EKOLOGIJA. 2006. Nr. 3. P. 122–127

Factors predetermining the abundance of fungi and mycotoxins in grain from organic and conventional farms

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In August–September 2003, samples of wheat and barley after harvest were randomly taken from 11 organic and 13 conventional farms in Lithuania. The results of mycological tests indicated that wheat from organic farms was contaminated with fungi by 70.5% ($p < 0.05$) more and barley by 24.8% ($p > 0.05$) less as compared with wheat from conventional farms. Grains taken from both types of farms were contaminated with fungi from the *Fusarium, Alternaria*, *Cladosporium*, *Penicillium*, *Aspergillus* and other genera. The most toxic fungi belong to the genera *Fusarium* and *Alternaria*. From wheat of organic farms 22 while from wheat of conventional farms 12 fungal species were isolated. Barley from organic farms 22 fungal species and from conventional farms 20 species.

DON and ZEN were most frequent in grain from different farms. 100% of wheat samples from organic and conventional farms contain DON and ZEN. Small amounts of aflatoxins and ochratoxins, up to 0.8 and 0.3 $\mu g\ kg^{-1}$ respectively, were found in samples of wheat from organic farms.

Key words: conventional farm, fungi, grain, mycotoxins, organic farming

INTRODUCTION

Organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain and enhance ecological harmony. The primary goal of organic agriculture is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people (DARCOF, 2000).

Cereals are the most important organic crop and represent about 70% of organic arable area in the European Union (European Commission, DG Agri, 2005). Fungi growth in grain is a normal occurrence in both the field and storage (Santin, 2005). Fungal contamination can occur at various stages of crop production: in the field, during transportation and storage (Steyn, 1995).

Fungi are known to produce a variety of secondary metabolites that seem to improve their competitiveness in nature (Steyn, 1998). Secondary metabolites are formed at the final stages of the exponential growth phase (Jay, 2000). Fungal metabolites exhibit an intrinsic toxicity even at low concentrations, resulting in their collective classification as mycotoxins (Jay, 2000; Steyn, 1995). Fungi produce mycotoxins under stressful conditions such as changes in temperature, moisture, or aeration and in the presence of aggressive agents (Fink-Gremmels, 1999; Bakutis, 2004). In animals and humans, mycotoxins can evoke cytotoxic, neurotoxic, immunosuppressive, teratogenic, mutagenic and carcinogenic effects (D'Mello et al., 1999; Hussein, Brasel, 2001; Fink-Gremmels, 1999). There are thousands of secondary fungal metabolites, the vast majority of which have not been tested for toxicity or unequivocally associated with disease outbreaks (Bryden, 2004).

Many researchers have divided fungal species into two groups: field fungi and storage fungi. Field fungi are those that invade the seed while the crop is still in the field and require high moisture conditions (20–21%). These include *Fusarium*, *Alternaria*, *Clodosporium*, *Diplodia*, *Gibberella* and *Helmintosporium* species (CAST, 2003).

Fungi of the genus *Fusarium* are common plant pathogens worldwide in a variety of crops, although they are mainly associated with cereals. *Fusarium* head blight, *Fusarium* foot rot and *Fusarium* seedling blight are the most destructive diseases of small-grain cereals in humid and semi-humid areas. The diseases are commonly caused by *Fusarium culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae* (Parry et al., 1995; CAST, 2003). The *Fusarium* grain infection decrease the germination and quality of kernels in connection with mycotoxic contamination. The most important fusariotoxins are trichothecenes: deoxynivalenol (DON), diascetoxyscirpenol (DAS), T-2 toxin, HT-2 toxin, nivalenol, zearalenone (ZEN), fumonisins (Brennan et al., 2002; Samson, 1988; Lugauskas et al., 2002). Environmental factors (mostly temperature, rainfall rate and humidity) have a significant influence on the *Fusarium* species occurrence and disease severity (Parry et al., 1995). Numerous field experiments were carried out for controlling head blight and subsequent grain infection. Great differences among fungicide efficacy were observed in the field (Matthies, Buchenauer, 2000; Jennings, Turner, 2000). The problems of head blight control are based on a wide spectrum of causal agents. The prevalence of *Fusarium* species is different in each plant tissue and depends on agroclimatic conditions (Samson, 1988). *F. graminearum* is the principal DON-producing fungus in grains, but *F. culmorum* is occasionally involved. *F. graminearum* produces zearalenone. *F. sporotrichioides* is the principle fungus responsible for the production of T-2 toxin, HT-2 toxin, DAS. Toxin production is most intensive under increased humidity and in a temperature range 6–24 °C (Samson, 1988; Lugauskas et al., 2002). *F. moniliforme* and *F. proliferatum* produce fumonisins. These toxins are less important in grain (Santin, 2005).

Species of *Alternaria*, *Cladosporium* are frequent on grain. C*ladosporium* spp. fungi are harmless saprophytes of cereals, while *Alternaria* is known to produce toxins, such as tenuazonic acid, alternariols, altertoxins. *Alternaria* species, *A. alternata* in particular, infected cereal grain under persistent rainfalls, although a high incidence has been observed also in relatively dry weather (Semaškienė et al., 2005).

Storage fungi are those that invade grains or seeds during storage. These need less moisture (13–18%). Storage fungi include *Aspergillus* and *Penicillium* species (Czerwieci et al., 2002).

Ideal conditions for fungal growth depend on the species, but normally fungi need a high temperature and moisture. Fungi grow at temperatures between 20–30 °C. Normally, fungi grow in storage conditions at a 13–18% moisture (Novošinskas et al., 2005; Tuite, 1994). Sometimes management conditions intended to reduce fungal growth actually increase the potential infection. Drying grain at high temperatures breaks the protective pellicle of the grain, thus opening the door to mould invasion of the exposed endosperm. Fungi utilize inter-granular water vapour as a moisture source inside the bin. The inter-granular water concentration is described as water activity (a_w). In practical situations, the storage bin is normally exposed to the sun, which increases the temperature inside the container as convection moves heat into the grain mass. This might carry water to cooler regions of the container. When water vapour moves from warmer to cooler regions in the bin, it condenses and may increase the moisture content to 18% when fungi can grow more easily (Chelkowski, 1991; Samson, 1988). After harvest, a grain is still a live entity and continues the metabolic process of respiration. In the presence of O_2 (aerobic conditions), respiration occurs by oxidation of carbohydrate and production of CO_2, water and energy in the form of heat. When O_2 access to the grain mass in interrupted, aneaerobic metabolism occurs. The final products are CO_2, gas and organic compounds (Santin, 2005). Most fungi need at least 1–2% O_2. However, *F. moniliforme* is an exception and is able to grow in an environment with 60% CO_2 and less than 0.5% O_2 (Tuite, 1994). Insects and mites make a significant contribution to fungal growth through physical damage of grain, which predisposes fungal invasion of the exposed endosperm. The metabolitic activity of insects and mites also causes an increase in both moisture content and temperature of the infested grain, which is favourable for fungal development. Insects and mites can also carry spores of fungi (Muir, Kalan, 1998).

The important mycotoxins produced by *Aspergillus* species include aflatoxin B_1, B_2, G_1 and G_2, ochratoxin A, sterigmatocystin and cyclopiazonic acid. Aflatoxins are produced mainly by *A. flavus*, *A. parasiticus* and *A. nominus*. Aflatoxins are produced at temperatures ranging within 12–40 °C at a pH from 3.5 to 8.0 and with a_w around 0.99. In addition, nutritional factors such as carbohydrate and nitrogen sources affect aflatoxin production, but the mechanisms by which these factors regulate aflatoxin biosynthesis are still unclear (ICMSF, 1996).

Ochratoxin A is produced by *A. ochraceus* and by some *Penicillum* species. *Aspergillus* spp. can produce ochratoxins at 12–37 °C at a minimum pH of 2.2 (Wheler et al., 1991). *Aspergillus* spp. can produce ochratoxins at a_w as low as 0.80. Ochratoxins also can be produced by *Penicillium* spp. at 4 °C with a_w as low as 0.86 (Sweeney, Dobson, 1998).

Penicillium species can produce 27 different mycotoxins, the most important being ochratoxins, patulin, citrinin. Ochratoxins can be produced by *P. verrucosum* in temperate climates. Patulin is produced by the fruit pathogen *P. expansum* which can grow at temperatures from –2 to 35 °C, a_w 0.95. Citrinin is produced at temperatures ranging within 15–37 °C, the optimum being 30 °C (Sweeney, Dobson, 1998).

In recent years some studies have been carried out to examine fungal and mycotoxic contamination in organic and conventional products (Czerwiecki et al., 2002; Malmauret et al., 2002; Jestoi et al., 2004). The results were quite inconsistent: some studies revealed no differences between organic or conventional products, while some have reported an increased risk from the consumption of organic products, and yet others have stated that conventional products pose a greater risk. In fact, most studies have concluded that more investigations are needed to assess the safety of products.

The purpose of the present study was to determine the fungi species most frequent on wheat and barley, the levels of mycotoxins in grain from organic and conventional farms of Lithuania and to compare the quality of grain from organic and conventional farms.

MATERIALS AND METHODS

In August–September 2003, random samples of wheat (*Triticum aestivum* L.) and barley (*Hordeum distichon* L.) were randomly taken from 11 organic and 13 conventional farms after harvest. All samples were sent to the Lithuanian Veterinary Academy.

The concentration of fungi was expressed as colony forming units per gram sample (cfu g^{-1}). Gram grain sample in water extract (1:1000) was spread onto standard Czapek agar and Malt Extract Agar (MEA) in Petri dishes according to Trojanowska K. (1991). The fungal contamination level of grain was determined by direct plating. 100 grains were laid onto standard Czapek agar and Malt Extract Agar in Petri dishes (Smirnova, Kostrova, 1989). The dishes were kept for 10 days in a thermostat at a temperature of 26 ± 2 °C. Pure fungal cultures were isolated, cultivated in standard Czapek agar and Malt Extract Agar (MEA) at a temperature of 26 ± 2 °C for 5–6 days according to manuals (Chelkowski, 1991; Samson, 1988; Lugauskas et al., 2002).

Meteorological data were obtained from the hydrometeorological service.

The mycotoxins deoxynivalenol (DON), T-2 toxin, zearalenone (ZEN), aflatoxins (AFL), ochratoxins were analyzed by the ELISA method. Neogen (USA) diagnostic tests were used for mycotoxin analysis.

Statistical analysis was processed using Prism 2.01 programme. Both ANOVA (analysis of variance) and the non-parametric Wilcoxon sum-rank test were used as a supplementary test. Differences were considered significant when both tests gave $p < 0.05$.

RESULTS AND DISCUSSION

In Lithuania, wheat and barley are a significant component of food and feed. The occurrence of fungi and mycotoxins in grain is mainly dependent on weather conditions after harvest. Wet, rainy, warm and humid weather from flowering and on promotes cereal infection by fungi and the production of mycotoxins. Climatic conditions in Lithuania in the summer of 2003 tended to be warmer (by 0.8 °C) than the average for 1998–2002, with less (by 16.2%) than average rainfall (Table 1).

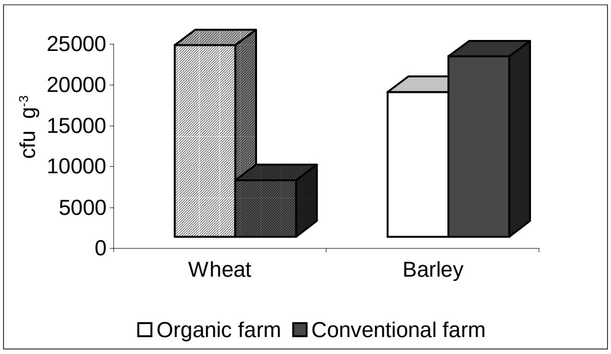

Figure. Mycological test of wheat and barley

The results of mycological tests indicate that wheat from organic farms was contaminated with fungi 23.4 × 10^3 cfu per gram ($p < 0.05$), i. e. by 70.5% more as compare with wheat from conventional farms (Figure). Barley from organic farms was contaminated with fungi 17.7 × 10^3 cfu per gram ($p > 0.05$), i. e. by 24.8% less as compared with barley from conventional farms (Figure). The mycological state of grain can be considered as good when the number of cfu is within the range 10^3–10^5 per gram (Chelkowski, 1991).

The grain samples taken from different types of farms were contaminated with *Fusarium*, *Alternaria*, *Cladosporium*, *Penicillium*, *Aspergillus* and other genera (Table 2). The most toxic fungi belong to the genera *Fusarium* and *Alternaria*. Up to 36% of wheat and 43% of barley from organic farms were contaminated with *Fusarium*, 44% of wheat and 40% of barley with *Alternaria*, the results for wheat in organic farms being *Fusarium* by 35% ($p < 0.05$) and *Alternaria* by 11% ($p > 0.05$) less compared to wheat from conventional farms. Wheat from organic farms was contaminated by *Penicillium* by 34% ($p < 0.05$) were compared to wheat from conventional farms. Contamination with fungi from the genera *Cladosporium, Aspergillus* was similar in wheat ($p < 0.05$) from different types of farms. The results for barley in organic farms were *Fusarium* 7% ($p > 0.05$), *Alternaria* 12% ($p > 0.05$), *Cladosporium* 18% ($p < 0.05$) less compared to barley from conventional farms. Barley from organic farms were contaminated by *Penicillium* 16% ($p < 0.05$) more compared to barley from conventional farms. Contamination with fungi of the genus *Aspergillus* wasn't found in barley from conventional farms. More various genera and species of fungi were found in grain from organic farms, including *Bipoaris*, *Botrytis*, *Septoria* and other genera. The results of our research agree with results presented by other authors (San-

Table 1. **Mean temperatures and precipitation in June, July, August and September in 1998–2003**

Years	June		July		August		September	
	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)
1998–2002[1]	15.6	62.1	17.6	73.8	16.6	73.4	13.1	63.7
2003	15.5	54.9	20.6	54.6	17.3	66.5	12.9	22.4

[1] Average.

Table 2. **Prevailing fungal genera in grain from organic and conventional farms**

Fungi genera	Grain			
	Wheat		Barley	
	Contaminated (%) in organic farm	Contaminated (%) in conventional farm	Contaminated (%) in organic farm	Contaminated (%) in conventional farm
Fusarium	36.0*	68.0*	43.0**	50.0**
Alternaria	44.0**	55.0**	40.0**	52.0**
Cladosporium	12.0*	10.0**	6.0*	24.0*
Penicillium	38.0*	4.0*	22.0*	6.0*
Aspergillus	1.0*	2.0*	2.0	0.0
Other	2.0**	1.5**	2.0**	1.7**

* Differences are significant ($p < 0.05$).
** Differences are not significant ($p > 0.05$).

Table 3. **Concentration of DON, T-2 toxin, ZEN, aflatoxins, ochratoxins (μg kg^{-1}) in wheat and barley from organic and conventional farms in Lithuania**

Mycotoxin concentration	Grain			
	Wheat		Barley	
	Organic farm	Conventional farm	Organic farm	Conventional farm
DON				
Positive[1]	100	100	82	100
Mean pos.[2]	115	138	138	110
Mean all[3]	115	138	113	110
Max.[4]	230	240	210	200
T-2				
Positive[1]	82	69	73	82
Mean pos.[2]	54	36	29	35
Mean all[3]	44	25	21	29
Max.[4]	189	113	100	186
ZEN				
Positive[1]	100	92	82	100
Mean pos.[2]	41	150	56	211
Mean all[3]	41	138	46	211
Max.[4]	68	500	100	450
AFL				
Positive[1]	27	54	27	77
Mean pos.[2]	3	4.6	1.8	2.5
Mean all[3]	0.8	2.5	0.5	2
Max.[4]	5	5.3	4	4.7
OT				
Positive[1]	27	15	18	0
Mean pos.[2]	1.2	4.5	1.4	0
Mean all[3]	0.3	0.7	0.3	0
Max.[4]	1.5	8	1.8	0

[1] Percentage of positive samples.
[2] Mean concentration of only positive samples.
[3] Mean concentration of all samples.
[4] Maximum concentration (μg kg^{-1}) detected.

tin, 2005; Semaškienė et al., 2005). Due to the different management systems, organic farms have advantages because cereal varieties with longer stems can be used and growth regulators are banned. This leads to a lower infection risk of the ears.

From wheat of organic farms 22 while from wheat of conventional farms 12 fungal species were isolated. Barley from organic farms contained 22 and conventional farms 20 fungal species. After harvest and excretion of mycotoxins, the most active fungal species were *Fusarium* and *Alternaria* (*F. graminearum*, *F. poae*, *F. culmorum, F. sporotrichioides, A. alternata, A. tenuissima*). Fungi of the latter species produce mycotoxins such as deoxynivalenol, zearalenone, toxins T-2, HT-2, nivalenol, etc. which strongly influence humans and livestock. The following toxic *Penicillium* species were isolated: *P. aurantiogriseum*, *P. verrucosum*, *P. funiculosum, Aspergillus* species: *A. flavus*, *A. sydowii*, etc. Fungi of the *Penicillium* spp. produce ochratoxins, while *Aspergillus* spp. fungi produce ochratoxins and aflatoxins.

Fungi can grow almost anywhere under a wide array of environmental conditions. Fortunately, not all fungi produce measurable levels of mycotoxins. In many instances, fungi thrive and produce molds, but little or no mycotoxins are formed. The synthesis of mycotoxins, more than fungal growth, is dependent on specific weather and environmental conditions (Santin, 2005).

Results of the mycotoxin analysis are summarized in Table 3. Fusariotoxins DON and ZEN were most frequent in grain from different farms. 100% of wheat samples from organic and conventional farms contained DON. In wheat from organic farms DON content was 115 μg kg^{-1}, and in wheat from conventional farm it was by 20% ($p > 0.05$) higher.

ZEN was found in 100% of wheat from organic farms, but ZEN content was by 72% ($p < 0.05$) less than in wheat from conventional farms. T-2 toxin was present in 82% of wheat samples from organic farms and in 69% of wheat samples from conventional farms. T-2 toxin content in wheat from organic farms was by 43% ($p < 0.05$) higher. Significant differences in DON levels in organic wheat were detected in 1997 and 1998 in Germany, reflecting the major differences in moisture conditions between the two years during wheat flowering. DON content in 1997 was 111 μg kg^{-1}, and in 1998 it reached 280 μg kg^{-1} (Birzele et al., 2000). In wheat, 69% of the conventional wheat samples tested positive for DON at a mean level of 1.54 μg kg^{-1}, while 54% of organic samples were positive, at a mean level of 760 μg kg^{-1}, in Germany. ZEN levels were compared in conventional (74 μg kg^{-1}) and organic winter wheat samples (47 μg kg^{-1}). Far fewer samples tested positive for ZEN compared to DON, and the mean level was far lower (Brandt, Leifert, 2005).

Small amounts of aflatoxins and ochratoxins (up to 0.8 and 0.3 μg kg^{-1}, respectively) were found in wheat samples from organic farms. In wheat from conventional farms, aflatoxins were detected in 54% and ochratoxins in 15% of samples (up to 2.5 and 0.7 μg kg^{-1}, respectively). The content of aflatoxins and ochratoxins was lower in wheat from organic farms, but wheat samples were contaminated with aflatoxins 27% more.

82% of barley from organic farms was contaminated with DON, its content was reaching 113 μg kg^{-1} 100% of barley from conventional farms was contaminated with DON at a concentration of 110 μg kg^{-1}. DON content was only by 2.6% ($p > 0.05$) higher in barley from organic farms. ZEN was found in 82% of barley from organic farms and in 100% of barley from conventional farms. ZEN content was 46 μg kg^{-1} in barley from organic farms, but it was by 78% ($p < 0.05$) higher in barley from conventional farms. T-2 toxin content was by 27% ($p > 0.05$) higher in barley from conventional farms. Aflatoxins were present in 77% of barley from conventional farms and in 27% of barley from organic farms. Aflatoxin concentration was low in barley, reaching 0.5 μg kg^{-1} in samples from organic farms and 2 μg kg^{-1} in samples from conventional farms. Ochratoxins were detected in 18% of barley from organic farms at a concentration of 03 μg kg^{-1}. Ochratoxins were not found in barley from conventional farms. In grain from organic farms in Germany and Italy ochratoxin A content did not exceed 5 μg kg^{-1} (Birzele et al., 2000; Biffi et al., 2004).

The most important factors – weather, site, conditions, and storage – impact both systems equally. The presence of mycotoxins in grain is an inevitable and unavoidable consequence of the existence of fungi. Mycotoxins in grain at levels posing risks to livestock and people are infrequent and preventable. Serious mycotoxin problems are most likely when adverse weather conditions strike a region close to harvest, when farmers have to dry grain quickly. The lack of a clean and dry storage space opens the door to fungal infection and the production of molds and mycotoxins.

Received 22 May 2006
Accepted 10 August 2006

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VEIKSNIAI, LEMIANTYS MIKROMICETŲ GAUSĄ IR MIKOTOKSINŲ GAMYBĄ GRŪDUOSE IŠ EKOLOGINIŲ IR CHEMIZUOTŲ ŪKIŲ

Santrauka

2003 m. rugpjūtį–rugsėjį po derliaus nuėmimo kviečių ir miežių mėginiai buvo paimti iš 11 ekologinių ir 13 chemizuotų Lietuvos ūkių. Mikologinių tyrimų rezultatai parodė, kad kviečiai iš ekologinių ūkių buvo užkrėsti mikromicetais 70,5% ($P < 0,05$) daugiau, miežiai iš ekologinių ūkių – 24,8% ($P > 0.05$) mažiau, palyginti su kviečiais ir miežiais iš chemizuotų ūkių. Grūdai, užauginti ir paimti iš skirtingų ūkių, buvo užsikrėtę *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. ir kitų genčių mikromicetais. Ant grūdų labiausiai buvo išplitę *Fusarium*, *Alternaria* genčių grybai. Iš kviečių ir miežių, paimtų iš ekologinių ūkių, buvo išskirti 22 rūšių, iš kviečių, paimtų iš chemizuotų ūkių, – 12 rūšių, iš miežių – 20 rūšių mikromicetai. DON ir ZEN dažniausiai buvo nustatyti grūduose iš skirtingų ūkių, 100% kviečių mėginiai abiejose ūkių sistemose buvo užteršti DON ir ZEN. Maži aflatoksinų ir ochratoksinų kiekiai (0,8 ir 0,3 $\mu g\ kg^{-1}$) buvo nustatyti kviečiuose, paimtuose iš ekologinių ūkių.

Raktažodžiai: chemizuotas ūkis, ekologinis ūkis, grūdai, mikotoksinai, mikromicetai