International Journal of Food Microbiology 167 (2013) 284–291

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Deoxynivalenol and other selected *Fusarium* toxins in Swedish wheat — Occurrence and correlation to specific *Fusarium* species

Mats Lindblad [a], Ann Gidlund [a], Michael Sulyok [b], Thomas Börjesson [c], Rudolf Krska [b], Monica Olsen [a], Elisabeth Fredlund [a,*]

[a] National Food Administration, P.O Box 622, SE-751 26 Uppsala, Sweden
[b] Center for Analytical Chemistry, Department IFA-Tulln, University of Natural Resources and Life Sciences Vienna, Austria
[c] Lantmännen Business Development, Grain, SE-531 87 Lidköping, Sweden

ARTICLE INFO

Available online 18 July 2013

Keywords:
Fusarium graminearum
Beauvericin
Enniatins
T-2 toxin
Fusarium tricinctum
qPCR

ABSTRACT

Wheat is often infected by *Fusarium* species producing mycotoxins, which may pose health risks to humans and animals. Deoxynivalenol (DON) is the most important *Fusarium* toxin in Swedish wheat and has previously been shown to be produced mainly by *Fusarium graminearum*. However, less is known about the co-occurrence of DON and *F. graminearum* with other toxins and *Fusarium* species in Sweden.

This study examined the distribution of the most important toxigenic *Fusarium* species and their toxins in winter wheat (2009 and 2011) and spring wheat (2010 and 2011). DNA from seven species was quantified with qPCR and the toxin levels were quantified with a multitoxin analysis method based on liquid chromatography/electrospray ionisation-tandem mass spectrometry (HPLC/ESI-MS/MS). The method enabled detection of many fungal metabolites, including DON, zearalenone (ZEA), nivalenol (NIV), T-2 toxin, HT-2 toxins, moniliformin (MON), beauvericin (BEA), and enniatins (ENNs).

It was found that *Fusarium poae* and *Fusarium avenaceum* were present in almost all samples. Other common *Fusarium* species were *F. graminearum* and *F. culmorum*, present in more than 70% of samples. Several species occurred at lower DNA levels in 2011 than in other years, but the reverse was true for *F. graminearum* and *Fusarium langsethiae*. The most prevalent toxins were ENNs, present in 100% of samples. DON was also common, especially in spring wheat, whereas ZEA and NIV were common in 2009 and in winter wheat, but less common in 2011 and in spring wheat. Only three samples of spring wheat contained T-2 or HT-2 above LOQ. Annual mean levels of several mycotoxins were significantly lower in 2011 than in other years, but the reverse applied for DON.

The strongest correlations between mycotoxin and *Fusarium* DNA levels were found between *F. avenaceum* and ENNs ($r^2 = 0.67$) and MON ($r^2 = 0.62$), and *F. graminearum* and DON ($r^2 = 0.74$).

These results show that several *Fusarium* species and toxins co-occur in wheat. The highest toxin levels were detected in spring wheat and DON and ENNs, the latter belonging to the group of so called “emerging toxins”, which were the most prevalent toxins and those occurring at the highest levels.

1. Introduction

Cereal-based products are important nutrient sources for animals and humans world-wide. Wheat is the most important small-grain cereal crop in the world, with a production quantity of almost 700 million tonnes per year (Anonymous, 2010). Unfortunately, cereal crops can be colonised by moulds, including species from the genus *Fusarium*, which cause significant reductions in crop yield and quality due to *Fusarium* Head Blight (FHB) disease and due to their ability to produce mycotoxins (Miller, 2008). *Fusarium* toxins commonly occurring in cereals include trichothecenes, zearalenone (ZEA) and fumonisins. Trichothecenes are divided into type A trichothecenes, including T-2 and HT-2 toxins, and type B trichothecenes, including deoxynivalenol (DON) and nivalenol (NIV) (Bottalico and Perrone, 2002; Miller, 2008).

Due to their negative health effects in both humans and animals, the European Commission has set maximum levels for these toxins in cereals (Commission Regulation 2006/1881). In unprocessed wheat, the maximum level for DON is 1250 μg/kg (durum wheat 1750 μg/kg) and that for ZEA is 100 μg/kg. A recommendation on monitoring the levels of T-2 and HT-2 toxins in cereals has recently been adopted by the European Commission (Commission Recommendation 2013/165/EC), with benchmark levels for the sum of T-2 and HT-2 toxins of 100 μg/kg in unprocessed wheat and 50 μg/kg in processed cereals other than oats and maize. For cereals intended for animal feed, the maximum recommended levels of DON and ZEA are 8000 μg/kg and 2000 μg/kg, respectively (Commission Recommendation 2006/576/EC). The toxic effects include vomiting, abdominal pain, nausea, reduced weight gain in animals caused by DON (Anonymous, 1999), fertility problems in pigs and

* Corresponding author. Tel.: +46 18 175788; fax: +46 18 105848.
E-mail address: elisabeth.fredlund@slv.se (E. Fredlund).

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http://dx.doi.org/10.1016/j.ijfoodmicro.2013.07.002

hormonal disturbances in humans caused by the high oestrogenic activity of ZEA (Anonymous, 2011). Of the metabolites covered by regulations, DON is the most prevalent *Fusarium* toxin in wheat. Three different chemotypes of DON-producing *Fusarium* isolates exist; the 15-ADON, 3-ADON and NIV chemotypes (Jennings et al., 2004). The DON-producing species *Fusarium graminearum* and *Fusarium culmorum* also produce ZEA, another common contaminant in cereals. Thirteen phylogenetically distinct species have been resolved within the *F. graminearum* clade, of which *F. graminearum* sensu stricto (clade 7) predominates on wheat in North and South America and in Europe (O'Donnell et al., 2000, 2004; Starkey et al., 2007; Yli-Mattila et al., 2009).

In addition to the regulated toxins, wheat cultivated in northern Europe is also reported to be contaminated by NIV (Bottalico and Perrone, 2002; Edwards, 2009b; Eskola et al., 2001; Nielsen et al., 2011; Pettersson et al., 1995; Yli-Mattila et al., 2008a, 2008b) as well as the so-called 'emerging mycotoxins', including beauvericin (BEA), moniliformin (MON) and enniatins (ENNs) (Jestoi, 2008; Jestoi et al., 2004a; Sharman et al., 1991; Uhlig et al., 2004, 2006; Yli-Mattila et al., 2006). However, no data on their prevalence have yet been presented for Sweden. Many *Fusarium* species have been described as producers of these toxins (Jestoi, 2008; Jestoi et al., 2008), but *Fusarium avenaceum* seems to be the most important producer of MON and ENNs, at least in the Nordic countries (Jestoi, 2008; Jestoi et al., 2004a, 2004b, 2008; Morrison et al., 2002; Uhlig et al., 2004, 2006; Vogelgsang et al., 2008; Yli-Mattila, 2010). *Fusarium poae* in particular, but also *F. avenaceum* and many others, has been described as producers of BEA (Bottalico and Perrone, 2002; Jestoi, 2008; Jestoi et al., 2008; Logrieco et al., 2002; Somma et al., 2010; Thrane et al., 2004; Uhlig et al., 2006). *F. poae* has also been reported to produce NIV (Jestoi, 2008; Jestoi et al., 2008; Langseth et al., 1999; Pettersson et al., 1995; Somma et al., 2010; Thrane et al., 2004; Vogelgsang et al., 2008). *F. avenaceum* is the most abundant *Fusarium* species in Northern Europe (Jestoi et al., 2004a; Kosiak et al., 2003; Langseth and Rundberget, 1999; Uhlig et al., 2007; Yli-Mattila et al., 2004). This species is closely related to *Fusarium tricinctum* and *Fusarium acuminatum* and differentiation between these species based on morphology and molecular markers has been shown to be difficult (Harrow et al., 2010; Kristensen et al., 2005; Yli-Mattila et al., 2002). *F. acuminatum* has recently been reported as a contaminant in wheat in Canada (Gräfenhan et al., 2013) and Spain (Marín et al., 2012), but is not described as an important contaminant in wheat in the Nordic countries (Eskola et al., 2001; Langseth et al., 1999; Yli-Mattila et al., 2008b).

Growth of *Fusarium* fungi and mycotoxin production is governed by climate factors such as temperature and humidity, but agricultural practices such as choice of variety, crop rotation strategies and tillage practices are also important (Schaafsma and Hooker, 2007). As a consequence of climate change, the levels of mycotoxins are expected to increase in north-western Europe (van der Fels-Klerx et al., 2012) and world-wide (Wu et al., 2011). Several authors have already ascribed the shift from the previous dominance of *F. culmorum* to *F. graminearum* in cooler areas such as Northern Europe to climate factors (Miller, 2008; Nielsen et al., 2011; Parikka et al., 2012; Wu et al., 2011), and models predicting DON contamination in wheat based on weather data and agronomic factors have been developed (Franz et al., 2009; Schaafsma and Hooker, 2007; van der Fels-Klerx et al., 2010). Schaafsma and Hooker (2007) found that environmental effects accounted for almost half the variation in DON levels in wheat, followed by variety and previous crop as significant explanatory factors.

LC–MS/MS is increasingly being used for simultaneous determination of large numbers of mycotoxins (Krska et al., 2008). The robustness of modern mass spectrometers permits accurate quantitative analysis of diluted crude wheat extracts (Desmarchelier et al., 2010; Spanjer et al., 2008; Sulyok et al., 2006). The main advantage of this 'dilute-and-shoot' approach is the reduction in costs and time consumption. At the same time, the risk of losing parts of the metabolite spectrum due to incompatibility with the chosen clean-up procedure is minimised.

Quantification of *Fusarium* species in food and feed using conventional methods requires specific expertise, experience and time, and is dependent on living propagules. Real-time PCR (qPCR) uses species-specific primers and probes to detect and quantify specific species without the need for isolation and cultivation of the fungi. It also gives an estimate of fungal biomass, which allows for better correlation between fungal growth and toxin production than methods such as plating of seeds, which only give percentage mould infection. PCR-based methods have been developed for specific amplification of DNA from several trichothecene-producing *Fusarium* species, including SYBR Green-based methods (Kulik, 2008; Nicolaisen et al., 2009; Wilson et al., 2004) and TaqMan-based methods (Demeke et al., 2010; Waalwijk et al., 2004; Yli-Mattila et al., 2008a).

The objective of the present study was to investigate the occurrence of *Fusarium* species and their toxins in wheat (winter and spring wheat), including emerging toxins not previously reported in Sweden. The study also looked for correlations between toxins and fungal species and examined annual differences in mould and mycotoxin content.

2. Materials and methods

2.1. Fungal strains and production of mycelium

F. poae strain VI 1226, *Fusarium langsethiae* strain VI 1272, *Fusarium sporotrichioides* strain VI 1310, *F. graminearum* strain IBT 1958, *F. culmorum* strain IBT 2303, *F. avenaceum* CBS 170.31 and *F. tricinctum* IBT 2015 were used as reference strains to produce DNA standards for the absolute quantification of *Fusarium* in the samples. These strains are deposited in the DTU culture collection in Lyngby, Denmark (IBT 1958, IBT 2303 and IBT 2015), in the CBS culture collection, Utrecht, Holland (CBS 170.31) and in the Norwegian Veterinary Institute in Oslo (VI 1226, VI 1272 and VI1310). To produce fungal mycelium, the isolates were grown in MEA broth (LP0039, Oxoid Ltd., Hampshire, UK) in a water bath (100 rpm) at 25 ± 0.5 °C for 5 days. The mycelium was washed twice in sterile tap water, centrifuged for 10 min at 4000 ×*g*, and then freeze-dried (Edwards Modulyo freeze dryer). The

Table 1
Wheat samples per year and region and weather conditions in July and August in 2009 through 2011.
Meteorological data from Malmö (South), Skara (West), and Västerås (East).

Year	Region	No. of samples		Mean temperature (°C)		Precipitation (mm)	
		Winter wheat	Spring wheat	July	August	July	August
2009	South	11	–	19.0	18.4	37	55
	West	8	–	16.6	16.1	207	54
	East	12	–	17.6	16.9	86	82
2010	South	–	7	20.5	17.4	19	209
	West	–	6	18.3	15.6	155	169
	East	–	15	20.1	16.5	82	77
2011	South	12	11	17.3	16.8	153	131
	West	3	3	17.2	15.6	108	185
	East	18	19	18.5	16.4	70	128