DEOXYNIVALENOL AND ZEARALENONE IN WINTER WHEAT GROWN IN ECOLOGICAL AND INTEGRATED SYSTEMS

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Abstract: *A long term trial comparing ecological (ECO) and integrated (INT) farming systems was carried out in western Slovakia from 1990 to 2009. The location has a continental climate, with average temperatures of 19,7 °C in July and – 1,7 °C in January and an average annual precipitation of 561 mm, the soil type is a Haplic luvisol. The ECO and INT systems were composed of a six stage crop rotation. Subplots were fertilised and unfertilised treatments. 25 g samples of w. wheat was placed into a 250 mL glass Erlenmayer flask and then 100 mL of 84+16 acetonitrile water were added. After stired extract was filtered and clean up by immunoaffinity column MycoSepTM 226 for ZON and 227 for DON. Purified extract was evaporate to dryness using RVO 400 and redislоved in 0,5 mL mobile phase. The concentration of deoxynivalenol (DON) and Zearalenone (ZON) was detected in the w. wheat grain samples by using of HPLC WATERS BREEZE equipped with binary pump WATERS 1525, RHEODYNE injector and UV detector WATERS 2487 was used with a stainless steel reverse phase 150x3,9 mm, 4 μm spherical particle C18 Nova-Pak column. UV detector set to 218 nm for DON and 238 nm for ZON. Mobile phase were mix of of A: water, B: acetonitrile with using gradient. In ECO system, the concentration of DON was lower by 46 % (average 192,4 μg.kg-1) compared with INT system (average 361,6 μg.kg-1). Fertilisation treatments enhanced the level of DON in both systems. Within INT system also pre-crop effect on DON was revealed, peas as pre-crop significantly enhance the concentration of DON. The effects of farming system and fertilisation were not significant for ZON, but level of ZON was influenced by weather conditions. Drier and warmer weather supported the production of ZON, more humid and warm weather enhanced the level of DON.*

Key words**:** *deoxynivalenol, zearalenone, ecological ,integrated system*

INTRODUCTION

The presence of mycotoxins, especially deoxynivalenol (DON) is a major concern for grain growers using any system. DON is a trichothecene mycotoxin produced by *Fusarium* moulds genera. Its biosynthesis runs during the growth of infected grain in the field. Contamination in feedstuffs can cause serious health problems and diseases. Even at low levels, DON may cause animals to refuse feed or, at higher levels, induce vomiting leading to growth depression, increased susceptibility to infections, diarrhoea and haemorrhage (HSU et al., 1972). DON has been implicated as a factor in the human disease ATA (alimentary toxic aleukia).

Zearalenone (ZON) is an estrogenic mycotoxin mainly produced by *Fusarium graminearum*, a species which colonizes a wide variety of grains. It develops as a consequence of prolonged cold or humidity and of the interaction of multiple factors, such as moisture content of the grains, infections, temperature, microbial interactions, and others (BENNET et al., 1979, MOSS 1991).

Review of the literature suggests, that the mycotoxin content of cereals is highly variable, regardless of the farming system. Organic grains might be expected to be more susceptible to mycotoxins because they are not treated with fungicides. This paper reports on the effects of ecological and integrated farming systems, fertiliser inputs on the mycotoxins contamination of winter wheat in a log-term field trial.

MATERIAL AND METHODS

Rotation and cropping system field experiments were carried out at Dolná Malanta, Western Slovakia (18°07´E, 48°19´N) from 1990 to 2009 on a Haplic Luvisol developed at proluvial sediments mixed with loess. The altitude of the experimental plots is 178 m. The location has a continental climate with an average temperatures 19,7 °C in July and - 1,7 °C in January, an average annual precipitation is 561 mm. A split – plot design was used with two main treatments, Ecological (ECO) and Integrated (INT) farming systems. The ecological system was composed of a six course crop rotation: beans + lucerne – lucerne – winter wheat – peas – maize – spring barley. The integrated system consisted of the crop rotation: winter wheat – peas – winter wheat – maize – spring barley – lucerne (3 years at the same plot).

Subplots were fertilised (+fert) and unfertilised treatments (-fert). The +fert treatment in ECO system was based on 40 tonnes of manure (192,0 N; 40,2 P; 208,2 K kg per rotation), while the INT system also received 40 tonnes of manure plus synthetic fertilisers. Treatments were replicated four times. Sowing and harvesting dates, rainfall and average temperature calculated for vegetative period of the crop, synthetic fertiliser inputs (kg.ha-1) applied in the INT system are shown in Table 1. Nitrogen fertilisers were applied in three split applications.

Table 1.

Crop management data for w. wheat, 2007-2008; climatic data during vegetative period, synthetic fertiliser inputs in INT system

Year	Sowing date	Harvest date	Rainfall (mm)	Average temperature (°C)	Nitrogen ($kg.ha^{-1}$)	Phosphorus ($kg.ha^{-1}$)	Potassium ($kg.ha^{-1)}$)
2007	9/10/06	2/07/07	347	10.0	58.0	28.3	56.6
2008	9/10/07	28/07/08	467	8.9	57.5	28.3	21.6

During 2007 and 2008 the concentration of deoxynivalenol (DON) and zearalenone (ZON) was detected in the w. wheat samples from ECO and INT systems, +fert and –fert variants.

Chemicals and reagents: Deoxinivalenol (DON) in acetonotrile (100 μg/mL) and Zearalenon (ZON) in acetonotrile (100 μg/mL) was obtained from BIOPURE (Tulln, Austria) MycoSep ™ 226 and 227 immunoaffinity colums was obtained from INTERTEC s.r.o. (B. Bystrica, Slovakia). Analytical and gradient grade methanol and acetonitrile were obtained from MERCK (Bratislava, Slovakia).

Instruments: The liquid chromatography WATERS BREEZE (Lambda Life, Slovakia) equipped with binary pump WATERS 1525, RHEODYNE injector and UV detector WATERS 2487 was used with a stainless steel reverse phase 150x3,9 mm, 4 μm spherical particle C18 Nova-Pak column (Waters, Milford, MA, USA). Rotary vacuum evaporator RVO 400 (INGOS, Prague, Czech republic) for evaporating samples was used.

Extraction: Weight out 25 g of ground sample was placed into a 250 mL glass Erlenmayer flask and then 100 mL of 84+16 acetonitrile water were added. The mixture was stired for 5 min. The extract was filtered trough a fluted filter.

Clean up by immunoaffinity chromatography: 8mL of final extract was placed into glass tube, push all trough MycoSep™ 226 for ZON and 227 for DON immunoaffinity column. Exactly 4 mL of purified extract was pipeted into a clean tube, evaporate to dryness using RVO 400 and redissolved in 0,5 mL mobile phase.

HPLC chromatography: 50 μL of the samples were injected into the HPLC column heated on 35°C and determined by UV detector set to 218 nm for DON and 238 nm for ZON. Mobile phase were mix of A: water, B: acetonitrile with using gradient: 0-5 min 10 % B, 6-16 min 10-25 % B, 1735 min 25-60 % B, 36-40 min 60 % B, 41 min 10 % B. Flow rate was 0,6 mL/min mobile phase. Retention time was for DON 13,4 min and for ZON 40,8 min.

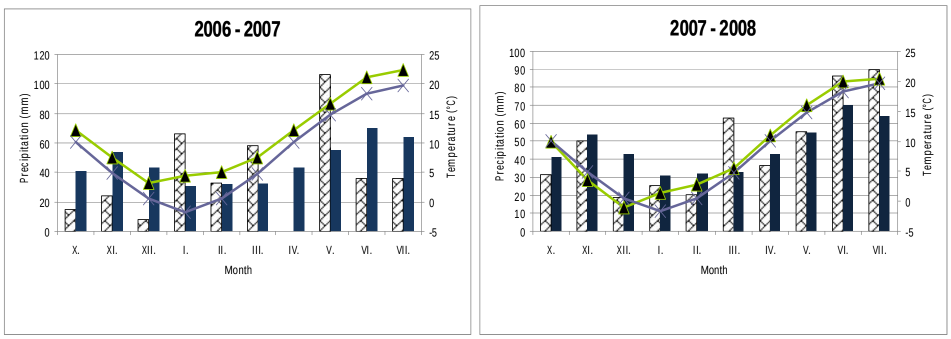

Figure 1- Detailed information on weather conditions during 2006-2007 and 2007-2008 growing seasons
Total monthly precipitation; Long-term average monthly precipitation; Mean monthly temperature; Long-term average monthly temperature.

RESULTS AND DISCUSSIONS

Effect of farming system on DON and ZON concentrations.

The effects of farming systems, years, fertilisation on DON concentration in winter wheat grain were significantly different. Average DON concentration in INT system was 361,6 $\mu g.kg^{-1}$, in ECO system its overall concentrations was lower by 46 % and reached an average of 192,4 $\mu g.kg^{-1}$ (Table 2). Fertilisation treatments enhanced the level of DON in both systems, overall concentration in fertilised treatments was 420,5 $\mu g.kg^{-1}$ and in non fertilised treatments comparatively lower level was detected, on average 189,9 $\mu g.kg^{-1}$. Not only farming system, but within INT system also pre-crop effect on DON concentration was revealed. Peas, as pre-crop for winter wheat, significantly enhance the concentration of DON, namely fertilised treatment reached the level of 601,5 $\mu g.kg^{-1}$, what was the highest concentration detected in the experiment. Weather conditions during two consecutive growing seasons significantly influenced levels of DON in winter wheat samples. More humid conditions during June and July in 2008, together with warm weather caused significantly higher production of DON than it was in 2007, when the weather conditions were different, with prevalence of very warm and dry periods, in April, June and July.

Table 2.

Concentration of DON ($\mu g.kg^{-1}$) in winter wheat samples.

Production system	**Pre-crop**	**Fertilisation**	**Year**		**Average**	**Average for system**
			2007	**2008**		
INT	peas	+Fert.	553,7	649,2	601,5	431,8c
		-Fert.	187,9	336,3	262,1	
INT	barley/lucerne	+Fert.	108,4	653,4	380,9	291,5b
		-Fert.	62,6	341,7	202,2	
ECO	lucerne	+Fert.	155,5	402,8	279,2	192,4a
		-Fert.	124,3	87,0	105,7	
average			198,7a	411,7b		

Numbers with different letters are significantly different (P=0,05).

However, within our experimental study, detected concentrations of DON and ZON did not exceed the limits allowed by EC regulation 1881/2006, for DON in cereals for direct human consumption up to 750 μg.kg-1 and for Zearalenon up to 75 μg.kg-1.

Statistical analysis of ZON concentration in winter wheat grain showed significant effect of years. The effects of farming system and fertilisation were not significant, but interaction system x fertilisation showed significant differences. Average ZON concentration in INT system was 7,43 $\mu g.kg^{-1}$, in ECO system the concentration was also low and reached 7,85 $\mu g.kg^{-1}$ (Table 3).

Table 3.

Concentration of Zearalenon ($\mu g.kg^{-1}$) in winter wheat samples.

Production system	Pre-crop	Fertilisation	Year		Average	Average for system
			2007	2008		
INT ES	peas	+Fert.	17,7	2,8	10,25c	7,50 n. s.
		-Fert.	8,0	1,5	4,75a	
INT ES	barley/lucerne	+Fert.	9,8	2,7	6,25ab	7,35 n. s.
		-Fert.	14,0	2,9	8,45bc	
ECO	lucerne	+Fert.	11,7	1,4	6,55ab	7,85 n. s.
		-Fert.	16,0	2,3	9,15bc	
average			12,87b	2,26a		

Numbers with different letters are significantly different (P= 0,05).

Fertilisation did not enhance contamination of grains, average ZON concentration in –fert treatment was 7,45 μg.kg-1, in +fert treatment 7,68 μg.kg-1. Pre-crop effect in INT system was also not significant, in spite of the highest concentration of ZON in INT system, +fert treatment, when peas was the pre-crop of winter wheat. Weather conditions caused significant differences in levels of detected ZON. During 2006-2007 growing season, warm and dry weather, namely in June and July supported the production of ZON, whereas more humid and warm weather in 2008 was less favourable for ZON. The tendency was opposite as compared to DON concentration.

In the absence of fungicides, organic wheat was more tolerant to Fusarium infection than conventional (DŐLL et al. 2002). *Fusarium* infection rate was consistently highest in wheat grown with mineral fertilizer without pesticides, intermediate in conventionally grown wheat with mineral fertiliser and pesticides, and lowest in organically grown wheat (BIRZELE et al., 2002). The type of fertility management is more important for the extent of Fusarium infection than the use of pesticides. The relatively low and/or slow nutrient supply in organic farming helps to trigger the plants´ natural defenses against infections. If a plat is allowed to grown unusually fast by providing it with an abundance of nutrients, the accumulation of defense compounds is reduced, as well as resistance to diseases and pests (BRANDT et al., 2006; STAMP 2003). On the basis of mycotoxin studies and the acceptance of the concept of hormesis (beneficial effects at low concentration level), Calabrese and Blain (CALABRESE et al., 2005) indicated that consumption of food with low levels of mycotoxins may strengthen the immune system rather than harming it.

CONCLUSIONS

Mycotoxin contamination (DON, ZON) in wheat was below the level considered safe for consumption. In ECO system, the concentration of DON was lower by 46 % compared with INT system. Within INT system, pre-crop effect on DON concentration was determined, when peas enhanced the level of DON in w. wheat grain. Level of ZON was influenced by weather conditions. Drier and warmer weather supported the production of ZON, more humid and warm weather enhanced the level of DON.

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