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Ecological and technological factors influencing the distribution of toxin producing micromycetes on oats and their products

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The article deals with investigation of micromycetes detected on oats grown for food and fodder under climatic conditions of Lithuania. Attention is focused on the oat varieties that are popular in Lithuania and whose grain is used for producing oat flakes and other products. The distribution of micromycetes spread on oats grown in various regions of Lithuania was investigated in 2003–2005. During the study period, fungi of *Alternaria, Fusarium, Cladosporium, Drechslera, Penicillium, Aspergillus, Acremonium, Rhizopus* and *Rhizomucor* prevailed on oats. Fungal contamination of grain brought to a processing factory increased, and fungi mentioned above also prevailed. More evident changes in fungal contamination were noted during grain processing where strong mechanical and thermal factors acted. The highest amounts of micromycetes on oats were associated with oat shells and debris. The contamination was high indoors of a processing plant. Fungi most widely spread on processed oat grain and products belonged to the genera *Fusarium, Alternaria, Penicillium, Aspergillus, Cladosporium, Rhizopus*, etc. Toxin assessment revealed that fungi growing on oats produced toxins such as nivalenole, T-2 toxin, zearalenone, aflatoxin β_1, ochratoxins, patulin and rugulosine.

Key words: oats, micromycetes, ecological and technological factors, toxins

INTRODUCTION

Oats (*Avena sativa* L.) of various varieties are grown in Lithuania. The variety 'Jaugila', nurtured in Lithuanian Institute of Agriculture, is considered to be a standard oat variety. The biological and production characteristics of this variety are compared with the properties of other ones involved into the national list of plant varieties. The following properties are taken into account: average grain yield (t/ha), grain size, weight of 1000 seeds, protein percentage in grain, shell content, height and resistance to lodging (in points), duration of vegetation and maturation, and resistance to mostly widespread plant diseases such as crown rust and septariosis. In 2003–2006, oat crop contamination by fungal propagules was investigated in several districts of Lithuania. The oat varieties 'Jaugila', 'Szakal', 'Migla', 'Ceval' and 'Flikmingsprof'' are popular in Lithuania; also, naked oat (*Avena nuda L.*), mostly 'Beloruskij golozernyj' nurtured in Belarus is cultivated. Oats from the beginning of their growth are contaminated by various micromycetes (Пидопличко, 1978; Branderburger, 1995; Špokauskienė, 1996; Дьяков и др., 2001; Handham, 1992; Lugauskas, Krasauskas, 2005). Micromycetes get onto oats from the environment where they are grown. Micromycetes develop intensively on oat seeds and later in the rhizosphere and on terraneous parts and thus influence the growth of oats, often deterring oat growth intensity, harvest abundance and quality. The species composition of micromycetes is determined first of all by plant biological peculiarities, soil agrochemical and agrotechnological properties and their control measures, meteorological conditions, particularly during harvesting, and transportation and storage ways. On matured, uncut and untrashed oats as well as on freshly trashed ones, the following fungi are found: the causative agent of oat-covered smut *Ustilago levis* (Kell. et Sev.) Magn., whose smut spores are usually accumulated between the kernel and shell; the causative agent of oat loose smut *Ustilago avenae* (Pers.) Jens, whose smut spores are established on kernel surface or beneath the shell. Together with soil and plant remnant particles, fungal propagules from the rhisosphere spread easily on oat grain. Such fungi usually belong to the following species: *Fusarium avenaceum* (Fr.) Sacc., *F. culmorus (*Wm. G. Sm.)

Sacc., *F. graminearum* Swabe, *F. moniliforme* J. Sheld., *F. poae* (Peck) Wolemv., *Penicillium biforme* Thom, *P. capsulatum*, Raper et Fennell, *P. clavigerum* Demelius, *P. corymbiferum* Westling, *P. crustosum* Thom, *P. cyclopium* Westling, *P. expansum* Link, *P. funiculosum* Thom, *P. griseofulvum* Dierckx, *P. viridicatum* Westling, *Olpidium agrostidis* Samps., *Aspergillus niger* Tiegh., *A. fumigatus* Fresen, *A. flavus* Link, *Chaetomium gibosum* Kunze, *Septoria avenae* Frank, *S. Sativa* Frandsen., *Drechslera avenae* (Eidam) Ito, *Ascochyta avenae* (Petr.) Spraque et A. G. Jonson, *Rhizoctonia solani* Kühn and species of many other genera (Brandenberger, 1985; Kozakiewicz, 1989; Klich, 2002; Lugauskas, Krasauskas, 2005).

Oats, in comparison with other grains, are characterized by a high shelling (up to 25–30% and more). Oat kernels have very thin fruit and seed coats and the aleuronic layer, and they all together make 9–11% of the grain mass. Therefore, oat products such as groats and flakes can be processed only from high quality grain, well matured and undamaged by fungi. Oats delivered to processing plants should meet the quality parameters for received oats.

Grain contaminated by fungi is often delivered to processing plants. Here grains and fungi established on them are affected by technological factors: increased humidity, temperature, hydrostatic pressure, oxygen regime change, and concentration of soluble substances, various chemical additives and biological factors. Under such conditions, abundance and species composition of micromycetes undergo changes. Fungi of some species do not survive or slow down their functional activity, while others start to function intensively and reproduce. Thus, metabolites produced by fungi and released into the environment change, too. Sometimes the ability to produce toxic secondary metabolites dangerous to human and animal health becomes more intensive (Nelson et al., 1983; Samson, Reenen-Hoekstra, 1988; Chelkowski, 1991; Dexter et al., 1997; Rabie et al., 1997; Svan, 1988; Filtenborg et al., 2000; Kiffer, Morfelet, 2000; Samson et al., 2000; Satton et al., 2001; Lugauskas, Krasauskas, 2005).

The aim of the present work was to investigate micromycetes contaminating oats, to assess their distribution and species composition in different oat processing stages, to find out their ability to synthesize toxic secondary metabolites and to evaluate the ecological and technological factors enhancing or limiting the spread and functioning of micromycetes.

MATERIALS AND METHODS

The investigation was conducted in 2003–2006 at the Laboratory of Biodeterioration Research and Laboratory of Phytopathogenic Microorganisms of Institute of Botany, and at Heat and Biotechnological Engineering Department and Experimental Station of Lithuanian University of Agriculture (LUA). Oat grains were taken from the Experimental Station of LUA situated in Kaunas district, and from private farm fields of Širvintai, Varėna, Alytus, Lazdijai, Kaišiadorys, Trakai, Kėdainiai and Vilnius districts. The following oat varieties were investigated: 'Jaugila', 'Szakal', 'Migla', 'Ceval' and 'Flikmingsprof'.

Meteorological conditions in 2003–2005 in Lithuania rather varied (Table 1). Conditions for oat sprouting and growth, especially for their initial development stage, were rather unfavorable: cold weather (2003), insufficient humidity (2004) and frequent heavy rainfall during harvesting in the end of July and the beginning of August (2003, 2004). Standard technological conditions for oat cultivation were used. It should be noted that in the study years the harvesting of oat was late.

Samples of oat grains and processed products were also taken at different oat processing stages at a plant. Oat processing may be divided into stages shown in Figure. During processing, grain undergoes altering temperature, pressure and humidity regimes, and this affects the survival of fungal propagules.

To eliminate impurities, grains are twice separated in separators and fractionated into heavy and light fractions. Then the fractions are processed using the hydrotechnical method, which is essential for complete shelling of oat grains. After this procedure kernel technological properties become better, shells are removed easier, the kernel becomes more resilient, properties of groats improve, cooking time shortens and the maintenance time of groats becomes longer.

Table 1. **Weather conditions during the vegetation period**

Month	Air temperature, °C				Precipitation, mm			
	Long-term average of 1924–2005	2003	2004	2005	Rate	2003	2004	2005
April	5.7	4.9	7.6	7.6	45	40	11.1	23.9
May	12.2	13.6	11.2	12.4	60	74	27.8	46.1
June	15.4	15.2	14.2	15.3	77	65	44.2	50.3
July	17.6	19.7	16.8	19.3	78	92	81.6	46.3
August	16.6	16.8	18.1	16.8	68	105	94.5	75.5
September	12.0	12.3	12.9	14.4	65	22	53.2	26.6

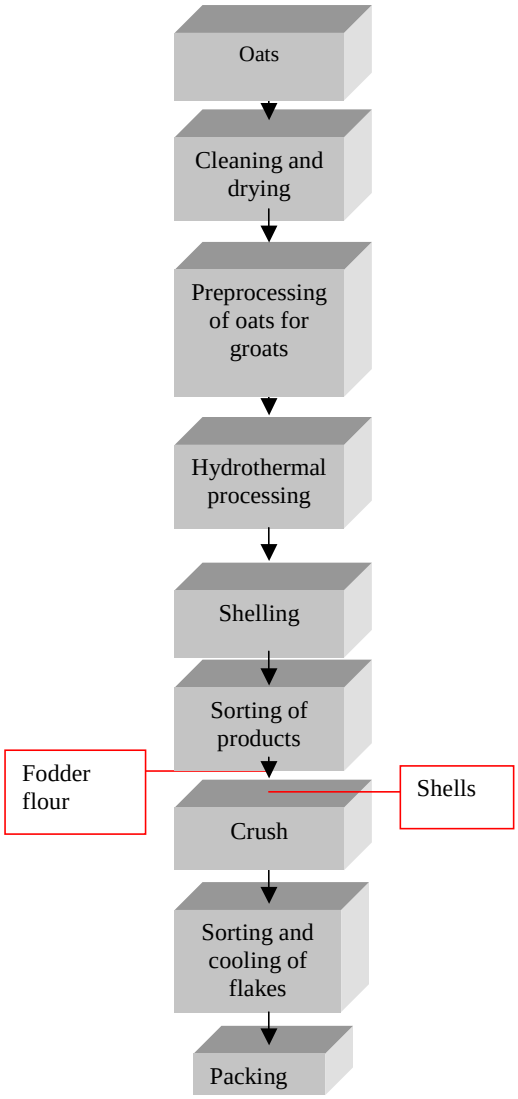

Figure. Oat processing stages

Oat grain is steamed in a vaporizer at 0.1–0.3 MPa for 5 min at 110–140 °C. Here grain moisture increases by 3–6%. Then, to achieve an equal distribution of moisture, oat grains are kept in ripening bins for 30 min. Grain mass is heated at over 100 °C and excess steam pressure. During steaming, grains absorb moisture and get warm, shells become more resilient and less fragile. The grains swell, enlarge and their coats break away easier.

After steaming, grains are dried in contact driers, where they touch pipes with circulating steam of 0.3–0.5 MPa. During this process a large part of water is removed from shells, they become fragile and easily break during shelling processes. The hydrothermical treatment of grains ends by cooling in coolers.

The above processes, especially temperature and moisture alterations, change the conditions for micromycetes established on grain. Some of fungal propagules do not survive, while the others develop further. When the grains in the next stage are processed with a scrub engine, micromycetes can occur on shells, in dust, in the air and on other surfaces.

Shelled products are sorted using two groups of sieves which differ in mesh size ranging of within 0.2–0.3 mm. After shelling, a mixture of the following fractions is obtained:

- the main fraction – shelled grain
- the second fraction – unshelled grain
- the third fraction – shells
- the fourth fraction – broken kernels
- the fifth fraction – fodder flour (small germs and shell parts)

After shelling oat groats are crushed with a roll engine with a smooth roll. Then the obtained oat flakes are directed to the sorting sieve, and here the light fraction is sorted out. At the same time oat flakes are dried to 12% moisture.

Samples for mycological investigation of oats and their products during processing were taken following selective sampling principles. In each investigation site 10 samples were taken. The samples were taken with sterile instruments, put into sterile glass vessels and tightly closed. The samples were analyzed next day or put in a refrigerator for a short time. Isolation and identification were performed according to the methods described by Кудряшова (1986), Rabie et al. (1997) and Samson et al. (2000). Analysis of each sample was performed in triplicate. Micromycetes were isolated from grain surface, placing grains directly onto malt extract agar (MEA) and, additionally, using the outwash-dilution method. To isolate fungi from the grain interior, grains were sterilized with 3% sodium hypochloride for 2 min and rinsed with sterile water, then drained with sterile filter paper and placed onto Petri dishes containing MEA. Micromycetes were cultivated in incubators at 26 ± 2 °C for 5–7 days. Aiming to purify and identify micromycete isolates, single spore cultures were cultivated on Czapek, malt and corn extract agars. The systematic position of fungi was determined according to Пидопличко (1978), Domsch et al. (1980), Kozakiewicz (1989), Roth et al. (1990), Howksworth et al. (1995), Kiffer et Morelet (2000), Samson et al. (2000), Саттон и др. (2001), Baliukonienė, Bakutis (2002), Klich (2002), Watanabe (2002), Дьяков, Сергеев (2003), Lugauskas et al. (2002), Chaverri, Samuels (2003), Samson, Frisvad (2004). The species occurrence frequency was calculated following Мирчинк (1988).

The primary screening of toxin-producing micromycetes was performed by methods proposed by Frisvad (1988) and Samson et al. (2000). Micromycetes were cultivated on Czapek, Czapek yeast extract (CYA) and yeast extract sucrose (YES) agars for 7–14 days. Potential toxin-producers were screened judging by a significantly changed color of colonies and intensive pigmentation on CYA and YES media. Capacities of selected micromycetes to produce toxins on oats were tested by the ELISA method (Chu, 1996; Samson et al., 1992). Mycotoxins extraction and tests were performed according to manufacturer's instructions. The VERATOX®, Alotox (total), VERATOX® DOH5/5,

Table 2. **Number of micromycetes and species diversity on pre-harvest oat grain in Lithuania, 2003–2005**

Oat cultivation location	Square, ha	Year	Number of micromycetes, cfu g^{-1}	Prevailing micromycete species
Kaišiadoriai district, private farm	~ 8	2003	273 ± 17	*Alternaria alternata, Penicillium corymbiferum, P. expansum, Acremonium fusidioides, Fusarium poae, F. sporotrichioides, F. graminearum*
Trakai district, private farm	~ 2	2003	312 ± 39	*Alternaria alternata, Drechslera sorokiniana, Penicillium vridicatum, P. corylophilum, P. expansum, Aspergillus repens, Fusarium poae, F. sporotrichioides*
Vilnius district, private farm	~ 4	2003	407 ± 42	*Alternaria alternata, Mucor racemosus, Fusarium poae, F. sporotrichioides, Penicillium vridicatum, P. corylophilum, P. expansum*
Šalčininkai district, private farm	~ 3	2003	279 ± 29	*Bipoliaris sorokiniana, Fusarium graminearum, F. poae, Aspergillus repens, Septoria avenae*
Varėna district, private farm	~ 2	2003	706 ± 43	*Alternaria alternata, Acremonium fusidioides, Fusarium equiseti, F.oxysporum, F. poae, Penicillium corymbiferum, Septoria avenae*
Lazdjai district, private farm	~ 6	2003	372 ± 15	*Alternaria alternata, Penicillium capsulatum, Cladosporium cladosporioides, Fusarium poae, F. sporotrichioides, Mucor hiemalis*
Alytus district, AB farm	~ 12	2004	324 ± 30	*Alternaria alternata, Drechslera sorokiniana, Cladosporium herbarum, Penicillium viridicatum, Penicillium funiculosum*
Marijampolė district, private farm	~ 6	2004	412 ±40	*Alternaria alternata, Cladosporium cladosporioides, Mortierella hyalina, Mucor circinelloides, Penicillium capsulatum, P. aurantiogriseum, Fusarium poae*
Kaunas district, training farm	~ 8	2004	203 ± 15	*Fusarium graminearum, F. culmorum, Penicillium expansum, P. corymbiferum, Aspergillus clavatus, A. niger*
Prienai district, private farm	~ 6	2004	345 ± 28	*Alternaria alternata, Rhizomucor pusillus, Penicillium claviforme, P. expansum, Fusarium poae, F. solani*
Kėdainiai district, private farm	~ 1	2005	215 ± 57	*Drechslera sorokiniana, Curvularia lunata, Fusarium sporotrichioides, Mucor mucedo, Penicillium oxalicum*
Kėdainiai district, experimental fields	~ 7	2005	285 ± 83	*Alternaria alternata, Drechslera sorokiniana, Fusarium graminearum, F. poae, Penicillium corymbiferum, P. brevicompactum*
Panevėžys region, private farm	~ 10	2005	314 ± 28	*Drechslera teres, Ulocladium botrytis, Penicillium expansum, P. viridicatum, P. rugulosum*
Ukmergė region, private farm	~ 4	2005	403 ± 29	*Alternaria alternata, A. tenuissima, Drechslera sorokiniana, Fusarium graminearum, F. poae, Penicillium cyclopium, P. palitans*
Vilnius region, private farm	~ 2	2005	312 ± 15	*Alternaria alternata, Fusarium oxysporum, F. equiseti, Septoria avenae*

VERATOX®-Ochratoxin A, Aflatoxin, T-2 toxin, and zearalenone test kits were used for the analysis.

RESULTS AND DISCUSSION

In Lithuania, the law legitimates the obligation of the state and its institutions to ensure that only safe products should be provided to the market. Recently in food processing enterprises a system of risk factors' analysis and important control centers (RFAICC) has been implemented. Significant attention in this system is paid to microbiological analyses as essential measures of an effective food control. The Lithuanian Hygiene Norm HN 15:2005 "Food Higiene" points out that the control of microorganisms should be performed regularly, controlling the ecological conditions that

Table 3. **Micromycetes isolated from oat grains delivered to a processing plant**

Micromycetes isolated from oat grain outwash: *Acremonium butyri* W. Gams, *A. strictum* W. Gams, *Alternaria alternata* (Fr.)Keissl., *A. dianthi* F.Stevens et G. Hall, *Cladosporium cladosporioides* (Fresen.) G. A. de Vrees, *Cladosporium herbarum* (Pers.) Link ex Gray, *Drechslera teres* (Sacc.) Shoemaker, *Fusarium avenace* (Fr.) Sacc., *F. graminearum* Schwabe, F. poae (Peck) Walenw., *F. sambucinum* Fuckel, *F. semitectum* Berk et Rev. *F. solani* (Mart.) Appel et Wollenv., *F. sporotrichioides* Sherb., *Hymenula cerealis* Ellis et Everh., *Olpidium brassiceae* (Wor.) Dang., *Sclerotinia sclerotiorum* (Li.) de Bary, *Septoria avena* Frank., *Mycelia sterilia*
Micromycetes isolated from grain surface: *Alternaria alternata* (Fr.) Keissl., *A. dianthi* F. Stevens et G. Hall, *A. radicina* Meier, Drechsler et E. D. Eddy, *Drechslera sorokiniana* (Sacc.) Subram. et Jain, *D. teres* (Sacc.) Shoemaker, *Fusarium avenace* (Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe, F. poae (Peck) Walenw., *F. sambucinum* Fuckel, *F.semitectum* Berk et Rev. *F. sporotrichioides* Sherb., *Hymenula cerealis* Ellis et Everh., *Olpidium brassiceae* (Wor.) Dang., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Sporotrichum aurantiacum* (Bull.) Fr., *Ulocladium botrytis* Preuss
Micromycetes isolated directly from grain interior: *Acremoniella atra* (Corda) Sacc., *Alternaria alternata* (Fr.) Keissl., *Arthrinium phaeospermum* (Corda), *Aspergillus (Eurotium) repens* de Bary, *A. niger* Tiegh, *Chrysosporium merdarium* (Link ex Grev.) J. W. Carmich., *Drechslera sorokiniana* (Sacc.) Subram. et Jain, *Fusarium graminearum* Schwabe, F. poae (Peck) Walenw., *F. proliferatum (*Matsushima) Nirenberg, *F. sporotrichioides* Sherb., *Mucor racemosus* Fresen., *Penicillium capsulatum* Raper et Fennell, *P. corymbiferum* Westling, *P. digitatum* Sacc., P. *expansum* Link, *P. viridicatum* Westling, *Talaromyces flavus* (Klöcker) Stolk & Samson, *Ulocladium oudemansii* E. G. Simmons

Table 4. **Micromycetes detected in dust and indoors in premises of an oat grain processing plant**

Samples	Premises or other surfaces	Number of micromycete propagules, $\times 10^3$ cfu g^{-1}	Species of isolated micromycetes
Dust	Grain shelling room	3000 ± 840	*Alternaria alternata, Ascochyta avenae, Botrytis cinerea, Cladosporium herbarum, Erysiphe graminis, Fusarium avenaceum, F. culmorum, Mortierella humicola, Mucor hiemalis, M. racemosus, Oidiodendron echinulatum, Penicillium fellutanum, P. viridicatum, Phoma exiqua, Sclerotinia sclerotiorum, Ulocladium botrytis, Verticillium alboatrum, Mycelia sterilia*
	Paper bags	3250 ± 490	*Alternaria alternata, Aspergillus amstelodami, Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, Drechslera tere, Erysiphe graminis, Fusarium moniliforme, Fusarium sambucinum, Mortierella humicola, Mucor sp., Phoma exiqua, Sclerotinia sclerotiorum, Verticillium alboatrum, Mycelia sterilia*
Indoor air	Grain shelling room	4260 ± 684	*Aspergillus amstelodami, Cladosporium cladosporioides, Penicillium brevicompactum, P. decumbens, P. nalgiovense, P. viridicatum, Saccharomyces cerevisiae, Mycelia sterilia*

determine microorganism occurrence and activity. It is important to know toxin-producing microorganisms and factors influencing the functional abilities and activity of such microorganisms in raw material and cereal products. According to data of Food and Agriculture Organisation (FAO), about 25% of food products in the world are contaminated with mycotoxins. Oat flakes and groats are considered to be a valuable dietary food. Such products are produced in Lithuania by several processing plants. Therefore, it is important to know what micromycetes as potential toxin producers contaminate oat grown under local conditions in stages of growth, harvesting, storage and processing.

Late harvesting and rainy weather in the study period could have influenced the contamination of oat grains by micromycete propagules. Contamination was caused mostly by the toxin-producing fungi of the genera *Fusarium, Aspergillus, Penicillium* and *Alternaria*. Often grain contamination reached 100%. The abundance and diversity of micromycete species are presented in Table 2. The data show that various micromycetes contaminate preharvested oats. The following fungal species were most frequently isolated: *Alternaria alternata, Drechslera solani, Fusarium graminearum, F. poae, F. sporotrichioides, Penicillium capsulatum, P. expansum, P. corymbiferum, P. viridicatum*. The number of micromycete propagules ranged from 215 to 706 cfu/g of grain. The highest counts of micromycetes were found on oats in Varėna region. No distinct differences in oat contamination in different years were noted.

Contamination of oat grains greatly depends on grain handling (harvesting, transportation and storage). When oat grains were delivered to a processing plant, the contamination of grains increased and reached 1.7×10^3 to 10.2×10^3 cfu g^{-1}. The composition of micromycete species also changed. To determine the most harmful species, exo- and endomycobiota were investigated (Table 3). The results show that both grain surface and interior were contaminated by fungi of a wide spectrum, among which species of the genus *Fusarium* prevailed. Many of these fungi are able to synthesize various toxic secondary metabolites (Roth et al., 1990; Cole, Schweikert, 2003; Cole et al., 2003; Lugauskas, 2005).

Long storage of oat grains in differently equipped storehouses or other premises in processing plants also influences the composition of fungal species. Fungi of some species are able to spread very quickly in grain mass, in the air and settle with dust on walls and other surfaces (Krysińka-Trasczyk et al., 2000; Shoug, 2003). Such spreading is characteristic of many species of the genera *Aspergillus* and *Penicillium, Mucor* and *Rhizopus*, *Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, Exophiala jeanselmei, Rhizomucor pusillus* and *Paecilomyces variotii*, etc. Thus, such fungi easily contaminate premises, grains and even can survive in grain products. In our investigation, huge numbers of micromycete propagules and a high species variety were detected in dust and indoor air collected in premises where grains were kept (Table 4).

Processing of oat flakes and groats is a complex process consisting of several technological stages (Figure). During technological processes, conditions for micromycete functioning change, often become stressful and force micromycetes to reorganize their functional processes to survive.

The first step of oat processing is cleaning when grains are separated twice, and together with impurities a lot of fungal propagules are removed. For hydrothermal processing, two oat grain fractions are prepared; their mycological stage was investigated and was found to be very similar. Here *Acremonium fusidioides, Alternaria alternata, Cladosporium herbarum, Drechslera erythospila, Fusarium avenaeum, F. graminearum, F. poae, F. proliferatum, F. semitectum, F. sporotrichiella, Mortierella hyalina, Rhodotorula rubra, Ulocladium chartarum* and some other fungi of the genera *Fusarium, Penicillium, Acremonium, Aspergillus* and *Drechslera* were detected.

During hydrothermal processes, which are performed for a better grain shelling, grains are kept in a vaporizer at 110–140 °C. This process increases grain moisture. Under such conditions many micromycete propagules die. The conidia of fungi more resistant to a high temperature can survive, and during the next process in ripening bins where moisture diffuses in whole grain mass they can recover after the stress and start to function. The further process is drying, when grain moisture decreases significantly. After the drying process, oat grain contamination was 48.0×10^3 cfu g^{-1}. Here the following fungi were detected: *Alternaria tenuissima, Alternaria spp., Cladosporium herbarum, Drechslera erytthrospyra, Fusarium avenaceum, Gonatobotrys symplex, Mucor racemosus, Penicillium viridicatum* and *Rhizopus oryzae.*

When grains were processed with a scrub engine, whole grains with shells were separated from shelled grains and directed to repeated shelling. Contamination of shelled grains was $0{,}6 \times 10^3$ cfu g^{-1}. In this processing stage the following fungi were detected: *Acremonium furcatum, Cladosporium herbarum, Fusarium avenaceum, F. equiseti, F. semitectum, Penicillium commune, P. stoloniferum, Sclerotinia sclerotiorum* and *Ulocladium chartarum*. A higher fungal contamination (up to $64{,}7 \times 10^3$ cfu g^{-1}) was detected on oat grain shells and debris. The following micromycetes were isolated and identified: *Cladosporium parasiticum, C. cladosporioides, C. herbarum, Fusarium avenaceum, Gonatobotrys simplex, Mucor racemosus, Ovularia pusilla, Penicillium commune, P. verrucosum, P. viridicatum, Rhizopus oryzae, Sclerotinia sclerotiorum, Septoria avenae, Spegazzinia deightonii, Trichobotrys fusca* and *Mycelia sterilia.*

Oat flour, consisting of small parts of germs and shells, was contaminated less ($32{,}5 \times 10^2$ cfu g^{-1}). Here the following fungi were distributed: *Alternaria alternata, Cladosporium herbarum, Fusarium graminearum, F. culmorum, F. heterosporum, Mortierella humicola, Mucor hiemalis, Penicillium velutinum, P. viridicatum, Sclerotinia sclerotiorum, Verticillium alboatrum* and *Mycelia sterilia.*

Shelled oat grain is crushed with a roll engine. In this processing stage, on broken grains only a few species were detected: *Aspergillus oryzae, Fusarium graminearum* and *Penicillium* spp. On freshly produced oat flakes, micromycetes *Aspergillus oryzae, A. clavatus* and *Penicillium* spp. were detected. During sorting, when light fractions are separated and flakes are cooled and dried to 12%, the number of fungal propagules and species composition increase. Additionally, here the following fungal species were found: *Rhizomucor pusillus, Mucor circinelloiodes, Rhizopus oryzae* and *Aspergillus niger.* It should be mentioned that packing bags were also contaminated. The fungi *Aspergillus clavatus* and *A.* (*Eurotium) repens*, various yeasts and spore-forming bacteria were found there.

During processing, fungal contamination of processed products varied. Most micromycetes remained in collateral products: bran, fodder flour, shells or did not survive during processing. Additionally, it should be stressed that the environment in which these processes are performed should be isolated from external infection, as well as products should be stored in appropriate temperature and humidity conditions.

To assess fungi contaminating oat products delivered to the market, oat flakes and groats were taken from shops where they were packed in cartoon boxes

Table 5. **Toxins produced by fungi of the genus *Penicillium* isolated from oats and grown on oat grain for 14 days**

Micromycetes	Sampling site of oats	Ochratoxins, μg kg^{-1}	Patulin, μg kg^{-1}	Rugulosine, μg kg^{-1}
Penicillium corymbiferum LK-13	Kaišiadorys district	0	0	28.6
Penicillium expansum LK-21	Kaišiadorys district	0	12.4	20.4
Penicillium viridicatum LT-53	Trakai district	28.6	0	0
Penicillium viridicatum LA-101	Alytus district	36.4	0	0
Penicillium aurantiogriseum LM-83	Marijampolė district	17.6	8.0	0
Penicillium claviforme LP-93	Prienai district	0	14.0	0
Penicillium oxalicum LK-62	Kėdainiai district	0	0	32.8
Penicillium variabile LP-94	Panevėžys district	22.4	16.4	0
Penicillium cyclopium LU-41	Ukmergė district	0	18	0

Table 6. **Fungi of the genus *Aspergillus* isolated from oats and their potential toxins (according to Cole, Schweikert, 2003; Roth et al., 1990; Chelkowski, 1991)**

Micromycetes	Source of micromycete isolation	Potential toxins
Aspergillus flavus L-ALXKS -3	Oat shells	Ditryptophenaline; aflavine: dihydroxyaflavinine; 20-hydroxyaflavinine; 24, 25-dehydro-10,11-dihydro-20-hydroxyaflavivnine; 10,12-dihydro-11,12-dihydro-20-hydroxyaflavinine; 14-epi—14-hydroxy-10,23-dihydro-24-25-dehydroflavinine; 10,23-dihydro-24-25-dehydro-21-oxoaflavinine; aflavazole; paspaline; aflatrem; a,a-dimethylallylpaspalinine; ß-aflatrem; aflatoxin B_1, B_2, B_{2a}, B_3, G_1, G_2, G_{2a}, M_1, M_2, D_1; parasiticol; O-methylsferigmatocystin; 6,7-dimethoxydifuroxonthone; dihydro-0-methylsterigmatoxin; 1,2-dihydro-6-methoxy-7-hydroxydifuro-xanthone; asportoxin; hydroxy-6,7-dimethoxydifuroxanthone
Aspergillus clavatus L-AK-132	Oat grain after thermal processing	Cytochalasin E, cytochalasine K_{ASP}; tryptoguivaline A, C^1, B^1, D^1, E, tryptoguivalone; nortryptoguivalone; nortryptoguivaline norisotryptoguivaline; deoxytryptoguivaline; deoxynortryptoguivaline; deoxynortryptoguivalone
Aspergillus niger LAGV-8	Interior of oat grains	Ergosterol; (24R)24-ethylcholestra-4,68(14),22-tetraen-3-one; malformin A; xanthoggmegnin; aspergilins; aspercyllones(+asperrubrol); dehydroflavinines; flaviolin; genisteins (+orobole); glutaconis acid; 4-hydroxymardelic acid; malformis; monoglucosyloxyoctadecanoic acid; naphtopyrones; neoechinulin A; nigerazines; nigragilin; orlandin; ubingensin A, B
Aspergillus repens LAGV-31	Interior of an oat kernel	Prechinulin; asperflavin; asperentins; aspergin; asperglaucide; auroglaucin; catenorin; erythroglaucin; flavoglaucin; physcion; echinulins; kotanins
Aspergillus terreus LAGV-451	Oat grain	Terretonin; patulin; claviformin; clavitin; clavicin; expansion; penicidin; mycoin; leucoin; tercinin; 4-hydrox4H-furo[3,2-c]pyran-2(6H)-one; citrinin; citreoviridin; territrems
Aspergillus oryzae LAGPT-10	Oat grain before crush	Cyclopiazonic acid; kojic acid

or bags. From oat products delivered to shops, the following micromycetes were isolated: *Aspergillus oryzae, A. clavatus, A. repens, A. fisheri, Paecilomyces variotii, Penicillium viridicatum, P. variabile, P. aurantiogriseum, P. puberulum, Mucor racemosus, M. hiemalis, Rhizomucor pusillus*.

While evaluating fungal contamination of food products, of importance is the ability of fungi found on grains or in products to produce toxic secondary metabolites, as well as their possible capabilities as pathogens to humans and animals. Species of fungi spread on grains or products often determine the level of toxicity of these products (Львова и др., 1992; Lund, Frisvad, 2003; Mills et al., 1995). It was noticed that mostly fungi of the genus *Fusarium* (*F. equiseti, F. solani, F. poae, F. graminearum* and *F. sporotrichioides*, etc.) contaminated oat grains harvested in rainy seasons and stored in bins. Toxicity analysis of these grains showed that the following mycotoxins were accumulated in the grains: nivalenole up to 0.186 mg kg^{-1}, T-2 toxin 0.192 mg kg^{-1} and zearalenone 0.198 mg kg^{-1}. Investigation of fodder flour in a private farm revealed that the flour contained propagules of fungi of the following genera: *Fusarium* 50%, *Penicillium* 2%, *Alternaria* 40%, *Cla-*

dosporium 12%, and other fungi 2%. In the same flour mycotoxins were tested, and the following toxins were detected: nivalenole <0.1 μg kg^{-1}, T-2 71.8 μg kg^{-1}, zearalenone 50 μg kg^{-1}, aflatoxin-B_1 2.9 μg kg^{-1}, ochratoxin A 1 μg kg^{-1}. When such a contaminated product is stored for a long time, toxin content can increase, especially if environmental humidity increases.

In order to reveal toxin-producing abilities on oat grain of potentially toxic fungi of the genus *Penicillium*, micromycetes of this genus isolated from oats were cultivated on oat grain and after a 14-day growth toxins in grain were tested (Table 5). Fungi of the genus *Aspergillus* are also known to produce toxins (Cole, Schweikert, 2003; Cole et al., 2003; Lugauskas et al., 2003; Kozakiewicz, 1989; Krysińska-Trasczyk, 2001). Several species of this genus – potential toxin producers – were detected on oat grain. Their potential abilities to produce toxins are presented in Table 6.

To conclude, our investigation revealed that oat grain was contaminated in all stages: growth, storage and processing. Increase of grain contamination was noted on grain delivered to a processing plant. It should be mentioned that the indoor contamination of a processing plant was high and could influence contamination of further processed products. The highest numbers of fungi were associated with shells and debris. Most fungi spread on oat grains and products belonged to the genera *Fusarium, Alternaria, Penicillium, Aspergillus, Cladosporium* and *Rhizopus*. Toxin assessment revealed that fungi growing on oats produced toxins such as nivalenole, T-2 toxin, zearalenone, aflatoxin B_1, ochratoxins, patulin and rugulosine.

Oat grain processing under proper environmental conditions can limit the spread of micromycetes in raw material and processed products. The suitable packing, storage and realization reduce the opportunities for later micromycete occurrance in products.

To limit the contamination of processed cereal food, it is important that as little as possible micromycetes occur on growing, harvested and stored crops, and this could reduce the occurrence of micromycetes in processing plants. During processing, a stricter control should be given to technological processes to limit the distribution of hazardous micromycetes.

The aim is to reduce the occurrence of oat grain fungi, first of all of the genus *Fusarium* as much as possible (Brennan et al., 2003; De Vries et al., 2002; Ellner, 1997; Елинов, 2002; Doohan et al., 2003; Magae et al., 2002; Malmauret et al., 2000; Mills, 1990; Morin et al., 1999; Muthomi et al., 2000; Naiker, Odhau, 2004). Especially the following species are undesirable: *F. poae, F. graminearum, F. sporotrichioides, F. oxysporum, F. equiseti, F. heterosporum, F. moniliforme* and *F. solani.* Fungi of other genera can also pose risk to consumers: *Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. ustus, A. versicolor, A. (Eurotium) repens, Penicillium aurantiogriseum, P. brevicompactum, P. chrysogenum, P. claviforme, P. corymbiferum, P. corylophilum, P. commune, P. cyclopium, P. expansum, P. janthinellum, P. nalgiovense, P. oxalicum, P. viridicatum, P. rugulosum* and *P. verrucosum*. Oat grains are deteriorated *by Rhizopus oryzae, Rhizomucor pusillus, Alternaria alternata, Drechslera sorokiniana, Cladosporium cladosporioides* and *C. herbarum,* etc. These fungi worsen the quality of processed products, shorten expiry, pose risk to human health, as micromycete propagules and their toxins can get from the air and food into the human organism and cause diseases (Mckenzie, 1990; Horner et al., 1995; Дьяков и др., 2001; Husein, Brasel, 2001; Manabe, 2001; Satton et al., 2001; Shoug et al., 2001; Gárny, Dutkiewicz, 2002; Fisher, Dott, 2003; Lugauskas et al., 2003; Shoug, 2003).

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EKOLOGINIAI IR TECHNOLOGINIAI VEIKSNIAI, LEMIANTYS TOKSINUS GAMINANČIŲ MIKROMICETŲ PAPLITIMĄ ANT AVIŽŲ IR IŠ JŲ PAGAMINTŲ PRODUKTŲ

S a n t r a u k a

Pateikiami duomenys apie mikromicetus, aptiktus ant avižų grūdų, auginamų maistui ir pašarui Lietuvos klimato sąlygomis. Ypač daug dėmesio skiriama toms avižų veislėms, kurios Lietuvoje yra plačiai auginamos ir kurių grūdai naudojami avižinių dribsnių ir kitų maisto produktų gamybai. Mikromicetų paplitimas ant avižų, auginamų įvairiuose Lietuvos rajonuose, tirtas 2003–2005 m. Visais tyrimo metais labiausiai ant avižų buvo paplitę grybai, priklausantys *Alternaria, Fusarium, Cladosporium, Drechslera, Penicillium, Aspergillus, Acremonium, Rhizopus* ir *Rhizomucor* gentims. Atvežtų į perdirbimo įmonę grūdų užterštumas grybais buvo didesnis, tačiau ir čia vyravo ankščiau minėti grybai. Ryškesni grybų taršos pokyčiai buvo pastebėti perdirbant grūdus, kai veikė stiprūs mechaniniai ir terminiai veiksniai. Didžiausi mikromicetų kiekiai aptikti ant avižų lukštų ir atliekų. Nustatyta didelė perdirbimo įmonės patalpų tarša mikromicetais. Ant perdirbamų avižų grūdų ir jų produktų buvo labiausiai paplitę grybai iš *Fusarium, Alternaria, Penicillium, Aspergillus, Cladosporiumi* ir *Rhizopus* genčių. Ištyrus mikotoksinus, nustatyta, kad grybai, augdami ant avižų, gamino nivalenolį, T-2 toksiną, zearalenoną, aflatoksiną B_1, ochratoksinus, patuliną ir rugulozinę.

Raktažodžiai: avižos, mikromicetai, ekologiniai ir technologiniai veiksniai, toksinai