

The impact of natural environment and technological measures on the mycological contamination of grain

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Grain contamination with fungi at the moment of harvesting depends upon many factors such as plant genotype, soil, plant rotation, the agrotechnical and agrochemical measures applied, climate. Micromycetes often damage grains while they still ripen in ears. In fields, most frequent on various grains are micromycetes of the genera *Alternaria* and *Fusarium*, which perish if the humidity of the nutritious substrate does not exceed 12–13% ($a_w = 0.65$) for a longer period of time. Micromycetes of the genera *Aspergillus* and *Penicillium* are less sensitive to humidity. These fungi can develop when the humidity of substrate is of the level allowed by the standards for grains or seeds to be admitted for storing. Therefore, the fungi of these genera also prevail on stored grains. Means to decrease grain contamination by fungi should be safe for man and animals both during production and application.

Key words: grain, contamination, micromycetes, drying, anolyte

INTRODUCTION

Studying grain and seed micromycetes, the majority of authors (Christensson, Kauffman, 1969; Chelkowski, 1991) divide them into two groups: field fungi and storage fungi. Such provisory division is mostly based on the varied needs for humidity of the substratum on which these fungi develop. Field fungi are known as mesophylls; this group includes *Alternaria*, *Cladosporium*, *Fusarium* and some species of other genera. Meanwhile, the majority of *Aspergillus*, some *Penicillium*, and species of other genera that demand by far less humidity are ascribed to the xerophiles. The latter can develop in such humidity of the substratum which is, according to the valid standards, acceptable to the storage of grains and seeds. These micromycetes may widely disperse in the conditions of a grain storehouse (Christensson, Kauffman, 1969; Lacey, Magan, 1991; Frisvald, 1995).

Grain ripening in the fields is frequently affected by micromycetes of the genera *Alternaria* and *Fusarium* (Krasauskas, 2002). During the harvest period in the temperate zone countries of Europe, in rainier years the contamination of crop by micromycetes of the genus *Alternaria* reaches 100%. *A. alternata* micromycetes prevail (Chelkowski, Grabarkiewicz-Szczesna, 1991). Meteorological conditions have a great impact on the contamination of crop with micromycetes (Obst et al., 1997a). Conditions for the development of *Fusarium* micromycetes are favourable in summer when the weather is humid and warm. *Fusarium graminearum*, *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. moniliforme* most frequently occur on various grains (Chelkowski,

1991; FAO, 1995). Wheat ears are infested with micromycetes of the genus *Fusarium* during the bloom, especially when the weather is humid and warm (Rintelen, 1997a). Due to the grain ear fusariosis caused by fungi, the fertility of grains, their weight and number in the ear is reduced and the quality of dough cooking is notably lowered (Mielke et al., 2000). During dry years there can be found a number of *Aspergillus*, *Penicillium*, *Cladosporium*, *Drechslera* and micromycetes of some other genera on ripening grains in the fields. *Fusarium* infection of wheat kernels does not only result in a reduction of crop yield, but also in a lower seed and grain quality, especially through the production of mycotoxins (Homdork et al., 2000).

Many recent reports demonstrate that NIV is produced by *Fusarium culmorum* (Abramson et al., 1993; Gareis and Ceyanowa, 1994; Mirocha et al., 1995).

The ability of fungi to produce mycotoxins is greatly influenced by environmental factors, mainly temperature and relative humidity (Tanaka et al., 1998).

In general, conditioned storage with controlled temperature and relative humidity (cool and dry) is required to maintain a good seed and grain quality and to delay seed deterioration (Homdork et al., 2000).

The abundance of micromycete propagules on stored grains depends on the raw material to be stored, on the primary impurity of the bin, the term and conditions of grain storage, especially temperature and humidity. In temperate climate regions, grains before harvest are just slightly contaminated by micromycete propagules, however, while reaping with a combine harvester the number of micromycetes increases sometimes even more than 250 times (Flanigan, 1987). If the humidity

in starchy grain crops (wheat, barley, oats or maize) does not reach 13.5% and 12.5% in albuminous soy, micromycetes do not develop in them.

A very important factor is different content of moisture in the mass of stored grains. Storehouse fungi grow in sufficiently humid places, and this is not necessarily related to the common level of grain mass moisture.

Due to air humidity, differences in the temperature of the storehouse constructions and the air, humidity may condense and fall on dry grain in the form of drops. For this reason, humidity conditions favourable for the proliferation of micromycetes emerge because of the spaces occurring in the mass of grains. The condensation of humidity can also be caused by insects feeding on grain. In the places where they pullulate, humidity condenses because of their breathing and metabolism, and the temperature increases. Such places in the mass of grains are called "hot spots". These spots provide a particularly friendly environment for micromycetes to spread (Pittet, 1998).

The quantity of moisture in grains is reduced by drying it so that fungi would not be able to proliferate when grains are stored. However, when the process is accomplished incorrectly the result is opposite. Too rapid heating of may split the grain hull and facilitate the penetration of fungi (Schmidt, 1991).

With regard to the chemical composition and morphological qualities of grains their drying temperature should be carefully chosen, otherwise overheating may cause the split of the hull and the grain itself. The temperature in the tank when drying wheat, barley and rye should not exceed 43 °C. Micromycetes proliferate faster in broken grains. Overheating may alter the development of fungus dependence on water accessibility and relative quantity, therefore, the colonising of grains with micromycetes becomes possible at a lower a_w index.

The fungi propagules start developing primarily on physically affected grains. Owing to the specific qualities and unique morphological structure of grain, it absorbs the humidity of the environment extremely rapidly. Therefore, fungi colonize them relatively quickly as a favourable nutritional environment.

Considering the fact that most micromycetes found on grains are capable of synthesizing and excreting into the environment various chemical toxic substances, an appreciable threat to grain consumers emerges. Many micromycetes found on grains can synthesize mycotoxins harmful to man and animals (Елинов, 2002). In regions of colder climate the quality of grain yield is conditioned by mycotoxins emitted by *Fusarium*, and in regions of tropical and subtropical climate it is affected by mycotoxins emitted by fungi of the genera *Aspergillus*, *Penicillium* and *Alternaria* (Ellner, 1997; Muthomi et al., 2000).

The possible means of prevention for grain after-yield contamination could be the optimum humidity and temperature when storing, during transportation, the use

of preservatives and adequate technologies of grain processing (Lopez-Garcia et al., 1999). It is impossible, however, to entirely avoid contamination by micromycetes. Researches have proved that 50 to 85% of harvested crops are moister in comparison with the basic (14%) storage humidity (Idler et al., 1998). During the after-harvesting period grains are usually infested with *Aspergillus* and *Penicillium* fungi which get onto grains together with soil dust, plant residues or from the surface of dirty harvesting machines (Abramson, 1998). It is important for grains to be mechanically undamaged during the harvesting period, because the risk of infestation for such grains is considerably greater. The quantity of the micotoxin fumonisin of damaged grains was 10 times larger than in whole grains (Murphy et al., 1993).

Antimicrobial agents should be safe for man and animals both during their production and application, as well as after the end of its employment as intended, i. e. in the period of degradational and destructive changes due to the environmental factors or as a result of bio-degradation processes in the human body; in other words, an antimicrobial agent and the products of its natural or artificial degradation should not contain xenobiotic substances (Bakhir et al., 2004).

One of the most efficient antimicrobial agents that demonstrate no toxicity at all to warm-blooded animals is electrochemically activated water – anolyte. When anolyte was used instead of plumbing water during the hydrothermal processing, the contamination of flour with microorganisms was reduced (Дубикова, 2001).

MATERIALS AND METHODS

Researches were carried out in Kaunas region in 2003–2005. The microbiological contamination of grains before harvesting, during harvesting and processing, and in the storehouse was investigated. Samples of grains from the field of crops were taken before harvest by cutting 20 crop ears 3 times, the grains were winnowed and the quantity of micromycetes was estimated. To assess grain contamination, during the harvesting period grain samples were taken from the combine harvester tank. The impact of transportation on grain contamination was assessed by taking samples from the bin immediately after grain supply. The prevention measures to reduce mycological contamination were surveyed. A grain-processing plant, which used a MEGA-ANTI 64M grain dryer (duty 24 t/h), was chosen for the assessment of the impact of the drying process on grain microbiological contamination.

Harvesting. Combine harvesters started working in the field of wheat when grain moisture reduced to 20%.

Part of wheat after the harvest was left in the trial field for further survey of grain microbiological contamination. The number of micromycete propagules in samples taken from growing wheat ears was assessed. Grain samples for the study were taken five times in

September (with three repetitions). All time of the study the relative air humidity, temperature and quantity of rainfall were monitored at the nearby Kaunas meteorological station.

Isolation and identification of fungi. Agarized media of Czapek (CA) and malt extract (AMA) were used for selection of fungi. Contaminated media were incubated in a thermostat at a temperature of $26 \pm 2^\circ\text{C}$. The growing fungi colonies were measured on 3, 5 and 7 days of development. The grown fungi were purified and identified according to cultural and morphological signs by the method of light microscopy.

For identification of dominant fungi (general and by species), the indicator A of detection frequency was used, calculated by the following formula:

$$A = \frac{B}{C} \times 100\%,$$

where B is the number of samples in which fungi of the same species were found, and C is the total number of samples.

Quantitative grain contamination by fungal propagules (fungal propagules = cfu = colony forming unit) was determined by the dilution method (Trojanowska, 1991).

RESULTS AND DISCUSSION

A number of micromycetes contaminate grains and reduce their quality. Field fungi invade the seeds before harvest while the crop is still in the field. Field fungi may affect the appearance and quality of seed or grain. Usually the damage caused by field fungi occurs before harvest. It can be detected by routine inspection and does not continue to increase in storage if grain is stored at the proper moisture content and temperature. Most field fungi are more prevalent when rainfall is above normal during grain fill and harvest. Invasion by field fungi may be more severe if the crop has been damaged by insects, birds or hail. Field fungi common on wheat grain in Lithuania include species of *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Fusarium*.

Storage fungi (also called storage molds) are the fungi that invade grains or seeds during storage. Storage fungi are usually not present to any serious extent before harvest. Small quantities of spores of storage fungi may be present on grain going into storage or may be present on spilled grain present in harvesting, handling and storage equipment or structures. Under improper storage conditions this small amount of inoculum can increase rapidly, leading to significant problems. Stored grain is contaminated by micromycete propagules through dust; additionally, they are spread by various insects and rodents. The most common storage fungi were species of *Aspergillus* and *Penicillium*. These fungi are widely distributed and almost always present. Owing to the specific morphological qualities of grain, it absorbs humidity from the environment ex-

remely rapidly, what enables the development of micromycete propagules using grain as a nutrient. The development of storage fungi is influenced by the moisture content and temperature of the stored grain, the condition of the grain going into storage, the length of time the grain is stored, and the level of insect and mite activity in the grain. Thus, humidity and temperature are the main ecological factors that foster or suppress the spread of micromycetes in grain.

Meteorological conditions, i. e. air temperature and relative humidity were monitored during crop ripening in July and August 2004 (Fig. 1).

The average weather temperature in July ranged from 15 to 22 °C (Fig. 1 a), meanwhile, the average air temperature in August varied from 13 to 23 °C (Fig. 1 b). On the 11th of August the average air temperature du-

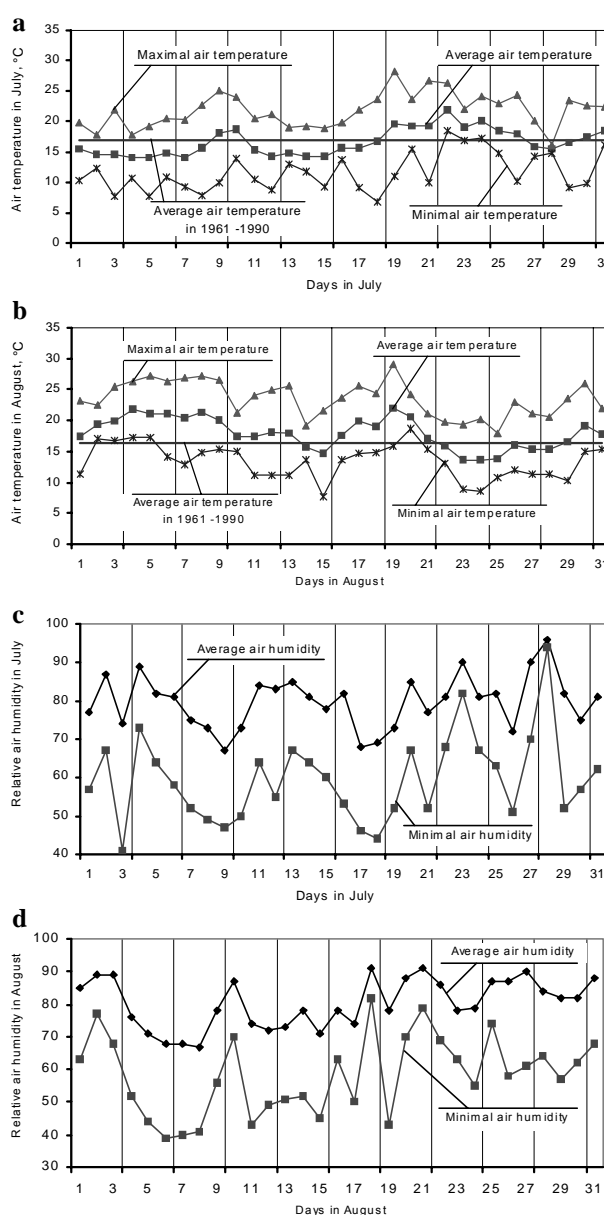


Fig. 1. Changes in air temperature and humidity: **a** – air temperature in July; **b** – air temperature in August; **c** – relative air humidity in July; **d** – relative air humidity in August

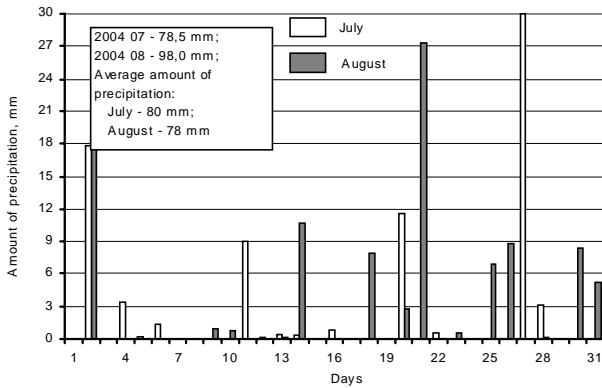


Fig. 2. Quantity of rainfall in July and August

ring the harvest was 18 °C. The average humidity in July ranged within 68–98% (Fig. 1 c). Humidity reached 98% on the 28th of July. The average humidity in August ranged from 68 to 90% (Fig. 1 d). During the harvest, humidity topped 73%. In the second half of August, however, the amount of rainfall increased (Fig. 2) and the average air temperature decreased. It varied from 13 to 17 °C. The crop was harvested under good weather conditions (there was no rainfall) on the 11th of August 2004 (Fig. 2).

At the end of August there was by 7.2 mm more rainfall than at the end of July. During the ripening period fairly humid and warm weather prevailed. The yield of wheat crops was harvested under rather favourable weather conditions – there was no rainfall in the middle of August. During the harvest grain moisture reached 21.0%. Samples contained micromycetes of the genus *Fusarium* mainly (*F. sporotrichioides*, *F. culmorum*, *F. tricinctum*).

Analysis of the microbiological contamination of grain showed that the quantity of micromycetes in grain cultivation technology from the field to the storehouse was increasing (Fig. 3).

The number of micromycete propagules in the tank of the combine harvester was by 30% greater than in wheat ears before the harvest, possibly because during the harvest the working elements of the combine harvester interacted with the crop, and the dust rising from the soil got into the tank of the combine harvester

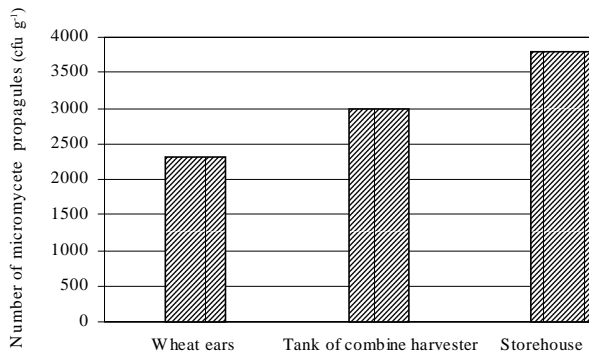


Fig. 3. Dynamics of micromycete propagule number during harvesting and transportation

together with grain. The cutting apparatus of the combine harvester should be adjusted to prevent the dust, including soil dust, getting into the combine harvester during the process. The cleaner of the combine harvester should ensure that only clean grain without admixtures would get into the tank.

Grain from the tank of the combine harvester was poured into a vehicle which brought it into the storehouse and placed into a bin. It was established that during the transportation grain was additionally contaminated by mould fungi, because the number of micromycete propagules in samples from the bin increased by 25%.

Studying the microbiological contamination of wheat crops, 8 species of micromycetes were found in grain ears, 12 species in the tank of the combine harvester and 16 species after transportation.

The prevailing species of micromycetes in the ears of wheat before harvesting were *Alternaria alternata*, *Fusarium culmorum*, *F. sporotrichioides* and *Penicillium verrucosum*; later, when grain passed through the working elements of the combine harvester and got into the tank, the above mentioned list of species was complemented by *Aspergillus oryzae*, *Arthrotrichy oligospora*, *Penicillium expansum* and *Fusarium tricinctum*, and, finally, during the transportation to the storehouse grain interacted with the environment and working surfaces of vehicles and was additionally contaminated by four more species of micromycetes which were not prevailing (Table).

Analysis of samples from the tank of the combine harvester revealed that there was one third less micromycetes on whole grain than on the mechanically damaged grain (Table). Due to the fact that damaged grain is more prone to be infested by mould fungi, the combine harvester whacking apparatus should be adjusted so as to avoid damage of grain as much as possible.

The quantity of micromycete propagules in grain of wheat ears left in the trial field depended on the meteorological conditions. At the beginning of September the number of propagules due to the humid weather and rainfall was increasing – from 3.0×10^3 cfu g⁻¹ (1 September) up to 7.1×10^3 cfu g⁻¹ (9 September) (Fig. 4). The conditions for the proliferation of micromycetes in grain were favourable, therefore, fungi were developing rapidly. In the middle of September, when dry weather came, field fungi experienced unfavourable conditions and their number in grain decreased to 4.9×10^3 cfu g⁻¹ (19 September). The second half of September was rainy again, however, the quantity of micromycetes in grain did not increase because of chilly weather; actually, the number slightly decreased to 3.8×10^3 cfu g⁻¹ (29 September).

The impact of the drying process on the microbiological contamination of grain was determined (Fig. 5). The number of micromycete propagules in wheat grain samples from the field was 3.0×10^3 cfu g⁻¹, whereas in barley it was 4.0×10^3 cfu g⁻¹.

Table. Grain contamination with micromycetes during harvesting and transportation

Place of sample picking	Grain moisture %	Number of isolated micromycete species	Micromycetes of prevailing species
From the grain ear	19.2	8	<i>Alternaria alternata</i> <i>Fusarium culmorum</i> <i>Fusarium sporotrichioides</i> <i>Penicillium verrucosum</i> <i>Rhizopus oryzae</i>
Whole grain from the tank of the combine harvester	21.0	12	<i>Alternaria alternata</i> <i>Arthrotrrys oligospora</i> <i>Aspergillus oryzae</i> <i>Fusarium sporotrichioides</i> <i>Fusarium culmorum</i> <i>Fusarium tricinctum</i> <i>Penicillium verrucosum</i>
Damaged grain from the tank of the combine harvester	20.0	16	<i>Alternaria alternata</i> <i>Arthrotrrys oligospora</i> <i>Fusarium sporotrichioides</i> <i>Fusarium culmorum</i> <i>Penicillium expansum</i> <i>Aspergillus oryzae</i>
Whole grain from the storehouse	19.1	12	<i>Alternaria alternata</i> <i>Arthrotrrys oligospora</i> <i>Aspergillus oryzae</i> <i>Fusarium sporotrichioides</i> <i>Fusarium culmorum</i> <i>Fusarium tricinctum</i> <i>Penicillium verrucosum</i>

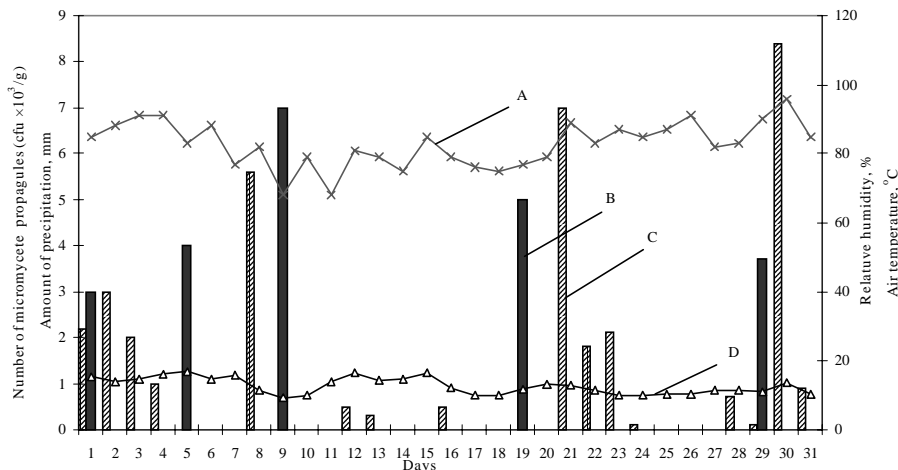


Fig. 4. Changes in micromycete propagules in wheat ears after harvest (in September): A – relative air humidity, %; B – number of micromycetes propagules, $\times 10^3$ cfu \cdot g $^{-1}$; C – amount of precipitation, mm; D – air temperature, $^{\circ}$ C

The number of micromycete propagules in the tank of the combine harvester slightly increased. It reached 5.1×10^3 cfu g $^{-1}$ in wheat and 5.3×10^3 cfu g $^{-1}$ in barley. In samples before drying the number of micromycete propagules was respectively 7.5×10^3 and 6.7×10^3 cfu g $^{-1}$. Grain from the combine harvester tank went to a vehicle which took it to the storehouse where it was placed into a bin before drying. The grain was additionally infested with micromycete propagules during transportation. The number of micro-

mycete propagules was increasing on the route from the field to the grain dryer. After drying the number of micromycete propagules reduced to 1.0×10^3 and 3.2×10^3 cfu g $^{-1}$.

The impact of the duration of grain drying in the shaft grain dryer on the microbiological contamination of grain was studied. The mathematical pattern for the estimation of micromycete propagule number during the grain drying process was worked out assessing the duration of drying (t_{d2} , min),

the primary number of micromycete propagules (M_0 , cfu g $^{-1}$) and the humidity of grain before drying (w_0 , %) (Fig. 6). The mathematical pattern demonstrates the impact of these factors on the number of micromycete propagules.

The primary moisture of grain prepared for drying ranged from 16 to 22.5%, the moisture of dried grain was 14%, the temperature of the drying agent was 78 $^{\circ}$ C and the temperature of grain heating varied from 34 to 37 $^{\circ}$ C.

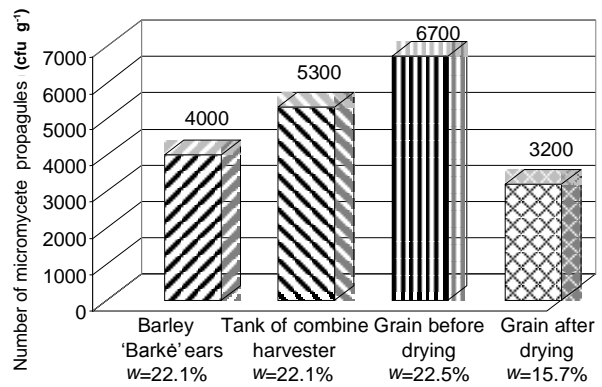
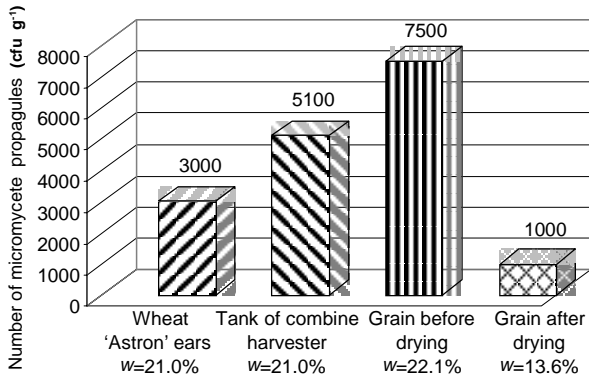


Fig. 5. Changes of the number of micromycete propagules during harvest and drying of wheat 'Astron' and drying of barley 'Barké'

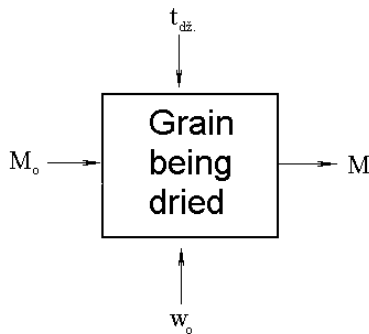


Fig. 6. Scheme of the mathematical pattern: t_{dz} – duration of drying; M_0 – primary number of micromycete propagules; w_0 – moisture content of grain before drying; M – number of micromycete propagules

For the analysis, the following regression equation was used:

$$M_{mod} = 5766,2 - 20,7t_{dz} + 0,1M_0 - 124,3w_0 - 0,4t_{dz} \cdot w_0 + 5,1w_0^2,$$

where t_{dz} is the duration of drying, M_0 is the primary number of micromycete propagules, cfu g⁻¹, and w_0 is the humidity of grain before drying, %.

Analysis the regression equation of the pattern has shown that the number of micromycete propagules may be considerably reduced by prolonging the duration of wheat grain drying. If drying takes more than an hour, e. g., 180 min instead of 120 min, the number of micromycete propagules reduces almost three times. The primary grain moisture had little influence on the increase in the number of micromycete propagules both during the drying process and in dried grain (Fig. 7).

The mycological contamination of the dried grain was proportional to the primary number of micromycete propagules. The more grain was contaminated before drying, the greater its contamination was after drying (Fig. 8).

Fig. 8. Impact of grain drying duration t_{dz} on the number of micromycete propagules after drying M : 1 – primary number of micromycete propagules in grain 1.0×10^4 ; primary moisture of grain 22.5%; 2 – primary number of micromycete propagules in grain 7.0×10^3 , primary moisture of grain 22.5%; 3 – primary number of micromycete propagules in grain 3.0×10^3 , primary moisture of grain 22.5%; 4 – primary number of micromycetes propagules in grain 3.0×10^3 , primary moisture of grain 16.0%

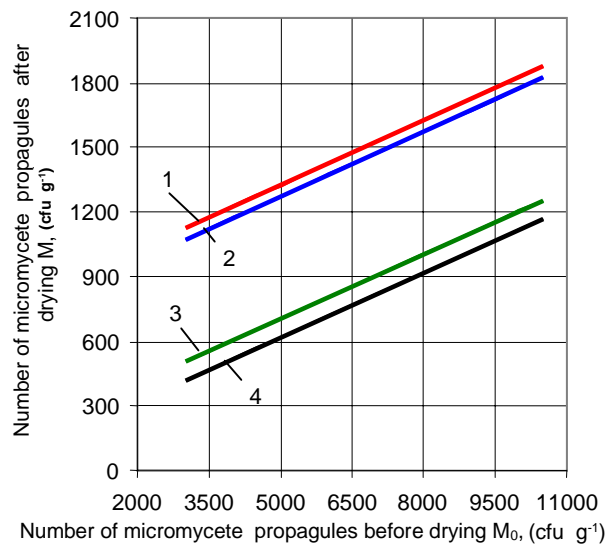
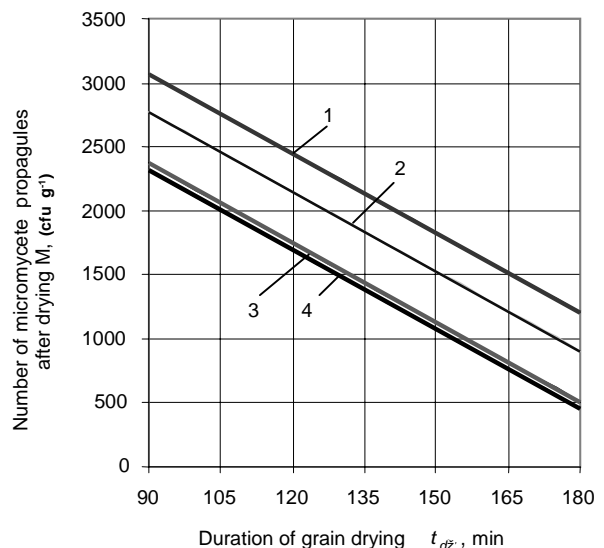


Fig. 7. Impact of micromycete propagule number M_0 before drying on their number after drying M : 1 – duration of drying 150 min, primary grain moisture 22.5%; 2 – duration of drying 150 min, primary grain moisture 16.0%; 3 – duration of drying 180 min, primary grain moisture 22.5%; 4 – duration of drying 180 min, primary grain moisture 16.0%



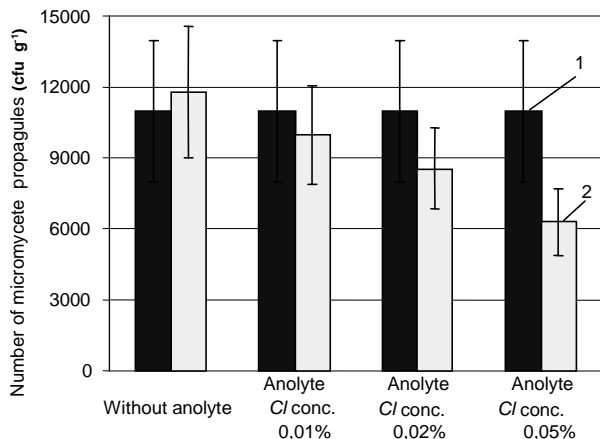


Fig. 9. Impact of anolyte on the mycological contamination of wheat 'Taurus': 1 – primary number of micromycete propagules in grain; 2 – number of micromycete propagules in grain after a 12-h exposure to anolyte

The difference between the number of micromycete propagules in dry grain and in grain processed by the anolyte is statistically reliable only if the concentration of chlorine is 0.05%. The impact of anolyte with a lower concentration of chlorine on the mycological contamination of grain was insignificant (Fig. 9).

CONCLUSIONS

1. On summarising the results of experimental studies, a mathematical pattern of micromycete propagule number dynamics during the drying process was worked out; the pattern assessed primary grain moisture, contamination by micromycete propagules and the duration of drying.

2. Analysis of the pattern regression equation has revealed that the number of micromycete propagules can be considerably reduced by prolonging the duration of grain drying in dryers. When the drying time was prolonged from 120 min to 180 min, the number of micromycete propagules in grain decreased almost three times.

3. The mycological contamination of grain while drying in all stages of drying remains proportional to the initial primary number of micromycete propagules in it. The initial grain moisture has little influence on the number of micromycete propagules.

4. In grain milling technologies, for moisturing grain it is advisable to use anolyte (a 0.05% Cl solution) instead of pure water. In this way the mycological contamination of grain is reduced almost by half.

5. Grains used for food and animal feed were intensely contaminated with propagules of various fungi species such as *Alternaria alternata*, *Arthrotrichy oligospora*, *Fusarium culmorum*, *F. sporotrichioides*, *Penicillium verrucosum*. Some of the isolated and identified fungi species possessed the ability to synthesize actively toxic materials and emit them into the environment.

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- Aurimas Krasauskas, Marija Railienė, Dainius Steponavičius, Algirdas Raila, Aušra Steponavičienė**
- GAMTINIŲ SĄLYGŲ IR TECHNOLOGINIŲ PRIEMONIŲ ĮTAKA GRŪDŲ DERLIAUS MIKOLOGINEI TARŠAI**
- S a n t r a u k a**
- Straipsnyje aptarti 2003–2005 m. Kauno rajone užauginto grūdų derliaus mikologinės taršos ir jos priklausomybės nuo aplinkos sąlygų tyrimų rezultatai. Tirtos įvairios technologinės prevencinės priemonės mikologinei taršai mažinti. Pateikti rezultatai, atspindintys džiovavimo proceso ir aktyvuoto vandens – anolito įtaką grūdų mikrobiologinės taršos lygiui. Nustatyta, kad subrendusių grūdų mikologinė tarša nuolat didėja nuo pat derliaus nuėmimo iki džiovavimo. Analizuojant iš kombaino bunkerio paimtus grūdų ėminius nustatyta, kad ant mechaniškai pažeistų grūdų mikromicetų rūšių trečdaliu daugiau nei ant sveikų. Sudarytas matematinis modelis mikromicetų pradų skaičiui nustatyti grūdų džiovavimo proceso metu, įvertinant džiovavimo trukmę, pradinį mikromicetų pradų skaičių ir grūdų drėgnį prieš džiovinimą. Matematinis modelis parodo veiksmų įtaką mikromicetų pradų skaičiui. Atlikus modelio regresinės lygties analizę nustatyta, kad mikromicetų pradų skaičių galima gerokai sumažinti ilginant kviečių grūdų džiovavimo trukmę. Ištyrus anolito įtaką mikromicetams, statistiškai patikimas skirtumas tarp mikromicetų pradų skaičiaus sausuose grūduose ir apdorotuose anolitu gautas tuomet, kai chloro koncentracija – 0,05%.
- Raktažodžiai:** mikromicetai, grūdai, tarša, džiovinimas, anolitas