of the seeds by the fungi concerned, time, and the activities of various grain-inhabiting insects and mites:

MOISTURE CONTENT. Federal regulations (54) specify maximum moisture contents of 14.5 per cent for hard red spring and durum wheats (those with 14.5–16 per cent moisture are graded " tough ") ; 14 per cent for other classes of wheat (those with 14 to 15.5 per cent are graded " tough ") ; 14.0 per cent moisture for No. 1 corn, and 15.5 per cent for No. 2 corn. The moisture content limits for wheats presumably were based on studies made in 1918 (2) and in 1925 (11), which indicated that wheat stored at a relative humidity below 75 per cent, or below an equilibrium moisture content of 14.5–15 per cent, did not respire measurably and thus presumably was safe for long time storage. Actually these tests, as well as some later ones, the results of which appeared to support the limits established earlier (33–35), involved storage for only two to three weeks, and the basis for determining whether fungi were developing, or whether deterioration was occurring, was mainly measurement of respiration. Two errors may have been involved: *a*) that tests over a period of three weeks would predict accurately the behavior of grain over a year or more; *b*) that growth of fungi or incipient deterioration could be detected, at moisture contents of 14–14.5 per cent, by measurement of respiration.

Actually there is no experimental evidence to prove that fungi growing in the germs of wheat whose moisture content is 14 per cent will respire at a rate sufficient to be detected by present technics. During the last five years we have encountered several species of fungi, all of them in commercial lots of seed in which deterioration was progressing, that are able to invade, kill and discolor germs of wheat at moisture contents of 13.5–14.5 per cent. *A. restrictus, A. ruber, A. amstelodami* and the unidentified species of *Aspergillus* recovered from germs of seed mentioned above, all appear to be able to invade and cause deterioration of wheat at moisture contents at or just below the limits now specified for long time storage. The moisture content specified for No. 2 corn (15.5 per cent) is known to permit at least moderately rapid invasion by some species of *Aspergillus* (29), and this is amply supported by unpublished work in our own laboratory. It seems that the present regulations concerning the moisture contents permissible for long time storage of grains are unrealistically high.

Several other complications enter into this. One is the means by which moisture content is determined. In the grain trade, moisture contents usually are determined by means of an electric moisture meter, on a supposedly average sample of the bulk being tested. There is good evidence (40) that moisture meters may at times give a reading as much as one per cent off, even when operated carefully. This appears to be true especially when the moisture contents are in the critical range. If not carefully calibrated and carefully operated, the margin of error may be greater. Recently, for example, the writer obtained a number of samples of soft red winter wheat that had deteriorated in storage during the winter of 1955–56. The moisture content of these samples, as determined by the elevator operator with an electric moisture meter, was 11.9 per cent. As determined in the laboratory, by oven drying according to methods specified by Cereal Laboratory Methods (1), the moisture contents ranged from 13.4 to 13.6 per cent. Apparently the same error crops up in the scientific literature. A 1956 paper (13), dealing with the development of flour beetles in grain, stated that wheat stored in equilibrium with a relative humidity of 75 per cent had a moisture content of 12.2 plus or minus

0.1 per cent. The moisture content in this case was determined by a Steinlite electric moisture meter; there was no mention of its accuracy having been checked against approved oven methods. There is ample evidence (11) that wheat in equilibrium with a relative humidity of 75 per cent has a moisture content of 14.5–15 per cent, or even slightly over 15 per cent, not 12.2 per cent. If an error of that size occurs in laboratory research, one may suppose that errors of equal magnitude sometimes appear in practical grain testing, where many samples are tested hurriedly.

It does not appear to be generally appreciated, especially by those outside the field of cereal chemistry, that moisture content in grains and grain products can be determined accurately only if the methods and equipment specified by those who have established the accepted standards are rigidly adhered to. Moisture content determinations in cereals are not to be taken lightly, and the evidence presented above suggests that data on moisture content determined by any but the approved procedures may often be regarded with suspicion or disbelief.

The use of average samples introduces another fruitful source of error. Granted that grain is bought and sold on a basis of average moisture content; in storing grain, the critical factor, so far as growth of fungi and its attendant deterioration are concerned, is not the average moisture content but the highest moisture content that prevails in any considerable portion of the bulk for any considerable length of time. Evidence from commercial bulks of grain will be instructive here.

In one case, a bin of hard red spring wheat, stored at an average moisture content of 12.8 per cent, began to heat after it had been in storage for three months. As the bin was emptied we obtained a number of samples from the belt near the hopper, put them into moisture-proof cans, and determined their moisture contents by oven drying in the laboratory. Over half the samples so taken and tested had a moisture content of 14 per cent or over, and a few had a moisture content in excess of 16 per cent. In another bin, containing winter wheat with an average moisture content of 13.0 per cent, we obtained samples by probe from depths of five, 15 and 30 feet in each of six different places in the bin, put them immediately into tight containers, and tested their moisture content in the laboratory. Nine of the 18 samples had moisture contents in excess of 13.5 per cent; four had moisture contents just over 14 per cent. The moisture contents of these samples were just above the point where molds might invade the grain, instead of just below, as the elevator operator supposed. In a third case we buried high grade, seed quality wheat whose moisture content was 11 per cent, in small cloth bags at known locations in the bin as the bin was filled. The wheat going into the bin had an average moisture content of 13.2 per cent. Some heating occurred in portions of the bulk after storage for about three months, and the bin was emptied. The bags were recovered on the belt near the hopper, the grain put into moisture-proof tins, and taken to the laboratory for moisture content determinations. About one third of these samples had moisture contents over 14 per cent, several others over 15 per cent. The average moisture content of these samples when the bin was emplied was 13.2 per cent, the same as the average for the entire bulk. Data on mold invasion of these samples indicated that at some time during storage, nearly all of them had for a while been at a moisture content that permitted heavy invasion by *A. glaucus, A. candidus* and *A. flavus.* The samples originally had been almost free of storage molds. The fact that so many of the seeds in so many of the samples had been rather extensively invaded by *A. candidus* and *A. flavus* indicated that these samples had been at moisture contents of 15 to 17 per cent for a long enough time to permit such invasion. The figure on average moisture content was accurate but certainly gave no accurate base for judging risk of deterioration (8).

We now have considerable additional evidence that the practice of testing only average samples for moisture contents gives only a very haphazard picture of what may be happening in some large bulks of stored grain. Closer attention to this and to the accuracy of moisture content determinations undoubtedly would help remove much of the mystery that up to this time has surrounded many cases of deterioration in commercial bins where the grain supposedly was at a moisture content safe for long time storage.

VARIATION IN MOISTURE CONTENT AND TRANSFER OF MOISTURE WITHIN A GIVEN BULK. It is well established that, in any moderately well enclosed bulk of grain in which differences in temperature are maintained for some time, moisture will be transferred from the warmer to the cooler portions of the bulk (37). Thus in a bulk where the moisture, at time of binning, is uniform throughout the mass of grain, it may become unequally distributed upon storage. There is a greater transfer of moisture with greater differences in temperature than with small differences in temperature between different portions of the bulk. Also, in the cereal grains, if the bulk originally contains a moisture content near the critical limit (i.e., about 13.5 per cent) or above, subsequent moisture transfer from one portion of the bulk to another is likely to be more rapid, and larger in extent, than if the moisture content is lower (around 11–12 per cent). This is explained by the relative humidity–moisture content relationship. As the relative humidity rises from 50 to 65 per cent, the equilibrium moisture content of the grain increases only slightly; as the relative humidity rises from 65 to 80 per cent, the moisture content of the grain increases rapidly (11, 46). In practice this means that if grain is stored at a relatively high temperature and a moisture content near the critical limit, in the fall when there are sharp changes in the daily temperature, temperature differentials are likely to be set up that lead to moisture transfer, mold growth, and to deterioration. If the moist grain is cool when stored in autumn, as often is the case with corn in the northern regions of the United States, no deterioration is likely through the winter. However, with the advent of spring weather and high outside temperatures, especially at the top and on the sunny side of the bins, the grain may be warmed up sufficiently to give rise to convection currents that allow rapid transfer of moisture, and, when the temperature of the moist grain becomes high enough for molds to grow rapidly, sudden spoilage ensues. In many bins, condensation occurs on the inner side of the top during early spring, and the water drips down on the grain in sufficient quantity to wet thoroughly the top layers (44). Often bins are so constructed that leakage may occur from rain, which of course leads to local moist regions (41). Condensation and leakage normally account for only minor wet regions within a large bulk of grain—the greater part of high moisture areas, at least in terminal bins, must be attributed to transfer of water vapor from one portion of the grain to another, with no condensation or leakage being involved.

Some studies now are available to show the pattern of moisture transfer within supposedly typical grain bulks (42); more such studies are in progress by commercial grain storage firms. Eventually enough data should be available to enable us to know much more exactly than we now do, the circumstances under which such transfer will be great enough to lead to serious spoilage, and to know more accurately its pattern within bulks of different size and shape. Such information would enable us to know the major danger areas within a given bulk under a given set of conditions; assuming that the location of such large-risk or deterioration-prone areas could be known beforehand within a given bulk, it would be a simple matter to remove samples from those areas periodically for testing, and thus anticipate and forestall deterioration without laborious sampling of the entire bulk.

VARIATION IN MOISTURE CONTENT BETWEEN SEEDS. It is common practice, from the farm to the final processor of grains, to blend different lots of grain of different moisture content, to achieve an average moisture content that is within a given grade limit. It is assumed that such mixing leads to uniform distribution of moisture among the seeds so mixed. However, more than 20 years ago (18) it was found that when wheats of different moisture contents were mixed together thoroughly and allowed to come to equilibrium, the seeds originally high in moisture content retained a higher moisture content than the theoretical average. It was stated: " From these five sets of tests the general conclusion is that when wet and dry wheats are mixed in equal proportions and allowed to lie together, moisture is transferred from the wet to the dry. The transference is complete in three days. Equality of moisture is not attained, there being a final difference of about 2% between the wheats ".

This problem requires more attention and investigation than has been given to it, if for no other reason than that the practice of such mixing is so firmly established and widespread. Unless the theoretical equilibrium aimed at in such mixing is below the supposed safe limits for storage, some of the seeds, even after thorough mixing, may retain a moisture content high enough to permit invasion of the seeds by molds, and consequent spoilage.

MOISTURE ABSORPTION FROM THE AIR. Grain may pick up moisture from the air relatively rapidly. In some of our own unpublished tests, wheats of 12 per cent moisture were stored in bags exposed to outdoor air, but protected from rain and snow; the moisture content of these increased to 15 per cent within three months during the fall, and remained so during the winter. According to the Monthly Weather Review, a relative humidity of 75 per cent and above is common during the winter months from Minneapolis to Buffalo. Presumably that portion of the grain exposed to air would come to equilibrium with the relative humidity of the air fairly rapidly. If there is much circulation of the air into and through the bulk, as we know there is in some cases, the grain may gradually accumulate moisture in humid weather. This supposedly is one reason why grain, even though originally dry, is difficult to store without deterioration in humid climates. Moisture content may also be increased drastically in the grain from activities of insects, as will be described below.

TEMPERATURE. Most of the fungi that invade stored grains grow best at temperatures of about 30° C. We have some isolates of *A. flavus*, however, that grow well up to 45° C., and some of *A. candidus* that grow at about 55° C. The growth rate of most of them is greatly reduced below 20° C., but some of the subspecies of the *A. glaucus* group will grow slowly down to 5° C., and *A. restrictus* appears to grow at about the same rate at 15 as at 25° C. Tests aimed at determining the temperature ranges at which these fungi will invade grain should be made with the fungi growing on grain whose moisture content equals that met in practice; tests on agar media often give results that can be applied only to growth of the fungi on agar media. Some strains of *A. repens, A. amstelodami* and *A. ruber*, for example, will not grow on agar media at a temperature above 35° C., but will invade wheat seeds at a moisture content of 15–16 per cent kept at 40–45° C. Much more extensive tests are needed to determine more accurately the relationship of temperature, both constant and fluctuating, to the invasion of seeds by these fungi, and the appearance of commercial damage as a result of such invasion.

TIME. Moisture content, temperature and time are all intimately related to the growth of molds in stored grains. Thus the higher the moisture and temperature, within the limits of growth of the fungi involved, the shorter the permissible storage time. Thus wheat at a moisture content of 14.5 to 15.0 per cent can be stored safely at 20 to 25° C. for a few months, but not for a year; while at the same moisture content and at a temperature of 10–15° C. it presumably could be stored for a year without serious damage from molds. Apparently the absolute lower limit of moisture content that will permit growth of storage molds in seeds over a period of one to two years at a temperature of 20–25° C. is that in equilibrium with a relative humidity of 70 per cent in the interseed air.

INSECT INFESTATION. There is considerable evidence in the literature to indicate that some of the common grain-infesting insects are able to establish themselves and develop through a normal life cycle in grain whose moisture content is in the range of 10 to 12 per cent (12). However, so far as can be determined by the writer, moisture contents of grain have not been determined after the insects have become established. In some of the tests, moisture content has been determined by the use of electric moisture meters that apparently were not accurately calibrated; a paper already cited, for example (13), dealing with moisture content in relation to growth of the confused flour beetle (*Tribolium confusum*), states that wheat in equilibrium with a relative humidity of 75 per cent had a moisture content of 12.2 plus or minus 0.1 per cent. Wheat in equilibrium with a relative humidity of 75 per cent should have a moisture content of 14.8 to 15.0 per cent, as was established long ago (11) and amply supported by many recent tests. Thus even at the beginning of the tests reported with this beetle, the moisture contents given presumably are in error; either the relative humidity was lower than stated or the moisture content was several per cent higher than stated. The moisture content at the end of the tests was not determined.

In recent cooperative work between the Departments of Entomology and Plant Pathology and Botany at the University of Minnesota with grain insects, such as the granary weevil (*Calandria granaria*), and the lesser grain beetle (*Rhizopertha dominica*), establishment of the insects in grain having an original moisture content of 12 to 15 per cent resulted in rather rapid increases in moisture content to an amount of several per cent. It also resulted in very rapid increase in some of the common storage molds associated with the development of sick wheat, especially *A. restrictus*. The relationship between some of the insects and mites known to infest grain and grain products, and the fungi known to cause damage of one kind or another to these products, apparently may offer a rather fruitful and hitherto unexplored field of research. It is anticipated that work now under way at the University of Minnesota, dealing with these relationships, will be continued. At present it seems very probable that even moderate infestations by insects in commercial bulks of grain may very greatly increase the hazard of deterioration from fungi, within and probably even at some distance from that portion of the bulk where the insects are established.

OXYGEN–CARBON DIOXIDE RATIO. In agar cultures a 30 per cent concentration of carbon dioxide stimulated *Penicillium,* and a concentration of 40 per cent was required to inhibit the germination of spores of *A. repens* (20). From results of tests with wheat stored at 18 per cent moisture and under different concentrations of oxygen and carbon dioxide, it was concluded that: ". . . mold growth, germ damage, fat acidity, and respiration rate all gradually decreased as the oxygen concentration was lowered. Some mold growth occurred at 0.2 per cent oxygen but the wheat maintained its viability; . . ." and "In the presence of 21 per cent oxygen, increasing levels of carbon dioxide had little effect on respiration rate until the concentration exceeded 13.8 to 18.6 per cent, when a very sharp and marked inhibition of respiration, mold growth, and development of fat acidity occurred. At large carbon dioxide concentrations (50 and 79 per cent), the viability of the wheat remained high and there was little or no germ damage" (39). The little evidence available indicates that even when molds are actively growing on and in seed in commercial bins, the composition of the interseed air remains about the same as that of outside air (41). So far as is known, no tests have been made to determine the effect of carbon dioxide concentrations on the fungi that cause deterioration of stored seeds, at moisture contents of 13.5–15 per cent, and for a period of several months to a year, which are the conditions under which deterioration often occurs in practice. This is rather important, especially since attempts now are being made to control or reduce deterioration in stored grain by keeping the grain in an atmosphere high in carbon dioxide (26, 49).

PREVIOUS INVASION BY STORAGE MOLDS. If seeds are moderately to heavily invaded by the fungi known to cause deterioration, then dried to a moisture content of 12–14 per cent, deterioration, as indicated by increase in fatty acids, decrease in non-reducing sugars and increase in amounts of " sick " wheat, will continue to develop (21, 47). This " carryover " effect has been observed only in laboratory tests, but it may help to explain some cases of deterioration of grain stored at supposedly safe moisture contents, since it is not unusual to find parcels of grain going into terminal storage with invasions by storage molds as heavy as those in some of the tests cited. Information concerning degree to which given parcels of grain have been invaded by the fungi known to cause deterioration, if obtained at intervals from harvest on, might well give more information on storability and deterioration hazard than any other tests now available.

EFFECTS OF STORAGE MOLDS ON SEEDS

Fungi growing in stored seeds can reduce germination of the seed, cause darkening of the germ or of the entire seed, increase the fatty acid content, cause or contribute to other biochemical changes, increase the moisture content of the seed, and, in extreme cases, cause heating. Each of these will now be discussed.

REDUCTION IN GERMINATION. Seeds of wheat and barley free of fungi that cause storage deterioration can be stored at moisture contents of 15 to 18 per cent and a temperature of 20–25° C. for weeks or months with little reduction in germination (21, 34, 52). This is fairly conclusive evidence that processes inherent in the seeds themselves are of very minor significance in death of seeds under those conditions; and it is good presumptive evidence that processes inherent in the seeds themselves are of minor significance in such deteriorations as are encountered in practice. There is an abundance of evidence that invasion of the germs of the seeds by those species of *Aspergillus* encountered in seed that has undergone deterioration, will result in rapid death of the germs (21, 52). These fungi, therefore, are at least facultative parasites. They differ from one another in their degree of pathogenicity, *A.*

restrictus, A. candidus and *A. ochraceus* apparently being more pathogenic than *A. repens.*[3]

Different isolates of *A. candidus* differ greatly from one another in the rate at which they kill wheat seeds at moisture contents of 16 to 17 per cent, and it seems likely that such pathogenic races occur within all major species of seed-invading fungi. The nature of this pathogenicity is mostly not understood; however, *A. flavus* is known to produce toxins that are lethal to wheat seeds (51).

DISCOLORATION OF THE GERM. Darkening of the pericarps toward the point of attachment of wheat seeds may be caused in the field, before or during ripening, by bacteria or by various fungi, for example, *Alternaria, Helminthosporium* and *Cladosporium,* which have dark mycelia and which sometimes invade the pericarps of the seed heavily during humid weather (23, 24, 31). This is quite distinct from the blackening of the germ itself, which is designated in the trade as " sick " or " germ damaged " wheat. Federal regulations specify the maximum percentage of damaged kernels permissible in each grade, and thus sick or germ-damaged seeds constitute a grading factor and a price factor in wheat, indeed in all cereals, including corn.

Development of dark germs in stored seeds was long considered by those in the trade to be caused by mysterious factors. Unfortunately, perhaps, fungi had been more or less excluded as a possible cause; first, on a basis of logic (whose premises were faulty), and second, on a basis of examination of sick seeds with the unaided eye by those entirely unfamiliar with fungi (48). Sick or germ-damaged seeds are detected only by removing the pericarps that cover the germ, to expose the germ surface. Examination of such seeds with the unaided eye is scarcely a method to which a mycologist would give any credence in determining whether fungi are or are not involved.

In recent years it has been found that the germs of seeds that have become sick in commercial storage are almost invariably heavily invaded by storage molds. In many cases the fungi cover the germ surface with masses of mycelium and spores. Inoculated into seeds which then are stored at moisture contents that permit

[3] G. C. Papavizas. Unpublished evidence, Dept. of Plant Pathology, University of Minnesota.

the molds to develop, these fungi cause typical sick seed (52). There now seems no doubt that, in practice, sick wheat commonly is produced by invasion of the germs of the seeds by storage molds. In the laboratory, what appears to be typical sick wheat (judged by brown or black color of the germs) has been produced by storing seed under carbon dioxide, under nitrogen, at temperatures of 35° C. or above and by overexposure to fumigants. Sick seed so produced is not likely to have the biochemical properties—high fatty acid content especially—of the sick seed encountered in commercial storage, and of the sick seed produced as a result of invasion by fungi. It has been supposed that there may be a number of types of sick wheat, one caused by invasion by fungi, others by other factors. All of the evidence from hundreds of samples obtained from commercial bins over the past few years, however, suggests that sick wheat normally is a product of invasion of the germs by species of *Aspergillus;* if other factors ever are involved, they have not yet been detected, and they presumably are of minor significance in producing sick wheat in commercial bulks of wheat. Recent evidence with corn indicates that there, also, invasion of the germ by fungi is the principal cause of development of dark germs. Had the germs of sick seed been inspected with the aid of a stereoscopic microscope and by men acquainted with fungi, when this sort of damage first became an important factor in grading, some 20 years or more ago, it is very likely that molds would at that time have been at least suspected to be a cause of the trouble.

BIOCHEMICAL CHANGES. A variety of biochemical changes accompany or follow invasion of stored seeds by fungi (3, 30, 32–34, 41). The more obvious are increase in fatty acids, increase in reducing sugars, decrease in non-reducing sugars, and increased respiration. At one time these changes were assumed to be produced mainly or solely by the activity of seed enzymes, but as microbiological and biochemical studies were undertaken together, the importance of fungi was gradually recognized, and now these changes are attributed mainly or solely to fungi. As stated recently (5), " The fat acidity and sugar values . . . show that the changes in these properties upon storage are associated with the growth of microorganisms rather than with the metabolic activity of the wheat itself ".

Attempts have been made to use the quantity of fatty acids present as a measure of deterioration and of grain quality. In rather extensive tests with stored corn it was found that if moist stored corn was aerated there was a fairly good correlation between increase in fatty acids and increase in storage molds (22), at least for so short a time as three weeks. However, if the corn was stored under essentially anaerobic conditions, certain molds increased without any appreciable increase in fatty acids. Different storage molds convert fats into fatty acids at different rates, and some consume fatty acids, being able to use them as their sole source of carbon (22). For these reasons it seems doubtful that the amount of fatty acids in a given sample would furnish any accurate evidence as to its microbiological history.

Almost no studies have yet been made on biochemical changes induced by some of the most common and prevalent storage molds in the *A. glaucus* group which grow in grain at moisture contents of 13.5 to 15 per cent. It is hoped that work eventually will be undertaken to determine some of the outstanding physiological characteristics of *A. restrictus, A. repens, A. amstelodami* and *A. ruber* under conditions that approach those at which these fungi grow in bins.

INCREASE IN MOISTURE CONTENT. As molds grow in stored grain they convert into water a part of the material they consume. In large bulks of grain this means that if molds are growing even slowly in certain portions of the bulk, they will contribute some moisture to the portion of the bulk where they are growing or, if the moisture is transferred by convection or diffusion, to other portions of the bulk. Even in laboratory tests in which moist grain in small lots is continually aerated with air of a given relative humidity, that is, in equilibrium with the original moisture content of the stored grain, the moisture content of the seed is likely to increase as the seed is invaded by molds (34). We do not know whether this is a factor of importance in commercial bulks, but it would be desirable to find out.

HEATING. The fact that microorganisms growing in organic materials can cause an increase in temperature was known before 1900 (10). Rather extensive tests of the growth of fungi, yeasts and bacteria in relation to the heating of materials, such as corn meal and straw, were made in the 1920's (28) and showed that rapidly growing microorganisms are capable of producing large amounts of heat. As long ago as 1912 there was fairly conclusive evidence (14) that moldy seeds produce more heat than non-moldy seeds when moistened sufficiently to germinate. In 1930 (19) molds were shown to cause heating of stored corn; this is amply substantiated by later work (9). Studies such as these apparently were unknown to, or disregarded by, practical men interested in grain storage, and it was widely believed that heating which occurred in the absence of insects was caused by the grain itself. Later studies with adiabatic devices (34, 41) proved that the respiration of the seeds themselves, at moisture contents below those necessary for germination, and at moisture contents ordinarily encountered in storage, does not contribute significantly to heating. To quote a modern view (5): "The data indicate that the heating of moist wheat in storage can be entirely accounted for by the energy released in the respiration of fungi present on and in the wheat kernels".

Heating of bulk stored seeds is caused by rapid increase in the quantity of certain insects among the seeds up to a temperature of 105 to 110° F. (about 40 to 43° C.), above which the insects cannot survive (12). Where insects have been eliminated, as is true of much grain stored in terminals, the sole cause of heating is the growth of storage molds in the grain.

Detectable heating often is considered by those who store bulk grain as the first evidence of deterioration, and commercial operators often assume that if a given bulk of grain is not heating (according to their methods of detecting heating) it is not deteriorating. This is not necessarily true.

Relatively dry grain is a good insulator, about one third as good as cork (37). Thus any heating that may occur is detectable only if it occurs in the immediate vicinity of the temperature-sensing element. In stored hay (38) the temperature in one portion of a bulk only one meter from the thermocouple was 90° F. higher than that at the thermocouple. Temperature detection cables often are at or near the center of bins; in a bin 15 feet in diameter, 25 per cent of the grain is in the outer foot, and 45 per cent of the grain is in the outer two feet. In a bin 21 feet in diameter, 18 per cent of the grain is in the outer foot, and 34 per cent in the outer two feet; and in a bin 30 feet in diameter, 13 per cent of the grain is in the outer foot, and 25 per cent in the outer two feet. In the outer portions of many bins rather drastic changes may occur, and considerable deterioration develop, before there are temperature changes at cables near the center.

Also, heating is not always, nor perhaps even usually, a good criterion of deterioration of stored grain by molds. Most of the deterioration caused by molds in commercial storage probably occurs without any detectable heating. For example, we stored wheat at moisture contents of 14 to 16 per cent in vacuum bottles wrapped in a layer of insulation about a foot thick, and there was no detectable rise in temperature within the grain, although it was slowly invaded, killed and discolored by *A. restrictus, A. repens, A. ruber* and *A. amstelodami*. At these moisture contents the fungi grew too slowly to produce any detectable rise in temperature. The actual heat and moisture produced by these fungi growing in wheat at a moisture content of 14 to 15 per cent never have been measured. We do know, however, from actual tests in bins, in which samples have been taped onto temperature cables when the bins were filled and recovered when the bins were emptied, that severe deterioration of the grain may occur without any detectable rise in temperature. Heating is likely to be the final and violent effect of mold invasion of the seed, not an indication of beginning deterioration. There are also other complications. It recently was stated (5): "*A. glaucus* sometimes produced different rates of respiration and heating on this moist dead wheat. These differences were associated with the type of spores produced. Production of ascospores was associated with moderate oxygen uptake and very little heat production. The appearance of conidia, on the other hand, coincided with higher respiratory measurements and more heating". As ordinarily measured in stored grain, heating is, in many cases, less a warning of impending trouble than proof that trouble already has developed.

CONTROL OF MOLDS

The three general methods that have been suggested or tested, either in the laboratory or in practice, to control the growth of storage molds in moist grain have been the use of fungicides, storage under toxic or inert gases, and drying the grain to reduce the moisture content to a safe level.

FUNGICIDES. There is some evidence that certain fungicides (chlorinated phenols), when added to hay in concentrations not obviously toxic to domestic animals, will control, or at least reduce, the molding of the hay (15). Even if this were permissible, adding chlorinated phenols to grain probably would not add to the sales appeal of the products. More than 100 fungicides were tested on wheat stored in the laboratory at moisture contents of 16 to 25 per cent (35); most of those that controlled the molds killed the seed; some of them reduced external growth and sporulation of the fungi and thus kept the seed outwardly clean, but allowed molds to grow internally; some had a differential effect on some of the molds, inhibiting certain species but not others. It has been claimed (40) that microbiological activity in moist stored seed was completely eliminated by treating the seeds with Ceresan-M (ethyl mercury phosphate, 3 per cent; inert materials, 97 per cent). No details were given as to how the seeds were tested for molds after storage. We have encountered cases in which a fungicide applied to seeds did not suppress molds on and in the seed during storage, but, when the seeds were cultured on agar afterwards, the fungicide prevented the fungi from growing out onto the agar. That is, the fungicide was not toxic to fungi on the stored seeds but was toxic to them on agar. Fungicides that depend for their effectiveness upon being dissolved in water are not likely to be toxic at seed moisture contents of 15 to 20 per cent because no free water is available. In tests of the effect of fungicides on molds it is essential to inactivate the fungicide, at the end of the storage tests, before the seeds are cultured. Evidence is now available (36) that Ceresan-M applied, in the concentrations reported above, to seeds that are stored for some time, has no fungicidal effect whatever at moisture contents below those where free water is available. This is supported by unpublished work in our own laboratory.

STORAGE UNDER TOXIC OR INERT GASES. Storage under carbon dioxide has been proposed as a relatively easy means of controlling the growth of molds in stored grain; it has been used in England (26) and France, and to some extent for rice in this country (49). A considerable body of data will be required before we are in a position to determine under what circumstances such storage will be effective and practicable.

At present the only effective method for control of molds in long-time storage is to keep the grain at a moisture content so low that no portion of the bulk is moist enough to permit damaging invasion by storage molds. As indicated above, some hazards may be involved in mixing lots of different moisture content to achieve a theoretical average. Also moisture may, for one reason or another, become unevenly distributed in the bulk of grain with time, or the grain, once dry, may pick up water. Thus control of moisture content is not simply a matter of drying the grain and leaving it in dead storage. Data should be obtained on such grain at intervals and in various portions of the bulk, to determine whether changes in moisture content, mold development, and biochemical and processing characters are taking place, which are of sufficient magnitude to endanger the quality. Under present practices much grain is needlessly shifted from one bin to another, while some which should be shifted is not. Tests that would furnish data on some of the essential characteristics within the bulk would permit more rational and economical handling of grain.

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