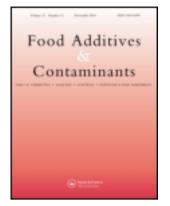
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H.J. Van Der Fels-Klerx^a, S. Klemsdal^b, V. Hietaniemi^c, M. Lindblad^d, E. Ioannou-Kakouri^e & E.D. Van Asselt^a

^a RIKILT - Institute of Food Safety, Wageningen University and Research Centre, PO Box 230, NL-6700 AE Wageningen, The Netherlands

^b Norwegian Institute for Agricultural and Environmental Research, Hogskoleveien 7, N-1432 Ås, Norway

^c MTT Agrifood Research Finland, Services Unit, FI-31600 Jokioinen, Finland

^d National Food Agency, PO Box 622, SE-751 26 Uppsala, Sweden

^e State General Laboratory of Cyprus, 44 Kimonos Street, 1451 Nicosia, Cyprus

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Mycotoxin contamination of cereal grain commodities in relation to climate in North West Europe

H.J. Van Der Fels-Klerx^{a*}, S. Klemsdal^b, V. Hietaniemi^c, M. Lindblad^d, E. Ioannou-Kakouri^e and E.D. Van Asselt^a

^aRIKILT – Institute of Food Safety, Wageningen University and Research Centre, PO Box 230, NL-6700 AE Wageningen, The Netherlands; ^bNorwegian Institute for Agricultural and Environmental Research, Hogskoleveien 7, N-1432 Ås, Norway; ^cMTT Agrifood Research Finland, Services Unit, FI-31600 Jokioinen, Finland; ^dNational Food Agency, PO Box 622, SE-751 26 Uppsala, Sweden; ^eState General Laboratory of Cyprus, 44 Kimonos Street, 1451 Nicosia, Cyprus

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This study aimed to investigate mycotoxin contamination of cereal grain commodities for feed and food production in North Western Europe during the last two decades, including trends over time and co-occurrence between toxins, and to assess possible effects of climate on the presence of mycotoxins. For these aims, analytical results related to mycotoxin contamination of cereal grain commodities, collected in the course of national monitoring programmes in Finland, Sweden, Norway and the Netherlands during a 20-year period, were gathered. Historical observational weather data, including daily relative humidity, rainfall and temperature, were obtained from each of these four countries. In total 6382 records, referring to individual sample results for mycotoxin concentrations (one or more toxins) in cereal grains were available. Most records referred to wheat, barley, maize and oats. The most frequently analysed mycotoxins were deoxynivalenol, 3-acetyl-deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin and zearalenone. Deoxynivalenol had the highest overall incidence of 46%, and was mainly found in wheat, maize and oats. Mycotoxins that showed co-occurrence were: deoxynivalenol and 3-acetyl-deoxynivalenol in oats; deoxynivalenol and zearalenone in maize and wheat; and T-2 toxin and HT-2 toxin in oats. The presence of both deoxynivalenol and zearalenone in wheat increased with higher temperatures, relative humidity and rainfall during cultivation, but the presence of nivalenol was negatively associated with most of these climatic factors. The same holds for both nivalenol and deoxynivalenol in oats. This implies that climatic conditions that are conducive for one toxin may have a decreasing effect on the other. The presence of HT-2 toxin in oats showed a slight decreasing trends over time, but significant trends for other toxins showed an increasing presence during the last two decades. It is therefore useful to continue monitoring of mycotoxins. Obtained results can be used for development of predictive models for presence of mycotoxins in cereal grains.

Keywords: statistical analysis; mycotoxins; mycotoxins - Fusarium; mycotoxins - trichothecenes; cereals; cereals and grain

Introduction

Mycotoxins are secondary metabolites produced by a wide variety of fungal species in a range of crops and fruits, particularly during cultivation and/or storage stages. They are chemically stable and – to a large extent – resistant against most of the feed and food processing steps (Kabak 2009), resulting in contamination of the final feed and food products. Consumption of contaminated cereal-derived feed and food products can cause harmful effects to humans and animals (International Agency for Research on Cancer (IARC) 1993). Trichothecenes, including deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2), and toxins like

zearalenone (ZEA) and fumonisins (FB) are major mycotoxins in worldwide cereal grains, in particular wheat, barley, oats, rice and maize (IARC 1993; Wu 2007). These toxins are produced by *Fusarium* spp., which are commonly found in the temperate areas of Europe, America and Asia (Creppy 2002). Trichothecenes can be divided into two groups, type A and type B. In general, toxins of the type B group, including DON and NIV, are the most common trichothecenes, but toxins of type A, such as HT-2 and T-2, are considered more toxic to animal and human health than type B trichothecenes (Joint FAO/ WHO Expert Committee on Food Additives (JECFA) 2001). Furthermore, fumonisins – a group of

^{*}Corresponding author. Email: ine.vanderfels@wur.nl

structurally related metabolites produced primarily by the fungi *Fusarium moniliforme*, *F. verticilloides* and *F. proliferatum* – have been found as worldwide contaminants of maize, particularly, fumonisin B_1 (FB1) fumonisin B_2 (FB2) and fumonisin B_3 (FB3) (Munkvold and Desjardins 1997; Curtui et al. 2004).

Other relevant mycotoxins of cereal grains include aflatoxins (AF) and ochratoxin A (OTA). Both toxins can be produced by certain species of Aspergillus spp. In Europe, AF are mainly produced by A. flavus which may form aflatoxin B_1 (AFB1) and aflatoxin B_2 (AFB2), and A. parasiticus which may form aflatoxin G_1 (AFG1) and aflatoxin G_2 (AFG2), in addition to the former two toxins. AFB1 is the most potent toxin out of these four. In Europe, AF are often found in cereals and cereal-derived products from Mediterranean countries due to the sub-tropical climate which favours the growth of A. flavus and A. parasiticus (Gursoy and Bicici 2004). OTA can be produced by several Aspergillus species, the most notable ones being A. ochraceus, A. alliaceus, A. albertensis, A. carbonarius and A. niger (Varga et al. 1996). These species produce OTA in the warmer and tropical parts of the world, but occurrence in cereal grains is relatively low (Pittet 1998; JECFA 2001). OTA contamination of cereals is mainly due to *P. verrucosum*; this species can occasionally lead to OTA contamination in cereals grown in Northern European countries (Lund and Frisvad 2003).

Within the European Union, regulatory limits have been set by the European Commission such to protect human from exposure to mycotoxins through cereal grain consumption (European Commission 2006a, 2007). These Commission regulations lay down maximum levels for the presence of DON, ZEA, FB, OTA and AF in unprocessed cereals as well as in cerealderived products and cereals intended for direct human consumption, with particular limits for baby food. In addition, European Commission regulation states that the presence of T-2 and HT-2 may also be of public health concern, and maximum limits should be defined; they are currently under discussion. Good agricultural practices are needed to limit the presence of fungi and mycotoxins in cereal grains during cultivation, followed by good manufacturing practices, particularly related to storage, during the further cereal production chain (Edwards 2004; van der Fels-Klerx and Booij 2010). Infection of cereal grains in the field with fungi and subsequent mycotoxin production is known to be affected by agronomic and climatic conditions. The various fungal species mentioned have their own optimal temperature and moisture conditions for infection of the cereals, defining their spread and occurrence over regions and years, and subsequent mycotoxin production (Magan et al. 2011). For instance, F. graminearum has been found to dominate in regions with warm and humid conditions, whereas F. avenaceum and F. culmorum are both associated with cool, wet and humid conditions (Bottalico and Perrone 2002). *A. flavus* and *A. parasiticus* are more prevalent in warmer parts of the world; they infect the cereals and produce AF especially during periods of droughts. Given the influence of local weather, climate change effects are expected to affect fungal distribution and mycotoxin occurrence, as cited in recent reviews (Miraglia et al. 2009; Magan et al. 2011; Paterson and Lima 2010, 2011). However, to make underpinned estimations on expected impacts of climate change effects on mycotoxin contamination, more detailed insights into the relationships between weather conditions and mycotoxin contamination are needed.

The aim of the current study was to investigate mycotoxin contamination of cereal grain commodities for feed and food products in North Western Europe during the last two decades, including co-occurrence and trends, and to assess the effects of climatic conditions on mycotoxin contamination. The underlying aim was to gather information on the influence of climate on mycotoxin presence.

Material and methods

Data collection

For the aims of the current study, analytical results related to mycotoxin concentrations in cereal grain commodities for feed and food production collected in the course of national monitoring programmes in Finland, Sweden, Norway and the Netherlands were gathered as well as historical weather data from these four countries. To ensure consistent data collection, a standardised Excel database format was prepared, including a description of the overall aims of the study, the parameters to be collected, and definitions of each of these parameters. Mycotoxin monitoring results and weather data were stored into two different Excel sheets. The table with mycotoxin data included separate records for analytical results per sample. The table with weather data consisted of records per year and region/country.

Mycotoxin data

Results from monitoring programmes on mycotoxins in feed and food commodities were collected in cooperation with the competent organisations in each of the four study countries. In the course of the Finnish monitoring programme representative oats, wheat, barley and rye samples were collected from farmer's silos after harvest during 1999–2009 from the two main cultivation areas in Finland. Swedish mycotoxin data were available from national monitoring programmes performed from 1999 to 2008 by the National Food Agency and by Lantmännen, a farmers' owned company, in three different cultivation regions in the country (central, southwest and southern Sweden). Mycotoxin data from Norway included results of different national food and feed monitoring programmes performed by the Norwegian Food Safety Authority during 1990–2009, except for the year 1999. Most grain samples originated from the central or southeast regions of Norway. In the Netherlands, mycotoxin data were collected in the course of two national monitoring programmes for contaminants in feed and food and their commodities, including the programme from the Dutch Product Board of Feed and from the Dutch Food and Consumer Product Safety Authority, starting in 1989. Most of the samples were analysed at the competent national laboratories.

Weather data

Observational weather data were obtained from national meteorological institutes, considering stations that were located in regions from where the mycotoxin samples originated. Data included daily temperature, rainfall and relative humidity during the period April-September of the years for which mycotoxin data from the particular region/country were available. In the Netherlands, weather data were obtained from the central meteorological station (located in the Bilt) of the Royal Netherlands Meteorological Institute as origin of the mycotoxin samples was not recorded (http://www.knmi.nl). Finnish weather data were obtained from the Finnish meteorological institute (http://en.ilmatieteenlaitos.fi/observations-in-finland). Weather data for the two regions of interest were calculated by combining individual weather stations in these regions into regional ELY-Centres (http:// www.ely-keskus.fi/en/frontpage/Sivut/default.aspx). Swedish weather data were obtained from meteorological stations in central, southwest and southern Sweden: Tullinge, Rångedala and Malmö. Norwegian

Sweden: Tullinge, Rångedala and Malmö. Norwegian weather data were obtained from Bioforsk's meteorological stations (http://www.vips-landbruk.no/ weather/we710s.jsp). Data were used from the three stations that are located in the main cereal cultivation area, including: Ås and Rygge in the south eastern part and station Kvithamar in the central part of the country.

Data processing

All collected data were stored in the database and checked for consistency. Since the type of wheat, i.e. spring wheat or winter wheat, was not recorded consistently, these two types were combined into one group, referred to as "wheat." Likewise, mycotoxin data for barley groats (20 records from Norway) were combined with those of barley. In case the limit of detection (LOD) of a particular mycotoxin varied between years and/or countries, the LOD was set to a fixed value. For this purpose, the highest LOD that was reported most frequently was selected. The resulting cut-off LOD per mycotoxin were: $30 \,\mu g \,kg^{-1}$ for 3-acetyl-deoxynivalenol (3-AcDON) and HT-2; $50 \,\mu g \,kg^{-1}$ for T-2, OTA, ZEA and NIV; and $100 \,\mu g \,kg^{-1}$ for DON. In case the LOD of a particular toxin was higher than the particular cut-off LOD, the result for the particular toxin was not considered in the analyses. Samples that contained a particular mycotoxin in concentrations above the cut-off LOD were considered 'positive' for the particular toxin. Results of samples that were recorded not to contain the particular mycotoxin or – in any case – below its LOD were set to zero.

Using the observed daily weather data, the following four climatic parameters were calculated on a monthly basis: average temperature, total rainfall, number of days with rainfall of 3 mm or higher (rainy days) and average relative humidity. These four parameters were calculated for April–September of the year on a regional basis, with three, three, two and one region for, respectively, Norway, Sweden, Finland and the Netherlands.

Data analyses

Statistical analyses were performed to obtain descriptive results for mycotoxin levels in the different cereal grain commodities and countries. For each mycotoxin, the distribution of the reported concentration was skewed and, therefore, data were log-transformed prior to further statistical analyses.

Data of each country comprised multiple mycotoxins, but not all cereal samples were analysed for all toxins. For each cereal type, correlations between concentrations of two individual mycotoxins were examined using all sample results, provided that at least one sample was positive for one of the two toxins. Pearson correlation coefficients were also calculated considering the positive samples (for one or both of the toxins) only. Correlation values of 0.5 or higher were considered relevant and further examined by plotting the concentrations of the two mycotoxins (log scale) in the particular cereal grain.

Trends in time were evaluated for those combinations of cereal types and toxins that had at least 20 observations. For each cereal type, individual sample results (log scale) were plotted on a yearly basis. Also, logistic regression analyses were performed for each cereal type-mycotoxin combination, using the percentage of positive samples per year as the response variable and year as the explaining variable. Trends of mycotoxin contamination over the study period were evaluated using the outcomes of both these analyses.

Possible relationships between monthly climatic parameters and individual mycotoxins in particular cereal grains were evaluated using the percentage of samples of the grain that contained the particular toxin. These percentages of positive samples were calculated per grain type, per year and per country in the case of at least 100 observations available. Monthly climatic parameters were linked to the toxin data based on year and country. Logistic regression analyses were performed to estimate the individual effects of the monthly climatic parameters on the per cent of positive samples, and the corresponding *p*-value was calculated. This was done both with and without considering separate regions for Sweden, Norway and Finland. In all analyses, p < 0.05 were considered as being statistically significant.

Results

In total 6382 records were gathered, referring to individual mycotoxin samples (one or more toxins) in cereal grains. Of this total, 807 records originated from Finland covering the period from 2000 to 2009, 1875 samples were from the Netherlands from 1989 to 2008, 2292 records were from Norway from 1990 to 2009 (except 1999), and 1408 records were from Sweden from the years 1999, 2001 and from 2004 to 2009. The majority of the samples were collected from the following cereal grain commodities: wheat, barley, maize, oats and rye. The majority of these five commodities were destined to be used for food production (55%), followed by feed production (42%), and other uses, such as energy (3%). Other cereal grain types, such as millet, sorghum and triticale, were less frequently tested for the presence of mycotoxins, and thus not included in further statistical analyses.

In total 17 different mycotoxins were analysed, but the number of different mycotoxins that were analysed per sample varied. DON was the most frequently analysed toxin, with 4899 records in total. This toxin was followed - ordered by number of available records - by NIV, T-2, HT-2, ZEA, 3-AcDON, OTA, diacetoxyscirpenol (DAS) and moniliformin (MON) (149 records). The numbers of samples that were analysed for the presence of AFB1, AFB2, AFG1 and AFG2, and FB1, FB2, FB3, and sterigmatocystine were very low, and levels of these mycotoxins were found to be below their registered LOD $(100 \,\mu g \, kg^{-1})$ for each of the fumonisins, and $5 \,\mu g \, k g^{-1}$ for each of the aflatoxins and sterigmatocystine) in all samples. Also, none of the 826 samples that were analysed for the presence of DAS contained levels above the registered LOD (of $25 \,\mu g \, kg^{-1}$) and none of the 1391 samples that were analysed for the presence of OTA contained this toxin (above cut-off LOD of $50 \,\mu g \, kg^{-1}$). Out of the 149 Norwegian records for MON, 28 samples of wheat, barley and oats were found to contain this toxin, with levels varying from 50 to $421 \,\mu g \, kg^{-1}$. Table 1 shows the summarised results for the presence of the remaining six mycotoxins in the cereal grain commodities. The highest overall incidence (for all cereals) was found for DON, as this toxin was reported to be present (>LOD) in 45.5% of the samples. This toxin was followed – in order from highest to lowest incidence - by HT-2 (26.7%), 3-AcDON (12.9%), T-2 (11.9%), ZEA (8.3%) and NIV (6.5%). Distributions of the levels of each of the six mycotoxins in the positive samples were truncated at the left and skewed to the right (Table 1). Considering all samples, median values of each of the six mycotoxins were zero as the incidences detected (above cut-off LOD) were all less than 50%. Considering only those samples with toxin levels

Table 1. Presence of several mycotoxins in samples (total of 6382) from cereal grain commodities cultivated in the Netherlands, Norway, Sweden and Finland in the period 1999–2009.

				All sample	s			Sample	es with a tox	in presence
Mycotoxin	N below the LOD ^a	N above the LOD ^b	N missing ^c	Incidence (%)	Mean level	75th percentile level	Maximum level	Mean level	Median level	75th percentile level
3-Acetyl-deoxynivalenol	1342	199	4841	12.9	22.0	0.0	1500	170	83.0	172
Deoxynivalenol	2671	2228	1483	45.5	257	272	10,000	564	310	660
HT-2 toxin	1324	482	4576	26.7	49.9	35.0	2400	187	105	210
T-2 toxin	1610	218	4554	11.9	18.7	0.0	1100	157	111	195
Zearalenone	1559	142	4681	8.3	25.0	0.0	1690	299	173	381
Nivalenol	2410	170	3802	6.5	8.0	0.0	644	121	93.0	150

Notes: ^aN represents the number of samples; The level of detection (LOD) was preset per mycotoxin at $30 \,\mu g \, kg^{-1}$ for 3-acetyl-DON and HT-2 toxin; at $50 \,\mu g \, kg^{-1}$ for T-2 toxin, zearalenone and nivalenol; and at $100 \,\mu g \, kg^{-1}$ for deoxynivalenol. Sample results below the LOD were set to zero for the particular toxin.

^bSamples that contained the toxin in levels above the preset LOD.

^cSamples not analysed for the particular mycotoxin.

above the LOD (positive samples), DON levels averaged 564 μ g kg⁻¹, with a median of 310 μ g kg⁻¹ and a maximum of $10\,000\,\mu g\,kg^{-1}$. This toxin was most frequently analysed and reported to be present in wheat and oats from all four study countries (Table 2). DON was reported to be present (above the LOD) in only three out of 139 rye samples (2%) originating from Sweden and Norway with a maximum level of $162 \,\mu g \, kg^{-1}$. Nearly all 147 maize samples that were analysed for DON originated from the Netherlands, and 83% of these sample were reported to contain this toxin (Table 2). 3-AcDON was determined in 1541 samples; besides 23 Norwegian barley samples, all samples originated from oats and wheat grown in Finland and Norway. Overall incidence was 12.9% (Table 1), with highest levels in Finnish oats.

In cereal grain samples that were positive for HT-2 (482 samples) and T-2 (218 samples), median concentrations were 105 and $111 \,\mu g \, kg^{-1}$, respectively (Table 1). These two toxins were analysed mainly in wheat and oats originating from Norway and Finland as well as barley from Norway, and reported to be present most often in oats (Table 2). Maximum concentrations in oats were $1576\,\mu g\,kg^{-1}$ for HT-2 and $770 \,\mu g \, kg^{-1}$ for T-2 in Norway, and $2400 \,\mu g \, kg^{-1}$ for HT-2 and $1100 \,\mu g \, kg^{-1}$ for T-2 in Finland. About 13% and 2% of the Norwegian barley samples (over 250 samples) contained HT-2 and T-2, respectively, with a maximum HT-2 level in all these barley samples of $462 \,\mu g \, kg^{-1}$. The majority of wheat samples (>99%) did not contain HT-2 and T-2. ZEA was analysed most frequently in wheat and oats, originating from all four study countries, as well as in maize from the Netherlands (Table 2). The highest percentage of positive samples (containing ZEA above its cut-off LOD) was found in maize from the latter country (37%), with a maximum ZEA concentrations in Dutch maize of $1000 \,\mu g \, kg^{-1}$. NIV was analysed most frequently in wheat and oats in nearly each of the four countries, as well as in barley from Norway. The highest percentage of positive NIV samples referred to oats; this percentage was 23% for Finland and 10% for Norway. Maximum concentrations of this toxin were 644 and $337 \,\mu g \, kg^{-1}$ in these two countries, respectively.

Correlations between toxins

The levels of 3-AcDON showed to be highly correlated to levels of DON in oats (r = 0.86 with all samples, and r = 0.89 with positive samples). In both barley and rye the number of samples that contained both mycotoxins (>LOD) were too low for such an evaluation. DON levels were correlated with ZEA levels in maize (r = 0.69 with all samples, and r = 0.80 with positive samples). In wheat, DON levels tended to be positively

correlated with levels of ZEA. In each of the four cereal grains. DON levels were either not significantly correlated to HT-2, T-2 and NIV, or could not be evaluated due to low numbers of samples. However, NIV and DON in wheat seemed to be negatively correlated to each other (r = -0.70 but based on only six positive samples, and r = 0.04 with all samples). Correlations between levels of two out of the other five mycotoxins, besides DON, could only be evaluated in oats, as in the other cereal grains the number of samples that contained the two mycotoxins were too low. Results showed that levels of HT-2 and T-2 in oats were positively related to each other (r = 0.58 for all samples, and r = 0.59 with positive samples). Based on over 300 oats samples which contained both toxins, the HT-2 concentration was on average 2.7 times higher than the T-2 concentration. For the remaining cereal types, the number of samples that were positive for HT-2 and/or T-2 were too low to evaluate such a relationship. Levels of each of these two toxins were not related to 3-AcDON, ZEA or NIV. Also, levels of the latter three toxins, based on a two-by-two evaluation, were not related to each other.

Mycotoxins trends over time

Results of logistic regression analyses showed that the percentage of 3-AcDON positive samples of oats significantly increased over time during the period 1991–2009 (p = 0.00) (Figure 1a). Also, the percentage of samples with DON in barley and oats (Figure 1b) showed a significant increase during this time period (p < 0.05), but for DON in wheat (Figure 1c) no significant trend in time could be observed (p = 0.50). The percentage of oat samples that was positive for HT-2 showed a significant – but slight – decrease over time (1996–2009) (p < 0.05). Although not significant (p=0.11), HT-2 in barley also showed a decreasing trend during the last decennium. For T-2 in barley, oats and wheat, no significant trend over time could be observed. The percentage of samples that contained ZEA significantly increased during the last 10 years in maize (Figure 1d) and wheat (p < 0.05), but in oats no significant trend could be observed. The percentage of oat samples with NIV showed an increasing trend during 1990-2009.

Relationships between climate and mycotoxins

Possible relationships between climatic parameters and mycotoxin presence could be examined for 3-AcDON, DON, T-2, HT-2, ZEA and NIV in one or more of the commodities of oats, wheat, barley and maize. Relationships between climatic parameters and presence of 3-AcDON could be examined in oats and wheat. Higher rainfall during July (both total rainfall

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Table 2. Detailed results for the presence of mycotoxins in samples collected from different types of cereal grain commodities in Finland, Sweden, Norway and the Netherlands during the period 1999–2009.

							Mycoto	Mycotoxin in cereal grain per country ^a	eal grai	n per co	untry ^a							
		Deoxynivalenol	ivalenol		H	HT-2 toxin	u	·T	T-2 toxin			Zearalenone	none			Nivalenol	enol	
Sample results ^b	FI	NL	NO	SW	FI	NL	NO	FI	NL	NO	FI	NL	NO	SW	FI	NL	NO	SW
Wheat																		
Number of samples	338	940	832	554	338	134	235	338	159	235	285	312	258	51	338	134	832	75
Incidence $(\%)^{c}$	29.9	71.4	29.4		0.6	0	0.9	0	0	0.4	0.7	8.7	0.4	0	0.9	0	1.3	0
Median level ^c	0	220	0		Ι	Ι	Ι	Ι	Ι	Ι	I	0	Ι	Ι	Ι	Ι	Ι	Ι
75th percentile ^c	120	480	120	0	I	I	I	I	I	I	I	0	I	I	I	I	I	I
Maximum ^c	5865	10000	1552	890	Ι	Ι	Ι	Ι	I	I	Ι	310	Ι	Ι	Ι	I	Ι	I
Oats																		
Number of samples	469	9	399	469	469	0	347	469	0	328	336	9	125	20	469	0	399	10
Incidence $(\%)^{c}$	64.8	I	46.9	73.8	49.9		59.7	23.9		30.2	12.2	Ι	7.2	0	23.5		10	0
Median level ^c	184.0	I	0	290.0	0		49.0	0		0	0	Ι	0	Ι	0		0	I
75th percentile ^c	508	ļ	375.2	632.5	123.5		124.0	0		58.0	0	I	0	ļ	0		0	I
Maximum ^c	8800	I	9076	1200	2400		1576	1100		770	1690	I	855	I	644		337	
Barley																		
Number of samples	0	126	291	189	0	0	271	0	27	259	0	89	53	0	0	0	291	0
Incidence $(\%)^{c}$		35.7	15.1	24.3			13.3		0	2.3		6.7	1.9				2.1	
Median level ^c		0	0	0			0		Ι	Ι		0	Ι				Ι	
75th percentile		150.0	0	0			0		I	I		0	I				I	
Maximum ^c		3184	1206	1100			462.0		I	I		0	I				I	
Maize																		
Number of samples	0	142	0	5	0	0	0	0	0	0	0	147	0	0	0	0	0	0
Incidence $(\%)^{c}$		84.5		40.0								37.4						
Median level ^c		500		0								0						
75th percentile ^c		988.0		187.5								100						
Maximum ^c		5000		420.0								1000						
Notes: ^a Mycotoxin is given per country: FI Finland: NI the	s oiven nei	r conntrv.	FI Finlan	d NI the		Inde: N	SWION C	Netherlands: NO Norway: and SF Sweden	F Swed	en								
^b Results are presented for all samples, considering the followin	e for all s	samples, co	insidering	the follow	ing prese	t level c	of detectic	on per my	vcotoxir	т: 30 µg k	ng preset level of detection per mycotoxin: 30 µg kg ⁻¹ for HT-2 toxin; 50 µg kg ⁻¹ for T-2 toxin, zearalenone and	IT-2 toxi	n: 50 µg l	kg ⁻¹ fo:	r T-2 to	xin, zea	ralenone	and
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nivalenol; and 100 μg kg⁻¹ for deoxynivalenol. ^cIncidence, median level, 75th percentile level and maximum level are given for the distribution of concentrations for the particular mycotoxin, considering the preset detection level.

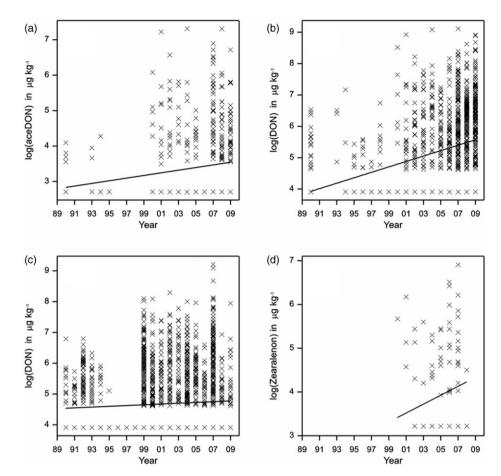


Figure 1. Trends of the presence of mycotoxins in cereal grains in North Western Europe over the period 1989–2009 for 3-acetyldeoxynivalenol (3-AcDON) in oats (a), deoxynivalenol in oats (b), deoxynivalenol in wheat (c) and zearalenone (ZEA) in maize (d). The presence of mycotoxins is indicated by the percentage of samples that contained the particular toxin above the level of detection, and expressed on a log scale (in $\mu g k g^{-1}$). One cross may indicate more than one value. Except for the presence of deoxynivalenol in wheat, the effect of year had a significant effect on the presence of the toxin (p < 0.05).

and number of rainy days) correlated positively with the presence of 3-AcDON in wheat, but a higher relative humidity during June correlated with a lower presence of this toxin in wheat.

Various climatic factors were found to have significant effects on the presence of DON in the four cereal grain types. The presence of DON in barley was positively correlated with a higher temperature and more rainfall (total rainfall and number of rainy days) during April. In oats, the presence of DON increased with higher temperatures during April, and decreased with higher wetness, including total rainfall, number of rainy days and relative humidity, during this month. The presence of DON in maize increased with a higher temperature during May. Many of the climatic factors were found to affect the presence of DON in wheat. Temperature had a significant increasing effect during April, May, June and September, and in August the increasing effect of temperature was almost significant (p=0.052). During July, temperature had no significant (increasing/decreasing) effect. More rainy days during June increased the presence of DON, as well as

a higher relative humidity during May and June, with a tendency towards such a positive effect for the month of July (p = 0.054).

The effects of climatic parameters on the presence of HT-2 and T-2 were investigated in barley, oats and wheat, but results showed only few parameters were significant. A higher temperature during June increased HT-2 presence in wheat. The presence of T-2 in oats increased with a higher rainfall during May (total rainfall and number of rainy days) and higher temperatures at the end of the growing season (July–September).

Possible relationships between climatic parameters and the presence of ZEA could be investigated in oats, maize and wheat. The presence of this toxin in maize was higher with increased relative humidity in September. All other climatic factors did not affect the occurrence of this toxin in maize. On the contrary, various climatic factors showed to have significant effects on the presence of ZEA in oats, including a negative effect of higher temperatures in both May and August, and positive effects of more rainfall in April, June and September (total rainfall and/or number of rainy days per month). Also, the presence of ZEA in wheat was affected by several climatic parameters during most months of the growing period, including increasing effects of higher temperatures during April, May, June and August and of relative humidity during May through September. More rain during May, including both total rainfall and number of rainy days, also positively affected the presence of ZEA in wheat.

The presence of NIV in barley was not affected by the weather factors considered. On the contrary, the presence of this toxin in oats was influenced by several parameters, all had a negative relationship; more rain during April (total rainfall and/or number of rainy days per month) resulted into lower presence of NIV in oats, as well as a higher relative humidity during four months (April, June, August and September). In wheat, the presence of NIV was negatively affected by a higher temperature in June and more rainfall during four months (April, May, August and September). In June, the relationship seemed to be the other way around as a higher relative humidity during this month increased the presence of NIV in wheat.

Discussion

This survey used mycotoxin data collected from major cereal grain types grown in four North Western European countries during the last two decades. It included a large number of records related to field grown cereals on private farms in these countries. Some other studies have been held in individual study countries, particularly in Finland and Norway, mostly covering several or only one year of out the series of 20 years covered in the current study (Eskola et al. 2001; Langseth et al. 2001; Hietaniemi et al. 2004; Jestoi et al. 2004; Yli-Mattila et al. 2008, 2009).

Obviously, DON was the most frequently analysed mycotoxin in cereals, and was found with the highest incidence (46%) and at the highest concentrations. It occurred at levels above the European Commission legal limits for food commodities in 66 samples of wheat (2.5%) and some barley samples (legal limit of $1250 \,\mu g \, kg^{-1}$) and in 127 oat and maize samples (8.5%) (legal limit of $1750 \,\mu g \, kg^{-1}$). Wheat and maize samples mainly originated from the Netherlands, and most oat samples were from Norway and Finland. In comparison with surrounding countries, the overall incidence of DON in wheat of 42.5% found in the current study was lower than DON incidences in commercial Lithuanian wheat grown during 2006 and 2007 $(LOD = 100 \,\mu g \, kg^{-1})$ and randomly selected wheat grown in Germany during 2000 and 2001 $(LOD = 7 \mu g k g^{-1})$, where both incidences were nearly

100% (Schollenberger et al. 2006; Mankevičienė et al. 2011). However, the LOD in the two German studies was much lower than in the current study, as were DON concentrations. In UK wheat grown during 2001-2005, the incidence of DON was 86% $(LOQ = 10 \,\mu g \, kg^{-1})$, with mean and median concentrations being comparable with the current study but having an even higher maximum (Edwards 2009a). In Denmark, the mean DON concentration in wheat samples varied from 153 to $916 \,\mu g \, kg^{-1}$ between 2003 and 2007, which - in general - was somewhat higher than findings from the current study (Nielsen et al. 2011). The incidences of DON in oats cultivated in Finland, Sweden and Norway, varying between 47% and 75%, were comparable with German oats grown during 1987-1992, which had an annual incidence of 49-85% (LOD = $1-5 \,\mu g \, kg^{-1}$) (Müller et al. 1998) and of 71% during 2000 and 2001 (LOD = $7 \,\mu g \, kg^{-1}$) (Schollenberger et al. 2006). In the current three study countries, however, DON concentration ranges were higher than in both the German studies (Müller et al. 1998; Schollenberger et al. 2006). In UK oats grown in the period of 2002–2005, both the incidence and concentrations of DON (LOQ = $10 \,\mu g \, kg^{-1}$) were much lower (Edwards 2009b) but, on the contrary, DON incidence in UK barley was higher (57%, $LOQ = 10 \,\mu g \, kg^{-1}$) than in the current study (around 22%) (Edwards 2009c). Concentration ranges were comparable. DON incidence and levels in maize samples - all collected from the Netherlands - were high, coinciding with results from Germany (Schollenberger et al. 2006). Maize from France grown between 2004 and 2007 showed a comparable high DON incidence $(LOQ = 10 \,\mu g \, kg^{-1})$ but lower concentration ranges (Scudamore and Patel 2009).

The acetylated versions of DON, including 3-acetyl-DON and 15-acetyl-DON, are co-contaminants of DON. They normally occur at a low percentage of the DON concentration, and as such are only normally detected when DON is present in high concentrations (Placinta et al. 1999). Indeed, the current study showed that 3-AcDON occurred at much lower incidence and levels as compared with DON, and correlations between the two toxins were very high in oats but not completely clear for wheat. However, Edwards (2009a) showed that, incidentally, acetylated DON can also occur at a high concentration in conjunction with low DON concentrations in wheat and, therefore, suggests that acetylated DON should be monitored in conjunction with DON, rather than relying on analysing DON only.

In European grown cereals, DON is mainly produced by *F. culmorum* and *F. graminearum*. This also accounts for ZEA, which is commonly associated to DON and its derivates (Jestoi et al. 2008). Indeed, the presence of ZEA was found to be positively associated with the presence of DON in maize. Out of the different cereal grain types, ZEA incidence was highest in maize, being nearly 40% (samples from the Netherlands only); contamination was much lower in the other cereal grains. NIV is a chemical structure closely related to DON. NIV can be produced by some isolates of F. culmorum and F. graminearum but also by F. poae, especially in Sweden and other North European countries, and by F. cerealis particularly in grains from Central to North East Europe (Jestoi et al. 2008; Yli-Mattila 2010). The tendency towards a negative relationship between the presence of DON and NIV in wheat (current study) could be explained by the different species having produced the two toxins. In the UK study on Fusarium toxins in wheat, DON and NIV also showed signs of mutual exclusion: when DON was present at high concentrations, NIV concentrations were low, and vice versa (Edwards 2009a).

As expected, the majority of T-2 and HT-2 positive samples were found in oats samples from Finland and Norway. Overall incidences in these two countries were around 54% for HT-2 and 27% for T-2, which was lower as compared with incidences of these toxins in oats (100%) from Germany in the years 2000 and 2001 (Schollenberger et al. 2006). Note, however, that the German study had a very low LOD of $3 \mu g k g^{-1}$ for HT-2 and $4 \mu g k g^{-1}$ for T-2. Concentrations of the two toxins were higher in Finland and Norway (current study) as compared with Germany. In the current study, HT-2 levels in oats were a factor of 2-3 higher compared with the levels of T-2, coinciding with previous studies (Gottschalk et al. 2009; Scudamore et al. 2009), although some other studies reported an even higher rate up to 7, as reviewed by van der Fels-Klerx and Stratakou (2010). Also, the positive correlation between T-2 and HT-2 in oats found in the current study is in line with previous studies (Gottschalk et al. 2009; Edwards 2009b). In both UK wheat and barley, a weak positive relationship between these two toxins was found. These cereal grains had much lower contamination levels as compared with UK oats (Edwards 2009a, 2009c). In the current survey, contamination of wheat and barley with HT-2 and T-2 was also much lower as compared with oats. In fact, numbers of samples that contained one or both of the toxins in these grains were too low to evaluate a possible relationship between HT-2 and T-2. Correspondingly, concentration levels of HT-2 and T-2 were found to be low in samples of grain from Denmark covering the period 2003–2007 for wheat and 2005–2007 for barley (Nielsen et al. 2011).

Results indicated that all barley, oat and wheat samples contained OTA levels below the cut-off LOD of $50 \,\mu g \, kg^{-1}$. This LOD is in between the European Commission legal limit of $5 \,\mu g \, kg^{-1}$ for unprocessed cereals destined for human food (European Commission 2006a, 2007) and recommendations for

maximum levels of OTA in cereals for feedstuffs range from 50 (feed for pigs) to $250 \,\mu g \, kg^{-1}$ (unprocessed) (European Commission 2006b). Data showed that none of the 508 samples that were analysed for OTA content with an LOD of $5 \,\mu g \, kg^{-1}$ contained this toxin. *P. verrucosum* may produce OTA in cereals grown in the temperate and cold climates of Northern Europe (Lund and Frisvad 2003), particularly post-harvest when improper drying occurs (Magan and Aldred 2007). Current low OTA levels in cereals grown in Northern Europe are, therefore, as expected.

MON was found with an overall incidence of 19% in 149 Norwegian barley, wheat and oats samples, collected in the years 2000 and 2001. Considering only the 48 wheat samples, this incidence was 68%. Uhlig et al. (2004) reported that the overall incidence of MON in 2002 had increased to 67% in 94 Norwegian grain samples (barley, wheat and oats). Considering only the 35 wheat samples of 2002, MON incidence was found to be 86%, with a maximum concentration of 950 μ g kg⁻¹. In Finland, MON was found in 74% of 38 grain samples harvested in 2001 and 2002. The highest level was 810 μ g kg⁻¹ in a spring wheat sample of 2001 (Jestoi et al. 2004).

Many of the investigated climatic factors showed significant effects on the presence of DON in wheat, all having a positive relationship, i.e. the presence of this toxin increased with higher temperature, rainfall and relative humidity in specific months of wheat cultivation. Wheat contamination with DON was thus significantly influenced by seasonal weather conditions in the various countries. Indeed, empirical models that predict DON levels based on climatic factors during the growing season (including temperature, rainfall and relative humidity) - in some cases added with agronomy factors - have been developed, and show good predictive performance (Hooker et al. 2002; Klem et al. 2007; Franz et al. 2009; van der Fels-Klerx and Booij 2010; van der Fels-Klerx et al. 2010). Several climatic factors also positively influenced the presence of ZEA in wheat during the last two decades. As both DON and ZEA in wheat seemed to be correlated to each other (current study), models that predict DON levels in wheat could be used as a starting point to predict ZEA levels as well, though the per cent of explaining variance must be considered. After all, climatic factors could have a statistical significant effects, but low impacts on toxin presence. First attempts to model the incidence of ZEA in wheat using climate data have been made recently (Edwards 2011). Contrary to DON, all climatic factors that significantly affected the presence of NIV in wheat had a negatively relationship with this toxin (current study). Consequently, increasing temperature and rainfall will increase the presence of DON in wheat, but decrease the presence of NIV, and vice versa. Possible effects of weather conditions on the presence of toxins in maize could be tested for DON and ZEA only, but only one of the climatic factors showed a significant effect. This probably explains why there are limited empirical models available to predict mycotoxin levels in maize (Schaafsma and Hooker 2007). Apparently, other factors play a role, for instance, corn hybrid had a very high influence on DON levels in maize (Hooker and Schaafsma 2005). All climatic factors that had a significant effect on the presence of NIV in oats showed a negative relationship with this toxin. However, the opposite was seen for T-2. This implies that weather conditions that increase the presence of T-2 in oats will decrease the presence of NIV, and vice versa.

Results of the current study showed that, except for HT-2 in oats, all significant trends over time were positive, i.e., the presence of mycotoxins in cereal grains has increased over the last two decades in the four study countries. It is, therefore, useful to continue monitoring of mycotoxins in cereal grains and further to develop models that predict mycotoxin levels based on seasonal weather, possibly in combination with other relevant factors. Mycotoxin predictions are helpful in setting up risk-based monitoring by food safety authorities and food industry and, in addition, can be used to help decision-making on fungicide application and purchasing commodities. The results of this study can be used as a first starting point in further developing models. Given the results for trends in time and the effects of climatic factors on toxin contamination, the focus could be on the occurrence of 3-AcDON, DON and NIV in oats as well as ZEA in wheat. Moreover, the available models for the prediction of DON in wheat could be elaborated upon for application in other Northern European countries (van der Fels-Klerx and Booij 2010).

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