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Natural *Fusarium* occurrence qualitative evaluation on cereals under conventional and organic system in the Czech Republic

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Cereals are the most growing crops under organic conditions. Cereals are supposed to be a source of mycotoxins produced by *Fusarium* species. Our research was led to describe the differences among natural *Fusarium* species occurrence in two localities and two growing systems – organic and conventional. The main differences were observed among years and cereal species. No statistically significant differences were determined between localities and farming systems.

Keywords: *Fusarium* natural occurrence, organic farming, conventional farming

1 Introduction

Fusarium species play an important role as a plant pathogen causing a number of different plant diseases such as head-blight, stem and root rot etc. Most *Fusarium* species occur was found to be toxigenic to human, animals, plants and microorganisms as well (Denijs et al., 1996). The content of *Fusarium* species and mycotoxins in the organic production is discussed internationally due to the fact that in organic agriculture is prohibited the use of any fungicides. In spite the fact that there are many studies on mycotoxin content, the study on natural occurrence of *Fusarium* species comparing the organic and conventional farming system in the Czech Republic is lacking. The main objective of this study was to assess the natural occurrence of *Fusarium* species in two localities and under organic and conventional system.

2 Material and Methods

2.1 Plant material

The precise field trials were carried out in University of South Bohemia in České Budějovice (CB) and in the Crop Research Institute in Prague (CRI). The spring forms of emmer, common wheat, and spelt wheat under conditions of conventional and organic system were grown. During the vegetation period only herbicides were used in conventional system. No fungicides were used not to influence the results. On organic part of trials the mechanical weeding was proceeded. The samples of grains were taken away after harvest. The qualitative and quantitative evaluation of presented fungi was carried out on dehulled milled grains.

2.2 DNA extraction

DNA both from a mycelium of all tested fungi and from infected grain samples was extracted using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer instructions. The quality and the concentration of extracted DNA were verified electrophoretically in 0.8% agarose gel. DNA was visualized by ethidium-bromide and detected under UV lamp. As a

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size and concentration standard Lambda DNA/HindIII Marker (Fermentas, Lithuania) was used. DNA extracted from infected seed samples was diluted to a concentration of 50 ng/µl using a GeneQuantPro spectrophotometer (Amersham, Cambridge, UK).

2.3 Species specific amplification

The markers specific to the species: *F. culmorum* (Fc), *F. graminearum* (Fg), *F. pseudograminearum* (Fpse), *F. poae* (Fp), *F. sporotrichioides* (Fsp), *F. equiseti* (Fe) and *F. avenaceum* (Fa) were borrowed from the literature (Parry and Nicholson, 1996; Doohan et al., 1998; Aoki and O´Donnell, 1999; Demeke et al., 2005; Leišová et al., 2006). PCR reactions were performed in 15 µl reaction mixture (0.3 µM of each primer, 170 µM dNTP, 1x PCR buffer, 2 mM MgCl2, 1U Tth DNA polymerase Biotools (DYNEX) and 50 ng of template DNA) in the cycler SensoQuest (Goettingen, Germany). The amplification products were separated in 1.6% agarose gel, stained with ethidium bromide and visualised under UV light. The size of the product was verified by comparing with the size standard GeneRulerTM 100bp DNA Ladder (Fermentas, Lithuania).

3 Results

The results from qualitative evaluation of natural occurrence of several species of *Fusarium* are shown in Table 1 and 2. The numbers represent the level of the infection observed by relative quantification. Although the evaluation is very aproximate it gives us a good idea about the level of *Fusarium* spp. infection. The differences were observed between localities. The larger *Fusarium* attack was determined in Prague (CRI). The differences were also among years. The largest attack of *Fusarium* in Prague was in year 2011 in comparison to 2012 and 2013. On the other hand the largest *Fusarium* occurrence was determined on common wheat in both localities. No differences were found between both systems. This fact was proved also by quantitative analyses where *F. culmorum* and *F. graminearum* were evaluated (p_{Fc} = 0,72; p_{Fg} = 0,07; data not shown).

The most widespread *Fusarium* species in our experiment were *F. avenae* and *F. poae*.

Table 1 Natural occurrence of *Fusarium* species in České Budějovice

Crop	Year	System	Fsp	Fg	Fc	Fe	Fa	Fp
Emmer	2011	organic	0	0	1	0	1	2
		conventional	0	0	1	0	2	2
	2012	organic	0	1	0	0	2	3
		conventional	0	0	0	1	2	3
	2013	organic	0	0	0	0	2	2
		conventional	0	0	0	0	1	0
Common Wheat	2011	organic	1	0	1	0	2	2
		conventional	1	0	1	2	3	2
	2012	organic	0	2	1	1	3	3
		conventional	0	2	0	3	3	3
	2013	organic	1	1	0	0	2	1
		conventional	1	0	0	0	2	1
Spelt Wheat	2011	organic	0	0	1	0	1	3
		conventional	0	0	1	0	2	3
	2012	organic	1	1	0	0	2	3
		conventional	1	1	2	0	3	3
	2013	organic	0	1	0	0	0	0
		conventional	0	0	0	0	1	1

Table 2 Natural occurrence of Fusarium species in Prague

Crop		System	Fe	Fc	Fsp	Fg	Fp	Fa
Emmer	2011	organic	1	1	3	3	2	2
		conventional	0	1	0	3	1	1
	2012	organic	0	0	0	0	1	1
		conventional	0	0	0	0	2	1
	2013	organic	0	0	0	0	1	1
		conventional	0	0	0	0	1	1
Common Wheat	2011	organic	2	2	2	3	2	3
		conventional	0	1	2	2	2	2
	2012	organic	1	1	3	1	3	3
		conventional	0	2	2	0	2	3
	2013	organic	0	0	0	1	1	1
		conventional	0	0	1	0	1	1
Spelt Wheat	2011	organic	0	1	2	0	2	2
		conventional	0	1	1	2	2	2
	2012	organic	1	0	1	0	3	2
		conventional	0	0	0	1	3	1
	2013	organic	0	0	0	0	1	0
		conventional	0	0	1	0	1	1

4 Conclusions

The organic farming system is debated as a source of *Fusarium* species producing the mycotoxins due to forbidden utilization of fungicides. However, from our research there are clear results that no differences between natural occurrence of *Fusarium* species on organic and conventional growing cereals were determined. The main differences were observed among years and cereal species. No statistically significant differences were determined between localities and farming systems.

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