

Occurrence of deoxynivalenol in Czech grain

I. Polišínská¹, S. Sýkorová², E. Matějová², J. Chrpová² and L. Nedomová¹

¹Agrotest Fyto, Ltd., Havlíčkova 2787/121, 767 01 Kroměříž, Czech Republic; ²Crop Research Institute, Praha 6, Ruzyně, Czech Republic; ivana.polisenska@vukrom.cz

Received: 28 June 2007 / Accepted: 14 February 2008

© 2008 Wageningen Academic Publishers

Abstract

A survey of deoxynivalenol (DON) content in cereals intended for human consumption was carried out in the Czech Republic (CR) over a seven-year period (2000-2006). Wheat, barley and rye samples of harvested cereals were collected directly from farmers where the selection was based on sample origin. In wheat, randomly sampled spikes and risk samples selected on the basis of visually scabby kernels (VSK) were also analysed. An immunochemical method, ELISA, was used for analysis. The maximum limit, according to Commission Regulation (EC) No. 1881/2006, for DON content (1,250 µg/kg) in unprocessed cereals for human nutrition was exceeded in random samples of wheat in 3.5% out of 345 analysed samples, of barley in 1.6% out of 498 analysed samples, and there were no samples of rye (out of 113) exceeding this limit. The analysis of spike samples and the samples that were selected based on VSK occurrence revealed much higher levels of DON content. Highly significant effects of the year, sample origin, and preceding crop on the DON content were found.

Keywords: wheat, barley, rye, deoxynivalenol

1. Introduction

Over recent years, a considerable change in consumers' preferences towards the quality and traceability of agricultural products has been recorded (Opara, 2003). A significant factor that affects food quality with regard to health and safety is the occurrence of mycotoxins and their producers, microscopic fungi, in agricultural commodities. Mycotoxins are characterised by both adverse acute and chronic effects on human and animal organisms that can express themselves in carcinogenic, immunogenic, organ specific, teratogenic and mutagenic forms. Mycotoxins, being secondary fungal metabolites, can contaminate agricultural crops, including cereals, during their growth and maturation in the field, or after harvest during non-conforming storage. Under the climatic conditions of Central Europe, the main mycotoxin producers in cereals in the field are pathogens of the genus *Fusarium*, which cause *Fusarium* head blight (FHB) – one of the most damaging diseases for cereals in regions with mild and humid climatic conditions (Cook, 1981). The most important

toxic products of these pathogens are A trichothecenes (HT-2 and T-2 toxins, diacetoxyscirpenol, neosolaniol), B trichothecenes (DON, nivalenol), zearalenones (ZEA) and fumonisins (Desjardins, 2006).

Under natural conditions, several *Fusarium* mycotoxins usually occur together. This can induce a synergic effect when the negative toxic actions of individual mycotoxins increase (Speijers and Speijers, 2004). Due to the clearly adverse effects of *Fusarium* mycotoxins on both human and animal health, limits for their maximum acceptable amounts in cereals and final products for human consumption or feeds have been adopted in a number of countries over the last few years. Commission Regulation (EC) No. 856/2005 sets the maximum limits for the content of some *Fusarium* mycotoxins in cereals for human nutrition for all countries of the European Union from 1 July 2006 (EC, 2005). These limits are 1,250 µg/kg for DON and 100 µg/kg for ZEA. Though new legislation was passed in 2006 (Commission Regulation (EC) No. 1881/2006, EC, 2006), limits for these two *Fusarium* mycotoxins remained unchanged.

The approved limits are continuously revised taking into account the progress in scientific and technological knowledge concerning these toxins in food (EC, 2006). Data on their occurrence from individual EU member countries is needed.

The main objectives of the present study were to estimate the level of grain contamination by DON in the Czech Republic (CR) and compare it with EU legislation limits; to compare different approaches to cereal sample selection, and to evaluate the effects of known factors on the level of contamination by this *Fusarium* toxin.

2. Material and methods

Cereal samples

Cereal samples grown in the CR during the period 2000-2006 were analysed. Three approaches for sample selection were applied. Approaches A and C were based on collecting about 1,000 wheat samples, 500 barley samples and 100 rye samples from all regions in the CR where these crops are grown. The grain samples were taken by farmers immediately after harvest according to instructions provided for proper sampling. These samples were used for making selection for further analyses. Approach A, which was applied for wheat, barley and rye, was based on the sample origin when a certain number of samples according to the growing frequency in a given region were chosen randomly from each of 13 regions of the CR. For approach C, all of the 1,000 wheat samples a year were evaluated for the number of visually scabby kernels (VSK). VSK evaluation was based on kernel colour (pink, chalky white or pale grey) and shape (thin or shrivelled). The DON content was then determined only in samples containing more than two (in 2003) or five (in 2004, 2005, 2006) VSK in 100 g. For wheat, spike samples were also obtained (25 randomly sampled spikes in a plot) from the 'watch-points' of the State Phytosanitary Administration defined beforehand (approach B). All spikes had been threshed and the grain was ground and prepared for analyses in the same way as grain samples selected using approach A or C.

Mycotoxin analyses

An immunochemical assay ELISA with the limit of detection (LOD) of 54 µg/kg for wheat, 12 µg/kg for barley and with the limit of quantification (LOQ) of 200 µg/kg for cereals in general was employed for DON analyses. The RIDASCREEN®FAST DON kits (R-Biopharm AG, Darmstadt, Germany) were used and manufacturer's guidelines were observed. Absorbances were measured with a spectrophotometer MRX II (Dynex, USA).

Statistical analyses

Distribution of DON data (Kalmogorov-Smirnov test, Shapiro-Wilks test) was evaluated for individual crops and sampling methods. The data on DON occurrence is presented using arithmetic means and medians. For statistical analyses, DON data below the LOQ was replaced by x_i values according to the formula $x_i = (100\% - A) \times \text{LOQ}$, where A = percentage of samples with a DON content below the LOQ for a certain crop, sampling method and year. Results were assessed using the Kruskal-Wallis test to measure the effect of individual factors (year, cultivar, location or preceding crop). Software STATISTICA 7 (StatSoft, CR) was used.

3. Results

Neither normal nor lognormal distribution of DON levels was confirmed for any of five sets of the analysed data. For the samples of wheat, barley and rye selected randomly (approach A) and wheat spike samples (approach B), more than half of the DON levels were below the LOQ in all years. Therefore, in these cases the median of sets equals the calculated x_i value.

Approach A

Wheat

A total of 345 randomly selected wheat samples were analysed for DON content during the five years; the legislation limit was exceeded in 12 samples, which is 3.5% (Table 1). In 2003 and 2004, analyses of randomly selected samples were not carried out. The highest detected level using this method was 4,590 µg/kg. The mean DON content was 203 µg/kg for all years under study.

Barley

In the period from 2000 to 2006, 498 barley samples were analysed for DON content (Table 2). The DON content was higher than the approved limit in eight cases (1.6% of the total number of samples). The maximum DON content was found in 2001 (3,770 µg/kg), which was the only above-limit sample in that year.

Rye

A total of 113 rye samples were analysed for the DON content (Table 3). Investigations of samples were not conducted in 2003 and 2004. No sample exceeding the approved limit was found. The maximum level was 1,100 µg/kg.

Table 1. Results of DON content determination – randomly selected wheat samples.

Year	Number of samples			Number of cultivars	DON content ($\mu\text{g}/\text{kg}$)		Maximum DON content ($\mu\text{g}/\text{kg}$)
	Total analysed	Above 200 $\mu\text{g}/\text{kg}$	Above 1,250 $\mu\text{g}/\text{kg}$		Mean	Median	
2000	56	13	0	21	107	46	740
2001	55	13	1	14	183	48	2,490
2002	95	13	2	19	98	28	1,450
2005	39	8	4	11	379	42	3,790
2006	100	20	5	10	247	40	4,590
Total	345	67	12				

Table 2. Results of DON content determination – randomly selected barley samples.

Year	Number of samples			Number of cultivars	DON content ($\mu\text{g}/\text{kg}$)		Maximum DON content ($\mu\text{g}/\text{kg}$)
	Total analysed	Above 200 $\mu\text{g}/\text{kg}$	Above 1,250 $\mu\text{g}/\text{kg}$		Mean	Median	
2000	33	15	0	7	210	90	550
2001	32	12	1	7	379	96	3,770
2002	30	12	0	5	214	72	1,150
2003	71	5	0	5	314	14	250
2004	73	14	1	6	114	38	2,400
2005	112	39	3	11	238	78	3,310
2006	147	19	3	8	108	26	1,560
Total	498	116	8				

Table 3. Results of DON content determination – randomly selected rye samples.

Year	Number of samples			Number of cultivars	DON content ($\mu\text{g}/\text{kg}$)		Maximum DON content ($\mu\text{g}/\text{kg}$)
	Total analysed	Above 200 $\mu\text{g}/\text{kg}$	Above 1,250 $\mu\text{g}/\text{kg}$		Mean	Median	
2000	22	5	0	6	112	46	460
2001	15	2	0	4	62	26	330
2002	32	1	0	6	40	6	1,100
2005	24	8	0	7	211	66	650
2006	20	1	0	5	52	10	850
Total	113	17	0				

Approach B

A total of 483 wheat spike samples were analysed for DON content over the four years. Of these, the legislation limit was exceeded in 44 samples, which is 9.1% (Table 4). In 2000, 2001 and 2002, analyses of wheat spike samples were not carried out. The highest detected level using this method was 27,500 $\mu\text{g}/\text{kg}$.

Approach C

Three hundred and fourteen wheat samples that had been selected based on the presence of VSK were analysed for DON content. Out of these, 117 samples (37.3%) had a DON content higher than 1,250 $\mu\text{g}/\text{kg}$ (Table 5). The highest detected level using this method was 18,300 $\mu\text{g}/\text{kg}$.

Table 4. Results of DON content determination – wheat spike samples.

Year	Number of samples			Number of cultivars	DON content ($\mu\text{g}/\text{kg}$)		Maximum DON content ($\mu\text{g}/\text{kg}$)
	Total analysed	Above 200 $\mu\text{g}/\text{kg}$	Above 1,250 $\mu\text{g}/\text{kg}$		Mean	Median	
2003	96	23	2	38	276	48	10,100
2004	139	35	14	37	923	50	27,500
2005	144	46	21	41	868	64	19,300
2006	104	21	7	37	261	40	6,800
Total	483	125	44				

Table 5. Results of DON content determination – wheat samples selected based on VSK.

Year	Number of samples			Number of cultivars	DON content ($\mu\text{g}/\text{kg}$)		Maximum DON content ($\mu\text{g}/\text{kg}$)
	Total analysed	Above 200 $\mu\text{g}/\text{kg}$	Above 1,250 $\mu\text{g}/\text{kg}$		Mean	Median	
2003	61	39	16	21	1,100	382	5,090
2004	50	48	35	23	2,771	2,014	18,300
2005	84	61	20	36	970	512	4,880
2006	119	74	46	37	1,161	419	4,437
Total	314	222	117				

Analysis of factor effect

The significance of the year, origin location and cultivar was analysed for all data sets using a non-parametric Kruskal-Wallis test (Table 6). In addition, the effect of the preceding crop was evaluated in wheat samples collected using approach C. Highly significant effects of the year were found in all analysed sets. A highly significant effect of the sample origin was confirmed in wheat (approach B and C). The effect of the cultivar was highly significant in barley only.

The proportion of maize as a preceding crop was 29.3% in all wheat samples (approach C) but it amounted to 54.7% of samples with a DON content above 1,250 $\mu\text{g}/\text{kg}$ (Table 7).

4. Discussion

To protect human health and to comply with relevant legislation, it is necessary to control cereals carefully at the point of their entrance into the food chain. One of the main problems arising from the practical introduction of regulations that limit the content of *Fusarium* mycotoxins is the number of and cost requirements for analyses if carried out across the whole country. Therefore, the development of new rapid, reliable and at the same time cost-effective methods is emphasised. In addition, it is important to gather information on the frequency of the occurrence of samples that do not conform with legislation limits. Since contamination of cereals by *Fusarium* mycotoxins depends

Table 6. Tests of significance (Kruskal-Wallis H test) of the relationship between DON content and the year, region, cultivar, and preceding crop.

	Wheat (A)		Wheat (B)		Wheat (C)		Barley (A)		Rye (A)	
	H	P-value	H	P-value	H	P-value	H	P-value	H	P-value
Year	170.761	0.0000	179.484	0.0000	34.731	0.0000	310.965	0.0000	88.528	0.0000
Region	17.259	0.1877	32.670	0.0019	37.780	0.0002	22.679	0.0457	20.334	0.0872
Cultivar	48.765	0.0484	65.524	0.3228	67.291	0.1890	53.463	0.0000	22.335	0.0339
Prec. crop	N		N		61.760	0.0000	N		N	

N: not evaluated.

Table 7. Comparison of proportions of individual preceding crops for all analysed samples and the samples with a DON content above 1,250 µg/kg, wheat samples selected based on VSK.

Preceding crop	Number and percentage of samples according to individual preceding crops			
	Samples in total		Samples with DON above 1,250 µg/kg	
Cereal	45	14.3%	7	6.0%
Maize	92	29.3%	64	54.7%
Others ¹	131	41.7%	28	23.9%
Unidentified	46	14.6%	18	15.4%
Total	314		117	

¹ The most common preceding crops in this category were oilseed rape (53), clover and lucerne (17), and poppy (16).

heavily on weather conditions in a given growing season (Cook and Christen, 1976; McMullen *et al.*, 1997; Obst *et al.*, 1997), multi-year observations are necessary.

For wheat, barley and rye, approach A, when a certain number of samples, according to the growing frequency in a given region, was chosen randomly from each of 13 regions of the CR. For wheat, DON values above the EU limit were found in 3.5% of all 345 samples obtained in 2000-2002 and 2004-2005. The highest detected level was 4,590 µg/kg, the mean value of all examined years was 203 µg/kg. The frequency of barley samples with the DON content above the EU limit level was lower than in wheat. Out of 466 analysed barley samples, collected in the seven year period from 2000-2006, the limit was exceeded in eight cases only (1.7% of a total number of samples). The maximum DON content in barley (3,770 µg/kg) is also slightly lower than that in wheat. Among the examined 113 rye samples there was none exceeding the limit level; the maximum detected level was 1,100 µg/kg. In a similar survey in Great Britain, Edwards and Ray (2005) found (on an average of 2001-2004) 2.6% of wheat samples with DON values above the EU limit from a total number of 1,298 analysed, a maximum detected level of 20,333 µg/kg and the mean value was 227 µg/kg. Čonková *et al.* (2006), who examined the DON content in wheat samples from Slovakia (60 samples) and Poland (45 samples), found maximum levels of DON of 2,770 and 970 µg/kg, respectively. The comparability of results obtained by individual authors is also influenced, besides various extents of examined sample sets, by the analytical method employed or its characteristics such as the LOD and LOQ, and a treatment way for mycotoxin levels below the LOQ or LOD. For instance, Kononenko and Burkin (2006), in their study of the occurrence of *Fusarium* mycotoxins in Russia, indicate a proportion of

samples with the content above the LOD, and then mean levels of the DON content only for samples above the LOD. This mean ranged from 224 µg/kg to 1,480 µg/kg depending on the individual regions.

The spike sampling method was used in wheat only. In comparison with the other sampling methods, the maximum levels of DON content were found in all years. The proportion of samples with a DON content above both the LOQ and the legislation limit of 1,250 µg/kg was always lower than with the sampling method based on the content of VSK (approach C), but higher than with the random selection (approach A).

Approach C, which was also used for wheat samples only, was based on the relationship between DON and VSK content. Numerous correlations between VSK and DON content in wheat have been reported (e.g. Trenholm *et al.*, 1981; Visconti *et al.*, 1986; Teich *et al.*, 1987; Perkowski *et al.*, 1991; Wong *et al.*, 1995; Ittu *et al.*, 2000; Dexter and Nowicki, 2003). Schaafsma *et al.* (2004) indicate that VSK and DON levels are positively correlated, but correlations can vary. One of the factors that can affect the VSK/DON relationship is that some samples may contain VSK but are negative for DON because the *Fusarium* spp. present on the kernel is not a DON producer (Schaafsma *et al.*, 2004). Some visual symptoms can also be induced or affected by factors other than *Fusarium* infection, e.g. by stress due to unfavourable environmental conditions. In our survey, however, such a sampling method based on VSK content proved very useful in identifying DON positive wheat samples. Out of the analysed 314 samples selected in this manner, 117 samples (37.3%) showed the DON content above the EU limit. The highest detected level was 18,300 µg/kg.

The sampling approach based on VSK content as a risk factor revealed that cereals with a high DON content occur in our country, but analyses of randomly selected samples (approach A) suggest that their frequency is rather low. The method of random sampling can provide an exact picture of DON presence if a sufficient number of samples are analysed. At lower numbers of analysed samples, the probability of catching high levels decreases. However, extending the tested set increases labour intensity and costs. If all required samples cannot be tested, sampling based on a known risk factor can detect samples with a high DON content and eliminate their entrance into the food chain. However, it does not objectively reflect the overall situation. Analyses of mycotoxins from collected spikes (approach B) are needed in practice particularly before the harvest in order to determine a level of contamination and/or to make decision about grain use. With spike sampling, it is difficult to obtain a sample that would characterise the entire stand. The combine harvest itself is part of the procedure of sample homogenisation. As indicated

by Whitaker (2003), usually only a small proportion of all kernels are contaminated by mycotoxins; however, these have extremely high levels of mycotoxin content. Therefore, it is necessary to stick to the given guidelines regarding both the number of collected spikes and the sampling method, so that their proportion in the sample corresponds to the proportion in the stand. Each of the sampling methods employed can be justified and can be appropriate for a defined purpose. However, due to the considerable effects on resultant findings, the sampling method should always be indicated and results should be interpreted in the context of the chosen method.

In addition to the weather, there are many factors influencing *Fusarium* mycotoxin content in cereals, particularly crop rotation, tillage, choice of cultivar, and application of fungicides (Beyer *et al.*, 2006). Schaafsma *et al.* (2005) indicate that in wheat environmental effects accounted for 48% of the variation in DON content, followed by cultivar (27%) and preceding crop (14 to 28%). A highly significant effect was confirmed for the year factor in all examined data sets. The sample origin (region) was highly significant only for wheat that had been collected using approach B and C. In our survey, the preceding crop was known only in wheat samples selected on the basis of VSK content (approach C) and its highly significant effect was statistically confirmed. The proportion of samples of maize as a preceding crop was 29.3% in set C within all samples, but this proportion was 54.7% in a subgroup of samples with DON content above the limit value of 1,250 µg/kg. However, the actual percentage of maize followed by wheat in the CR is lower, on an average of all regions about 10-12% (F. Tichý, personal communication 2007). Its higher proportion in the examined set was probably caused by the previous selection being based on VSK presence. Recent studies on *Fusarium* head blight of wheat confirm that maize residue is a much more important source of *F. graminearum* inoculum than wheat residue (Schaafsma *et al.*, 2001). Maize is now regarded as the most dangerous preceding crop in the context of FHB epidemics and DON contamination. The increased occurrence of *Fusarium* spp. worldwide is also ascribed to wider use of reduced soil tillage practices, which prolong residue retention on the soil surface (e.g. Sturz and Johnston, 1985; Miller *et al.*, 1998; Dill-Macky and Jones, 2000). The fact that soil tillage practices were not considered in our study since there was no data available, may be one of the possible reasons as to why the cultivar did not exhibit significant effect. Beyer *et al.* (2006) states that comparisons of genotypes grown at different sites, in different years, after different preceding crops or in different tillage systems would bias a fair assessment of genotype susceptibility. Also, a great number as well as various wheat cultivars in various years in our sets of the selected samples of wheat may play a role. In barley, where a number of cultivars were much lower, the highly significant effect of the cultivar was confirmed.

Acknowledgements

The results were obtained within research projects No. QG60047 and QG50041 supported by the National Agency for Agricultural Research, project No. 0002700602 of the Ministry of Agriculture of the Czech Republic, and research project No. MSM 2532885901 supported by the Ministry of Education, Youth and Sports.

References

- Beyer, M., Klix, M.B., Klink, H. and Verret, J.A., 2006. Quantifying the effects of previous crop, tillage, cultivar and triazole fungicides on the deoxynivalenol content of wheat grain – a review. *Journal of Plant Diseases and Protection* 113: 241-246.
- Cook, R.J., 1981. *Fusarium* diseases of cereals in Western Europe. In: Nelson, P.E., Toussoun, T.A. and Cook, R.J. (eds.). *Fusarium: Diseases, biology and taxonomy*. The Pennsylvania State University Press, University Park and London, pp. 56-63.
- Cook, R.J. and Christen, A.A., 1976. Growth of cereal root rot fungi as affected by temperature-water potential interactions. *Phytopathology* 66: 193-197.
- Čonková, E., Laciaková, A., Štyriak, I., Czerwiecki, L. and Wilczyńska, G., 2006. Fungal contamination and the levels of mycotoxins (DON and OTA) in cereal samples from Poland and east Slovakia. *Czech Journal of Food Sciences* 24: 33-40.
- Desjardins, A.E., 2006. *Fusarium* mycotoxins. The American Phytopathological Society, St. Paul, Minnesota, U.S.A., 260 pp.
- Dexter, J.E. and Nowicki, T.W., 2003. Safety assurance and quality assurance issues associated with *Fusarium* head blight in wheat. In: Leonard, K.J. and Bushnell, W.R. (eds.). *Fusarium head blight of wheat and barley*. The American Phytopathological Society, St. Paul, Minn., pp. 420-460.
- Dill-Macky, R. and Jones, R.K., 2000. The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. *Plant Disease* 84: 71-76.
- Edwards, S.G. and Ray, R., 2005. *Fusarium* mycotoxins in UK wheat production. In: Congress Proceedings, The BCPC International Congress Crop Science and Technology 2005, 31 Oct-2 Nov 2005, Glasgow, Scotland, UK, pp. 437-440.
- European Commission (EC), 2005. Commission Regulation (EC) No 856/2005 of 6 June 2005 amending Regulation (EC) No 466/2001 as regards *Fusarium* toxins. Official Journal of the European Union, L 143/1.
- European Commission (EC), 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 amending Regulation (EC) setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L 364/5.
- Iltu, M., Grabarkiewicz-Szczeszna, J., Kostecki, M. and Golinski, P., 2000. Deoxynivalenol accumulation and other scab symptoms in six Romanian wheat genotypes inoculated with *Fusarium graminearum*. *Mycotoxin Research* 16: 15-22.
- Kononenko, G. and Burkin, A., 2006. Fusariotoxins: the large-scale research of cereal grain contamination in Russia. In: Book of Abstracts, European *Fusarium* Seminar, EFS9, 19-22 September 2006, Wageningen, The Netherlands, 2006, p. 33.

- McMullen, M., Jones, R. and Gallenberg, D., 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease* 81: 1340-1348.
- Miller, J.D., Culley, J., Fraser, K., Hubbard, S. and Meloche, F., 1998. Effect of tillage practice on *Fusarium* head blight of wheat. *Canadian Journal of Plant Pathology* 20: 95-103.
- Obst, A., Lepschy-Von Gleissenthal, J. and Beck, R., 1997. On the etiology of *Fusarium* head blight of wheat in south Germany – preceding crops, weather conditions for inoculum production and head infection, proneness of the crop to infection and mycotoxin production. *Proceedings of the Fifth European Fusarium Seminar. Cereal Research Communications* 25: 699-703.
- Opara, L.U., 2003. Traceability in agriculture and food supply chain: a review of basic concepts, technological implications, and future prospects. *Food, Agriculture & Environment* 1: 101-106.
- Perkowski, J., Chelkowski, J., Blazczak, P., Snijders, C.H.A. and Wakulinski, W., 1991. A study of the correlation between the amount of deoxynivalenol in grain of wheat and triticale and percentage of *Fusarium* damaged kernels. *Mycotoxin Research* 7A Part II: 102-114.
- Schaafsma, A.W., Tamburic-Ilincic, L., Miller, J.D. and Hooker, D.C., 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. *Canadian Journal of Plant Pathology* 23: 279-285.
- Schaafsma, A.W., Savard, M.E., Clear, R. and Dexter, J., 2004. Methods and issues regarding detection of deoxynivalenol, *Fusarium*-damaged kernels, and *Fusarium* spp. in commercial grain in Canada. *Canadian Journal of Plant Pathology* 26: 443-452.
- Schaafsma, A.W., Hooker, D.C. and Miller, J.D., 2005. Progress and limitations with respect to pre-harvest forecasting of *Fusarium* toxins in grains. *Phytopathology* 95: S123.
- Speijers, G.J.A. and Speijers, M.H.M., 2004. Combined toxic effects of mycotoxins. *Toxicology Letters* 153: 91-98.
- Sturz, A.V. and Johnston, H.W., 1985. Characterization of *Fusarium* colonization of spring barley and wheat produced on stubble of fallow soil. *Canadian Journal of Plant Pathology* 7: 270-276.
- Teich, A.H., Shugar, L. and Smid, A., 1987. Soft white winter wheat cultivar field-resistance to scab and deoxynivalenol accumulation. *Cereal Research Communications* 15: 109-114.
- Trenholm, H.L., Cochrane, W.P., Cohen, H., Elliot, J.I., Farnworth, E.R., Friend, D.W., Hamilton, R.M.G., Neish, G.A. and Standish, J.F., 1981. Survey of vomitoxin contamination of the 1980 white winter wheat crop in Ontario, Canada. *Journal of the American Oil Chemists Society* 58: A992-A994.
- Visconti, A., Chelkowski, J. and Bottalico, A., 1986. Deoxynivalenol and 3-acetoxydeoxynivalenol-Mycotoxins associated with wheat head fusariosis in Poland. *Mycotoxin Research* 2: 59-64.
- Whitaker, T.B., 2003. Standardisation of mycotoxin sampling procedures: an urgent necessity. *Food Control* 14: 233-237.
- Wong, L.S.L., Abramson, D., Tekauz, A., Leisle, D. and McKenzie, R.I.H., 1995. Pathogenicity and mycotoxin production of *Fusarium* species causing head blight in wheat cultivars varying in resistance. *Canadian Journal of Plant Science* 75: 261-267.