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FACTORS DETERMINING ACCUMULATION OF MYCOTOXIN PRODUCERS IN CEREAL GRAIN DURING HARVESTING

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Abstract: During the meteorologically contrasting period of 2003-2005, the contamination of winter wheat, malt barley and fodder barley grain with micromycetes during grain harvesting and preparation for storage was investigated. Micromycetes of over 70 species ascribed to 16 genera were isolated and identified, the density of their populations in grain was determined. Micromycetes with a population density of >50% were attributed to dominant species. Short biological characteristic, ecological peculiarities of the dominating micromycetes are provided; factors determining intensity of their development and abilities to synthesise and excrete toxic metabolites are indicated. The importance of grain drying for stabilisation of its contamination with micromycete propagules is highlighted. It is noted that in grain dried in shaft dryer using air at 90°C the number of cfu (colony forming units) was reduced from 2.2 to 8.2 times. When active ventilation is applied, conditions favourable for the development of micromycetes remain longest in the upper layers of the mound. The airflow passing through the layer of damp grain inhibits the development of micromycetes, but an increase of comparative air flow for more than 500 $m^3 \cdot (t \cdot h)^{-1}$ did not reduce the abundance of micromycete cfu. After drying *Alternaria alternata*, *Fusarium avenaceum*, *F. culmorum*, *Penicillum verrucosum* dominated in wheat grain; *Aspergillus flavus*, *Bipolaris sorokiniana*, *Fusarium chlamydosporum*, *F. culmorum*, *F. tricinctum* in malts barley grain; *Fusarium avenaceum*, *F. culmorum*, *F. tricinctum*, *Alternaria alternata* in fodder barley grain. It has been determined that all micromycetes recorded on grain after drying are potential producers of toxic metabolites, i.e. are hazardous to human health.

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INTRODUCTION

Recently, particular attention has been given to the control of microbiological contamination of food and mycotoxins accumulating in foodstuffs. Safety of food raw materials and final products is evaluated by common physical, chemical and microbiological criteria provided by the EU directives. The process of microbiological contamination of food raw materials starts with the beginning of ripening of grain and seeds and continues untill the consuming of the final product. Under natural environmental conditions the main infectors of plants are microscopic fungi, characterised by the ability to synthesize toxic materials of various chemical composition that are hazardous to the health of humans and animals. Toxic secondary metabolites synthesises and excreted by microscopic fungi can cause functional, cancerous, psychical health disorders; as indicated by the specialists worldwide [2, 8, 22, 29, 47, 72].

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The severity of the impact depends not only on the amount of the toxic substance entering the organism but also upon the frequency of the uptake. Small doses of mycotoxins continuously getting into the organisms of humans, especially infants or people with a weak immune system, can cause severe health disorders [2, 5, 23, 26, 52]. It should be mentioned that the majority of mycotoxins are chemically stable substances, resistant to temperature changes, technological impacts and chemical detoxicants. Therefore, prevention of microbiological contamination of food raw material is the most reliable way to protect it [1, 6, 27, 79, 80]. Under conditions of temperate climate, contamination of plant raw material with propagules of microscopic fungi before harvesting is usually moderate and very much depends upon meteorological conditions of a particular year [2, 18, 22, 24, 32, 35, 42, 43, 52].

Development of microscopic fungi on grain and seeds depends upon ambient humidity and temperature. Under conditions of temperate climate more than 85-95% of the harvested grain is characterised by humidity higher than 14-15% [40, 67, 92]. In such grain conditions the development of microscopic fungi are favourable, therefore, in order to suppress their development the grain must be quickly dried or conserved applying other physical or chemical measures [66, 93]. Presently, the most widely used method of grain conservation is drying up to critical humidity: crop grain to 14%, oilseed to 9%. A low amount of humidity in grain is the main factor limiting the development of microscopic fungi. Therefore, timely and technologically appropriate drying of grain allows preservation of the nutritious value of grain, avoidance of economic losses and ensures food safety [6, 9, 33, 35, 44, 60]. However, inappropriate drying technologies and regimes can lead to negative results. Too fast heating of grain can cause seed-shell cracks and thus facilitate the penetration of microscopic fungi inside grain [78]. In the temperate climate zone, majority of micromycetes recorded on grain are mesophiles. The most favourable temperature for their development is 15-30°C [12, 14, 15]. Still, some micromycetes species can develop at a temperature below 0°C (*Cladosporium herbarum*, *Fusarium nivale*, *Fusarium avenaceum*). Micromycetes of other species, e.g. *Rhizomucor pusillus*, *Rhizopus oryzae*, some *Aspergillus* and *Mucor*, intensively develop untill the temperature of grain reaches 60°C [7, 12, 41].

Plants of the *Poaceae* family, to which belong wheat, barley, etc., are frequently infected with fungi of the *Fusarium* genus. During the last few decades, outbreaks of plant diseases caused by these fungi were recorded in Germany, USA, and Canada [59]. Some authors [87] state that micromycetes of the *Fusarium* genus most frequently infect grain in the field as they excrete deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, HT-2 toxin, zearalenon, fumasin. The grain is usually infected with toxins of the trichothecene group, especially deoxynivalenol (DON); its concentration exceeds by several times the concentrations of other toxins (T-2 toxin, nivalenol (NIV), zearalenone, etc.). *Fusarium graminearum* and *F. culmorum* are regarded as the most active toxin producers [55].

There is an opinion [16] that in temperate climate regions the yield of crops is mostly determined by *Fusarium* micromycetes, while in the regions of tropical and subtropical climate – by *Aspergillus*, *Penicillium* and *Alternaria* fungi.

However the results obtained by other authors do not support this opinion [10, 11, 43, 69, 90]. They state that a lot of micromycetes recorded in grain are able to synthesise mycotoxins that are hazardous to humans and animals.

The research has shown that while the grain is harvested by combine harvester its contamination with micromycetes significantly increases, sometimes up to 250 times [18]. It has been noticed that if in starchy wheat, barley and other grain the humidity does not reach 13.5%, and in protein-rich grain does not reach 12.5%, the microscopic fungi do not develop in the grain. It is essential that the humidity would be evenly spread in the whole mass of the stored grain, because microscopic fungi start developing in places of humidity accumulation, and later gradually spread through the whole mound of the dried grain. The condensation of humidity is induced by pest developing in grain. In places, where they settle and excrete metabolites, the temperature rises, humidity condensation intensifies, the so-called “heating spots” form [63, 66, 74]. Food raw material contaminated with fungal metabolites is no longer safe; it is hazardous to consume the contaminated grain, even after thermal treatment, because in high temperature the fungi perish sooner than their toxic metabolites. Prevention measures for the storage of the threshed grain must be planned beforehand. The complex of prevention measures should include modern systems of grain moisture regulation, storage, environmental cleaning and ventilation, as well as periodic control of mycological state and toxin contamination [13, 40, 65, 93].

The aim of the present research was to evaluate the mycological condition of cereal grain during initial phases of harvest processing, depending upon meteorological conditions and employed technologies, to identify dominating micromycetes infecting grain during this period and the hazard they impose upon food safety.

MATERIALS AND METHODS

The research was performed in the period of 2003-2005. Agrometeorological conditions during grain maturing and harvesting were evaluated based on the data of Kaunas meteorological station. The indices of grain quality were estimated and reported by the Lithuanian State Plant Varieties Testing Centre.

For the investigations of microbial contamination, the grain samples were taken before harvesting, in the course of harvesting and during grain drying. The grain for investigations were sampled at 3 sites in a field, 20 equally developed ears were cut at each site; later, they were shelled

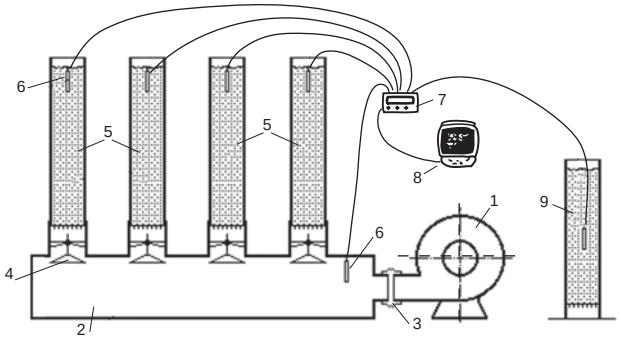

Figure 1. Principal scheme of shaft drier: 1 – ventilator; 2 – constant static pressure chamber; 3 – flexible joint; 4 – valve; 5 – ventilated cylinders filled with grain; 6 – temperature and humidity sensors; 7 – secondary ALMEMO meter; 8 – computer; 9 – natural ventilation cylinder filled with grain.

and their contamination with micromycetes was determined, evenly placing the shelled grain on acidified malt agar medium. With the aim of determining the contamination of grain with micromycetes in the course of harvesting and transportation, the samples were taken directly from the bunker of a combine harvester or granary intended for grain storage.

The grain was dried in a shaft drier applying active ventilation. The mentioned drying technologies differed by temperature of drying agent, ventilation intensity and condition of the layer of dried grain. The grain drying in the shaft drier was carried out on a private farm in Kaunas district. A "Cimbria" shaft drier of 20 t × h^{-1} capacity was used. The duration of grain drying was 4 days. Every day, the humidity of the dried grain differed while the temperature of the drying agent remained constant, i.e. 90ºC. Humidity of grain, as well as abundance and diversity of microscopic fungi before and after drying, were recorded. Air temperature, temperature of drying agent and of grain from the dryer were measured using ALMEMO sensors FH A646-21 and ZA 9020-FSK; error of temperature measuring devices was ± 0.1ºC.

Winter wheat 'Širvinta', malts barley 'Barke' and fodder barley 'Henni' were used for the research.

Other investigations on grain drying were performed using a device designed at the Lithuanian University of Agriculture (Fig. 1). This consists of a centrifugal ventilator (1) and four 0.18 m diametre and 1.2 m height cylinders (5) connected to a chamber with constant static pressure (2) by a demountable joint. The grain was dried simultaneously in all the cylinders, only ventilation intensity varied. Before each grain drying stage the shaft drier was regulated using valves (4).

During the research, the weight of the dried grain, drying duration, ambient air temperature and temperature of air blown by the ventilator (1) and relative humidity in the constant static pressure chamber (2) and in the upper layers of grain mounds were recorded by periodic weighing of the cylinders (5). Air temperature and relative humidity were measured using ALMEMO sensors FH A646-21 (error of temperature measuring devices was ±0.1ºC, relative humidity error is ±2%). Measurement results were taken every 10 min and stored in the secondary device ALMEMO 3290. The grain was ventilated until the average grain moisture in the cylinder fell to 14%. In this case, we took some grain at 5-10 cm depth of the mound for the establishment of mycological pollution.

To compare the results, we performed analogical research in a cylinder with natural ventilation (9), where natural convection took place and the rate of air filtration was close to zero. The natural ventilation cylinder stood in the same place next to the shaft drier, set on a 10 cm trellis.

Grain samples were analysed according to the methods described [46]. Analyses were conducted in 3 replications. Direct isolation of micromycetes and imprint methods were used. The surface of the investigated grain was disinfected with 70% ethanol for 2 min, washed with sterile water, and grain placed in Petri dishes filled with sterile agar medium of malt extract supplied with chloramphenicol (50 mg × l^{-1}). The inverted Petri dishes were incubated at a temperature of 26±2ºC.

In order to determine the abundance of microscopic fungi on grain, the dilution method was used. 1-2 g of grains were placed into 10 ml of sterile water and shaken for 15 min. A dilution series was prepared from the obtained suspension. 1 ml of suspension from each dilution series was poured into sterile Petri dishes of 9 cm diameter and poured over with 15 ml 48ºC agar medium of malt extract supplied with chloramphenicol. Aiming to purify and identify micromycete isolates, they were inoculated on standard agar Czapek, malt and corn extract media, and cultivated at 26±2ºC for 5-7 days [47].

The isolates were ascribed to taxonomic groups following Ainsworth and Bisby's Dictionary of Fungi (Hawsksworth *et al.*, 1995). Micromycetes were identified according to various manuals [43, 91].

The research results were processed using the MS Excel program.

RESULTS AND DISCUSSION

In the years 2003-2005, meteorological conditions during grain maturing and harvesting varied – illustrated by the data presented in Fig. 2.

The beginning of summer in 2003 was unsettled, with strong temperature variations during separate stages of grain growth and maturing (Fig. 2 A).

However, average daily temperature was by 1.1ºC beyond the many-year average. The summer of 2004 was also marked by considerable temperature variations. In the period of intensive development of plants, i.e. from June 9 – July 18, the average daily temperature was by 2.1ºC below the many-year average. Particularly low temperature occurred during the nights, some nights reaching only 1.5ºC. Considerably warmer weather occurred at the end of July and during August, when the temperature exceeded the many-year average by 1.5ºC. In 2005, low temperatures

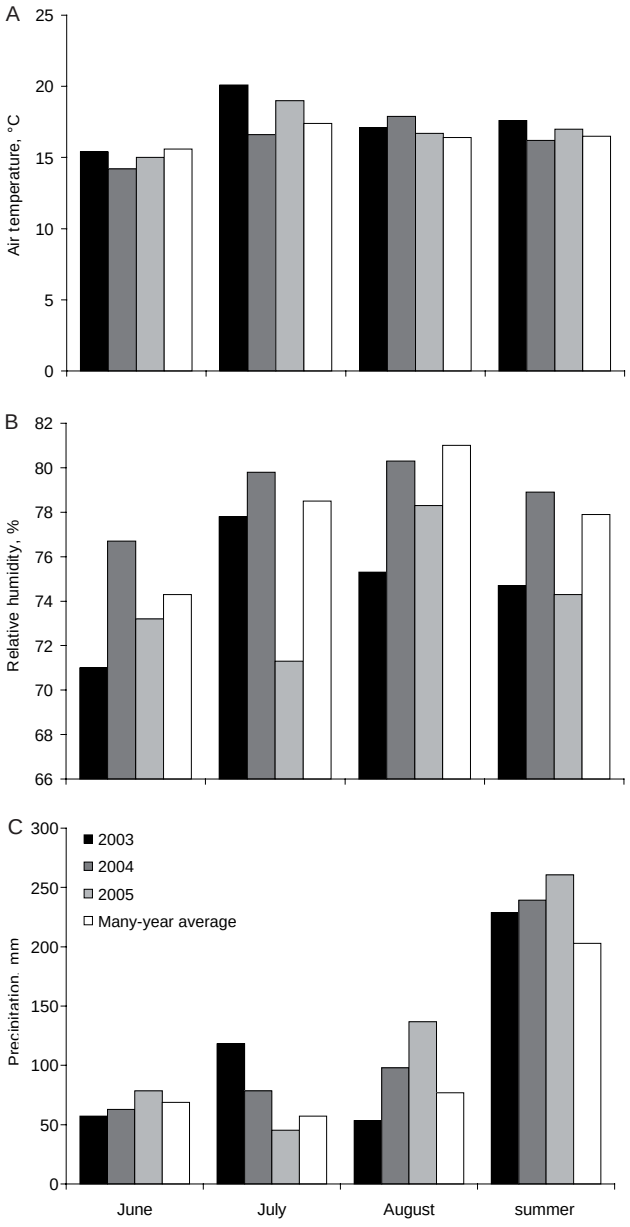

Figure 2. Meteorological conditions during grain maturing and harvesting, 2003-2005: A – air temperature; B – relative humidity; C – precipitation.

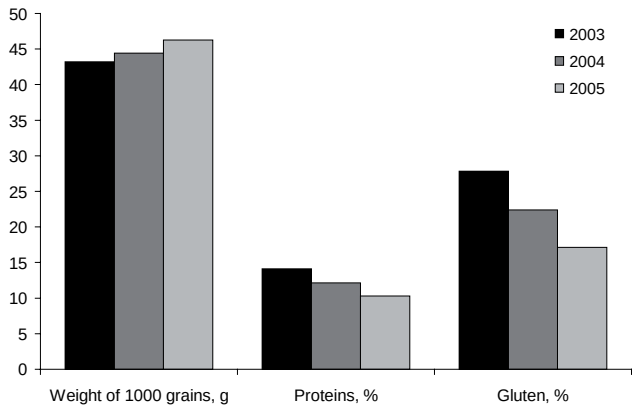

Figure 3. Quality indices of winter wheat 'Širvinta' grain cultivated under different meteorological conditions (2003-2005).

prevailed only during the first decade of June. Later, the weather warmed up and in July it was by 2 degrees higher than the many-year average of 17.7±0.3ºC. In August, the weather was again by 0.5-0.7ºC below the many-year average, but at nights the temperature did not drop below 10.5ºC, and the daytime temperature reached 20ºC.

During the whole period of grain investigation, relative air humidity was close to the many-year average and varied from 71-80.3%. The summer of 2004 was more humid. It was predetermined by lower air temperature in June and July as well as plenty of rainy days.

In all the research years the amount of precipitation exceeded the usual annual limits: in 2003 the amount of precipitation reached 228.7 mm constituting 113% of the many-year average; in 2004, this amount was 239.4 mm constituting 118%; and in 2005, the amount was 260.6 mm, constituting 129% of the many-year average. Crop growth and grain maturing were conditioned by the frequency and distribution of precipitation. In 2003, the rainy period lasted from June 21 – July 31 and through the second part of August. Average precipitation was 39.4±5.8 mm during those 10 days. The distribution of precipitation was very uneven. Usually, more than half of the precipitation amount of a decade would fall during one day. The first half of August was the driest – relative air humidity reached only 69.1±4.6%. Almost every day if rained during the last week of August; during a shower on August 30, 15.8 mm of precipitation were measured; this constituted 29.6% of the many-year August average. The grain harvesting was performed under complicated conditions and this affected the mycological state of the grain.

In 2004, average decade amount of precipitation was 266±14.7mm. The first decade of June was the driest with only 2.3 mm of precipitation, and the third decade of August was the most humid with 57.2 mm of precipitation. This constitutes 58.4% of the total precipitation in August. It should be noted that in 2004 the number of rainy days were distributed very unevenly: in June – 21 rainy days, in July and August – 14 rainy days in each. Rains were less frequent during the third decade of July, as well as in first and second decades of August. The precipitation amount was close to the many-year average.

There were 19 rainy days in June 2005; precipitation amount – 78.5 mm. July was dry. During the first half of the month, only 2 days were rainy with only 0.7 mm of precipitation. Average relative air humidity reached only 67.8±6.6%, being by 10% lower than the many-year average. During the second half of July the precipitation was more abundant, and 44.6 mm fell during 11 rainy days. The highest amount of precipitation was measured during the first decade of August (126.1 mm); the many-year precipitation average was exceeded by 4.5 times. During 6 rainy days, 92.2% of the many-year average August precipitation fell.

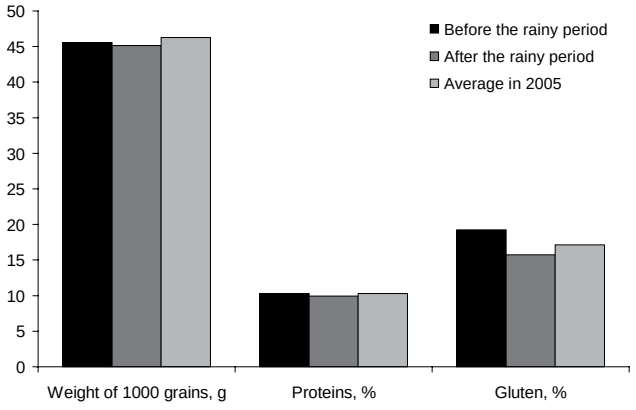

Figure 4. Impact of the rainy period upon wheat quality.

Meteorological conditions predominating during grain maturing and harvesting have the determinant influence on the grain quality, evaluated by the weight of 1,000 grains, percentage of proteins and gluten. The research data on the dependence of the quality indices of winter wheat 'Širvinta' upon environmental conditions is presented in Figure 3.

Significant changes in quality indices of wheat are noticed after rainy periods. This is evident when comparing quality indices of grain of the year 2005 before and after rainy periods when the many-year precipitation average was exceeded by 4.5 times (Fig. 4).

All indices defining the grain quality worsened, especially the percent of winter wheat gluten – from 19.26% before the rainy period to 15.75% afterwards. This index was lower than the annual average (17.15%).

As the quality of winter wheat worsens and relative air humidity increases, conditions form for the hospitable development of microscopic fungi and more intensive contamination of grain. The fungi can more easily penetrate inside a grain and consume the substances accumulated there because the protective properties of grains considerably weaken after environmental stress.

Before harvesting, the contamination of the ears with microscopic fungi was unequal and strongly depended upon meteorological and other environmental conditions.

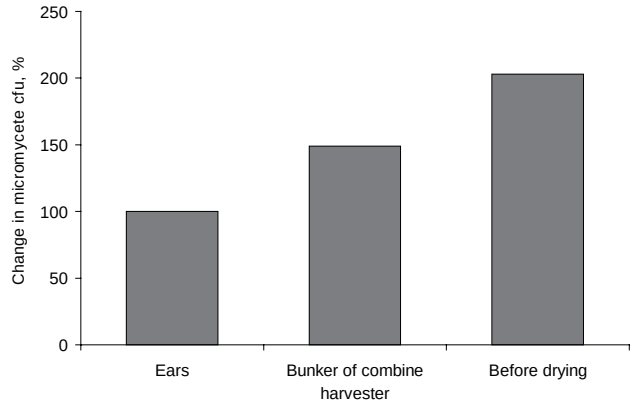

Figure 5. Abundance of micromycetes (cfu · g^{-1}) in grain during harvesting.

However, the abundance of fungi in the ears never exceeded the abundance of fungi on freshly threshed grain from the combine harvester bunker or on grain brought in for storage (Fig. 5).

In the combine harvester bunker the number of micromycete propagules was by 30% higher than in the ears before harvesting. This can be explained by the fact that in the course of harvesting, as operating parts of the harvester touch the grain and soil surface, the dust rises and part of it gets into the bunker together with grain. The operating parts of the combine harvester have to be regulated so as to allow the least possible amount of dust and, consequently, microscopic fungi propagules, to get inside the harvester. The purifier should ensure that only clean grain free of any additional impurities get into the bunker. From the bunker, the grain gets into transportation vehicles and later into granaries where additional contamination sources are present, i.e. grain surface, walls and flooring of granaries, and other equipment.

Analysis of winter wheat from the bunker revealed that on mechanically damaged grains the abundance of microscopic fungi is by one-third higher than on the intact grain. *Aspergillus oryzae*, *Penicillium expansum*, *Alternaria alternata*, *Fusarium sporotrichioides*, *F. culmorum*, *F. tricinctum* prevailed on the damaged grain. Therefore, in order to ensure the safety of food raw materials, it is essential to ensure appropriate grain harvesting, protecting it from mechanical damage.

In the course of the research, microscopic fungi of 56 species ascribed to the *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Acremonium*, *Cladosporium*, *Rhizomucor*, *Rhizopus*, *Bipolaris*, *Paecilomyces*, *Oidiodendrom*, *Mucor*, *Mortierella*, *Myrothecium*, *Humicola*, less frequently other genera were isolated from grain of winter wheat "Širvinta" (Tab. 1). Later, they were grouped according to the density of population according to Mirčink (1988). Fungi with the population density >50% were ascribed to dominant ones (*Alternaria alternata*, *Fusarium culmorum*, *F. avenaceum*, *F. tricinctum*, *Penicillium chrysogenum*).

Micromycetes of 85 species were isolated from grain of malt barley 'Barke'. Together with the above-mentioned fungi, *Absidia*, *Acremoniella*, *Athrobotrys*, *Aureobosidium*, *Botrytis*, *Circinella*, *Colletotrichum*, *Cunninghamella*, *Culvularia*, *Dorotomyces*, *Exophiala*, *Fulvia*, *Geomyces*, *Geotrichum*, *Gliocladium*, *Monilia*, *Phoma*, *Scytalidium*, *Sporotrichum*, *Ulocladium*, *Verticillium* were also recorded. *Aspergillus flavus*, *Fusarium culmorum*, *F. tricinctum*, *F. clamydosporium*, *Bipolaris sorokiniana* dominated.

Thirty-six species of fungi of similar systematic affiliation were isolated from fodder barley 'Henni'. The above-mentoned list was supplemented with fungi of the *Rhodotorula*, *Athrinium*, *Chrysosporium*, *Sepedonium*, *Thamnidium*, *Volutella* genera. *Alternaria alternata*, *Fusarium culmorum*, *F. tricinctum*, *F. avenaceum* were dominant (Tab. 1.)

According to the data of the Ministry of Agriculture of the Republic of Lithuania [49], in the stored winter wheat

Table 1. Contamination of freshly threshed grain before drying by active ventilation, cfu·g^{-1}.

Grain	Grain moisture, %	Propagule abundance	Species number	Dominant micromycetes, distribution frequency >50%
Winter wheat 'Širvinta'	16.0 ± 0.053	$1.8 \times 10^3 \pm 390$	14	*Alternaria alternata, Fusarium culmorum, F. avenaceum, F. tricinctum, Penicillium chrysogenum*
Malts barley 'Barke'	18.7 ± 0.096	$5.5 \times 10^3 \pm 690$	12	*Aspergillus flavus, Fusarium culmorum, F. tricinctum, F. clamydosporum, Bipolaris sorokiniana*
Fodder barley 'Henni'	17.9 ± 0.071	$1.6 \times 10^4 \pm 1{,}580$	11	*Alternaria alternata, Fusarium culmorum, F. tricinctum, F. avenaceum*

grain of the year 2004 and in grain cultivated in 2005, deoxynivalenol was recorded. From 30.9-60.3% of the sampled grain was contaminated. Most heavily contaminated (146.8 μg × kg^{-1}) were grain of the most frequently cultivated winter wheat 'Zentos'. In grain of other winter wheat varieties the concentrations of deoxynivalenol were lower – 'Bussard' – 92.3 μg × kg^{-1}, 'Širvinta I' – 95.4 μg × kg^{-1}, 'Lars' – 32.2 μg × kg^{-1}. Aflatoxins were recorded in 47.7% of barley samples. In some barley samples, ochratoxins exceeded the allowable limits. In 2004-2005, the contamination of oats with T-2 toxin constituted 100%. In some samples of oats, its concentration exceeded a hazardous concentration of 121.5 μg × kg^{-1}. Recently, in seaside regions of the country, a tendency towards the increase of aflatoxin in grain has been observed. This is related with warm and rainy vegetation periods and intensive spread of *Aspergillus flavus* fungi. Aflatoxin was recorded in grain of fodder barley 'Henni' (1.3 μg × kg^{-1}).

In many cases, during storage the accumulation of toxins in grain continues or even intensifies. This is due to active functioning of microscopic fungi on the stored grain and indicates the need of preventive measures insuring the safety of food raw material. The most popular way of grain conservation is drying in shaft driers or granaries with active ventilation, where excess water is removed from grain and conditions unfavourable for the development of microscopic fungi are formed.

In a shaft drier, a grain mound slowly moves downwards blown by a drying agent. Temperature of the drying agent

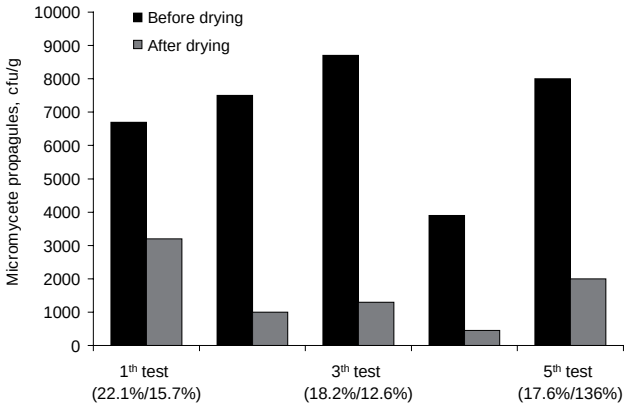

Figure 6. Changes in the micromycete abundance (cfu · g^{-1}) in the course of grain drying in thermal dryer (grain moisture before and after drying).

depends upon the grain, their initial moisture and purpose. This is usually not lower than 75°C. High temperature of the drying agent ensures intensive drying and rapid suppression of mould vitality. Still, high temperature is a risk factor influencing the indices of grain viability, resistance and quality.

During the research on grain drying in a shaft drier, the grain was dried using ambient air heated to 90°C. The moisture of the dried wheat did not exceed 14.5% (Fig. 6), except for the case when grain with 22.5±0.076% moisture had been brought in. Because of an improperly adjusted drier, even after drying the amount of moisture in grain still remained favourable for the development of microscopic fungi, i.e. 15.7±0.065%.

After drying in a shaft drier, the abundance of micromycetes in grain decreased from 2.07 to 8.67 times. The effect of drying upon the abundance of microscopic fungi depends on the initial grain moisture (determination coefficient 0.872):

$$N_i = \frac{674}{e^{0.1975 \times \omega_0}},$$

here N_i – decrease intensity in a number of microscopic fungi (cfu·g^{-1}), %/%; ω_0 – initial grain moisture, %.

The smaller the coefficient, the more efficient the drying process, i.e. a decrease in their number [93]. This is predetermined by higher temperature of grain. Temperature measurement of grain discharged from the drying zone of the drier indicated that in the dryer grain it warms up to 34.3±3.5°C. This, by a few degrees exceeds the optimal temperature for the development of mould fungi spread on grain. Under such conditions, the development of most fungi slows down considerably, and some of them perish. Simultaneously, intensively vaporizing humidity prevents more humid grain from heating. Therefore, viability of mould fungi present on dryer grain is influenced not just by low moisture of grain, but also by relatively high temperature. Thus, the effect of drying becomes more efficient.

Another method is also used for grain drying: drying in granaries with active ventilation, usually used on medium and small farms. It is an effective method, but its success very much depends upon meteorological conditions and process control. Contrary to the shaft driers, here, grain is usually ventilated by ambient air or ambient air warmed by a few degrees. The removal of humidity from grain and the

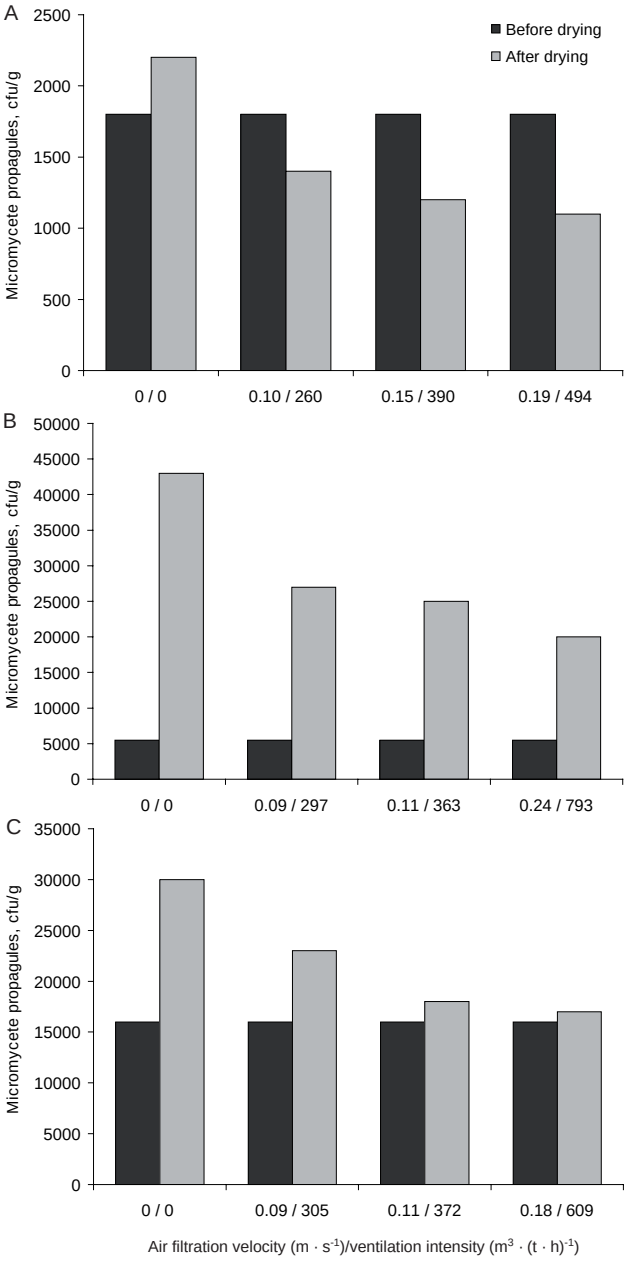

Figure 7. Grain drying duration and contamination with micromycete propagules in top layer of the grain mound before and after drying: A – winter wheat, B – malts barley, C – fodder barley.

process of their conservation are therefore slow. Besides, grain is usually dried in a stationary mound, and the drying takes place only in its small layer, the so-called drying zone. This shifts slowly in the direction of the air flow motion, and involves more and more new layers of humid grain untill it reaches the top of the mound and the grain there. Because of slow and uneven drying in the top layers of the grain mound, conditions hospitable for micromycete development remain untill the end of the drying process. In the top layers of the dried winter wheat, malt barley and fodder barley grain, moisture of 14-14.5% is achieved from 5-43 hours later than on the average in the whole mound. It is important that the grain should dry quicker than it is damaged by microscopic

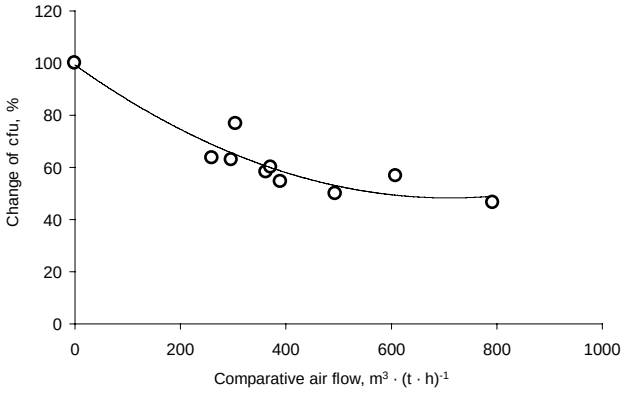

Figure 8. Impact of ventilation intensity on the number of micromycete propagules in grain.

fungi. Only then can drying in active ventilation granaries be successful, and grain safe to consume.

The grain drying rate usually depends on the ambient air temperature and relative humidity, ventilation intensity, and correct control of the drying process. In the course of investigations on the application of active ventilation, the worst conditions were during the drying of malt barley – the air temperature of the environment and relative air humidity were 19.7±2.0°C and 79.9±12.2%, respectively. High relative humidity and incorrect control of the drying process created such conditions that, while ventilating, the grain was not just dried but simultaneously also moistened due to very high relative humidity. The moisture absorbed by malt barley made from 45.7% (at v=0.24 m·s^{-1}) to 56% (at v=0.09 m·s^{-1}) of the vaporized water amount. Therefore, the grain drying protracted, and the medium hospitable for micromycete development remained in the mound for rather a long time. After drying, even in ventilated grain, the number of microscopic fungi was more than 3.5 times higher than before drying, while in non-ventilated grain (at v=0 m·s^{-1}) this number increased by more than 7.8 times. A similar situation was recorded while drying fodder barley. After drying, in all cases, the number of micromycetes increased. The still lower initial moisture of fodder barley, more favourable environmental conditions (67.2±7.8%) and shorter drying period insured the increase in micromycete number from 1.1 to 1.4 times in ventilated and 1.9 times in non-ventilated grain. An increase in the number of microscopic fungi was avoided only in the drying process of winter wheat. Low initial moisture and favourable environmental conditions (air temperature of 21.1±2.0°C, relative air humidity of 68.5±9.3%) insured that grain was rapidly dried to 14-14.5% moisture, which is unfavourable for micromycete development. The abundance of microscopic fungi propagules in ventilated grain was reduced by 1.6 times (Fig. 7). However, in non-ventilated winter wheat grain (at v=0 m·s^{-1}) the number of mould fungi increased by 1.2 times, similar to the trials with malts and fodder barley.

Air filtration through grain mound suppresses the micromycete development (Fig. 8). At the comparative air

Table 2. Contamination of grain with micromycetes after application of active ventilation.

Air filtration velocity	Number of propagules		Species number	Dominant micromycetes distribution frequency >50%
$m \cdot s^{-1}$	$cfu \cdot g^{-1}$	reduction, %	n	
Winter wheat 'Širvinta'				
0	$2.2 \cdot 10^3 \pm 320$	100	7	*Alternaria alternata, Fusarium culmorum, Fusarium avenaceum, Penicillium chrysogenum*
0.1	$1.4 \cdot 10^3 \pm 300$	63.6	9	
0.15	$1.2 \cdot 10^3 \pm 180$	54.5	10	
0.19	$1.1 \cdot 10^3 \pm 160$	50.0	10	
Malts barley 'Barke'				
0	$4.3 \cdot 10^4 \pm 6{,}100$	100	8	*Aspergillus flavus, Bipolaris sorokiniana, Fusarium avenaceum, Fusarium culmorum, Fusarium chlamydosporum, Fusarium tricinctum*
0,09	$2.7 \cdot 10^4 \pm 4{,}900$	62.8	10	
0.11	$2.5 \cdot 10^4 \pm 3{,}100$	58.1	9	
0.24	$2.0 \cdot 10^4 \pm 2{,}800$	46.5	11	
Fodder barley 'Henni'				
0	$3 \cdot 10^4 \pm 4{,}100$	100	10	*Altrnaria alternata, Fusarium avenaceum, Fusarium culmorum, usarium tricinctum*
0.09	$2.3 \cdot 10^4 \pm 3{,}600$	76.7	11	
0.11	$1.8 \cdot 10^4 \pm 2{,}000$	60.0	12	
0.18	$1.7 \cdot 10^4 \pm 1{,}600$	56.7	11	

flow of 500 $m^3 \times (t \cdot h)^{-1}$ the number of propagules was by 48% lower than in non-ventilated grain. The consequent increase in ventilation intensity had no significant impact on the decrease in the number of micromycetes in grain.

Data on grain contamination with micromycetes after application of active ventilation are presented in Table 2.

The use of the active ventilation does not guarantee that the number of micromycete propagules will decrease after the drying process. The correct control of the drying process is an extremely important point to consider. Ventilation under unfavourable conditions moistures the grain. Thus, the drying time is increased, and at the same time mould activity is induced. The amount of micromycete propagules in the grain after the drying decreased only in winter wheat. An increase in the amount of propagules in fodder barley was insignificant – 6.25% (at v=0.18 $m \cdot s^{-1}$), 12.5% (at v=0.11 $m \cdot s^{-1}$) and 43.8% (at v=0.09 $m \cdot s^{-1}$). There was a significant decrease in malt barley quality. The number of microscopic fungi on these grain increased from 3.64-4.9 times. Drying of malt barley was the longest. The species diversity of the fungi recorded during the drying process slightly varied, the species shown in Table 2 dominated.

Dominance of the mentioned fungal species on the harvested grain is due to conditions hospitable for their development and abilities to suppress the development of other fungi. Therefore, the mentioned fungi damaged and contaminated 100% of winter wheat, malt and fodder barley grain. Based on the obtained results and reference sources, the main cultural, morphological properties and some biological features influencing the potential hazard of these fungi to food safety and human health should be presented.

Alternaria alternata (Fr.) Keissl. Syn. *Alternata tenius*. Nees. Brown to black growth consisting of conidia in long

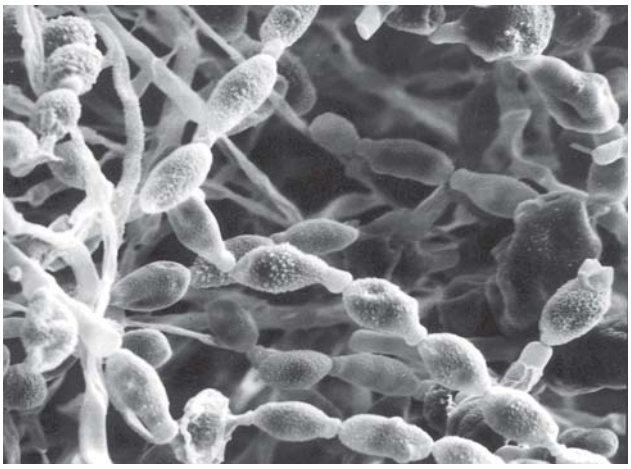

Figure 9. *Alternaria alternata* general view of micromycete conidiogenesis, ×750.

Figure 10. *Bipolaris sorokiniana* general view of micromycete conidiogenesis, ×200.

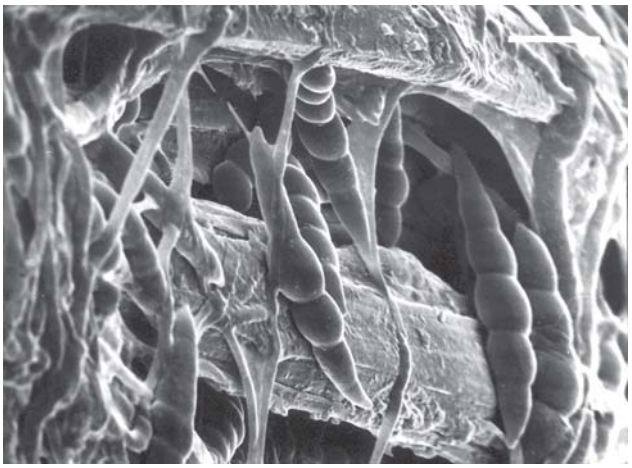

Figure 11. *Fusarium avenaceum* general view of micromycete macroconidia, ×1000.

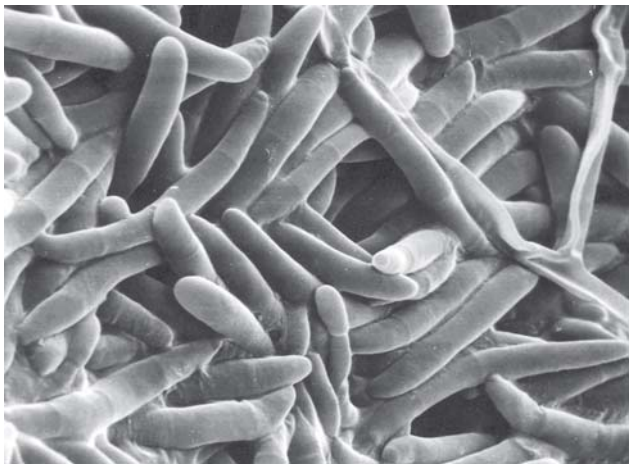

Figure 12. *Fusarium culmorum* general view of micromycete macroconidia, ×1500.

chains (as seen in Fig. 9 and in the line drawing) is characteristic of the species. The chains are usually simple, but can also be branched. Conidia polymorphous, short to long, olive brown to dark brown, highly variable in scope, oval to cylindrical, size and number of septation: transverse, longitudinal and oblique, 10-58 × 7-19 μm [56]. Conidial beaks also show variation, short to long, mostly of the same colour as the main body or a little lighter, 2-19 μm in length. Total length of conidia with beak – 10-71 μm [50].

Micromycetes of this species are recorded in plant rhizosphere soil, plant remnants; they are sometimes isolated from air or technical substances of diverse chemical composition. Optimal growth temperature – from 22-30ºC, maximum – 31-35ºC. The fungus is sometimes found at a temperature of 2-5ºC, or in juice after heating up to 65ºC. The minimum humidity for vegetative growth is at 210 bars water potential, for sporulation – 70 bars, for conidial germination – 100 bars or dew deposition [62]. The most favourable conditions for growth and development are at substrate pH 4.0-5.0, but certain strains can function at substrate pH 2.7-8.0. The fungi are resistant to various chemical substances and physical factors. In darkness, the formation of conidia is more intense. The species is able to accumulate heavy metals Ca, Zn, Cu, Fe, Mn, etc., intensively destructs lignin and cellulose rich substrates, pectin, easily adapts to different substrates, produces various enzymes [43]. The fungi of this species are able to synthesise and excrete various toxic metabolites: dehydroaltenusin, alternariol, altenuisol, altertenuol, altenusin, altenuic acid I, II, III, ergosterol, ergosta – 4, 6, 8 (14), 22-tetraen-3-one, altertoxin I, II, III dihydroalterperylenol, tenuazonic acid [10, 11, 69]. There are strong (numerous) evidence of the development of *A. alternata* in human skin and hypodermic tissues [3, 4], especially in case of AIDS or other diseases related to immune deficiency [39, 43, 88]. *A. alternata* fungi can infect bones, eyes, prosthetic eyes [71], ears, sinuses and the ureter [70, 77].

Aspergillus flavus Link. The distinction between *A. flavus*, *A. parasiticus*, *A. oryzae* and other similar species is often difficult to observe because integrating strains may occur [74]. Colonies on Czapek agar at 25ºC attaining a diameter of 3-5 cm within 7 days, usually consist of a dense felt of yellow-green conidiophores. Conidial heads typically radiate, later splitting into several loose columns, yellow-green, becoming dark yellow-green. Conidiophores hyaline, coarsely roughened, up to 1.0 mm or more in length. Vesicles globose to subglobose. Phialides borne directly on the vesicle or on the metulae, 6 × 10 × 4-5.5 μm. Metulae 6.5-10 × 3.5 μm. Conidia globose to subglobose, 3.6 μm in diameter, pale green, echinulate. Sclerotia often produced in fresh isolates, variable in shape and dimension, often brown to black.

A. flavus was isolated from barley, rye, corn, corn fodder, ground nuts, spices, oilseeds, occasionally from died fruits [47]. Important toxic metabolites: various aflatoxins, kojic acid, 3-nitropropionic acid, cyclopiazonic acid, aspergillic acid, ditryptophenaline, aflavinine, dihydroxyaflavinine, aflavazole, paspalinine, aflatrem, parasiticol, aspertoxin *et al.* [11].

Due to their toxicity, these fungi arouse interest worldwide. The diseases caused by *A. flavus* in humans, animals and birds are widely known; it is an occasional agent of pulmonary or disseminated infection in the immuno-compromised individuals. Cases of sinusitis and onychomycosis have also been reported. In animals, it may be an agent of respiratory diseases, bronchomycosis, etc. [43, 51, 91]. This fungus is responsible for granuloma of lymph nodes in humans, called soil aspergilloma [70].

Bipolaris sorokiniana (Sacc). Shoem. Syns. *Helminthosporium sativum* Sacc. *Drechslera sorokiniana* (Sacc.) Subram. et Jain (Fig. 10). Conidiophores of the fungus are brown, short, erect, in most cases single, bearing 1-6 conidia aeropleurogenously arranged at short distances in the upper half. Arrangement of shiny conidia or conidiophores is characteristic of the species and appears “palm tree-like”. Hardly any recognizable mycelium is produced on the infected grain or seed. Often, the growth of the fungus covers the entire grain or seed, and in a few cases the growth

may extend to the blotter where conidia are produced in the same manner as on seed or grain. Conidia ellipsoid, dark brown to black, smooth, mostly straight or slightly curved, wall thick but less so towards the ends, broadest in the middle, ends rounded, sear clearly seen within the basal cell, 3-12 distoseptate (mostly 6-12), 40-130 μm (mostly 60-100 μm) long, 17-28 μm (mostly 18-23 μm) vide.

These fungi are known to infect barley and other grain, as well as causing crop root rots, but they can develop on various plants and cause root and stem diseases. The degree of virulence of various strains is unequal in different plant species. The relation between strain virulence and ability to synthesise the mentioned toxins was not established [86]. Systematic position of the fungi of this genus is not clear. Outspread of the fungi on grain widely used for food and fodder should be mentioned: on wheat, barley, oats, oats and vetch mix, rye, triticale, wheat flour, barley flour, barley bran, barley groats, potatoes. Therefore, these fungi could be treated as potential pathogens [31, 61]. Under natural conditions, some strains of this species produce toxic secondary metabolites: curvularin, cynodontin, bipolaramide, sterigmatocystin, *trans*-sativenediol, isosativenediol, dihydroprehelminthosporol, helminthosporol acid, helminthosporic acid [11]. Terebinthine toxins produced by these fungi are also known: helminthosporol and longifolene. The mentioned biological peculiarities of this fungus make the contaminated raw food materials potentially hazardous to human health.

Fusarium avenaceum (Fr.) Sacc. (Fig. 11). On malt extract agar (Difco) growth is rapid, the aerial mycelium is dense and white but frequently varies in colour from tan to reddish-brown. Orange sporodochia may be present as the culture ages. The undersurface colouration varies from carmine red to dark brown. Macro conidia are generally scarce, oval-shaped, 0-3-septate, elongate spindle-shaped. Macro conidia are very long, slender, thin-walled, with an apical cell that is elongate and may be bent. The basal cell is foot-shaped or notched. Conidiophores are unbranched and branched monophialides. Chlamydospores absent. Sclerotia 60-80 μm in diameter, dark bluish, yellowish, dark purple, brown, yellow, white, sometimes absent [57, 58].

Fungi of this species are widespread on various substrates, therefore considered cosmopolitan. They infect plants of about 160 species, most frequently rotting roots and stem base. Stem of grain become grey, rusty, necrotic. The fungi intensively develop on vegetables, fruit, seeds, grain. They were also recorded on barley, wheat, oats, barley flour and bran [47]. These fungi are reported to be toxic [43, 53, 57, 83]. According Cole & Schweikert [11], Cole *et al.* [10] *Fusarium avenaceum* produces and excretes neosolaniol, solaniol, HT-2 toxin, 4-acetyl-T-2-tetraol, 15-deacetylneosolaniol, moniliformin. Fungi of this species are pathogenic to animals, especially horses and pigs [76]. There is some evidence that these fungi can be the reason for leucopenia in people [19].

Fusarium chlamydosporum Wollenw. & Reinking Syn. *F. fusarioides* (Frag & Cif.) Booth, *F. sporotrichioides* Sherb. var *chlamydosporum* rum (Wollenw & Reinking) Joffe, *F. tricinctum* Corda emend Snyd et Hans [25]. On malt extract agar (Difco) growth is rapid with dense, white to pink to brown aerial mycelium. Sporodochia are rare. The undersurface is generally carmine red but may as well be tan to brown. Microconidia are abundant, 0-1-septate, mostly spindle-shaped but no globose. Macroconidia are generally rare, typically sickle-shaped, 3-5-septate, and have a basal cell that is foot-shaped. Microconidiophores are unbranched and branched monophialides and polyphialides. Chlamydospores present and abundant, rough walled, occur in pairs chains or clumps. Chlamydospores may occur in such large quantities that they cause the mycelium to become brown in colour.

F. chlamydosporum is cosmopolitan, but can be regarded as a relatively rare soil inhabitant. The minimum temperature for growth is 5ºC, the optimum about 27ºC and the maximum 37ºC. It can cause wilting of bean seedlings *in vitro* [25]. During the research, these fungi were often recorded on heating barley, oats, wheat grain in storehouses. Under such conditions these fungi can synthesise moniliformin with an LD_{50} of $mg \cdot kg^{-1}$ in day old cockerels (160 $\mu g \cdot (40\ g)^{-1}$ cockerel). Gross and histology lesions in cockerels were ascites with edema of the mesenteries and small haemorrhages in the proventriculus gizzard, small and large intestine and skin [10]. There are references about the pathogenicity of this fungus, ability to infect lymph nodes and other organs in humans [28, 30].

If we agreed with the opinion of Ukrainian scientists [70], that *F. chlamydosporum* Wollenw. & Reinking Syn. species are identical to *F. sporotrichiella* (Bilai 1965), the description of its pathogenic features should be much wider. Additional ecological, systematic, physiological and pathological investigations performed with applying modern methods are necessary.

Fusarium culmorum (W.G. Smith) Sacc. (Fig. 12). Syn. *Fusarium cerealis* Cooke 1878.

On malt extract agar (Difco) growth is rapid, with dense aerial mycelium, generally white but often yellow to tan toward the base of the slant. Orange to red-brown sporodochia appear as the culture ages. The undersurface is carmine red. Microconidia are absent. Macroconidia are stout, distinctly septate, thick-walled, and have curved ventral and dorsal surfaces. Conidiophores are unbranched and branched monophialides. Chlamydospores generally form abundantly and quickly, they may occur singly, in chains or in clumps [57].

The fungi of this species are widespread on various plants, infect roots of many outdoor and greenhouse plants, are considered among the most hazardous agents of plant root rots. The optimal temperature for growth is 25ºC, the maximum 31ºC, and the minimum 0ºC. Optimal growth an agar occurs at pH values between 4-8; the minimum water potential is at 130 bars. *F. culmorum* is osmo-tolerant, autotrophic for growth substances an can grow in atmosphere

with 3-7% CO_2, good development is also possible under reduced O_2 partial pressure [12]. It actively absorbs nitrates, destroys cellulose, lignin, simazine and other pesticides, individually and sensitively reacts towards the changing substrate composition. *F. culmorum* is cosmopolitan. The major part of the isolated range of the *F. culmorum* strains can potentially synthesize secondary metabolites ascribed to the trichothecene. The trichothecenes show a wide range of biological activity as antibacterial, antiviral, antifungal and cytostatic agents; some are phytotoxic, and all show some degree of animal toxicity, including insecticide activity. Biochemically they are potent inhibitors of protein and DNA synthesis in whole-cell and cell-free systems. General gross clinical signs reported for trichothecene are vomiting, diarrhoea, anorexia, ataxia, haematuria, and leukocytosis, soon followed by severe leukopoenia, inflammation of the gastrointestinal tract, degeneration and haemorrhaging of cardiac muscle, and lesions of lymph nodes, testes, and thymus [12, 47]. These fungi can cause skin keratonosis and mycetoma, infect leg joints, and settle in the nasal cavity [28, 58].

Fusarium tricinctum (Corda) Sacc. (Syn. *F. sporotrichioides* Sherb. var. *tricinctum* (Corda) Raillo, 1935, *F. sporotrichiella* Bilai var *tricinctum* (Corda) Bilai 1955). On malt extract agar (Difco) growth is rapid, and the aerial mycelium is dense and white, with orange sporodochia appearing at the culture edges. The undersurface is carmine red. Microconidia are abundant and are lemon- to pear-shaped or spindle-shaped, 0-1-septate, and of ten have a papilla at the base. Macroconidia are also abundant, sickle-shaped. The basal cell is distinctly foot-shaped or notched. Conidiophores are unbranched and branched monophialides. Chlamydospores present and formed singly or in chains [57]. Occurrence of that fungus in cultivated soil, forest and grassland has been reported from different countries. It has been isolated from cereals: wheat, barley, barley flour, and barley bran [12, 43, 47].

The optimal temperature for growth is 22-27ºC, the maximum 35ºC and the minimum 2-7ºC. The humidity minimum for vegetative growth is at 170 bars, but half the optimal growth occurs at 55 bars water potential [20]. The fungi of this species are potential producers of toxins of the trichothecenes group: NT-1 toxin; 4,15 diacetoxyscirpenol; anquidine; T-2 toxin; isoneosolaniol; acuminatum; 8a,15-diacetoxy-T-2tetraol; 8-acetoxyneosolaniol; 15-deacetylneosolaniol; HT-2 toxin; 4-acetyl-T-2 tetraol [10, 37, 38]. Considerable difference among the isolated strains of the fungus should be mentioned, some of them cause clearly seen oedemas and intradermal hosmorrharges in rot skin, the impact of others is less pronounced. Pathogenicity is characteristic of all the fungi of the *Fusarium* genus ascribed to *Sporotrichiella* [85]; therefore, their spread on food raw materials is unwelcome and hazardous.

Penicillium chrysogenum Thom. Diagnostic features after Frisvad and Samson [73]: roguefortine C and D, chrysogine, penicillin F and G, globose to subglobose to broadly elipsoidal smooth-walled conidia, relatively short phialidis with short broad collula, high growth rate on YES (yeast extract sucrose) with a yellow reverse and strong sporulation. Conidiophores: bi-, ter- and quarterverticillate both appressed and divergent rami born from aerial and subsurface hyphae. Conidia: smooth-walled, globose to subglobose to broadly ellipsoidal, 2.5-4 × 2.3-3.5 µm. Phialides, cylindrical, with short broad collula. Metulae and rami cylindrical. Colony texture: floccose to velutinous. Conidium colour on CYA (Czapek yeast autolysate): blue green to green, exudates droplets often present, copious, yellow, reverse: cream, yellow, rarely brown on CYA and lemon yellow on YES. Yellow pigment often produced. Odour and volatile metabolites: 3-octanone; 1-heptene: 1,3-octadiene; 3-heptonone; 1-nonene; 1-octen–3-ol; 3-octanol (pineapple odour at low water activities) Larsen et Frisvad [36].

Growth has been observed at water activities between saturation and 350 bars, at temperature of 5ºC, but not at 37ºC, with the optimum at 23ºC. Extrolites: penicillins, roquefortine C and meleagrin, chrysogine, xanthocillins, secalonic acids, sorrentanone and sorbicilin, PR-toxin. As-mycotoxins have been recorded: roquefortine C, secalonic acids, PR-toxin, penicillic acid, cyclopiazonic acid, patulin, ochratoxin A [10, 11, 69]. Some strains of this species are characterised by pathogenicity [43, 45, 70]. They often cause endocarditis – inflammation of the endocardium [84]. The fungi of this species can be recorded in the ear orifice as the causative agent of otomycosis [89], they infect eyes causing endophthalmitis [17], inflammation of the cornea – keratitis [64]. There is some evidence for this fungi causing necrotic esophagus inflammation in patients with AIDS [21]. In pathologic material this fungus is often recorded together with the fungi of the *Fusarium*, *Pythium*, *Candida* genera [91]. Food grain contaminated with this fungus could be attributed to raw materials unsuitable for consumption and hazardous for health. It should be noted that conidia of this fungus easily detach, and together with dust can get on food raw material; such air is inhaled by people maintaining and processing the contaminated raw materials [54, 82].

Penicillium verrucosum Dierckx. Diagnostic features after Samson and Frisvad [73]: Production of ochratoxin A, citrinin, verrucolone, verrucins, smooth-walled conidia, red brown reverse on YES, slow growing on all media. A red-brown to terracotta reverse. Conidiophores: terverticillate, appressed elements, borne from surface or subsurface hyphae. Conidia rough-walled, globose to subglobuse 2.6-3.2 µm. Phialides: cylindrical tapering to a distinct collulum. Metulae and rami: cylindrical. Stipes: rough-walled. Colony texture on CYA: velutinous to floccose to weakly fasciculate, conidium green. Exudate droplets copious, clear. Odour and volatile metabolites: 2-pentanone, 2-butanone, isobutanol, isopentanol, 3-octanone [36, 81]. Most common in cold temperature regions on barley, oats rye, wheat, cheese – deteriorate cereals and produce brown spots on cheese [48].

Conidia germinate at temperatures between 4-37ºC, minimum water potential at 270 bars. Some varieties of *P. verrucosum* can grow within a temperature range of (-2) or +4ºC to 30 or 35ºC, with the optimum between 21-23ºC. The thermal death point for *P. verrucosum* was found to be 63ºC for 30 min in apple juice. Fungi are sensitive to β-irradiation.

Some authors ascribe many micromycetes recorded on wheat grain at various stages of their processing to this species, e.g. *Penicillium corymbiferum* Westling, *Penicillium cyslopium* Westling, *Penicillium puberulum* Bainier, *Penicillium palitans* Westling, *Penicillium terreste* Jensen, *Penicillium aurantiovirens* Biourge, *Penicillium olivinoviride* Biourge, *Penicillium crustosum* Thom. [12, 68]. However, such attribution is rather doubtful because micromycetes of the listed species considerably differ from *P. verrucosum* by morphological characteristics and physiological abilities. It mostly applies to *P. commune*, *P. cyclopium*, *P. palitans*, *P. viridicatum*. The already mentioned R.A. Samson and J.C. Frisvad [73] are of the same opinion, and classify species of the subgenus Penicillium based on morphology, growth pattern, ecology, extrolites and partiol β-tobulin sequences. Based on our research results, we grouped the above-mentioned fungi of the *Penicillium* genus the authors grouped into sections and series. Thus, *Penicillium cyclopium* and *P. viridicatum* micromycetes were ascribed to the section *Viridicata* series *Viridicata*, while *Penicillium verrucosum* – to the section *Viridicata* series *Verrucosa*; *P. commune* and *P. palitans* micromycetes were ascribed to the section *Viridicata* series *Camemberti*. It should be noted that a separate series *Corymbifera* has been isolated in the section *Viridicata*; 7 species are ascribed therein. *Penicillium hirsutum* Dierckx is mentioned among them; it is identified by the authors with *Penicillium corymbiferum* Westling, which is frequently recorded on grain and seeds, and *Penicillium verrucosum* var. *corymbiferum* (Westling) Samson, Stolk, Hadlok [75]. Time will show if these assumptions are correct. At present, many pros and cons are being discussed. Therefore, *P. verrucosum* fungi described in this paper should be regarded as a separate group of distinctive individuals, conditionally not belonging to the mentioned species.

Micromycetes ascribed to *Penicillium verrucosum* are able to synthesise ochratoxin A, penicillic acid, citrinin, verruculogen. Samson and Frisvad [73] state that *Penicillium verrucosum* was a possible candidate to be involved in the Balkan endemic nephropathy. However, because *P. nordicum* is much more prevalent in the Balkan countries and grows on dried meat products, this species may play a contributing role to Balkan endemic nephropathy in combination with other species in the series Viridicata.

While estimating the hazard caused by the spread of these fungi on food raw materials and final foodstuffs, their abilities to produce and excrete into the environment a wide range of toxic substances, as well as their parasitic properties and role in causing various diseases, should be considered [34].

CONCLUSIONS

Results of the research performed in 2003-2005 allow the conclusion that the number of fungal propagules on freshly threshed grain strongly depends upon meteorological conditions, which precondition the intensity of plant growth and development, maturity and quality of grain.

Under contrasting meteorological conditions, the tested grain was not equally mature and the quality indices (weight of 1,000 grains, percentage of proteins and gluten) differed in the course of harvesting.

Analytical expression of the dependence of decrease intensity in the abundance of microscopic fungi upon initial grain moisture was determined; its determination coefficient equals 0.872.

Air filtration through the grain mound suppresses the viability of microscopic fungi, but it does not protect the humid grain against fungal contamination. Technological measures ensuring the correct control of the drying process and rapid reduction of grain moisture to the level (14-14.5%) unfavourable for micromycete development are proposed.

The success of grain drying depends on the plant species, i.e. winter wheat grain is easier to dry, while the drying of malt barley is considerably more complicated. It plays a significant role in grain contamination with fungi after the drying. After application of identical drying technologies, the number of microscopic fungi propagules in barley grain was 3-4 times higher than in winter wheat grain.

Micromycetes of over 70 species ascribed to 16 genera were isolated and identified, the density of their populations in grain was determined. Micromycetes with a population density of >50% were attributed to the dominant. During research, species diversity of micromycetes recorded on other grain as well as dominant species changed slightly. Short biological characteristic of the dominating micromycetes are provided; factors determining intensity of their development and abilities to synthesise and excrete toxic metabolites are indicated.

After drying, *Alternaria alternata*, *Fusarium avenaceum*, *F. culmorum*, *F. tricinctum*, *Penicillium verrucosum* dominated in winter wheat grain; *Aspergillus flavus*, *Bipolaris sorokiniana*, *Fusarium chlamydosporum*, *F. culmorum*, *F. tricinctum* – in malt barley grain; *Alternaria alternata*, *Fusarium avenaceum*, *F. culmorum*, *F. tricinctum* – in fodder barley grain.

It has been determined that all micromycetes recorded on grain after drying are potential producers of toxic substances, and, therefore, considerably worsen the raw material quality and are hazardous to human health. Factors influencing the synthesis of toxic metabolites in fungi are not yet clear. Literature references confirm the parasitic features of fungi prevailing on the tested grain. Therefore, the abundance of these fungi on food raw materials is unwelcome and hazardous to human health, and constant attention and all possible preventive measures should be employed.

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