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PRINCIPLES OF MYCOTOXINS FORMATION IN GRAIN AND FEEDS UNDER NATURAL CONDITIONS

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AND FEEDS UNDER NATURAL CONDITIONS

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1. The factors which influence the formation of mycotoxins

The development of toxicogenic fungi and the formation of
mycotoxins on vegetable products under natural conditions is
governed by a number of external and internal factors. These
may be subdivided into three groups :   physical, chemical and
biological. Jarvis B. (1971) has summarized these factors in
relation to aflatoxins; however, the same factors also deter-
mine the biosynthesis of most known mycotoxins (Table 1).

Physical factors operate at every stage of production of
vegetative material, while biological and chemical factors,
primarily during the maturation and storage stages.

In this lecture we will consider the effects of some
of such factors on mycotoxin formation under natural condi-
tions.

2. Farm products as the substrate for mycotoxin formation

Mycotoxins, under natural conditions, contaminate a variety
of farm products. Aflatoxins are particularly wide-spread.

Oil-bearing crops like peanuts, cotton, copra and nuts
with a high oil content,   are contaminated with aflatoxins
more often than other crops. An analysis of more than 50,000
samples of oil-bearing crops, nuts, formula feeds taken from
different continents and carried out by the Institute of Tro-

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Factors affecting the formation of mycotoxins (after Jarvis B., 1971)

Table 1

Physical factors

Physical factors	Field	Harvesting	Storage
Humidity	+	+	+
Rate of drying	+	+	+
Humidation	+	+	+
Relative air humidity	+	+	+
Temperature	+	+	+
Mechanical damage	+	+	+
Mixing of grain of differing humidity	-	+	+
Self-heating	-	-	+
Time	+	+	+

Chemical factors

Chemical factors	Field	Harvesting	Storage
Gas medium	-	-	+
Substrate features	+	+	+
Mineral substances	+	-	+
Chemical treatment	+	-	+

Biological factors

Biological factors	Field	Harvesting	Storage
Number of plants	+	-	+
Number of animals	+	-	+
Infection of fungi	+	-	+
Sort peculiarities	+	-	+
Strain peculiarities	+	-	+
Spore load	+	+	+
Microbiological interactions	+	-	+

NOTE. + factor is present
- factor is absent

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pical Products has shown that in case of oil-bearing crops
(peanuts, in the main) the contamination level of Asian samples
was 98%, South American — 56% and European — 90% (Bainton J.,
Jones B., 1977).

As for grain crops, the occurrence of aflatoxins is some-
what more rare and at lower concentrations. The highest risk
crop is maize. Research done by American scientists indicated
that, as a rule, the level of aflatoxin contamination of samples
of marketable maize was within 2-3% (Shotwell O. et al., 1969,
1971). On some years, however, the contamination level of
maize in South-Eastern regions of the United States was as
high as 35% (Shotwell O. et al., 1973). The risk of growth of
toxins increases in countries with tropical and subtropical
climate; thus, in Uganda aflatoxins were found in 40% of samples
of maize, in Thailand — in 35%, in the Philippines — in 97%
(Stoloff L., 1976).

Aflatoxins are found rather rarely in wheat and in other
small grain crops. Shotwell O. et al.(1968) reported the pre-
sence of toxins in 9 out of 1,368 samples of wheat, sorgum
and oats in the USA. Aflatoxins were found in less than 2%
of samples of 400 samples of rice taken from markets of Africa,
the Philippines and Thailand.

An analysis of more than a thousand samples of grain of
different crops (1972-1987 harvest) grown in different zones
of the Soviet Union has shown that grain which was stored under
proper conditions was free from mycotoxins.

The legumes seem to be least of all inclined to aflatoxin
contamination. Aflatoxins are detected primarily in South-East
Asia and Africa. In most cases low concentrations of aflatoxins

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grow in legumes. <u>Ochratoxin A</u> (Canada) and penicillic acid
(USA) were found in moulded beans alongside with aflatoxins.

The danger is quite real in relation to contamination of
grain crops with ochratoxin A and fuzariotoxins (zearalenone,
vomitoxin, toxin T-2, diacetoxiscirpenol); ochratoxins are
found rather frequently in wheat, rye, barley, oats, maize in
the moderate climate zone in North America and Europe (Shot-
well O. et al., 1969, 1976; Krogh P. et al., 1973; Balzer J.
et al., 1977 and others). In the United States, ochratoxin was
found at a concentration of up to 29 μg/kg in 14% of 159 sam-
ples of barley, in Yugoslavia — in 26% out of 191 samples of
maize (concentration 490 μg/kg).

Fusariums can affect ears and kernels both in the field
and in storage. There are reports about their natural spread
on grain crops in North America, Europe, Asia and Africa.
Occurrence of zearalenone in grain crops, maize primarily, was
reported from North America, Europe and Africa. The greatest
level of maize contamination has been revealed in the United
States in years with an unusually humid weather in spring during
the sowing and in autumn at the time of harvesting.

Fusariotoxins have been practically absent in other
crops.

Thus we can clearly see differing susceptibility of indi-
vidual crops to contamination with toxigenous fungi and the
formation of mycotoxins.

3. <u>Conditions favouring the formation and spread of</u>
   <u>mycotoxins on a plant and in the field</u>

Contamination with aflatoxins and other mycotoxins can
occur not only during harvesting, transporting, storing and

processing of plant material but also in the field during plant growth.

3.1. Fusariotoxins

Fusariums, which are obligatory parasites, affect plants during all stages of their development, including the ear and the kernel ("scab"). The source of primary infection may be soil, plant remains and seeds. Fusarioses lead to considerable losses of harvest, worsening of grain quality and, in a number of instances, to the accumulation of fusariotoxins — zearalenone, deoxinivalenol (vomitoxin), toxin T-2 and other trichothecene cycotoxins. Active development of fusariums on plants is not always accompanied with the growth of mycotoxins, though Canadian researchers have demonstrated close relationship between the amount of vomitoxin and the content of kernels with a pink colouring affected with fusariosis (r - 0.755) (Martin R. et al., 1982).

The formation of fusariotoxins in the field is associated with the toxicosis known as spur of rye or ergot and spread in the 1910's in several regions of the Far East (the agent - F. graminearum), and also of alimentary toxic aleukia (ATA). In case of ATA the cause of mass death of people and animals was wheat and millet grain which overwintered in the field. Studies of Soviet scientists (A. Sarkisov, Y. Rubinstein, V. Bilai, T. Kvashnin) demonstrated the etiological role of F. sporotrichiella in the onset of the disease. It has been shown in recent years that strains of F. sporotrichiella — the agents of ATA — are capable of forming toxins T-2, PT-2 and diacetoxisoirpenol (Jagen B., Joffe A., 1976).

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A number of fusarial epiphytias occurred in recent years in the northern areas of the American continent, in the USA and Canada. In 1980 an unusually humid weather in Southeastern parts of Canada led to an outbreak of fusariosis of grain crops - scab caused by F. graminearum (Gibberella Zeal). Scab affected winter and spring wheat, oats and barley. From 37 to 55 cases grain contained vomitoxin at a concentration reaching 1 mg/kg, some samples of grain contained 5.7-8.5 μg/kg vomitoxin (Trenholm H. et al., 1981). As a result of this fodder wheat from Ontario and spring wheat from Quebec were found to be inapplicable for food application.

A similar outbreak of scab was registered in 1982 in the United States. Its centre was the state of Nebraska. Approximately 3.5% of the gross harvest of red grain winter wheat were affected with scab. The prevailing part of grain was contaminated with vomitoxin.

Another serious problem associated with grain fusariosis is its contamination with zearalenone in the field. Zearalenone producents are, as it is known, many species of fusarias, in the main , F. graminearum (F. roseum).

Zearalenone contaminates maize primarily in the southern parts of Canada and in the corn belt of the United States. In 1971-1973 in 17% of all cases maize, in the area of the co n belt, contained zearalenone at a rate of 0.1-5.0 mg/kg. In Ontario, from 1972 to 1978 from 9 to 29% of grain samples were contaminated with zearalenon. The largest contamination was found on years with abundant rainfall during the flowering stage (1975 and 1977). The presence of zearalenone in ripening maize has also been found in Europe (Italy, Poland, Austria, Yugoslavia).

American researchers, however, believe that zearalenone accumulates more often than not during the storage of humid and wet grain, specifically if in the ripening period it was intensively affected by fusariums. Maize contaminated in the field with toxigenic races of fusariums, demonstrated 50 times higher concentrations of zearalenone after storage than at the time of harvesting (Caldwell R., Tuite J., 1970).

The attempts at finding sorts or evolving maize hybrids resistant to zearalenone thus far have not been successful (Shannon G. et al., 1980).

Outbreaks of mass fusarioses of the ear are facilitated by prolonged rainfall during the ripening and moderately warm weather. The temperature of 20-30°C. at the time of infection favours the growth of vomitoxin. Canadian researchers (Sutton J. et al., 1980) have found, on the basis of statistical data for six successive years (1972-1978) that there is a close relationship between the spread of zearalenone in maize and the number of rainy days at the time of tasseling (r = 0.99±0.09).

3.2. Aflatoxins

Numerous studies have found the spread of A. flavus and the growth of aflatoxins in maize, peanuts and cotton seed prior to harvesting, in the period of ripening.

Though A. flavus belongs, in the main, to storage fungi, Taubenhaus found as far back as in 1920 the growth of this species upon maize in the field. Subsequently, in connection with the problem of aflatoxins, wide spread of A. flavus was determined in some years in maize during ripening, specifically in southern districts of the United States. Kernels were affected more often than not at the stage of late milky - early wax

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ripeness , though contamination could be observed also in other stages of development and lead to considerable growth of toxins. Thus, for instance, the presence of spores of A. flavus was found in maize stigmas in seven out of 48 samples, collected in different states of the USA.

what facilitates the formation of aflatoxins in the field? First of all - drought and the action of increased temperatures at the time of ripening (the so-called temperature stress). Precisely the influence of this factor has entailed the unusually wide spread of aflatoxins in the Southern regions of the USA in 1971, 1975 and 1977.

Wrong farming practices which entail plants weakening also may also lead to intensive growth of aflatoxins. This can be the excessive thickening of sowings, insufficient subfeeding, wrong time of sowing, late harvesting.

One of the main conditions for A. flavus infection and the formation of aflatoxins has been damage to the hull of a kernel which has been facilitated primarily by insect damage to the kernel, and specifically damage caused by corn borer and the weavel. Up to 84% of all aflatoxins damaged by insects (28% of the grain mass) was accumulated in maize kernels (Shotwell O. et al., 1977).

A. flavus has been found to damage peanut and cotton seed in the field also. Inasmuch as A. flavus is a soil fungus, it frequently infects peanuts prior to their removal from soil. In many instances the penetration of the fungus is facilitated by damage caused by insects, nematoda, termites and by cultivating implements.

Though the growth of A. flavus on peanuts is observed also in humid soil, drought favours fungus infection and the growth of aflatoxins (Pettit R. et al., 1971), so that irrigation during the ripening period lessens the danger of the aflatoxins formation.

Treatment of soil and plants with fungicides, the application of insecticides and insect resistant varieties of peanuts may be viewed as a real way of reducing contamination of peanuts with aflatoxins.

A. flavus is the agent which causes the rot of cotton balls. The green fluorescence (BGF) of the fibre is one of the symptoms of this rot and an indicator of the potential presence of aflatoxins in cotton seed (Ashworth L. et al., 1968). The insect and A. flavus damage caused to cotton bolls occurs prior to full opening of the bolls and is the largest at a temperature of $30-35^{\circ}$C. Aflatoxins may accumulate in undamaged bolls only at the period preceding their opening and complete drying of seed. Humid weather which inhibits complete opening of bolls, just as damage by insects, facilitates contamination with aflatoxins.

As a measure of aflatoxin control on cotton fields, early last wetting is used as well as insecticides which reduce the insect numbers.

4. Contamination of grain and seed with mycotoxins in storage

Grain and seed may be used directly after harvesting or may be stored for months and years.

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An analysis of information on the distribution of myco-
toxins in nature, in common vegetable products shows that myco-
toxins generally form owing to microbiological damages caused
by wrong storage practices. Thus, according to Hanseen E.,
Yung M. (1973) from 20 to 80% of moulded samples of peanuts
and nuts were contaminated with aflatoxins in amounts of up
to 100 μg/kg.

Aflatoxins were found but in one out of 169 samples of
wheat grain without signs of any microorganisms, whereas the
presence of aflatoxins was 17.4% of the studied samples of
wheat heavily affected by microorganisms (Lvova et al., 1976).

The state and development of microorganisms in grain and
the preservation of grain quality is decisively influenced by
humidity and temperature of grain in bulk, the extent of its
aeration, intactness of grain and the state of its hulls and al-
so the amount and content of admixtures (E. Mishustin, L. Tris-
vyatsky, 1963).

The same factors are decisive for the possibility and in-
tensity of mycotoxin formation in storaged products.

4.1. Humidity and temperature. Aflatoxins

A. flavus belongs to mesophilous organisms with cardinal
temperature points being the following 6-8°, 36-38° and 44-46°C.
The lowest limit of relative air-humidity for the growth of
these fungi ranges from 80 to 85%, it is 85% for spore forma-
tion.

The minimum and optimum and the highest possible tempera-
tures for aflatoxin synthesis, under laboratory conditions are
12, 24-35 and 40-42°C, respectively.

With the increase in temperature there is a growth of the relation of aflatoxins B_1 and B_2 to G_1 and G_2. This may be explained by higher optimum temperatures for the formation of B_1 ($35^{\circ}C$) that for G_1 ($18-25^{\circ}C$) (Diener U., Davis N., 1968; Schroeder H., Hein H., 1967). Preliminary incubation for 24 hours at room temperature has led in experiments conducted by van Walbeek et al. (1969) to a noticeable growth of toxins at $7-10^{\circ}C$. They reached $0.9-10.4$ µg/kg. Any preceding, even if short, development of A. flavus at high temperatures facilitated subsequent accumulation of aflatoxins at $0-5^{\circ}C$. A delay of maize cooling for 20 and 40 hours led to the development of A. flavus and the formation of toxins (Sauer B., 1972).

West S. et al. (1973) cultivated A. flavus in experiments which simulated real storage conditions with gradually increasing temperatures. It was increasing from 15 to $28^{\circ}C$ for six days and it has increased the yield of aflatoxins four-fold compared with cultivation at $28^{\circ}C$.

Stutz H. et al. (1976) considering the temperature variation in the development of fungi under natural conditions, suggested to describe temperature conditions of the development of A. parasiticus using a temperature coefficient (temperature x time). If $K < 208$, A. parasiticus does not grow and does not produce aflatoxins, at $K = 208-270$ there was a vegetative growth, at $K > 270$ — sporulation and the formation of aflatoxins.

The bottom limit of relative air humidity which allows for the formation of aflatoxins was RH = $83-85\%$.

Conventionally, the optimum conditions are $95-99\%$ (Northolt M. et al., 1976; Frank H., 1974). For starchy grain this corresponds to $18-18.5$ and 22% humidity, for oil-bearing — $9-10$ and $15-18\%$ for pulses — $18-19.5$ and $22-23\%$.

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The least time necessary for the growth of mycotoxins is
of great practical importance. Calderwood D. and Schroeder H.
(1968) observed in a commercial experiment, in freshly harvested
rice paddy, the formation of aflatoxins on the second-third day
of storage in aerated silages. Aflatoxins were found in peanuts
after the passage of 48 hours of storage given good aeration
(Dickens J., Pattee H., 1966). For wheat the lag was 4-8 days
(Spicher G., 1977; Stubblefield R., 1967). The lag value depends
both upon the crop and environmental conditions. In optimum con-
ditions this period for wheat grain was 2-3 days, whereas with
a decreasing humidity and temperature it went up to 6-45 days
(Lvova L. et al., 1975).

4.2. Humidity and temperature. Other mycotoxins

On the whole, higher humidity and comparatively moderate
temperatures are necessary for the development of toxigenous
species of penicilli and fusariums and for the synthesis of the
appropriate mycotoxins. Experiments with maize, rice, barley
and wheat have shown that even at temperatures ranging from 1°C
to +15°C the grain was found to develop F. cyclopium, P. marten-
sii, P. palitans and P. puberulum and there was a noticeable de-
velopment of penicillic acid (Ciegler A., Kurtzman G., 1970;
Kurtzman C., Ciegler A., 1970). The blue-eye disease of maize
caused by P. martensii is frequently accompanied with contamina-
tion of the grain with penicillic acid but manifests itself in
untreated maize stored at a temperature close to 5°C. P. martensii
is capable of synthesizing penicillic acid at temperatures of
5-32°C (maximum being at 15-20°C). If there is lower temperature
(5°C) this fungus forms within 88 days up to 12,700 µg/kg
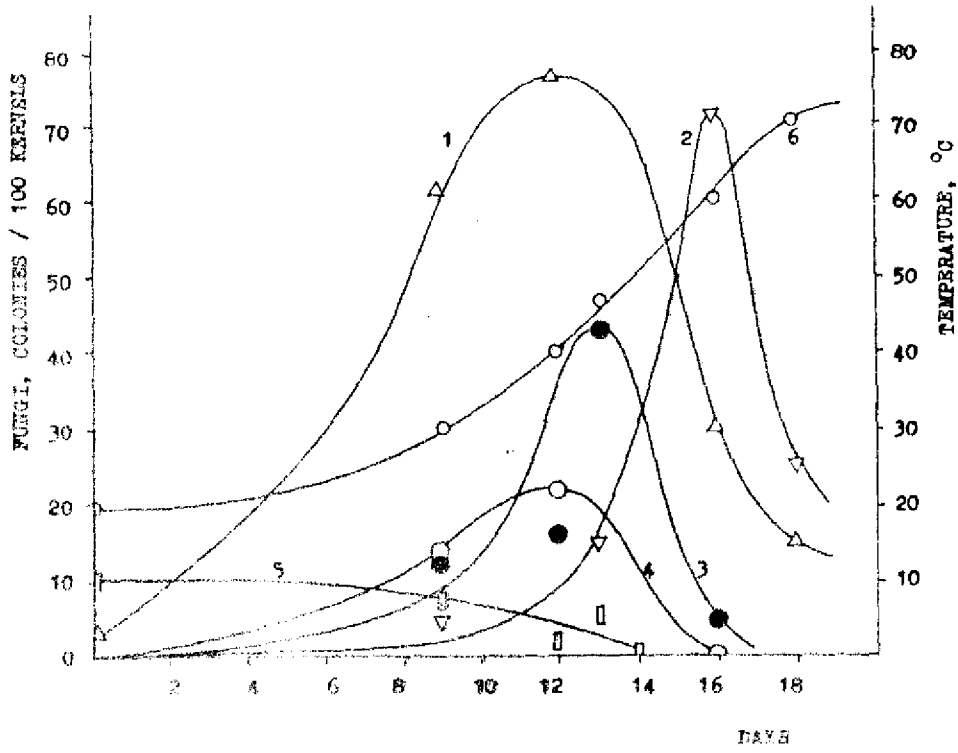of penicillic acid. An increase in temperature in excess of

Fig.1. Changes in the numbers of the main fungi in
maize selfheating (W = 31.6%).

1 - A. flavus; 2 - A. fumigatus; 3 - Mucor spp.;
4 - Penicillium spp.; 5 - Fusarium spp.; 6 - tem-
perature.

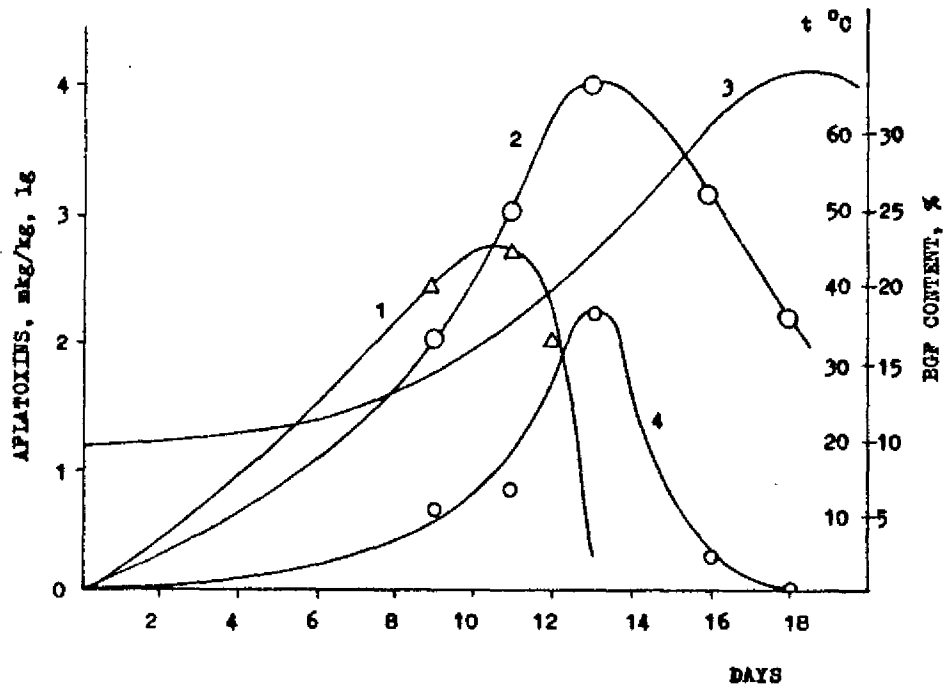Fig.2. Formation of aflatoxins B_1 and G_1 and BGP
kernels in selfheating of maize (W=31.6).

1 - aflatoxin G_1; 2 - aflatoxin B_1; 3 - t ^{o}C;
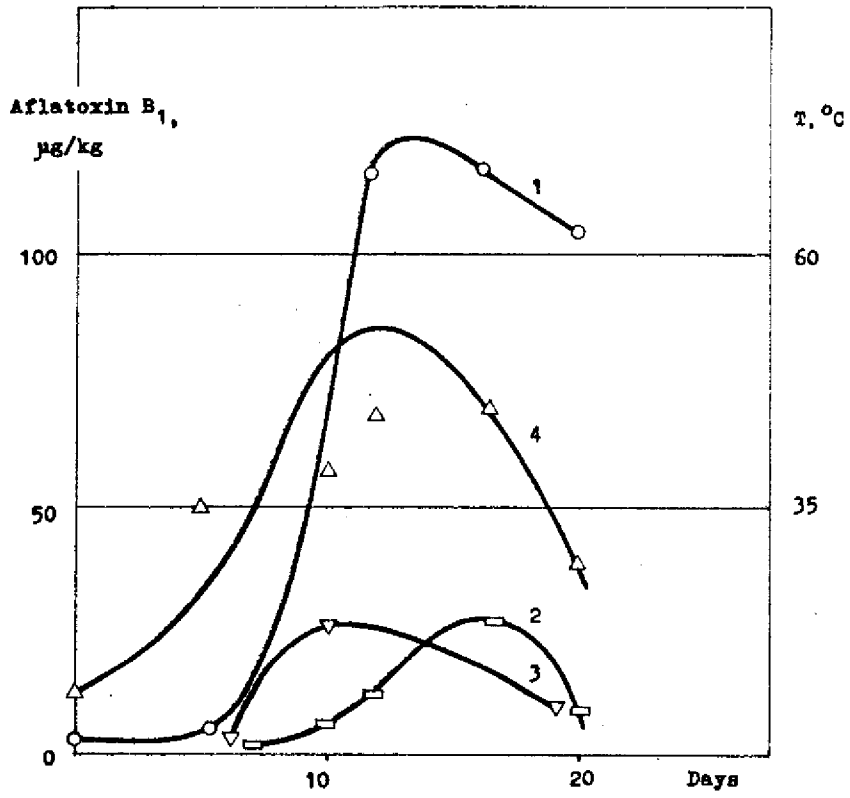4 - BGP content.

Fig.3. Aflatoxin B_1 production in different
layers of paddy bulk caused by spontaneous
heating.
1 - upper layer, 0-30 cm; 2 - layer 30-50 cm;
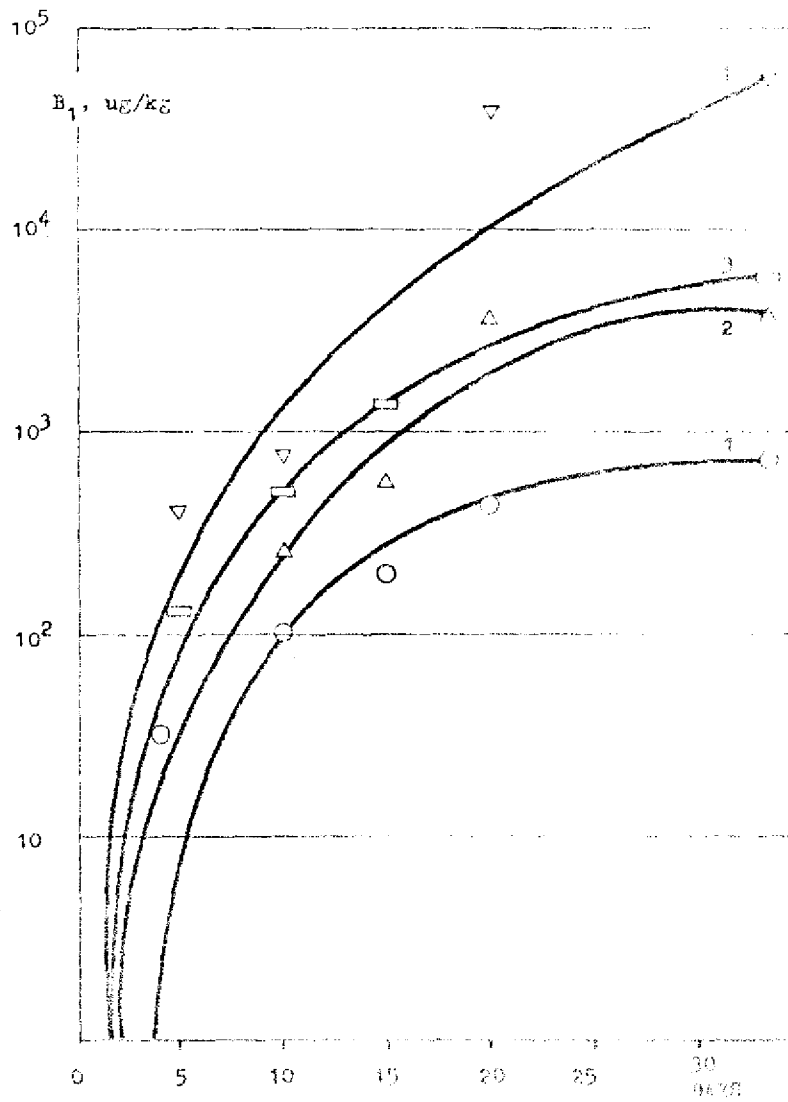3 - layer 50-100 cm; 4 - temperature of paddy
layer 30-50 cm, $^{\circ}C$.

Fig.4. The influence of broken kernels on aflatoxi· B₁
accumulation in grain rice (RH = 95 ... ...
The content of broken kernels: 1 - ... %, ... 4 - ... .

25°C strongly inhibits the synthesis of the toxin, the growth of the fungus discontinues at 32°C.

In a relatively high air humidity (RH >90%) we may observe a development of P. viridicatum and the synthesis of ochratoxin A and citrinin on grain crops even at a temperature of 5°C (Harwig J., Chen Y., 1974).

The level of humidity and temperature which allow for the growth of fungi and the synthesis of toxins are mutually related. According to Northold M. et al. (1977) the maximum levels of ochratoxin A. have been accumulated at RH = 95% and at a temperature of 24°C, however, ochratoxin A may be formed also at a temperature of 4°C and RH = 99%. Thus, humidity at about 19% allows for the formation of ochratoxin in barley and wheat given sufficiently high temperature (24°C). In case of high water content (29-31°) the toxin may be formed in the same grain at a temperature of 4°C.

Maize is less inclined to ochratoxin A contamination unlike barley and wheat since this toxin is formed upon maize at a higher humidity.

Intensive development of F. roseum and the synthesis of zearalenone was observed in stored grain at lower temperatures (12-18°C). The yield of the toxin increased provided the temperature went up to 27°C in the concluding stages of fermentation (Christensen C. et al., 1965; Mirocha C. et al., 1968). There are suppositions that the action of low temperatures which are unfavourable for the growth (temperature stress) is necessary for the activation of enzymatic systems which govern the synthesis of zearalenone (Mirocha C. et al., 1968, 1974). However, there are strains of fusariums which are capable of

forming large volumes of zearalenone at higher temperatures
(25°C) than at lower and variable temperatures (Schroeder H.,
Hein H., 1975; Naik D. et al., 1978). A similar regularity is
seen also in the producents of trichothecene mycotoxins --
F. poae, F. tricinctum, F. sporotrichiella. The temperature
optimum for the synthesis of toxin T-2 and diacetoxiscirpenol
is in the area of low temperatures 8-15°C (Smalley E., Strong
F., 1974). The highest toxicity of F. poae and F. tricinctum
according to Joffe A. (1974) was seen at 5-8°C. Some strains
were capable of producing maximum volumes of toxins at a tem-
perature of 15-20°C.

Though the optimum for the formation of patulin is 25°C it
accumulates but slowly also at zero temperatures (from -1 to +5°C).

Thus, the areas of spread of toxigenous species of fusa-
riums and penicilli and of the toxins of these species of fungi
are primarily in the moderate climate areas.

4.3. Self-heating of grain and mycotoxins

Grain and seed, in all countries, are usually stored at
a humidity which precludes the emergence of active microbiolo-
gical developments, i.e. below the critical humidity. The ap-
proximate limits of this humidity are given in Table 2. In case
of long-term storage humidity is lowered by 1-2% compared with
the critical.

The limits of humidity which allow for the development of
aflatoxins and other mycotoxins are much higher than those which
are conventionally used in storage. These conditions, however,
may be developed in the following instances:

a) during the harvesting of grain and seed of increased
humidity;

b) during secondary humidation owing to rainfall and sorption of water vapour;

c) as a result of thermomoisture diffusion in bulk grain which is comparatively dry, due to the changes in temperature;

d) during the mixing of lots of grain which are not even by their humidity;

e) during self-heating of grain.

Thus, the development of A flavus and the formation of aflatoxins was seen in maize grain at a humidity of 13.5% owing to thermomoisture transfer in uneven heating (Sellam M., Christensen C., 1976).

Table 2

Critical humidity and humidity necessary for the formation of aflatoxins in different crops

Crop	Critical humidity, %%	Humidity necessary for aflatoxin formation	
		minimum (RH=85%)	optimum (RH=95%)
Cereal (maize, wheat, rye, barley, oats, sorgum)	13.5-15.5	18-18.5	22
Oil-bearing (peanuts, nuts, sunflower, copra)	7-8	9-10	15-18
Legumes (beans, peas, lentil, soya-beans)	12.0-16.5	17.5-19.0	22-23

Aflatoxins were accumulated in a mixture of humid (26.6-27.9%) and dry (9.8%) maize at an average humidity of the mixture being 14%. Though in eight weeks dry maize was not being moistened above 13%, up to 500 μg/kg of aflatoxin B_1

was formed in it (Lillehoj E. et al., 1976).

Krogh P. (1979) indicates the possibility of formation
of aflatoxin in lots of grain with a low average content of
water ensuring good storage of grain. In limited areas within
such a lot of grain owing to the accumulation of immature grain
we may see the development of an increased humidity and ochra-
toxin A may develop there after the passage of one-three weeks
of storage. The deviations of temperature and humidity from
the safe average standard make it possible for mould fungi
to develop in local points of bulk lots creating foci
where self-accelerating self-heating commences. Self-heating
plays a particular part in contaminating grain and seed with
mycotoxins in areas of moderate climate. This process may occur
both in humid and wet as well as in dry grain. The cause is
intensive emission of warmth and moisture in the respiration
of life components of the grain mass, in the main, microscopic
gungi, and low heat conductivity of grain (Mishustin A., Tris-
vyatsky L., 1969). Every kilogram of dry substance of the stored
product expended for respiration by microorganisms yields a
heat effect which on the average is 4,400 kcal and it stimulates
the development of 1.54 kg of CO_2 and 0.58 kg of water which
moistens the product. When only 1% of the dry substances of the
grain is expended for respiration, the temperature should go
up 8^o(without considering the heat loss into the environment)
(Bauder H., 1971). Thereby, conditions are created for self-
acceleration and spatial spread of the process.

We may observe in self-heating a change of more xerophytic
species of fungi by hydrophilic and mesophilic species —
thermotolerant and thermophilic. This succession of species

is a result and at the same time an indispensable condition of further development of self-heating. Sooner or later conditions would develop in the self-heated product which favour the development of toxigenic species of fungi and the synthesis of mycotoxins.

Thus, at a relative air humidity of 68-72% fungi A. halophilieus and A. restrietus may develop in grain. But their development is comparatively slow and they are incapable of increasing grain temperature to a high level. If grain humidity is in an equilibrium its RH being 75-80% the main activity is shown by fungi of the A. glaucus group. As a result of this activity grain humidity grows to limits which correspond to relative air humidity of 80-85%. The main activity beyond this limit is shown by A. candidus and A. flavus. These fungi are quickly developing and are capable of increasing grain temperature to 50-55°. In the final stages termophilic bacteria, mucoric fungi and A. fumigatus are included in the self-heating process (Christensen C., Kaufmann H., 1977).

Unlike laboratory experiments, the development of toxigenic strains of fungi in self-heating occurs with grain which is viable and possesses active immunity, in continually changing conditions of temperature and humidity and in an intricate interaction with other epiphytic microorganisms of grain and seed. Therefore the ecological conditions of the growth of A. flavus and the synthesis of mycotoxins in self-heating differ considerably from the dimensions found in laboratory conditions.

The main groups of fungi which develop in self-heating reached their top numbers under the following temperatures.

Penicillium spp. - 25-40°C; _A. flavus_ - 35-50°C, _Mucor spp._- 45-60°C; _A. fumigatus_ - 60-65°C, i.e. the temperature level optimum for the development in natural conditions proved to be much higher than the one fixed in laboratory experiments.

Though the best possible for the synthesis of aflatoxin one should consider the humidity of 20-25%, aflatoxins have been growing in the grain mass with the average initial humidity of of > 16%, when there were zones of increased humidity which more often than not were in the upper part of the bulk lot. The commencement of toxinoformation was at an increase of grain temperature to 25-30°C. The largest amounts of aflatoxins were observed in a temperature range from 35°C to 50°C. Further temperature increase, above 50°C, led to the death of fungi and the lowering of aflatoxin content.

A. flavus developed primarily in grain layers where the temperature was 30-40°, adjacent on the periphery of the focus of self-heating, in the main, at a depth of 10-50 om from the surface. Within this zone it dominated _A. glaucus_, _A. candidus_, _Penicillium spp._, it developed practically in a monoculture and formed considerable amounts of aflatoxins. In the grain layers which were higher and lower, the prevailing species were penicilli and other species of _aspergilli_ and the aflatoxin content was much lower.

As for the central part of the bulk lot, the highest temperatures were maintained in self-heating of wet and humid grain (up to 60-70°C). Aflatoxins either did not grow or were in insignificant quantities owing to their destruction.

During the commencement of self-heating in grain of increased humidity, aflatoxins may be found in maize   on the

third-fourth day, in wheat -- on the seventh-eighth day, in
rice paddy -- on the ninth-fourteenth day.

Self-heating may occur in wet grain stored prior to drying
by forced ventillation if the specific air supply is inade-
quate for the removal from the grain mass of surplus moisture
and heat, the latter develop due to the activity of biological
components of the grain mass. Self-heating of poorly ventilated
wet grain mass is particularly dangerous in terms of contamina-
tion with aflatoxins. In this case the slow temperature rise
ensures prolonged maintenance of optimum temperatures (30-45°)
for the synthesis of toxins. Heat has been evenly distributed
in the ventilated bulk mass of grain with air currents and con-
ditions of aeration and temperature which favour the formation
of toxins develop almost everywhere in the mass of grain.

In case of self-heating of maize with a humidity of 32.2%
in non-ventilated conditions, on the seventh day temperature
went up to 70°C, aflatoxins were found after a drop of tempe-
rature to 50°C only on the 18th day of storage (75 μg/kg).
In a poorly ventilated lot of maize (W = 31.6%) there was 130
μg/kg of aflatoxin B_1 on the 8th-9th day, its content by the
13th day exceeded 5,000 μg/kg. During the same period we ob-
served the highest BGF value (27.5%) and that of the fungi num-
ber. A temperature rise in excess of 50°C led to a loss of ger-
mination capacity, a decrease in the number of fungi and content
of aflatoxins and BGF.

In self-heating of maize, species of _Fusarium_ --zearale-
none producents -- developed in the layers of bulk cobs with
a temperature of 30-45°C. Zearalenone was distributed in a si-
milar way. Increased temperatures in the centre of the self-

heating focus (50-60°C) entailed the death of fusariums and no
zearalenone was found in the kernels.

4.4. State of grain and seed

The rate of aflatoxin accumulation within the limits of
each crop is influenced by (1) the specific features of the given
variety (chemical composition, seed structure); (2) the ripen-
ing degree, harvesting humidity; (3) mechanical damage; and
(4) insect damage.

Considerable variations in terms of stability to A. flavus
damage are known among different varieties, determined by dis-
tinctions in the chemical composition or by the structure of
external protective layers of seed (Nagarajan V., Bhat R.,
1967 , Rao K., Tulpule P., 1967). The thickness of the enve-
lopes protecting the seed against the invasion of fungi also
controls the accumulation of aflatoxins (Calwert O. et al.,
1978). The accumulation of aflatoxin B_1 was less in late ripen-
ing maize hybrids than in the quick ripening ones (La Prade J.
et al., 1977).

The extent of ripeness , post-harvesting ripening of seed
and mechanical damage, ensuring the access of fungi to inter-
nal highly nutritive parts of the kernel also influence the
intensity of toxin formation.

Unripened peanut kernels even at high humidity remained
intact prior to harvesting, whereas a delay of harvesting en-
tailed intensive damage of peanut with moulds still in the field
and the emergence of toxins (McDonald D., 1964). A decrease in
the resistance of seed is explained by a lowering of their phy-
siological activity at the time of complete ripeness . Peanut
seed and beans following the first year of storage were affect-

ed with A. flavus more intensively than freshly harvested. The development of A. flavus and the accumulation of aflatoxins was more intensive on stored maize than in freshly harvested maize of the same humidity (Trenk H., Hartman P., 1970).

The studies of American researchers (Goldblatt L., 1968; Shozwell O. et al., 1972, 1977) indicated that aflatoxins are primarily concentrated in crushed and mechanically damaged maize kernels.

Rice paddy which was manually harvested and had an undamaged glume did not show any accumulation of aflatoxins notwithstanding the intensive development of A. flavus on the surface. A mechanical damage of the glume, as well as its removal in crushing, unavoidable in harvesting and post-harvesting treatment of rice, greatly weakened its resistance to aflatoxin contamination. The rate of aflatoxin accumulation increased manifold in rice samples which contained from 5 to 8% of husked kernels.

4.5. Microbiological interactions

Grain mass in storage is an intricate ecological system where microorganisms unavoidedly enter into differing relationships with species which occupy similar ecological niches (antagonism, synergism, etc.).

Relationships of competition of A. flavus and epiphytic microflora of maize have led to a lessening of aflatoxin formation from 24 to 117 times on non-sterile maize compared to sterile maize (Hunter, 1969). A. niger and Rhizoctonia solani on peanuts and a liquid nutritive medium, limited the development of A. flavus and the isolation of the toxin into the sub-

strate (Ashworth L., Langley B., 1964). Different species of
Penicillium were capable of decreasing the yield of toxins if
they were grown with A. flavus (Wildman J. et al., 1967). Simi-
lar results have been demonstrated for Rhizopus oryzae. When
RH < 86% the growth of xerophytic species of A. amstelodami
prevented the growth of A. flavus and the formation of aflato-
xins, at RH = 88% there was an accumulation of toxins but there
occurred antagonism between A. flavus and A. niger (Denizel T.
et al., 1976). Similar relationships were also demonstrated be-
tween A. parasiticus and A. chevaliery (Boller R., Schroeder H.,
1973).

Competitive relations between the toxigenous strains of
A. flavus and A. parasiticus led to a considerable suppression
of the latter in a joint culture so that former occurred much
more often on damaged maize than A. parasiticus did (Calwert O.
et al., 1978).

The relationship of the number of toxigenic and non-toxi-
genic strains of A. flavus influences the intensity of toxi-
genesis. An increase in the share of non-toxigenic strains
(9 fold) in the inoculum evolved a drop in the aflatoxin forma-
tion (70 fold) (Jacquet J. et al., 1979).

The value of spore load of toxigenic strains is also a
factor determining the intensity of toxigenesis.

Thus, in natural conditions, even if we have the necessary
temperature and humidity, an available substrate and the presence
of toxigenic strains for the accumulation of aflatoxins we have
to have the following conditions (1) prevalence of A. flavus
over other species of fungi; (2) prevalence of toxigenic strains
in an A. flavus population and (3) a definite value of the ini-
tial spore road for each case.

5. Conclusion

Preservation of food products   is the most real and effective way of controlling their contamination with mycotoxins.

This calls for a clear understanding of the factors facilitating mycotoxin contamination in natural conditions. Making use of this information one can develop a strategy of control of mycotoxins on all the stages of obtaining, storing and processing of plant material, turning it into food and feed products.