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ECOLOGY OF MICROFUNGI

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INTRODUCTION

Many microfungi develop on stored organic materials. These fungi decompose organic substrata with the aid of secreted enzymes. The development of fungi on various products, on the one hand, results in huge losses the true dimensions of which are difficult to establish. Some idea of the energy of decomposition caused by the action of these fungi can be gathered from the findings of Nagel and Semeniuk (1947). They determined that corn infected with *Aspergillus flavus* Link over a four-week period at a humidity of 32 per cent lost 44 per cent of its dry weight and 25 per cent of its nitrogen, whereas corn infected with *Aspergillus niger* van Tieghem and *Penicillium ehrysogenum* Thom over the same length of time lost 39 per cent of its dry weight and 16 per cent of its nitrogen. On the other hand, fungi are important ecological factors because they break down dead organic materials to liberate carbon, nitrogen, phosphorus, and other elements, as well as water. They are also utilized in industry to obtain organic acids, alcohol, antibiotics, and various food products. It is therefore difficult to classify them as either harmful or beneficial.

Most microfungi, usually known as molds, are cosmopolitan and can be found wherever man has stored his supply of organic materials.

A decisive role in the development of these fungi is played by such factors

as temperature, humidity, intensity of light, the chemical composition and reactivity of substrata, composition of the atmosphere, and others.

TEMPERATURE EFFECTS ON THE DEVELOPMENT OF FUNGI

As noted by Wolf and Wolf (1947), temperature cannot be separated from such other ecological factors as relative humidity of air, chemical composition and reactivity of substrata, etc., since the effects of temperature are expressed within the ecological complex.

As a rule fungi are more tolerant of low than high temperatures since the latter coagulate cell proteins. Most fungi develop at temperatures from 5° to 35° C., with the optimum being 25° to 35° C.

As Deverall (1965) noted, *Neurospora crassa* Shear & Dodge at 4.5° C. grows at a rate of 0.07 mm. per hour, whereas above 35.7° C. its growth stops. He explains this inability of fungi to develop at certain temperatures by their inability at these temperatures to synthesize certain essential vitamins and amino acids.

With regard to temperature, fungi as well as other microorganisms are divided into psychrophile, mesophile, and thermophile. Since this classification does not reflect the dynamics of interrelation of organisms with their natural environment, Panasenko (1944) considers as more correct a terminology of psychrotolerant, mesothermotolerant, and thermotolerant. The first group would include those species of fungi which can develop at temperatures below 0° C. The existence of this group was established by Schmidt-Nielsen (1902), and it was later extensively studied in conjunction with the storage of food products at low temperatures. This group was studied also by Brooks and Hansford (1923), Berry and Magoon (1934), Gieske (1938), Semeniuk and Ball (1937), Chistjakoff and Bocharova (1938), Panasenko and Tatarenko (1940).

Some of the cited authors, however, consider as psychrotolerant the fungi which do not develop below 0° C., such as species of *Aspergillus* and *Mucor pusillus* Lindt. Panasenko and Tatarenko, studying psychrotolerant fungi of food products in the Kharkiv city freezer (Ukraine), obtained 120 pure cultures which were identified as representing 40 species of which 65 per cent belonged to the genus *Penicillium.* For most of these fungi minimal developmental temperatures ranged from -2° to -4° C. Isolates from the air of the subsidiary rooms did not belong to the psychrotolerant group.

Garric (1965), studying the flora of discolored snow, found among the algal "snow flora" three microscopic fungi: *Chionaster nivalis* (Bohlin) Wille, *C. bicornis* Kol, and *Selenotila nivalis* Lagerheim. These species have a very simplified morphological structure, with little branched, short mycelia on which were formed conidia without clearly defined conidiophores. According to Panasenko (1944), low temperature either completely inhibits the development of fungi, or else it alters their cultural and morphological characteristics. For example, *Penicillium chrysogenum, P. italicum* Wehmer, and *P. puberulum* Bainier at 0° C. form white, floccose, almost conidialess colonies. P. *italicum* develops good coremia at 5° C. but none at 20° to 25° C. *Fusarium tricinctum* (Corda) Saccardo at 0° C. in two and a half months formed only a weakly

developed plectenchymic stroma without aerial mycelium or conidia. Thamni $dium$ elegans Link at -2° C. in 25 days formed very low colonies. Its sporangiophores instead of being dichotomous were monopodially branched, and instead of normal sporangioles it had formed sporangia 2-2.5 times as large and containing numerous spores. Thus, adaptation to lower temperatures led to evolutionary regressive changes.

Fungi of the mesothermotolerant group develop within temperature limits of 5° to 35° C, with an optimum of 20° to 25° C. Most fungi belong to this group.

The thermotolerant group includes fungi which develop at temperatures of 20° to 60° C. This numerically small group of fungi is of great economic importance: some species tend to raise the temperature of stored hay, grain, cotton, etc., sometimes to 55° to 60° C. Losses run into millions of dollars.

Fungi of this group are used industrially to manufacture compost, to ferment tobacco, cocoa, etc. Some of them cause diseases of men and livestock. Cooney and Emerson (1964) in their monograph indicate 13 species of fungi in this group. They state that thermotolerant fungi do not develop at temperatures lower than 20° C.

Thermal tolerance is better known among bacteria than among fungi. Clegg and Jacobs (1953) report that Bartolomew and Rittenberg had isolated thermotolerant bacterial cultures from ocean bottom deposits where the temperature is always below 10° C. This indicates that under certain natural conditions a "thermophile" can exist as a "mesophile."

Morrison and Tanner (1922, 1924) arrived at the conclusion that thermophilic bacteria can develop at low temperatures if they are kept at these temperatures for a sufficient length of time.

It may be assumed that the adaptation to high temperatures is reversible in some species under certain condition. Such species as *Aspergillus fumigatus* Fresenius, *Absidia liahtheimii* (Lucet & Constantin) Lendner, or *Paecilornyaes varioti* Bainier, show thermotolerant adaptation quite clearly, whereas it is less readily seen in other species and after a short time becomes difficult to observe at all.

Sie, Sobotka, and Baker (1961), working with thermotolerant aerobic bacteria, established that the bacterial cells in an active growth stage create some "factors" which can change mesophilic properties to thermophilic ones. It is sufficient to add 5 micrograms of such a factor to 1 ml. of medium to cause such alteration. This induced thermotolerance then becomes inherited over an indefinite period of time. According to the authors this factor is of nucleoproteinic nature. It can be deactivated by the action of deoxyribonuclease, ribonuclease, pepsin, and papain, but tripsin, chemotripsin, and carboxypeptidase do not affect it. The factor is not stable in aqueous solutions and can tolerate one minute of exposure to 100° C. temperature. Addition of *Saccharomyces cerevisiae* Hansen extract to the medium also makes possible the growth of mesotolerants at 55° C.; however, this induced thermotolerance is not inherited.

Temperature plays an important role in the development of fungi, acting not only on their biochemical processes but also on their morphological and colony characters such as pigmentation, type of sporulation, etc. Temperature limits for vegetative development of fungi are much wider than those for sporulation.

Weimer and Harter (1923) determined that the temperature limits for the development of eleven species of *Rhizopus* were from 1.5° to 44° C., whereas the temperature parameters for their sporulation were from 10° to $30-35^{\circ}$ C.

The germination of fungal spores depends especially on temperature. Ames (1915) states that spores of *Rhizopus stolonifer* (Ehrenberg ex Fries) Lind at $3^{\circ} - 4^{\circ}$ C. germinated in 200 hours, at 20° C. they germinated in 16 hours, and at 37.5 \degree C. in six hours. Conidia of *Penicillium digitatum* Saccardo at $3\degree$ - 4 \degree C. germinated in 90 hours while at 20° C. they germinated in eight hours. Conidia of *Trichothecium roseum* Link at $9^{\circ} - 10^{\circ}$ C. germinated in 96 hours, while at 20° C. it took them seven hours.

Pierson (1966), investigating the influence of temperature on the growth of *Rhizopus stolonifer,* determined that low temperature can be detrimental to germinated spores. According to his data, the rate of growth of this fungus at 18.3 \degree C. was 1.68 mm. per hour. When it was kept for seven days at $0\degree$ C. it did not show evidence of further growth for two days after being transferred to a higher temperature, and on the third day it grew at the rate of 0.42 mm. per hour.

In some fungi the type of sporulation depends on temperature. According to Coy and Tuveson (1964), *Aspergillus rugulosus* Thom & Raper at 37° C. forms numerous cleistocarps but no conidia. At 25° C. it does not form any cleistocarps but develops numerous green conidia. A culture grown from these conidia at 37° C. again forms numerous cleistocarps.

Concerning the dependence of pigmentation on temperature, Carbone and Johnson (1964) determined that *Aspergillus urnbrosus* Bainier & Sartory, having an optimal growth temperature of 20° – 24° C., forms the greatest amount of pigment per gram of mycelium at $16^{\circ} - 20^{\circ}$ C.

Cardinal temperature points of fungi depend on the substratum on which the fungi are forming. Christensen (1957) indicates that certain strains of *Aspergillus amstelodami (Mangin)* Thorn & Church, *A. repens* de Bary, and *A. ruber* (Konig, Spiek., & Brem.) Thorn & Church on wheat with a moisture content of 15 - 16 per cent (wet-weight basis) can develop at temperatures as high as $40^{\circ} - 45^{\circ}$ C, whereas on agar media the maximal temperature is 35° – 37° C. Some strains of *Aspergillus candidus* Link can grow on grain at a temperature of 55 $^{\circ}$ C., whereas *A. flavus* develops on this substratum at 45 $^{\circ}$ C.

Cardinal temperature points are closely related to relative humidity. According to Bonnet (1948), *Aspergillus niger* at 93 per cent relative humidity has an optimal temperature of 40° C., but at 100 per cent relative humidity the optimal temperature is less than 30° C. Thus, cardinal temperatures are of relative significance. In Table I Panasenko (1944) gives the cardinal temperatures for the development of some species of fungi.

Fungi as well as their spores are extremely sensitive to subminimal and supramaximal temperatures which either lower their viability or kill them outright. Smith, Miller, and Bassett (1965) placed germinated and nongerminated spores of *Rhizopus stolonifer* on Potato-Dextrose Agar (PDA) at -1.1° , 0°, 2.2°, 3.3°, and 4.4° C. for periods of 2.5, 7, and 10 days, respectively.

CARDINAL IEMPERATURES OF SOME SFECIES OF FUNOI, IN DEOREES CENTIORADE			
Species	Minimal	Optimal	Maximal
Aspergillus amylovorus	3	$20 - 25$	$35 - 37$
A. candidus	$3 - 4$	$20 - 24$	$40 - 42$
A. clavatus	$5 - 6$	$20 - 25$	42
A. flavus	$6 - 8$	$35 - 37$	$42 - 45$
A. fumigatus	$10 - 12$	37	$52 - 55$
A. glaucus	$4 - 5$	$24 - 25$	37
A. nidulans	$6 - 8$	$35 - 37$	$46 - 48$
A. niger	$6 - 8$	$35 - 37$	$45 - 47$
A. oryzae	$7 - 9$	$35 - 37$	$45 - 47$
A. repens	$4 - 5$	$25 - 27$	$38 - 40$
A. versicolor	$4 - 5$	$25 - 30$	$38 - 40$
Alternaria tenuis	0	$20 - 25$	
Botrytis cinerea	-2	$22 - 25$	$30 - 33$
Beauveria bassiana (Bals.) Vuillemin	$3 - 5$	$25 - 27$	$36 - 38$
Cladosporium herbarum	$-5, -7$	$24 - 25$	$30 - 32$
Mucor erectus Bainier	$3 - 4$	$20 - 25$	$30 - 32$
M. griseo-ochraceus Naumov	$4 - 6$	$20 - 25$	35
M. plumbeus Bonorden	$4 - 5$	$20 - 25$	35
M. pusillus	20	$37 - 40$	$56 - 58$
M. racemosus	$-3, -4$	$20 - 25$	$30 - 33$
Penicillium arenarium Shaposhnikov & Manteifel	-2	$35 - 40$	$45 - 48$
P. casiae Staub	-3	$24 - 25$	$30 - 35$
P. chrysogenum	-4	$25 - 28$	$32 - 33$
P. digitatum	-3	$20 - 25$	$32 - 35$
P. expansum	-3	$25 - 26$	$33 - 35$
P. glauco-griseum Sopp	-3	$20 - 24$	37
P. italicum	-3	$22 - 24$	$32 - 34$
P. luteum	-2	$20 - 24$	35
Rhizopus arrhizus Fischer	$6 - 8$	$35 - 36$	$43 - 44$
R. stolonifer	$5 - 6$	$26 - 29$	$32 - 34$
R. nodosus Namyslowski	$5 - 6$	$36 - 38$	$43 - 45$
R. oryzae Went & Gerlings	$7 - 9$	$36 - 38$	$43 - 45$
Sclerotinia libertiana Fuckel	-1	$25 - 27$	$37 - 40$
Stachybotrys atra Corda	$2 - 3$	$25 - 27$	$37 - 40$

TABLE I

TEMPERATURES OF SOME SPECIES OF FUNGI¹ IN DEGREES CENTIGRADE

After such an exposure the spores were transferred to a temperature of 23.9° C. and were compared to a control which had been kept at 23.9° C. all along. **Exposure of non-germinated spores for 2.5 days had no effect on the ger**minating percentage. Seven days' exposure to temperatures of -1.1° , 0° , and **3.3 ~ C. decreased the quantity of germinating spores. Those which had already** germinated were much more sensitive. Spores which had been kept at -1.1° C. for 2.5 days did not grow any further after they had been transferred to 23.9° C. Germinated spores that were exposed to 0° and 2.2° C. for seven days all had **been killed also. On the whole, low temperatures are more suited for preserving viability of microorganisms since they only slow up life processes.**

Greaves and Jones (1944) investigated the influence of temperature on microbial soil flora. They kept four types of soil at 10°, 20°, 30°, and 40° C. **for 24 months. During this time many microorganisms developed in the soil** that had been kept at 10° C., but were less frequent in the soil that had been kept at 40° C.

Fungi and their spores are most sensitive to supramaximal temperatures. Smith, Miller, and Bassett's (1965) report of *Rhizopus stolonifer* spores which had been heated for five minutes at 49° C. in bouillon, indicated that only 18 per cent germinated. Of spores which had been heated at 54° C. for two minutes, only 2 per cent germinated.

Lethal action of heated air on spores of *R. stolonifer*, according to Smith, is dependent on the relative humidity of the air. Heating them for 30 minutes at 49° C. and 50 per cent relative humidity resulted in 90 per cent of all spores germinating, whereas heat at 54° C. resulted in 83 per cent germination. However, 30 minutes heating at 47° C. and 30 per cent relative humidity resulted in only 11 per cent germination, and at 54° C. only 2 per cent.

Mazur (1956) showed that the lethal action of low temperatures depends on speed of thawing. He exposed the conidia of *Aspergillus flavus* to temperatures as low as -70° C. When he thawed them at the rate of 0.9° C. per minute, only 7 per cent would be able to germinate. When the warming rate was 700° C. per minute, 75 per cent would germinate. He determined the lethal action of slow thawing to be most effective between 0° and -20° C. However, low temperature is suitable for storing living cultures. Deverall (1965) kept viable cultures of *Botrytis* sp. at -20° C. for a year.

Temperature determines not only the rate of development of fungi but also the special composition of a particular flora. Barton-Wright and Tomkins (1940) determined that at 5° C. and 97 per cent relative humidity, fungi appear on flour in 20–25 days, but at 20° C. they appear in 8–9 days.

Schnathorst and Halisky (1960) and Halisky, Schnathorst, and Shagrun (1961) reported that *Aspergillus flavus* and *A. niger* seldom appear on cotton bolls in Central California where summer temperatures are about 25° C.; but they are very widespread in Southern California where summer temperatures average 32° C. A similar relationship was noticed by Panasenko (1936). In the Ukraine, species of *Aspergillus* on cotton bolls were almost absent, but in Azerbaidzhan, where the summer temperatures were much higher, *Aspergillus niger, A. flavus,* and *A. [umigatus* were very widespread on cotton bolls.

EFFECTS OF HUMIDITY ON THE DEVELOPMENT OF FUNGI

Humidity has a greater effect on the development of fungi than temperature. Humidity limits within which growth of fungi is possible are not so wide as temperature limits. Thus, *Aspergillus niger* can develop at temperatures from $6^\circ - 45^\circ$ C. while its relative air humidity range is $88 - 100$ per cent. *Botrytis cinerea* Persoon has a temperature range from -2° to 33° C. and a humidity range of 92 – 100 per cent; *Rhizopus stolonifer* has a thermal range of 3° – 34° C., a relative humidity range of $92 - 100$ per cent.

Concerning the possibility of fungal development at various relative humidity levels, Heintzeller (1939) proposed the following groups: 1) xerophilic, develop at relative humidity levels below 80 per cent; 2) mesophilic, develop at relative humidity range of $80 - 90$ per cent; 3) hydrophilic, develop at relative humidity above 90 per cent.

Panasenko (1944) proposed the following groupings: 1) xerotolerant,

whose members can develop within a relative humidity range of $65 - 75$ per cent; 2) mesohygrotolerant, develop at relative humidity of $80 - 90$ per cent; 3) hygrotolerant, develop at relative humidity above 90 per cent.

There is especially close relationship between the relative humidity of the air and the germination of fungal spores. Snow (1949) investigated the germination of conidia of 22 species of fungi at variable relative humidities. According to his data, conidia of *Aspergillus echinulatus* (Delacr.) Thorn & Church at 66 per cent relative humidity germinated in one year, conidia of *A. tuber* at 71 per cent germinated in four months, conidia of *A. restrictus* Smith at 71 per cent germinated in 15 days, and conidia of *A. versicolor* (Vuill.) Tiraboschi at 78 per cent germinated in 30 days. Conidia of most species of *Peniciilium* need 85-90 per cent relative humidity for germination, whereas species of *Rhizopus, Botrytis, Cladosporiurn,* and *Trichothecium roseum* were able to germinate only at 90-100 per cent.

Armolik and Dickson (1956) indicate that germination of conidia of *Aspergillus glaucus* Link requires relative humidity of the air above 70 per

TABLE II RELATIVE HUMIDITY REQUIREMENTS OF SOME FUNGI FOR DEVELOPMENT AND FORMATION OF CONIDIA OR ASCI

cent, of *Penicillium* 79-80 per cent, and of *Fusarium moniliforme* Sheldon 87 per cent.

Teitel (1958), investigating the effects of relative humidity on the viability of conidia of *Aspergillus flavus,* determined that within a range of 32-73 per cent the conidia remain dormant. However, 75 per cent produces a startling effect on conidia of *A. flavus:* at this humidity level and with a temperature of 29° C., 82 per cent of the conidia had lost their viability by the sixth day, and after 13 days 100 per cent of the conidia evidenced a loss of viability. Teitel explains this by the fact that a 75 per cent relative humidity is just sufficient for germination of the conidia but not for their further growth, thus resulting in death. A similar effect on this fungus is shown by 81 per cent and 45 $^{\circ}$ C.

Humidity is very important for the viability of conidia of fungi. McCrea (1923) reported that he observed germination of conidia of *Aspergillus oryzae* (Ahlb.) Cohn and of the spores of *Rhizopus stolonifer* after keeping them for 22 years in a dehydrated condition.

Relative air humidity not only determines the germination of conidia but also has an important effect on the rate of mycelial growth. Tomkins (1929) gives the following data of relative humidity effects on mycelial growth of *Alternaria citri* Ellis & Pierce, *Trichothecium roseum,* and *Fusarium lateritium* Nees: at 100 per cent and 25° C. their rate of growth was 7.7 microns per hour, at 94 per cent it was 1 micron per hour, and at 91 per cent only 0.3 micron per hour. Panasenko (1944) reports the correlation between relative humidity and development of certain fungi in Table II. These relative humidity values, like most data of this type, were obtained under controlled laboratory conditions on nutritive media. Under natural conditions they may differ somewhat depending on variability and complication of environmental factors.

COMBINED EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON THE DEVELOPMENT OF FUNGI

The biological action of relative air humidity is a function of temperature. Groom and Panisset (1933), while investigating the influence of temperature and relative humidity on germination of conidia of *Penicillium chrysogenum,* established that at 10° C. and 83-85 per cent relative humidity, 1056 hours are needed for germination to occur. At the same relative humidity but at 30 $^{\circ}$ C., only 189 hours are needed. At a temperature of 10° C. and at 100 per cent relative humidity, 96 hours are needed, whereas at 30° C. and the same humidity germination occurred in 34 hours. In general, the higher the temperature at a given relative humidity the less time is required for germination to take place. However, this relationship does not hold true when the maximal temperatures for a given species are approached.

In the storage of various products and materials, the main role is played not by the water content of the product itself but rather by the hygroscopic capacity of the product and the relative humidity of the storage room; water content of the product wll change until a hygroscopic balance has been established. The subject of hygroscopic balance of different grains at various relative humidity levels has been studied by numerous investigators.

Coleman and Fellows (1925) examined the relationship between the moisture content of various grains and flax seed and the relative air humidity (at 30, 45, 60, 75, 90, and 100 per cent). Larmour, Sallans, and Craig (1944) determined the hygroscopic balance of sunflower seed, flax seed, and soybeans at 31, 43, 51, 62, 71.2, 81, and 93 per cent.

Christensen (1957) and Christensen and Lopez (1965) investigated this question in their studies. Table III represents the hygroscopic balance of

TABLE III THE MOISTURE CONTENT (IN PERCENTAGE) OF VARIOUS GRAINS AT

several kinds of grains at various relative humidity levels expressed on a dry weight basis.

Relative air humidity and temperature of storage spaces are the factors which determine the composition of the fungal flora on any type of product. Kennedy (1964), while investigating the effects of fungi on soybeans, determined that at a relative humidity of 62 per cent the soybeans over a period of 74 days acquired a water content of 10 per cent; at a level of 93 per cent this rose to 17 per cent over the same length of time, whereas in 150 days this content had risen to 22 per cent.

Koehler (1938) determined that on corn, which had a moisture content of 14.3 per cent, only species of the *Aspergillus glaucus* group developed, whereas when the moisture content had been increased to 18 per cent *A. flavus* and A. *niger* were also observed. *Penicillium notatum* Westling developed on corn with a moisture content of 16.7 per cent; *P. palitans* Westling was observed at a moisture content of 19.5 per cent.

Barton-Wright and Tomkins (1940) reported that on flour, bran, and grits, which had a moisture content of 13 per cent, *Aspergillus repens* developed, but when the moisture content was increased to 16 per cent *A. niger* appeared. At 18 per cent, which corresponds to 90 per cent relative humidity, *Trichoderma* and *Botrytis* were observed.

Tuite and Christensen (1955, 1957), studying the storage of barley and wheat, determined that when the moisture content of the grain is 13 per cent no

fungi develop on it; if the moisture content is raised to 13.6 per cent, then *Aspergillus restrictus* and *A. arnstelodami* appear, followed by *A. repens* and *A. tuber* when the moisture content is 15-16 per cent. As a result of the biochemical action of these fungi, the moisture content rises even higher, enabling *A. candidus, A. flavus,* and other fungi to develop.

Christensen (1957) determined that such fungi as *Aspergillus repens, A. amstelodami,* and *A. ruber,* which do not appear on agar media at 35[°] C., will develop on wheat with a moisture content of 15-16 per cent at a temperature of 45° C. Papavizas and Christensen (1958) showed that wheat with a moisture content of 15-15.5 per cent at a temperature of 5° or 10° C. can be stored for as long as 12 months without any fungi appearing on it and without decreasing its germination potential. However, if its moisture content is raised to 16-16.5 per cent over the same length of time at the same temperatures, fungi develop and the percentage of germination decreases.

Qasem and Christensen (1958, 1960) reported that in their investigations corn with a moisture content of 12 per cent was stored for eight months at 5° , 10° , 15° , 20° , and 25° C. without any fungal formation. On corn with a moisture content of 14 per cent *Aspergillus repens* developed exclusively, whereas on grain with a moisture content of 16 per cent *A. candidus* developed. When the moisture content was raised to 18 per cent *A. flavus* also appeared. All of these fungi formed at temperatures above 10° C.

Christensen and Lopez (1965) investigated the development of fungi on rice in relationship to moisture content of the rice. According to their data, at 13-13.8 per cent moisture content *Aspergillus restrictus* developed on rice, at 14.5-15.5 per cent *A. repens* and *A. tuber* appeared, and at 17 per cent A. *candidus* and *A. ochraceus* Wilhelm were reported. Grain with this last moisture content, after being stored for 420 days, had lost all viability.

Fields and King (1962) investigated the roles of temperature and moisture in the development of certain species of fungi on stored peas. They started with fungus-free peas having a moisture content of 10 per cent and exposed them to temperatures of 10° , 20° , and 30° C. at relative air humidities of 75.5, 85.5, and 92.5 per cent. After some time, peas at 75.5 per cent acquired 15 per cent moisture content, at 85.5 per cent a moisture content of 17 per cent, and at 92.5 per cent a level of 21 per cent moisture. The peas were inoculated with *Aspergillus flavus, A. tuber, A. amstelodami, A. candidus,* and *A. restrictus. The* peas were stored for six months. They determined that of five *Aspergillus* species *A. flavus* was the most pathogenic to the peas and *A. restrictus* the least. Other species were about equally pathogenic. The most unfavorable storage conditions turned out to be at 30° C. with the relative humidity over 85 per cent. Under these conditions the peas lost all ability to germinate in three to six months; the uninoculated controls germinated to an extent of 97 per cent.

Kennedy (1964) determined that moving air at 90 per cent relative humidity increased the moisture content of soybeans to 20 per cent four times taster than did non-moving air at 93 per cent. Aeration of grain, raising the respiratory rate, increases its moisture content and thus its susceptibility to fungal infection.

The role of fungi in the process of heating was studied by numerous

researchers. Carter and Young (1950) thoroughly investigated heating of wheat, and according to their data losses in the United States alone average two million dollars. They determined that this process begins in wheat at a moisture content of 14 per cent with the appearance of *Aspergillus* species of the *glaucus* group. When the moisture content rises, *A. flavus, A. candidus,* and *A. niger* also appear. Inoculating sterile wheat at a moisture content of 20 per cent with pure cultures of *A. glaucus, A. candidus, A. flavus,* and *A. niger,* Carter and Young determined the role of each of these species in the process of spontaneous heating of wheat. The most active was found to be *A. candidus* which raises the temperature of the wheat $20^{\circ} - 26^{\circ}$ C. above that of the storage room, up to $50^{\circ}-55^{\circ}$ C. A. *candidus* was also the most active consumer of $oxygen$, utilizing up to 112.7 mm.³ per gram of dry weight. Less heat-productive were *A. flavus* and *A. niger*, which raised the temperature 13.7° C. and 15.3° C. above that of the storage area. The least heat-productive was *A. glaucus,* raising the temperature of wheat 11.9° C. above the storage room temperature.

Christensen and Gordon (1948) determined that corn with a moisture content of 22 per cent after 12 days heated up to 50° C. as a result of the development of *Aspergillus glaucus* and *A. candidus.* Corn with a moisture content of 26 per cent in 12 days reached the temperature of 53° C. as the result of development of *A. fumigatus.*

Gaskill and Gilman (1939) investigated the role of nitrogen in fungal thermogenesis. They showed that adding nitrogen to alfalfa hay increased the amount of heat generated and the loss of dry weight of hay.

Milner and Geddes (1946a, b), investigating the storage of soybeans with moisture contents of 14 and 17 per cent, found it infested with *Aspergillus glaucus* and *A. flavus* which raised the respiratory level and increased the temperature to 50° -55° C. This process of heating destroyed all sugars in the soybeans.

Block (1953) established that fungi develop at different relative humidities on different materials, i.e., on leather at 76 per cent, on hide at 80 per cent, on wool at 88 per cent; on cotton fungi develop only to a slight extent at 96 per cent relative humidity.

EFFECTS OF LIGHT AND RADIATION ON THE DEVELOPMENT OF FUNGI

Light energy affects organisms only when it is absorbed by a certain photoreceptor pigment. The absorbed quantity of light activates an electron in the pigment which causes a chemical chain reaction. The character of a photochemical reaction depends on the type of pigment as well as the types of enzymes connected with it. The most commonly encountered pigments in fungi are carotenes which may be yellow, orange, or red. Carotenes are most effective in absorbing the blue band of the light spectrum.

There are numerous frequently contrasting views in the literature regarding the role of light in the development of fungi. Christensen (1961) expressed the opinion that light is a secondary factor since the species grow equally well in light and in the dark.

Barnett and Lilly (1950) showed that while light is of little importance to mycelial growth of *Choanephora cucurbitarum* (Berkeley & Ravenel) Thaxter, it does regulate the formation on this fungus of conidia which do not develop in either uninterrupted light or darkness but require alternating periods of both. Page (1965) demonstrated that light inhibits the mycelial growth of *Coprings lagopus (Fries)* Fries, the mycelium of which when exposed to light continues to grow for only 5-10 minutes. When it is returned to a darkened area growth is resumed.

Kaiser (1964), investigating the influence of light on growth and sporulation of *Verticillium albo-atrum* Reinke & Berthold, demonstrated the effect of various bands of color of the spectrum on the development of this fungus. The mycelium developed intensely under the influence of blue, green, and white light. However, it did not form any microsclerotia under blue light, but rather under yellow, orange-yellow, and red light, as well as in darkness. Conidia were formed in great abundance under blue light.

Tatarenko (1954) confirms that light inhibits the mycelial growth and the ascogenous stage formation of *Aspergillus* and *Penicillium,* but stimulates the production of conidia. According to her observations, cultures which had been kept in darkness for a long time had lost the ability to form conidia. Her data show that the inhibitory light band is blue, whereas Page (1965) indicates that it is the blue band which stimulates the development of fungi.

Zaccharia, Hansen, and Snyder (1956), studying environmental influences on cultures of *Fusariurn,* determined that all of their species formed more mycelium in darkness than in light, but such cultural features as color, zonation, type of colonies, presence or absence of sporodochia, size and shape of macroconidia, number of septations in them, and the appearance of perithecia depend on light. Pigments are formed by some species better in darkness, by others in light.

Reid (1958) also confirms that light inhibits the development of mycelium of *Fusarium,* but stimulates the formation of conidia. Growing *Fusarium* in darkness and then exposing it to light, he noticed that lowering the intensity of the light decreases the number of macroconidia. A similar manifestation was established by Riedhart and Porter (1958) for *Penicillium herquei* Bainier & Sartory: light inhibited its mycelial growth but stimulated conidial formation.

Aragaki (1964) observed that light and higher temperatures inhibited conidial formation in species of *Alternaria, Sternphylium,* and *Helminthosporium.* However, *Aspergillus niger, A. oryzae,* and *Rhizopus stolonifer* at 20[°]-27[°] C. form spores equally well in light and in darkness.

Miller and Reid (1961) investigated light stimulation on conidial formation by *Trichoderma lignorurn* (Tode) Harz. They found the violet and blue bands of the spectrum to be especially effective, the green band less so, and the yellow and orange bands completely ineffective. A similar effect of light on sporulation of *T. viride* Link ex Fries was observed by Galun and Gressel (1966).

Lukens (1963) studied light influence on the development of conidiophores by *Alternaria solani* (Ellis & Martin) Sorauer which turned out to be positively

phototropic. There were far fewer conidiophores in darkness and they were diversely oriented. *A. solani* did not produce any conidia in light; their formation was stimulated only by the red band of the spectrum.

A completely opposite light effect on mycelial growth was revealed by the studies of Cantino and Horenstein (1956). They established that *Blastoeladiella emersonii* Kanouse grown under unvarying light conditions produced from 60 to 141 per cent more mycelial growth than in darkness.

The above data pertain to the visible part of the light spectrum with a range of waves of 4000-6000 Å. Numerous workers investigated the action of the invisible parts of the spectrum on fungal growth.

Leach (1959, 1961, 1962) irradiated 34 species of fungi with nearultraviolet rays (3100-4000 A.). This irradiation stimulated and speeded up sporulation in *Ascochyta pinodella* L. K. Jones, *A. pisi* Libert, *Alternaria ehrysanthemi* Schmidt, *A. tenuis* Nees, *A. zinniae* Pape, *Botrytis einerea, Fusar- ~um oxysporum* Schlechtendahl, *F. nivale* (Fries) Cesati, *F. solani* (Martius) Appel & Wollenweber, *F. roseum* (Link) Snyder & Hansen, *Stemphyliurn botryosurn* Wallroth, *Helminthosporium avenae* Eidam, and *H. oryzae* V. Breda de Haan. *H. oryzae* formed conidia when a period of darkness ensued after irradiation. *H. sativum* Pammel, King, & Bakke developed conidia well in both light and darkness. Alternating 12-hour periods of light and darkness resulted in zonation. Leach considers that near-ultraviolet light plays an important role in the process of sporulation.

Stevens (1928) studied the effects of ultraviolet light on cultures of *Glomerella cingulata (Stonem.)* Spaulding & vonSchrenk, and *Coniothyrium* sp. Irradiation of 15 seconds was lethal to aerial mycelium and spores of *Glomerella.* Whereas colonial growth was inhibited, perithecial formation was stimulated.

Ramsey and Bailey (1930), while irradiating with ultraviolet light the conidia of *Maarosporium* and *Fusarium,* determined that conidial pigmentation protects the conidia from the lethal action of ultraviolet radiation. The conidia of *Macrosporium* turned out to be more resistant than those of *Fusarium*. Ultraviolet irradiation of fungal conidia increased the rate of mutation.

Kosurina (1961), through irradiation of *Aspergillus niger* conidia by ultraviolet light of 2537 Å wavelength, obtained a white mutant with a small number of conidia. Grebeshova (1962) obtained a mutant of *A. niger* which had a high citric acid productivity and a heightened amylolytic activity. Imshenetskii and Ulyanova (1962), as a result of irradiation of *Fusarium moniliforme,* obtained a mutant which differed morphologically and biochemically from the original culture. Hollander *et al. (1945)* determined that for *Neurospora erassa* an ultraviolet radiation of 2650 A wavelength is most effective from a mutagenic viewpoint.

Hawker (1957), confirming the complexity of light effects on fungi, divides the species into four groups: 1) fungi which develop spores in darkness, but not in light; 2) fungi which form spores both in darkness and in light; 3) fungi which develop no spores in the dark; 4) fungi whose sporulation is inhibited by light at certain stages of their development.

THE INFLUENCE OF HIGH SUGAR AND SALT CONCENTRATION ON THE DEVELOPMENT OF FUNGI

Osmotolerant and halotolerant fungi are isolated most frequently from sugared or salted food products, from concentrated fruit juices, and from damp sugar and salt. Sugaring and salting of food products are old, well tried methods of food preservation that create ecological niches in which osmotolerant and halotolerant fungi can develop without competition.

The property of developing in high sugar and salt concentrations has been acquired by these fungi through an adaptation of their enzymes which enables them to function under conditions of high osmotic pressure. The greatest fungal tolerance to high concentrations of sugars and salts is found at optimal temperature and hydrogen-ion concentration (pH).

Ingram (1957) divides microorganisms according to their adaptation to high sugar and salt concentration as: non-tolerant, facultatively tolerant, and obligately tolerant. Ingram opposes loose employment of the terms "osmophilic" and "halophilic" and has expressed the doubt that obligately osmophilic yeast exist. Isolation of these fungi is rare.

Malevich (1936) isolated from salted fish a species of *Torula* which grew best on culture media with 15-20 per cent NaCI and which developed very well in 65-70 per cent sucrose solution. It did not form on media which contained less than 5 per cent NaC1.

Beyma (1933) isolated from crystals of NaC1 a fungus which he named Oospora halophila van Beyma since it developed only on those media which had a NaC1 content above 50 per cent.

Christensen, Papavizas, and Benjamin (1959) described *Aspergillus halophilicus* which was isolated from wheat and which did not develop on media with less than 10 per cent NaCl or 50 per cent sucrose, but did very well on media with 15 per cent NaC1 or 70 per cent sucrose. The fact that the fungus developed on wheat with a moisture content of 13-14 per cent indicates that the species is as xerotolerant as halotolerant.

More widely distributed are fungi which develop in high sugar concentrations. Browne (1918), studying the spoilage of Cuban raw sugar, isolated from it a series of fungi among which are encountered most frequently a *Torula* and two species of *Monilia* that he named *M. nigra* C. A. Browne and *M. [usca* C. A. Browne.

Rudakoff, Harzstein, and Sheveleva (1940) isolated from concentrated sweet milk *Torula pulchra* Tonywall which developed well at a sucrose concentration of 63 per cent but was inhibited by a sucrose content of 65.5 per cent.

Ingram (1950) isolated from concentrated orange juice a yeast belonging to *Zygosaccharomyces* which developed at a sugar concentration of 70 per cent. The species could ferment dextrose, levulose, and mannose but not sucrose.

Maltschewsky (1955b), investigating fungal damage to marmalade and candy, isolated from these materials nine species of fungi and tested their osmotolerance on Czapek's medium with sugar contents of 42, 45.5, 48.9, 52, 55.3, 58.4, 62, 64.6, and 67.5 per cent. All of the fungi grew for three weeks on all media up to and including 62 per cent. At a sugar level of 64.6 per cent only *Penicillium [requentans* Westling, *P. waksmani* Zaleski, and *Aspergillus flavus* developed well and formed conidia. At 67.5 per cent sugar content these three produced only a mycelium without any conidia, while the other fungi showed slight signs of development.

Tanaka and Miller (1963), investigating the spoilage of dried prunes in California, isolated yeast of the genera *Saccharomyces, Torulopsis, Candida,* and *Pichia,* and numerous filamentous fungi which belonged to the species *Aspergillus chevalieri* (Mangin) Thorn & Church, *A. repens, A. tuber, A. niger, Penicillium* spp., *Alternaria* spp., *Monilia* spp., *Chaetomella* sp., and *Mucor* sp. Of the isolated species, the most osmotolerant were of the *Aspergillus glaucus* group which developed at a sucrose concentration of 60 per cent. The others were less osmotolerant, with only one strain of *Mucor* developing well at 40 per cent sucrose.

As has been shown by indicated examples, osmotolerance and halotolerance among fungi are quite widespread. They are noticed, however, only when some fungus is placed in a corresponding ecological niche.

EFFECTS OF AERATION ON THE DEVELOPMENT OF FUNGI

Fungi are aerobic organisms and their development depends on the presence of air. Some fungi when they encounter partly anaerobic conditions are able to go over to an anaerobic mode of existence, oxidizing sugars and forming carbon dioxide, water, and various organic substances. In these circumstances fungi change morphologically.

Panasenko (1944) studied the development of 16 species of fungi in Czapek's liquid medium under a coating of sterile vaseline oil, and on malt-agar in an atmosphere of hydrogen in desiccator jars. In the liquid medium all fungi on the fifth day formed flocks of white mycelium without any indication of reproductive organs, and carbon dioxide blisters formed under the oil layer. After 16 days all species grew along the test tube walls above the oil layer and formed conidia.

In the hydrogen atmosphere after 35 days the best developed fungus was *Aspergillus clavatus* Desmazières; it formed on the agar surface a powdery growth which consisted of reduced conidiophores with sterigma but without conidia. *Mucor racemosus* Fresenius produced slimy colonies which by their external appearance resembled yeast colonies. Under microscopic investigation it was seen that the mycelium had numerous septations, sporangiophores were reduced, non-ramified, with reduced terminal sporangia which had two to three spores. All other fungi merely formed slimy colonies 4-5 mm. in diameter. It is noteworthy that a normal, completely developed colony of *Penieillium lilaeinum* Thorn, on a malt-agar medium in a test-tube, when placed into a hydrogen atmosphere turned into a slimy mass after 35 days, as a result of autolysis.

After 35 days all cultures were transferred from the hydrogen atmosphere to the incubation chamber at 25 ~ C. It was found that all species of *Peniaillium* survived but all species of *Aspergillus* were dead. *Penicillium expansum* Link developed normal coremia and conidia; *P. italicum* formed a white aerial mycelium but very few conidia; *P. lilaeinum* produced only a white aerial, sterile mycelium.

Brown and Kennedy (1966) reported that *Pythium* was alive but did not

grow after remaining for seven days in a nitrogen atmosphere. This fungus turned out to be very tolerant of a small amount of oxygen in the air.

Burges and Fenton (1953) consider that the distribution of fungi in the soil depends on their adaptation to partial anaerobic conditions and their tolerance of carbon dioxide. They differentiate the fungi into three groups: 1) completely aerobic, which develop on the surface of soil or not deeper than 5 cm. beneath the surface; 2) carbon-dioxide tolerant and partially anaerobic, which are found deep beneath the surface; and 3) indifferent to aeration, which are found at all soil depths. According to their data, carbon dioxide in a concentration of over 5 per cent inhibits the development of *Penidllium nigricans* (Bainier) Sartory but has little effect on *Zygorhynchus vuillerninii* Namyslowski.

Durbin (1955, 1959) also confirms that the distribution of fungi in soil depends on the concentration of carbon dioxide, which varies from 2 to 10 per cent.

Goos and Tschirsch (1962) investigated the toxicity of carbon dioxide for *Gloeosporium musarum* Cooke & Massee. They determined that in a carbondioxide atmosphere the colonies of this fungus were 2 cm. in diameter after 14 days, whereas in an atmosphere of nitrogen they were 7 cm., and in air, 9 cm.

Barnett and Lilly (1955) proved that the accumulation of carbon dioxide in enclosed cultures inhibits or completely stops sporulation of fungi. On the other hand, Cantino and Horenstein (1956) reported that carbon dioxide, far from inhibiting the development of the aquatic fungus *Blastocladiella emersonii,* actually stimulates it because the mycelium of this species is capable of fixing carbon dioxide in its biochemical action. Developing in light with carbon dioxide present in the medium, this fungus formed 100-150 per cent more mycelium than it did without the carbon dioxide. In darkness the presence of carbon dioxide increased the amount of mycelium by only 25 per cent. The presence of glucose in the medium decreased the carbon-dioxide fixation by this fungus.

Even more toxic to fungi is sulfur dioxide, explaining why it has been used for such a long time as a fungicide. Kvasnikov and Raev (1939) determined that conidia of *Aspergillus niger* died after being treated for a 30-minute period with a 0.05 per cent water solution of this gas.

Couey and Uota (1961) report that conidia of *Botrytis cinerea* perish from 100 p.p.m. SO2 at *96* per cent relative humidity of air, but at 75 per cent this concentration is not effective. Couey (1965) determined that 100-400 p.p.m. $SO₂$ at 90 per cent relative humidity lowers the viability of conidia of *Alternaria,* whereas at a lower relative humidity the concentration of SO_2 must be raised to 1000 p.p.m.

INFLUENCE OF REACTIVITY OF THE MEDIUM ON DEVELOPMENT OF FUNGI IN STORED PRODUCTS

Fungi develop in a constantly changing medium to whose changes they themselves contribute. This variability of media is reflected in the growth and functioning of the fungi. One of the variables in the environment is its hydrogen-ion concentration (pH). Fungi can develop in relatively wide pH ranges; however, their enzymatic activity which regulates their metabolism takes place within a much narrower range. Nobuyoshi (1951), while cultivating *Aspergillus oryzae,* determined that at pH 3.2-3.4 the fungus showed no amylolytic activity; at a pH of 4.8 such activity showed up to a considerable extent, but stopped at a pH of 6.2.

Most fungi can develop within pH limits of 2.0-10.2. The following data give the pH limits for some species:

Tanrikut and Vaughan (1951) cultivated *Sderotinia sderotiorum* on media ranging in pH from 2.0 to 10.0. The cultures developed well on media with a pH from 2.4 to 9.6. Growth was inhibited by a pH of 10.0 and completely stopped by a pH of 10.6.

The pH is quite important for formation and germination of fungal spores. Diener (1955) determined that *Stemphylium solani* forms conidia best in a pH range of 4.0 to 7.5. Sebek (1952) reports that growth of *Fusarium lycopersid* (Saccardo) Wollenweber is barely noticeable at pH 2.2; at pH 1.8 its conidia do not form at all. Optimal pH for its development is 7.0-7.2 with the maximal pH value being 8.7. For *Fusarium vasinfectum* Atkinson the optimal pH is $6.0-7.5$; at a pH of 1.4 its conidia do not germinate.

Norton (1954a), investigating blue damage of peanuts by *Aspergillus niger, A. flavus, Rhizopus stolonifer,* and *Sclerotium rolfsii* Saccardo, determined that this damage could take place only in a pH range of 2.2-7.0, as a result of the formation of oxalic and kojic acids.

Maltschewsky (1955b) investigated the development of fungi which she isolated from confectionery products and maintained on culture media with 48.9 per cent sugar and at a pH ranging from 3.0 to 7.0. At pH 3.0 the best developing fungus was *Penicillium frequentans* and to a much lesser extent *P. waksmani.* The optimal pH for these fungi was 6.0.

The optimal pH for the enzymes of fungi differs from that for their development. According to Small (1946), the optimal pH for the activity of invertase of *Aspergillus niger* is 2.5-3.5; for its amylase 3.5-5.5. In *A. oryzae* the optimal pH values are: for dextrinase 3.0-3.5, for lipase 5.0, for protease 5.1, for maltase 3.0-3.5, and for amylase 4.8-5.5.

Thus, even though fungi may develop at a pH range of 2.0-10.0, their sporulation and enzymatic activity occur within a much narrower range.

ANTAGONISTIC INTERACTIONS OF FUNGI

When investigating fungi it is frequently possible, even with the naked eye, to observe an almost pure culture. This phenomenon can be explained either by rapid growth of the fungus, by physical conditions of the medium such as osmotic pressure and humidity, or by the reaction of the medium.

For some species, however, this can also be explained by the fact that the fungus is forming substances which inhibit the growth of other individuals. Similar substances are known as antibiotics, which can be formed by fungi, actinomycetes, bacteria, lichens, and even algae. It can be stated that most plants produce substances toxic to other plants.

Tokin (1960) in 1928 determined that some plants (such as onions, garlic, radishes, horseradish, mustard, etc.) produce antibacterial substances which he named phytoncides (phytocides). The antibacterial action of these plants depends on the presence of ethereal oils, mustard oils, allicin, etc.

Following Fleming's 1928 discovery of penicillin, antibiotic properties were observed in numerous microorganisms. Brian (1957a) indicates that of the over 10,000 species of Fungi Imperfecti nearly 190 produce antibiotics. Most of the antibiotic producers belong to such genera as *Aspergillus, Penicillium, Fusarium,* and others.

Species of *Penicillium* produce about 25 antibiotics and around 20 toxic organic acids. Species of *Aspergillus* produce around 15 antibiotics. The number of new antibiotics grows daily. However, from this large number only about 35 are of practical importance in medicine and technology. They are utilized not only to control bacterial diseases but also to preserve such products as meat and fish. Unfortunately, in this second role they are frequently undesirable because some of them are toxic to man and others cause allergic responses. For food preservation aureomycin, terramycin, and neosin are used in dosages of about 7 p.p.m.

Antagonistic action of antibiotics is better studied in the soil. Gottlieb, Siminoff, and Martin (1952) discovered in the soil the presence of trichothecin, patulin, and clavicin; even though these substances rapidly disappeared in non-sterile soil, species tolerant to them still had the growth advantage.

Luke and Johnson (1953) isolated from soil a *Penicillium* which formed an antibiotic that inhibited the development of *Rhizoctonia, Helminthosporium*, *Fusarium, Sclerotinia, and Alternaria.* Heating for 40 minutes at 100° C, did not destroy it. Norton (1954b) established proof of antagonism in the soil between *Macrophomina phaseoli* (Maublanc) Ashby and some soil fungi.

Brian, Elson, and Love (1956) isolated the antibiotic patulin from apples rotting due to *Penicilliurn expansum.* From 600 to 1000 mg. of the antibiotic were isolated per liter of apple juice. This concentration is quite sufficient to inhibit the development of other microorganisms.

Brian (1957b) reports the development in sterile and non-sterile soils of patulin, citrinin, griseo-fulvin, gliotoxin, trichothecin, frequentin, and viridin. Schippers and Schermer (1966) report that *Penicillium* sp. and *Cladosporium* sp. inhibited the development in soil of the parasitic species of *Verticillium.* Lindsey (1965) demonstrated the competition between some soil fungi.

Christensen (1962), investigating the infection of wheat by *Aspergillus*

ochraceus, determined that this fungus did not infect grain on which A. glaucus was found. The studies of Tyner (1966) on *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler, *Aspergillus niger, Trichoderma viride, Gliocladium roseum* (Link) Thorn, and *Alternaria tenuis* showed that an extract from *C. sativus* inhibited development of all of the others, whereas extracts from *T. viride* and *G. roseum* inhibited *C. sativus.*

The above data indicate that antibiotics and toxic substances play important roles in development and distribution of fungi.

SUBSTRATUM GROUPS OF FUNGI

The development of fungi is determined not only by physical environmental conditions but also by the chemical composition of the substrata, as was reported by Byrde (1965). The interrelationship of ecological factors which govern the development of fungi is incredibly complex, explaining the fact that the same fungi are frequently found on different kinds of substrata under different conditions. Only the more common groups are included here.

Fungi o[Grain and its Products

The most characteristic property of this group is its amylolytic activity. Christensen (1957) described in detail the conditions under which the species develop on grain. The pioneers are always a group of *Aspergillus glaucus* which appear on grain with a moisture content of $13-14$ per cent. As a result of metabolic activity they raise the moisture content to 15-16 per cent, which enables *A. cwndidus, A. ochraceus,* and *A. flavus* to develop. With a further increase in moisture content *A. versicolor, A. niger,* and species of *Penicillium* appear. On the grains of various cereals over 50 species of fungi have been reported.

Carter and Young (1950) consider as frequent on wheat the following species: *Aspergillus glaucus, A. candidus, A. flavus, A. niger, A. tamarii* Kita, *A. fumigatus, A. clavatus, A. terreus* Thorn, *Penicillium expansum,* as well as species of *Rhizopus* and *Mucor.* They experimentally determined the roles of separate species of fungi in the process of spontaneous heating of wheat. According to their data, *A. candidus* on wheat is tolerant up to 55° C.

Koehler (1938) reported the following fungi on corn: *Aspergillus glaucus, A. ochraceus, A. tamarii, A. versicolor, A. wentii* Wehmer, *Fusariurn monili- [orme, Penicillium expansum, P. notatum, P. oxalicum* Currie & Thorn, P. *palitans,* and *P. viridicaturn* Westling.

Panasenko and Moskovetz (1933) isolated from corn *Aspergillus glaucus, A. candidus, A. flavus, A. niger, Fusarium rnoniliforme, Penicillium chrysogenum, P. commune* Thorn, *P. expansurn, Rhizopus stolonifer,* and *Trichothecium roseum.* Between 1930 and 1933 in the Ukraine, corn was so damaged by fungi that it resuked in mass disease and death of the livestock which was fed on it. Panasenko (1964a, b) isolated and described from wheat and potato starch and from macaroni *Aspergillus amylovorus* Panasenko, Penicillium *ucrainicum* Panasenko, *Phaeoscopulariopsis atrogrisea* Panasenko, and *Monilia alboviolacea* Panasenko.

Fungi on Sugar and Sugared Products

Fungus damage to sugar was reported in literature in the middle of the 19th Century. According to Maltschewsky (1955a), Payen determined fungal damage to sugar in France in 1843. He isolated from sugar fungi which Montagne identified as *Glycophilia erythrospora* Montagne and *G. eleospora* Montagne. The first discolored sugar to a red shade, the second to a greenish-brown shade.

A more detailed investigation of fungi infesting sugar was begun in the 20th Century. The primary agent responsible for such damage was held to be *Penicillium glaucum* Link (a name that was being used for many blue-green species of *Penicillium),* with a secondary role being ascribed to *Aspergillus* spp. Browne (1918) described *Monilia nigra* and *M. fusca* which had been isolated from sugar.

Kopeloff, Perkins, and Welcome (1921) found *Aspergillus sydowi* (Bain. & Sart.) Thom & Church especially harmful to sugar because it hydrolyzes sucrose, forming slimy and gummy substances.

Scarr (1951) isolated from sugar a yeast and *Aspergillus glaucus.* Such sugar-rich substances as jelly, candy, and marmalade are also damaged by fungi.

Panasenko (1941) isolated from confectionery goods *Aspergillus repens, A. terreus, A. varians* Wehmer, *Penicillium chlorophaeum* Biourge, *P. chrysogenum, P. citreo-viride* Biourge, *P. colurnnare* Thom, *P. italicum, P. luteurn, P. rnelinii* Thom, *P. puberulum* Bainier, *P. purpurogenurn* Stoll, and *P. rugulosum* Thorn. From candies the following fungi were isolated: *Aspergillus amstelodami, A. mangini (Mangin)* Thorn & Raper, *A. repens, Penicillium sanguineurn* Sopp, *P. spinulosum* Thorn, and *Torula pulchra.*

Maltschewsky (1955b) isolated from confectionery goods *Aspergillus flavus, Penicillium frequentans, P. spinulosum, P. citrinum, P. waksmani, P. chrysogenum,* and *P. gladioli* Machacek. From condensed sweet milk in which fungi formed button-like clumps, Harris (1933) isolated *Aspergillus glaucus, Torula pulchra,* and *Monilia nigra.* Rudakoff, Harzstein, and Sheveleva (1940) also isolated *Torula pulchra* from sweet milk.

Fungi of Meat, Fish, and Eggs

Meat is damaged mostly by bacteria, as stated by Jensen (1954). Fungi are secondary damage agents and their number on meat is small. Inasmuch as meat is refrigerated, important fungal damage agents are cold tolerant, and can develop at temperatures below 0° C. *Cladosporium herbarum* is the most frequently encountered species, forming maroon spots on the meat.

Brooks and Hansford (1923) determined that fungi can develop on meat even at temperatures of -6° to -10° C. They isolated from meat *Cladosporium herbarum, Chaetostylum fresenii* van Tiegham & Le Monnier, *Mucor racemosus, M. mucedo* (Linne) Brefield, *Penicillium expansum*, *P. asperum* (Shear) Raper & Thorn, *Thamnidium elegans, Sporotrichurn carnis* Brooks & Hansford, *Torula botryoides* Brooks & Hansford, and *lVardomyces anomala* Brooks & Hansford.

Semeniuk and Ball (1937) isolated from meat *Aspergillus repens, A. chevalieri, A. tuber, Cladosporium herbarum, Penicilliurn chrysogenum, P. rnelmii, P. notatum, P. puberulum,* and *Thamnidium elegans. Penicillium* *commune* frequently develops on sausage, forming a white powdery coating at 75 per cent reative humidity and a bluish-green coating at 80 per cent.

Kikkiwa and Kosugi (1937) isolated *Aspergillus repens* and *A. tuber* from dried fish which was being actively broken down by these two fungi. Kita (1914) isolated from dried fish *Aspergillus glaucus, A. rnelleus* Yukawa, A. *ochraceus, A. oryzae, A. candidus, A. versicolor, A. sulphureus* (Fres.) Thom& Church, *A. wentii, Cladosporium herbarum, Mucor racemosus,* and *Torula fuliginea* (Saito) Berkhout. The most frequently encountered fungi on fish are species of the *Aspergillus glaucus* group.

From eggs Kossovicz (1913) isolated *Aspergillus glaucus, A. niger, Cladosporium herbarum, Absidia lichtheimii, Scopulariopsis brevicaulis* Bainier, and *Rhizopus stolonifer.* Gieske (1938) investigated fungal development on refrigerated eggs. From there he isolated *Aspergillus candidus*, *Chaetostylum fresenii*, *Claclosporium herbarum, Mucor mucedo, M. racemosus, M. pusillus, Panicillium glaucum, Scopulariopsis bravicaulis, Rhizopus elegans* Eidam, *Thamnidium elegans,* and *Verticillium* spp. Of these fungi only a few can develop at low temperatures, most having been in the eggs before they were refrigerated.

Fungi of Butter and Fats

Butter contains a large number of spores of fungi, frequently up to several tens of thousands per gram of butter. However, spores in butter not only do not germinate but actually lose their viability, according to Korolev (1932), since they are in anaerobic conditions. Spores on the surface of butter grow when the relative air humidity of the storage area is not less than 80 per cent.

Such early research works as those by Jensen (1902) and Kühl (1910) report the following on butter: *Cladosporium butyri* Jensen, *Oidium lactis* Fresenius, *Penicillium glaucum,* and *P. roqueforti* Thom. Bisby, Jamieson, and Timonin (1933), having investigated 858 butter samples, isolated numerous fungi which they identified as belonging to 65 species. Of these, species of *Penicillium* comprised 37 per cent and species of *Aspergillus* 10 per cent. Most frequently they isolated *Penicillium chrysogenum* and *P. terrestre* Jensen. Most fungi isolated from butter are "soil fungi," with some of them being plant pathogens.

Ridder and Neill (1936) isolated from butter *Cladosporium herbarum, Pullularia pullulans* (deBary) Berkhout, *Penicillium expansum,* and *Muaor* sp. Jensen (1954) isolated from bacon *Aspergillus glaucus, A. niger, Alternaria* sp., *Monilia* sp., *Botrytis* sp., *Mucor racemosus,* and *Rhizopus stolonifer.*

Fungi of Cellulose

Fungi which decompose cellulose are of economic importance as destroyers of cotton, cotton goods, and stored books. They also develop on packing materials from which they contaminate goods.

De Bary (1884) pointed out that infection of plants by such fungi as *Sclerotinia* and *Botrytis* is possible because they are able to destroy cell walls of plants. Behrens (1897) confirmed this experimentally.

Van Iterson (1904) isolated from the air onto filter paper 35 species of

fungi which decomposed cellulose. Waksman (1916, 1944) carried out a detailed investigation into their role of decomposing cellulose in soil. According to his data, the most active cellulose decomposers are *Aspergillus fumigatus, Trichoderma koningi*, and *Basisporium gallarum* Dale.

Panasenko (1938) investigated the decomposition of cellulose by 22 species of fungi. Of these, *Fusarium oxysporum* after 30 days decomposed 51 per cent of the cellulose, *Helminthosporium sativum* 48 per cent, and *Trichoderrna lignorum* 36 per cent.

Siu (1951) divides cellulose fungi into three groups: 1) very active decomposers: *Chaetomium, Myrothecium, Sordaria, Aspergillus fumigatus, Botrytis, Fusarium, Glomerella, Graphium,* and *Humicola;* 2) active decomposers: *Stachybotrys, Trichoderma;* 3) slow decomposers: *Aspergillus nidulans* Eidam, A. *luchuensis* Inui, *A. ochraceus, Penicillium atramentosurn* Thorn, *P. pwrpurogenum, P. soppii* Zaleski, *P. waksmani,* and *Verticillium.* According to his data, the optimal pH for cellulose decomposition is: for *Aspergillus flavipes* 6.5, *A. [umigatus* 5.6, *Penicillium* spp. 4.2, *Trichoderraa koningi* 4.3.

The role of fungi which decompose cellulose is quite important in fodders on which they can form substances toxic to livestock; for example, *Stachybotrys alternans* Bonorden developing on straw caused the death of 30,000 horses in the Ukraine.

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