Designing a model for calculating the risk of mycotoxins presence in grain

Heino Lõiveke

Estonian Research Institute of Agriculture

Heino Lõiveke, *Department of Plant Sciences, Estonian Research Institute of Agriculture, 13 Teaduse St., 75501 Saku, Estonia*

Introduction

Toxic strains of the mould fungi *Cladosporium, Alternaria, Fusarium, Penicillium, Helminthosporium, Mucor and Rhizopus* often found in Estonian grain may be the causes for the contamination of grain with mycotoxins (Lõiveke et al., 2004). Less frequently, *Aspergillus* species may occur as well. Moulds with dark mycelium cause sooty mould on grain growing in fields in the autumn. *Fusarium* fungi cause Fusarium head blight on the ears and vegetative parts of cereals and the infection of grains. Of mycotoxins produced by moulds, mainly ochratoxin and zearalenon, more seldom aflatoxin and vomitoxin (Monitoring of mycotoxins…), and, in the course of the latest research (Lõiveke, 2008), also T-2 and HT-2 toxins have been found on grain grown in Estonia. The production of mycotoxins may begin already in the field, particularly in the case of *Fusarium* infection if there are favourable conditions for that, but it may also start in the storehouse. The most important preconditions include a sufficient amount of moulds and the existence of unbound water in grain (moisture content above 13%). There are many factors contributing to the process in a storehouse: moist weed seeds, the presence of pests, poor cooling of grain after drying it, greater thickness of the grain layer than allowed, the generation of condensed water, etc. – all factors are not yet known. Moulds with their biological functioning impair the sowing properties of grain, the quality of food and feed and, by producing mycotoxins, make grain dangerous as human food and animal feed. Moulds have evolved their production of mycotoxins as a means in the fight for a feeding environment with competing microorganisms. Mycotoxins are odourless, colourless and tasteless. Their existence in grain cannot be detected visually, but indirectly by means of biotests or directly by using relevant gas or liquid chromatographic methods. The latter methods provide accurate data, but are expensive and time-consuming. To assess the potential of mycotoxin generation in a certain grain batch (risk analysis) there must be data on the meteorological and agro-technological conditions and the phytosanitary state during the growing and harvesting of the grain and on its processing and organoleptic properties. All the conditions are evaluated in risk points, based on their effect on the development of mould fungi. Factors contributing to the development of moulds are, according to literature data, also favourable for the generation of mycotoxins (Tischner, Doleschel, 2003), which is also taken into account while compiling a model for calculating the risk of mycotoxins. A risk analysis of mycotoxins designed for a grain batch enables the determination of potential presence of mycotoxins in the batch and, based on that, the possibilities for its use.

Material and methods

Grain samples needed for the research (à 2 to 3 kg) were collected from producers from different areas of the country and from grain tests carried out by the Estonian Research Institute of Agriculture (ERIA) in its experimental fields at Kuusiku, Üksnurme and Kõbu over the period from 2006 to 2007. In the case of samples from production, data on agro-technological factors were collected from certain producers and meteorological data from the Estonian Meteorological and Hydrological Institute. As regards samples collected from tests, agro-technological data were fixed in the course of the tests whereas meteorological data were obtained by means of automatic weather stations. Until analysis, the samples were stored in paper bags in laboratory conditions where they could not become moist. The microbiological analysis of the samples was performed at the Plant Health and Microbiology Laboratory of the Agricultural Research Centre and the analysis of mycotoxins at the Residue and

Contaminant Laboratory (ochratoxin and zearalenon) of the same insitute and at the National Institute of Chemical Physics and Biophysics (deoxynivalenon, T-2 and HT-2).
Taken into account in designing the risk analysis of mycotoxins described below were views presented in literature and the results of the analysis of samples collected by us. In the United Kingdom, Sweden and Finland (Mycotoxin risk assessment…, UK; Branschriktlinjer …., 2006; veli.hietaniemi@mttt.fi) all meteorological and agro-technical conditions present during the growing and harvesting of grain are evaluated in risk points (+ or –). Also taken into account are the quality of grain drying and processing, the presence of pink grains, the colour and smell of grain. The model is based on the position that factors contributing to the development of moulds also increase the risk of mycotoxins presence in grain. However, the presence of toxicants in the form of moulds does not always result in the generation of mycotoxins (Eskola et al., 2001). The factors increasing the risk are marked with a '+' and the factors decreasing it with a '-'. Based on test data obtained from the ERIA, Agricultural Research Centre, Finland, Sweden, Germany and Denmark, the effectiveness of the risk factors was evaluated on a scale from 0 to 3, and, as an exception, to 5 risk points with a pre-crop in May. The indicators evaluated in the risk points are presented in Table 1. The sum of the risk points (maximum 34) gives an idea of the level of safety of the crop. The sum of points up to 11 = low risk, 12–21 = medium risk, 22 and above = high risk of mycotoxins.
If the risk of mycotoxins is high, the grain batch must not be used either for food or feed until additional research has been carried out. In such a case, it is necessary to establish the possibilities for the use of the grain by determining the general toxicity of the grain sample by means of a biotest using either *Paramaecium caudatum* or *Stylonichia mythilus.*

Results and discussion

The species and varieties of grain did not differ much in their susceptibility to the mould fungi *Cladosporium, Penicillium, Alternaria, Mucor, Rhizopus*, however, more susceptible to *Fusarium* spp (Fusarium head blight) were oats and triticale (table). According to data from the Agricultural Research Centre, varieties differ in their susceptibility to head blight in our conditions as well. When grain is grown in an area with unsuitable soils and climate (lack of water during the germination and sprouting, early night frost in the autumn, etc.), the yields are smaller and their microbiological quality is worse. Grains may become contaminated with *Fusarium* fungi causing head blight already during flowering. The level of infection depends primarily on precipitation. The same applies to the spread of the infection with *Fusarium* and *Cladosporium, Penicillium, Alternaria, Mucor, Rhizopus* and other moulds (sooty mould) prior to and during harvest. Grain can be more likely infected with *Fusarium* fungi and contaminated with mycotoxins if the pre-crop is infected with fusariosis and the infection retains its viability. The most unsuitable pre-crop is maize which is highly susceptible to fusarioses. Cereals themselves are not very suitable either. The most suitable are potato, crucifers, papilionaceous plants and grasses, which are rarely damaged by fusarioses. Infected harvest residue left on the ground is one of the sources of the infection also in the case of moulds. All cultivation methods which leave diseased plant parts on the soil increase the spread of the diseases. Compared to classic ploughing, minimum tillage and direct drilling are less recommended. The lodging of crops in the field increases their contamination with moulds and the risk of mycotoxins. The earlier the lodging occurs, the greater is the risk. The incidence of mycotoxins increases sharply (high risk) if the crop lodges more than 6 weeks before harvest (Mycotoxin risk assessment…, UK). The use of growth regulators guarantees the lodging resistance of the crop and is a prerequisite for quick harvest. Spraying with fungicides before and during flowering is the most important method for restricting the spread of Fusarium head blight and sooty mould and decreasing the contamination with *Fusarium* fungi. According to literature data (Tischner, 2004), more effective fungicides may reduce the incidence of mycotoxins in grain by 50 to 70%. With delayed harvest, the quality of the grain decreases, while at the same time the ripe grain is becoming intensively infested with Fusarium fungi and sooty moulds *(Alternaria, Macrosporium, Clodosporium, Mucor, ect.),* including toxicants. The golden tone of the grain turns grey. Grain harvested during a rainy season is damper and infested with moulds to a greater extent. With delayed harvest as well as with rain during the harvest period, there arises the risk of the generation of mycotoxins already in the field. The risk of mycotoxins can be considerably reduced by quick drying and processing. The shorter the period between harvest and drying and pre-cleaning, the lesser is the possibility for moulds to reproduce and produce toxins. If the generation of mycotoxins has already started in the field, quick drying may stop both the increase in the amount of moulds and further production of mycotoxins. EU regulation No 1572/2006 lays down the maximum allowable moisture content of wheat and barley, 14.5%, for intervention buying. The experience of researchers (Kallas, 2001) of the ERIA and Finnish growers has shown that the moisture content should be below 14% for the grain to be preserved well. Weed seeds, which, according to our data, have a moisture content often 4 to 5 five times higher than that of grain may, if they are abundant, increase the average humidity of grain by 5% and initiate the development of moulds already in 24 hours after the harvest. Damaged grains are a good feeding substratum for moulds and they also respire more actively, releasing moisture and warmth necessary for starting the spoiling process. In accordance with the requirements set out in EU regulations No 824/2000 and No 1572/2006, the percentage of miscellaneous impurities may not exceed 3% and that of broken grains 5%. According to the standard EVS 743:1998 (wheat), the total allowable percentage of grain impurities, including shrivelled grains, is 5%; according to the standard EVS 757:1998 (barley), the maximum allowable percentage of thin grains is 5% and that of weed seeds 1%. Thin grains are often infected with fusariosis and thus a risk factor. Grains contaminated with *Fusarium* fungi are of pink colour, matt, lustreless and shrivelled or husky. Due to their lighter specific weight, at least part of them can be separated by means of a winnower, reducing thereby the risk of mycotoxins. The amount of pink grains must not exceed 0.5%. The loss of the normal colour of grain and its unpleasant smell are the signs of spoilt grain (see Estonian standard EVS 677:1955. Cereals, legumes and products made from cereals. Determination of organoleptic properties). Grain infected with moulds has the characteristic smell of mould or must; the colour characteristic of the variety has faded or disappeared. Deviations from the normal colour and the existence of the smell of mould are a sign indicating the risk of mycotoxins.

Summary and conclusions

On the basis of the initial data obtained from studying the samples with the described risk factors the following tendencies can be observed. Mycotoxins (DON, T-2 and HT-2) were most often found in grain harvested during a period of heavier precipitation or if the harvest had been delayed due to rainy weather. Of cereal species, oat and triticale samples contained the mentioned mycotoxins most often while wheat, barley and rye contained them less. Due to the short research period and insufficient research material, the initial data and tendencies cannot be considered final or sufficiently proven. The initial model for calculating the risk of mycotoxins presence in grain, which has been described here, needs further development and supplementation on the basis of a more comprehensive database. The application of this model can still provide grain growers with the information necessary for the use of a specific grain batch and avoid unnecessary and large expenditures on the determination of mycotoxins as well as offer information to livestock farmers regarding the adding of mycotoxin binders to grain used as animal feed.

Table 1. Mycotoxins risk factors in points

No	Risk factors	Explanation	risk points
1.	Species of cereal	oats, triticale	+3
		winter and spring wheat	+2
		barley, rye	+1

2.	Susceptibility of variety	high	+2
		moderate	+1
		low	0
3.	Region by the compatibility	compatible	0
		less compatible	+2
4.	The amount of precipitation before and during the flowering (01.07-15.07)	less than 25 mm	0
		25-35 mm	+1
		35-55 mm	+2
		more than 55 mm	+3
5.	The amount of precipitation before and during the harvesting (01.08-15.09)	less than 30 mm	0
		30-75 mm	+1
		75-120 mm	+2
		more than 120 mm	+3
6.	Precrop	maize	+5
		cereals	+3
		other crops	0
7.	Cultivation method	ploughing	-3
		minimized cultivation	+2
8.	Presence of lodging before the harvest, use of retardants	more than 6 weeks	+3
		2-6 weeks	+2
		less than 2 weeks	+1
		no lodging occurred, retardants were used	-2
9.	Use of fungicides	are used	-2
		are not used	+2
10.	In-season presence of Fusarium head blight and smooty molds	diseases are presence	+2
		no diseases registered	-2
11.	Time and conditions of harvesting	well-timed harvest, good conditions	0
		badly timed harvest, bad conditions	+2
12.	Quality of drying and processing	good (grain moisture content less than 14%, litter and broken grains in range of limit)	0
		bad (grain moisture content more than 14%, litter and broken grains exceed limit)	+2
13.	Presence of pink grains (with fusarioses)	pink grains up to 0.5%	0
		pink grains more than 0.5%	+1
14.	Colour and smell of grain	colour and smell accord to variety	0
		colour darkened, smell of mould or staling	+2

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