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Associations between *Fusarium* species and mycotoxins in oats and spring wheat from farmers' fields in Norway over a six-year period

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RESEARCH ARTICLE

Abstract

During the last ten years, Norwegian cereal grain industry has experienced large challenges due to *Fusarium* spp. and *Fusarium* mycotoxin contamination of small-grained cereals. To prevent severely contaminated grain lots from entering the grain supply chain, it is important to establish surveys for the most prevalent *Fusarium* spp. and mycotoxins. The objective of our study was to quantify and calculate the associations between *Fusarium* spp. and mycotoxins prevalent in oats and spring wheat. In a 6-year period from 2004-2009, 178 grain samples of spring wheat and 289 samples of oats were collected from farmers' fields in South East Norway. The grains were analysed for 18 different *Fusarium*-mycotoxins by liquid chromatography – mass spectrometry. Generally, the median mycotoxin levels were higher than reported in Norwegian studies covering previous years. The DNA content of *Fusarium graminearum, Fusarium culmorum, Fusarium langsethiae*, *Fusarium poae* and *Fusarium avenaceum* were determined by quantitative PCR. We identified *F. graminearum* as the main deoxynivalenol (DON) producer in oats and spring wheat, and *F. langsethiae* as the main HT-2 and T-2-toxins producer in oats. No association was observed between quantity of *F. graminearum* DNA and quantity of *F. langsethiae* DNA nor for their respective mycotoxins, in oats. *F. avenaceum* was one of the most prevalent *Fusarium* species in both oats and spring wheat. The following ranking of *Fusarium* species was made based on the DNA concentrations of the *Fusarium* spp. analysed in this survey (from high to low): *F. graminearum* = *F. langsethiae* = *F. avenaceum* > *F. poae* > *F. culmorum* (oats); *F. graminearum* = *F. avenaceum* > *F. culmorum* > *F. poae* = *F. langsethiae* (spring wheat). Our results are in agreement with recently published data indicating a shift in the relative prevalence of *Fusarium* species towards more *F. graminearum* versus *F. culmorum* in Norwegian oats and spring wheat.

Keywords: deoxynivalenol, *Fusarium avenaceum, Fusarium graminearum, Fusarium langsethiae*, HT-2.

1. Introduction

Fusarium head blight is an important fungal disease in cereals caused by a range of *Fusarium* and *Microdochium* species (Parry *et al.*, 1995). During the 1980's, 1990's and at the beginning of the 21st century, *Fusarium avenaceum, Fusarium tricinctum, Fusarium culmorum* and sometimes *Fusarium poae,* were reported as the most prevailing *Fusarium* species in Norwegian wheat, barley and oats (Henriksen and Elen, 2005; Kosiak *et al.*, 2003; Uhlig *et al.*, 2004). In addition, *Fusarium graminearum, Fusarium langsethiae, Fusarium equiseti,* and *Fusarium cerealis* were often found (Henriksen and Elen, 2005; Kosiak *et al.*, 2003; Uhlig *et al.*, 2004). In 2002-2004, an increased prevalence of *F. graminearum* was registered in Norwegian grains of barley, oats and wheat (Bernhoft *et al.*, 2010). This seems to be the trend in other European countries as well (Fredlund *et al.*, 2013; Nielsen *et al.*, 2011; Waalwijk *et al.*, 2003; Xu *et al.*, 2005).

During the last ten years, Norwegian cereal grain industry has experienced large challenges due to *Fusarium* spp. contamination of cereals. In 2009, more than 30% of the oat seed lots from southern Norway were not suited as seed due to poor germination, mainly caused by *Fusarium* spp. infestation (http://www.kimen.no). Moreover, several *Fusarium* species are known to produce mycotoxins, which may reduce the food and feed quality of the grains. Recent analyses have confirmed high prevalence and relatively high concentrations of both type A and B trichothecenes in Norwegian cereals (Aamot *et al.*, 2012, 2013; Norwegian Scientific Committee for Food Safety, 2013; Uhlig *et al.*, 2013). Consequently, all Norwegian produced oat grain lots have been analysed for the content of deoxynivalenol (DON) at delivery since 2011. However, the more toxic trichothecenes T-2 and HT-2 toxin (T-2 and HT-2) are also often recorded, particularly in barley and oats (Aamot *et al.*, 2013; Bernhoft *et al.*, 2010; Langseth *et al.*, 1999). A poor association is often observed between the concentration of HT-2 + T-2 and DON in oats (Bernhoft *et al.*, 2010; Edwards, 2009; Fredlund *et al.*, 2010), which may indicate that the HT-2 and T-2 producers have different environmental requirements and/or epidemiology than the DON producers (Edwards, 2009; Xu *et al.*, 2014). In addition to the above mentioned trichothecenes, *Fusarium* toxins such as zearalenone (ZEA), moniliformin (MON), enniatins (ENNs) and beauvericin (BEA) are also frequently detected in Norwegian grains (Bernhoft *et al.*, 2010; Uhlig *et al.*, 2004, 2006, 2013). For many of these mycotoxins, consequences of chronic exposure have not been explored in detail (Tedjiotsop Feudjio *et al.*, 2010).

To prevent severe contaminated grain lots from entering the grain supply chain, it is important to identify the most prevalent *Fusarium* spp. and mycotoxins so relevant surveys can be established. During the last ten years, no study has been published on the prevailing mycotoxin producing fungi in Norwegian grain. Furthermore, analyses on the association between *Fusarium* DNA and important mycotoxins are scarce. The objectives of the present study were therefore: (1) to identify and quantify the DNA of *Fusarium* species and mycotoxins prevalent in oats and spring wheat from farmers' fields in South East Norway in a 6-year period from 2004-2009; (2) to calculate the associations between present *Fusarium* species and mycotoxins; and (3) to calculate the probability of mycotoxin contamination of a grain sample based upon the occurrence of selected *Fusarium* species.

2. Material and methods

Sample collection

Grains of spring wheat (178 samples) and oats (289 samples) were collected from farmers' fields in South East Norway, representing the main cereal districts, in a period from 2004-2009. The majority (75%) of the samples were collected in years 2006-2008. The collection of grain samples was done either by the agricultural extension service units at the farm during harvest, or at grain delivery by the use of automatic samplers at the elevators. The grain samples were dried to a water content of 10-14% and cleaned of impurities and dust by gentle air blowing before samples of about 350 g were obtained by passing the larger samples through a riffle type divider (Rationel Kornservice AS, Esbjerg, Denmark). The equipment was cleaned by compressed air between samples. The samples were then grinded to 1 mm particles of wheat and 2 mm particles of oats, using a Laboratory mill 3303 (Perten Instruments AB, Hägersten, Sweden). Grinded samples were stored at minus 20 °C until usage.

Analyses of fungal DNA

All spring wheat samples and 284 of the oat samples were analysed by quantitative PCR (qPCR) to determine the content of *F. graminearum, F. culmorum, F. avenaceum,* and *F. poae,* according to methods described in Halstensen *et al.* (2006), Reischer *et al.* (2004), Waalwijk *et al.* (2004), relative to plant DNA (Divon *et al.*, 2012). DNA was extracted according to Divon *et al.* (2012). Five of the 289 oat samples were not included in the analysis due to a low level of extracted DNA. The content of *F. langsethiae/Fusarium sporotrichioides* were analysed in all spring wheat samples according to Halstensen *et al.* (2006). This qPCR method does not discriminate between *F. langsethiae* and *F. sporotrichioides*, but as *F. sporotrichioides* is only sporadically found in Norwegian cereals (Bernhoft *et al.*, 2010; Kosiak *et al.*, 2003), we will refer to *F. langsethiae* further on when presenting these results. For all the above-mentioned analyses, qPCR was performed according to Divon *et al.* (2012).

In addition, DNA of *F. langsethiae* was quantified in a subset of 277 of the in total 289 oat samples by a species-specific assay (Waalwijk *et al.*, 2009). The probe and primer sequences for this qPCR-assay were: probe: 5`-6FAM-CAC ACC ***C***AT A***C***C TAC ***G***TG TAA-TAMRA-3` (bold italic lettering represents locked nucleic acid nucleotides), forward: 5`-GTT GGC GTC TCA CTT ATT ATT C-3`, reverse: 5`-TGA CAT TGT TCA GAT AGT AGT CC-3`. In this qPCR, the analysis of *F. langsethiae* DNA and plant DNA was performed in duplex reactions. qPCR was performed in a total volume of 25 µl that consisted of 4 µl genomic DNA (mean of 23 ng plant DNA), 300 nM of each *F. langsethiae* primer, 75 nM of each plant primer, 100 nM of each probe, and 1×iQ™ Multiplex Powermix (BioRad, Hercules, CA, USA), in a C1000 Touch Term Cycler combined with a CFX96TM Real-Time System (BioRad). Following parameters were used: 95 °C for 3 min followed by 45 cycles of 95 °C for 10 s and 60 °C for 30 s. The probe for detection of plant DNA was in this case labelled with VIC, and primer concentrations optimised to

enable reliable duplex detection were used. We evaluated the specificity of this qPCR assay by analysing a selection of strains of *F. langsethiae* and closely relates species, as well as for *Fusarium* species common in Norwegian cereals: 22 strains of *F. langsethiae*, 10 of *F. sporotrichioides*, 3 of *F. equiseti*, 9 of *F. poae*, 2 of *F tricinctum*, 5 of *F graminearum,* 4 of *F. culmorum,* 3 of *F. avenaceum*. As expected, this *F. langsethiae* qPCR assay gave positive signals for *F. langsethiae* only.

The fungal content is presented as pg fungal DNA per ng plant DNA (pg/ng).

Analyses of mycotoxins

All spring wheat samples were analysed for 18 different *Fusarium*-mycotoxins by liquid chromatography – mass spectrometry (LC-MS/MS) (Kokkonen and Jestoi, 2009) at the Finnish Food Safety Authority (Evira, Helsinki, Finland). The following mycotoxins were included in the analysis: HT-2, T-2, diacetoxyscirpenol, neosolaniol, DON, 3-actyldeoxynivalenol (3-ADON), nivalenol, fusarenon-X, ZEA, enniatin A (ENN-A), enniatin A1 (ENN-A_1), enniatin B (ENN-B), enniatin B_1 (ENN-B_1), BEA, MON, fumonisin B_1, fumonisin B_2 and antibiotic Y. In oats, the contents of the above mentioned mycotoxins, except HT-2 and T-2, were analysed for all the 289 samples at Evira (same method as described above). The content of HT-2 and T-2 in oats were analysed by LC-MS/MS, either at NIBIO (Ås, Norway) according to the method described in Aamot *et al.* (2013), or at Institute of Sciences of Food Production (ISPA) in Bari, Italy (description below). Mycotoxin concentrations are presented as µg of the respective mycotoxin per kg grain (µg/kg).

At ISPA, the sample preparation was done according to Lattanzio *et al.* (2008), with some modifications. Ground samples (10 g) were extracted with 50 ml acetonitrile:water (84:16, v/v) for 60 min on an orbital shaker (model 711 VDRL, Asal, Milan, Italy). The extract was filtered through filter paper (Whatman No. 4, Maidstone, UK), and 5 ml of filtrate (equivalent to 1 g sample) were evaporated to dryness at 50 °C under a stream of air. The residue was dissolved with 1 ml of methanol:water 30:70 (v/v) and cleaned up through an Oasis® HLB column (Waters, Milan, Italy) activated and conditioned according to instructions of the manufacturer. The sample extract was passed through the column, then the column was washed with 1 ml methanol:water, 55:45 (v/v) and dried. Thereafter, T-2 and HT-2 were eluted with 1 ml methanol. The eluate was dried, at 50 °C under a stream of air, and redissolved with 1 ml LC mobile phase (methanol:water, 60:40, v/v, containing 5 mM ammonium acetate) and added with an appropriate amount of $^{13}C_{24}$-T-2 and $^{13}C_{22}$-HT-2 (Biopure Referenzen substanzen GmbH, Tulln, Austria) each. 20 µl (equivalent to 20 mg sample) were analysed by LC-MS/MS performed on a QTrap® MS/MS system, from Applied Biosystems (Foster City, CA, USA) equipped with an APCI (atmospheric pressure chemical ionisation) interface and a 1100 series micro-LC system from Agilent Technologies (Waldbronn, Germany). All relevant instrumental conditions were performed as previously described (Pascale *et al.*, 2011).

The limit of detection (LOD) values for the different mycotoxins analysed are listed in Table 1. At Evira, the limit of quantification (LOQ) was set to 2× LOD. The highest standard points for enniatins in the analysis were: ENN-A 60 µg/kg; ENN-A_1 400 µg/kg; ENN-B 380 µg/kg; and ENN-B_1 1,080 µg/kg. To obtain cost-efficient analyses, no dilution of the samples exceeding these concentrations were conducted, and consequently concentrations above these levels can only be considered as semi-quantitative. From the analysis of HT-2 and T-2 in oats (at NIBIO and ISPA), LOD=LOQ (Table 1). The median concentration of mycotoxins were only calculated for those mycotoxins where more than 50% of the grain samples had a mycotoxin concentration above LOQ (Table 1).

Statistics

The relationship between the amount of *Fusarium* and concentration of mycotoxins in cereal grains was studied using two different methods: ordinary linear regression and binary logistic regression. We used Minitab version 16 (Minitab, Inc. 2009, Coventry, UK) for the analysis.

Ordinary regression analyses, to study relationships between occurrences of different *Fusarium* species, were performed on those species with a fungal DNA content of ≥0.1 pg fungal DNA per ng plant DNA in more than 10% of the grain samples. We also used ordinary regression analysis to study the relationship between occurrences of different mycotoxins, but only when more than 50% of the grain samples had a mycotoxin concentration above LOQ or when maximum values detected were below the highest standard points in the analysis. In the ordinary regression analysis, the natural logarithm (ln) of *Fusarium* DNA (pg fungal DNA per ng plant DNA) and mycotoxin concentrations (µg per kg grain) were used. All qPCR values were added the value 1 prior to ln transformation. For mycotoxins, a value <LOD was replaced by LOD/2; when LOD < value < LOQ the value was replaced by LOD + (LOD + LOQ)/2.

A binary logistic regression analysis was used to calculate the probability of a grain sample to contain above 100 µg/kg of a specific mycotoxin based upon the occurrence of selected *Fusarium* species. The probability P(A), where A is the event that a grain sample contain ≥100 µg/kg of a specific mycotoxin (or a sum of mycotoxins), was calculated as a function of the occurrence (≥0.1 pg *Fusarium* DNA per ng plant DNA) of *Fusarium* species. For grain samples

Table 1. Mycotoxin content of oats (n=289) and spring wheat (n=178) grain samples collected from farmers' fields in SouthEast Norway during 2004-2009.[a]

	Oats (n=289)				Spring wheat (n=178)			
	LOD (µg/kg)	≥LOD (%)	≥LOQ (%)	max conc. (µg/kg)	LOD (µg/kg)	≥LOD (%)	≥LOQ (%)	max conc. (µg/kg)
HT-2 toxin	20 (4)[b]	67	67[c]	2,040	50	2	0	<LOQ
T-2 toxin	3 (2)[b]	88	88[c]	864	15	2	0	<LOQ
Diacetoxyscirpenol	40	1	0	<LOQ	12.5	0	0	<LOD
Neosolaniol	200	0	0	<LOD	200	0	0	<LOD
Deoxynivalenol	50	90	66	30,000	45	92	71	16,000
3-acetyldeoxynivalenol	50	71	35	5,100	45	40	6	540
Nivalenol	100	1	1	310	100	0	0	<LOD
Fusarenon X	80	0	0	<LOD	80	0	0	<LOD
Zearalenone	50	21	11	2,300	30	39	13	350
Enniatin A	1.5	62	25	300[d]	1	85	17	36
Enniatin A_1	2.5	80	46	2,400[d]	1	94	63	260
Enniatin B	2.4	95	71	6,800[d]	1.5	100	100	5,400[d]
Enniatin B_1	2.7	91	61	6,200[d]	2.7	99	80	2,200[d]
Beauvericin	5	81	29	980	5	65	2	23
Moniliformin	200	47	3	1,200	150	69	11	1,600
Fumonisin B_1	40	0	0	<LOD	40	0	0	<LOD
Fumonisin B_2	40	0	0	<LOD	40	0	0	<LOD
Antibiotic Y	100	65	4	1,600	60	74	7	1,300

[a] LOD = limits of detection; LOQ = limits of quantification. LOQ are 2× LOD values for mycotoxins analysed at Evira.
[b] LOD values for HT-2 and T-2 toxins analysed at NIBIO (and ISPA).
[c] LOQ = LOD for HT-2 and T-2 toxins analysed at NIBIO (and ISPA).
[d] Concentrations above the highest standard.

with mycotoxin content <LOQ, the value LOQ/6 was used. The binary logistic regression model is:

$$P(A) = \frac{e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p}}{1 + e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p}} \quad (1)$$

In Equation 1, x_i, i = 1,2, ... , p is the DNA content of a specified *Fusarium* species, *i*. Occurrence of a *Fusarium* species was defined as ≥0.1 pg *Fusarium* DNA per ng plant DNA. We used x_i = 1 if *Fusarium* DNA ≥0.1 pg/ng, and x_i = 0 if *Fusarium* DNA <0.1 pg/ng. β_0, β_1,β_2, ..., β_p are regression coefficients to be estimated using the available data.

The Goodman-Kruskal Gamma was used as a measure of the predictive power of the different models. Its values ranges from -1.0 (100% negative association) to 1.0 (100% positive association between observed and predicted values). A value of zero indicates the absence of association.

3. Results

Occurrence of *Fusarium* spp. in oats and spring wheat

Of the five *Fusarium* species analysed for in this study, *F. graminearum, F. langsethiae* and *F. avenaceum,* were the species most frequently detected in oats. In spring wheat, *F. graminearum* and *F. avenaceum* were most commonly detected (Figure 1).

F. graminearum DNA ≥0.1 pg per ng plant DNA was registered in 69% of the oat samples and in 62% of the spring wheat samples (Figure 1). Concentrations of *F. culmorum* DNA ≥0.1 pg per ng plant DNA were only detected in 3 and 10% of the oat and spring wheat samples, respectively. 35% of the oat samples and 24% of the spring wheat samples had concentrations of *F. graminearum* DNA ≥1 pg/ng. In contrast, concentrations of *F. culmorum* DNA ≥1 pg/ng were registered in less than 1% of the oat and spring wheat samples.

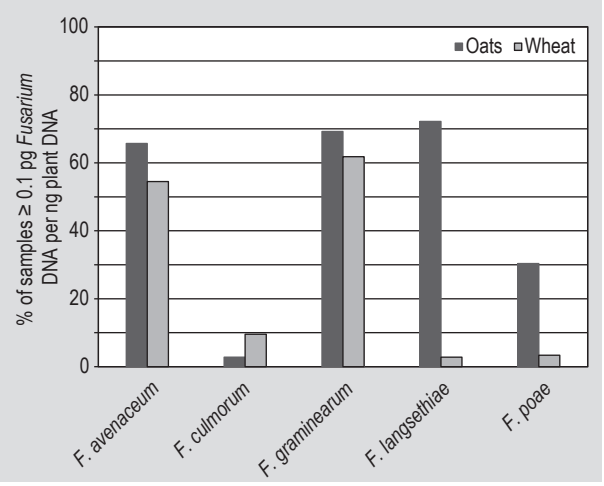

Figure 1. Occurrence of *Fusarium* species in grain samples of oats and spring wheat collected from farmers' fields in South-East Norway years 2004-2009. The percentage of spring wheat (n=178) and oat samples (n=284, except for *Fusarium langsethiae* where n=277) with a *Fusarium* DNA content ≥0.1 pg per ng plant DNA are shown. DNA concentrations were determined by quantitative PCR.

F. langsethiae was most frequently detected in oats, and 72% of the oat samples contained ≥0.1 pg fungal DNA per ng plant DNA (Figure 1). In wheat, on the other hand, only 5% of the samples contained *F. langsethiae* DNA ≥0.1 pg/ng. The proportion of the oat samples containing *F. langsethiae* DNA ≥1 pg/ng was 24%, whereas this concentration was not exceeded in any of the spring wheat samples.

A content of *F. avenaceum* DNA ≥0.1 pg per ng plant DNA was registered in 66% of the oat samples and in 55% of the spring wheat samples (Figure 1). Higher concentrations (≥1 pg/ng) of *F. avenaceum* DNA were registered in 19% of the oat samples, but only in 9% of the spring wheat samples.

F. poae was most frequently detected in oats where 30% of the samples contained *F. poae* DNA ≥0.1 pg per ng plant DNA (Figure 1). 2% of the oat samples had a *F. poae* DNA concentration ≥1 pg/ng. In spring wheat, 6% of the samples contained *F. poae* DNA ≥0.1 pg/ng, and no samples had a concentration of 1 pg fungal DNA per ng plant DNA or higher.

The following ranking of *Fusarium* species in Norwegian oats and spring wheat was made based on the DNA concentrations of the selected *Fusarium* spp. analysed in this survey (from high to low): *F. graminearum* = *F. avenaceum* = *F. langsethiae* > *F. poae* > *F. culmorum* (oats); *F. graminearum* = *F. avenaceum* > *F. culmorum* > *F. langsethiae* = *F. poae* (spring wheat).

Occurrence of mycotoxins in oats and spring wheat

Of the in total 18 different *Fusarium*-mycotoxins analysed in this study, DON, 3-ADON, ENNs, BEA, MON, and antibiotic Y were frequently detected in grain samples of oats and spring wheat. In addition, HT-2 and T-2 occurred commonly in oats (Table 1). Mycotoxin levels ≥100 µg/kg were frequently detected for DON (wheat and oats), 3-ADON (oats), ENNs (wheat and oats), MON (wheat and oats), antibiotic Y (oats), and HT-2+T-2 (oats) (Figure 2). About 10% of the oat and spring wheat samples contained ZEA levels ≥100 µg/kg. The maximum concentration detected for the different mycotoxins varied from not detected for the least common mycotoxins (such as the fumonisins), to above the highest standard points in the analyses for others (ENNs).

DON was detected (above LOD) in 90% of the oat samples and in 92% of the spring wheat samples (Table 1), and DON concentrations ≥100 µg/kg were registered in 66% of the oat samples and in 70% of the spring wheat samples (Figure 2). A maximum DON concentration of 30,000 µg/kg was recorded in oats, whereas the maximum DON concentration registered in the spring wheat samples was 16,000 µg/kg. Median DON-concentrations were 360 µg/kg in oats, and 290 µg/kg in the spring wheat samples analysed. 20% of the oat samples had DON concentrations above legislation limits in grains intended for human consumption (1,750 µg DON/kg (EC, 2006). The legislation limit of 1,250 µg/kg was exceeded in 9% of the spring wheat samples.

Detectable amounts of 3-ADON were most commonly recorded in oats, where 71% of the samples had 3-ADON concentrations ≥LOD. In spring wheat, 40% of the samples had 3-ADON concentrations ≥LOD (Table 1). 3-ADON concentrations ≥100 µg/kg were registered in 35% of the oat samples and in 5% of the spring wheat samples (Figure 2). Since less than half of the grain samples of oat and spring wheat had 3-ADON concentrations exceeding LOQ, median 3-ADON values could not be calculated. A maximum 3-ADON concentration of 5,100 µg/kg was recorded in oats. In contrast, a maximum 3-ADON concentration of only 540 µg/kg was recorded in spring wheat. In oats samples with 3-ADON concentrations above 100 µg/kg, the relative content of 3-ADON to DON varied between 5 and 100%, with an average of 14%. Due to the low amount of spring wheat samples containing 3-ADON above 100 µg/kg, the relative content of 3-ADON was not calculated. In oats, the median concentrations of DON + 3-ADON were below 500 µg/kg in most years except the last two years of the survey, 2008 (1,305 µg/kg) and 2009 (2,275 µg/kg). In spring wheat, the median concentrations of DON + 3-ADON were below 500 µg/kg in most years except the last year of the survey, 2009 (978 µg/kg). The content of 15-ADON was not analysed.

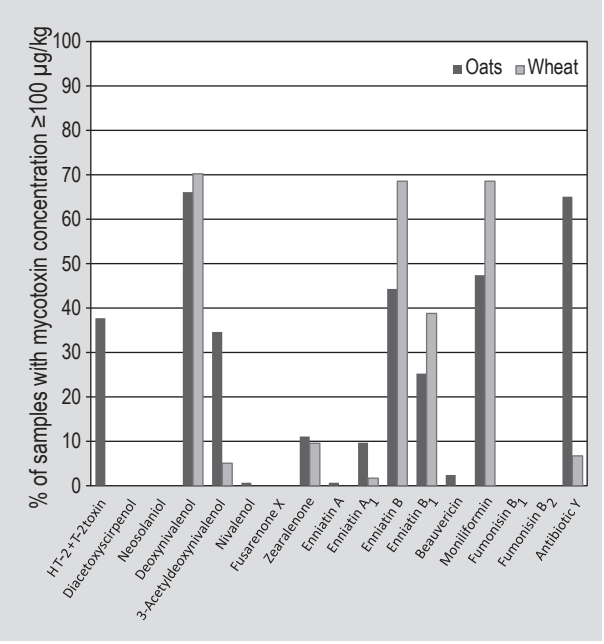

Figure 2. Mycotoxin contents in grain samples of spring wheat (n=178) and oats (n=289) collected from farmers' fields in SouthEast Norway during a 6-year period from 2004-2009. The percentage of samples with mycotoxin concentrations ≥100 µg/kg are shown for most mycotoxins, except moniliformin, fusarenon-X, neosolaniol ,where the percentage of samples above the limit of detection values are shown. For antibiotic Y, the percentage of samples with mycotoxin concentrations above the limit of quantification values are shown for spring wheat.

ZEA was detected in 21% of the oat samples and in 39% of the spring wheat samples (Table 1). Concentrations of ZEA above the legislation limits in unprocessed wheat and oats intended for human consumption (100 µg/kg; EC, 2006) were registered in 11% of the oat samples and in 10% of the spring wheat samples (Figure 2). A maximum ZEA concentration of 2,300 µg/kg was detected in oats. Furthermore, the maximum ZEA concentration recorded in spring wheat was 350 µg/kg. The regression equation between the natural logarithm of DON + 3-ADON and ln ZEA concentrations in oats and spring wheat was significant ($R^2_{(adj)}$=36%, P<0.001). In five samples of oats and seven samples of spring wheat with DON levels below legislation limits for grains intended for human consumption (1,250 µg/kg in wheat and 1,750 µg/kg in oats), ZEA concentrations above the legislation limits for human consumption (100 µg/kg) were detected. The concentration of ZEA in these samples was up to 570 µg/kg in oats and 240 µg/kg in spring wheat.

HT-2 and T-2 were detected in 67 and 88% of the oat grain, respectively (Table 1). In spring wheat, HT-2 or T-2 were only detected in 2% of the samples. 38% of the oat samples contained a sum of HT-2 and T-2 of 100 µg/kg or more (Figure 2). The maximum level of HT-2 + T-2 recorded in oats was 2,433 µg/kg, while the calculated median sum of HT-2 + T-2 in these 289 oat samples was 59 µg/kg. The yearly median concentrations of HT-2 + T-2 varied from 170-279 µg/kg during the six-year period. 6% of the oat samples contained a sum of HT-2 + T-2 above 1000 µg/kg.

ENNs were detected in 62-95% of the oat samples and 85-100% of the spring wheat samples (Table 1). ENN-B was most frequently detected. In oats, the percentages of grain samples with a mycotoxin content ≥100 µg/kg were: 1% for ENN-A, 10% for ENN-A_1, 44% for ENN-B, and 25% for ENN-B_1 (Figure 2). In spring wheat, the percentages of grain samples with a mycotoxin content ≥100 µg/kg were: 0% for ENN-A, 2% for ENN-A_1, 69% for ENN-B, and 39% for ENN-B_1 (Figure 2), respectively. No median values were calculated due to concentrations of enniatins exceeding the highest standard points used in the analysis for many of the samples (see material and methods for details).

BEA was detected in 81% of the oat samples and in 65% of the spring wheat samples (Table 1). Concentrations of BEA ≥100 µg/kg were registered in only 2% of the oat samples, whereas none of the spring wheat samples had concentrations of BEA ≥100 µg/kg (Figure 2). MON was detected in 47% of the oat samples and in 69% of the spring wheat samples (Table 1 and Figure 2). Concentrations of MON ≥LOQ were registered in 3% of the oat samples (LOQ=400 µg/kg). In comparison, 8% of the spring wheat samples had concentrations of MON ≥400 µg/kg. Furthermore, concentrations of MON ≥LOQ were registered in 11% of the spring wheat samples (LOQ=300 µg/kg, Table 1).

Nivalenol and diacetoxyscirpenol were only detected in 1% of the oat samples, and neither of these mycotoxins was detected in spring wheat (Table 1 and Figure 2). The mycotoxins fusarenon-X, neosolaniol, fumonisin B_1 and fumonisin B_2 were never detected.

The association between mycotoxin content and *Fusarium* spp.

A significant and high positive association ($R^2_{(adj)}$=68.6%, P<0.001) was found between concentration of DON + 3-ADON (ln(µg mycotoxins/kg grain)) and *F. graminearum* (ln(pg DNA/ng plant DNA + 1)) in oat and wheat grains (Figure 3). On the contrary, no significant association was found between DON + 3-ADON concentrations and *F. culmorum* DNA in these samples. Still, the binary logistic regression analysis, fitted model (Supplementary Equation S1), showed that the probability of detecting DON at a level ≥100 µg/kg was highly related to the occurrence of both *F. graminearum* and *F. culmorum* DNA (Goodman-Kruskal Gamma = 0.85). The highest predicted probabilities (P=0.99 for oats and 1.00 for wheat) of DON levels ≥100 µg/kg were

calculated for grain samples where both *F. culmorum* and *F. graminearum* DNA levels were ≥0.1 pg/ng (Table 2). High probabilities (*P*=0.82-0.92) of DON levels ≥100 µg/kg were also calculated if either *F. graminearum* or *F. culmorum* DNA were present at concentrations ≥0.1 pg/ng. None of the other *Fusarium* spp. had a significant influence on the DON concentration in the model.

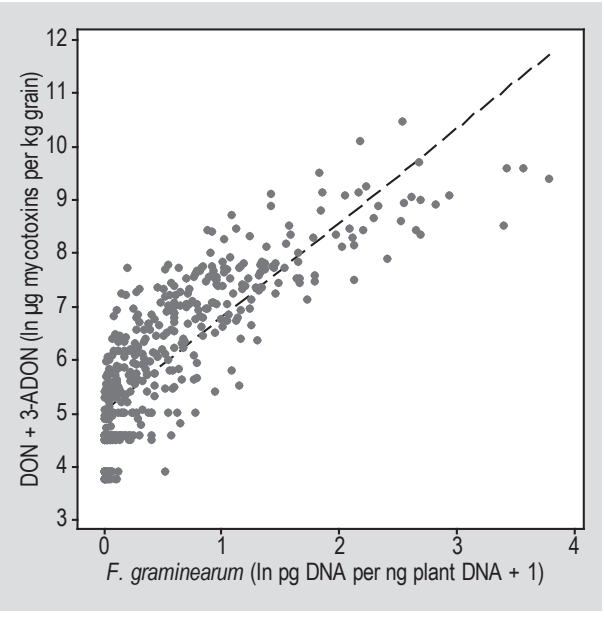

Figure 3. The association between ln(µg deoxynivalenol (DON) + 3-acetyldeoxynivalenol (3-ADON)/kg grain) and ln(*Fusarium graminearum* pg DNA/ng plant DNA + 1) in spring wheat (n=178) and oat (n=284) grains from farmers' fields in South East of Norway during years 2004-2009. $R^2_{(adj)}$=68.6%.

According to the fitted binary logistic regression model (Supplementary Equation S2), the probability of detecting ZEA was related to the occurrence of DNA from *F. graminearum*, *F. culmorum* and *F. avenaceum* in the grain sample. Generally, the probability of ZEA levels from 100 µg/kg and above seems to be low. Grain samples had the highest predicted probability (*P*=0.39) to contain ZEA levels from 100 µg/kg and above, if both *F. graminearum*, *F. culmorum* and *F. avenaceum* DNA levels were ≥0.1 pg per ng plant DNA (Goodman-Kruskal Gamma = 0.82, Table 3). According to the model, neither cereal species nor the other *Fusarium* spp. that were quantified, had a significant influence on the ZEA concentration.

A significant positive association ($R^2_{(adj)}$=51%, *P*<0.001) was found between ln(HT-2 + T-2) and ln(*F. langsethiae* DNA + 1) content of oat grains (Figure 4). According to the fitted binary logistic regression model (Supplementary Equation S3) (Goodman-Kruskal Gamma = 0.72), oat samples had the highest predicted probability (*P*=0.65) to contain HT-2 + T-2 levels from 100 µg/kg and above, if DNA levels of *F. langsethiae* were ≥0.1 pg/ng combined with DNA levels of *F. graminearum* <0.1 pg/ng (Table 4). None of the other *Fusarium* spp. had a significant influence on the concentration of HT-2 + T-2. As HT-2 + T-2 concentrations ≥100 µg/kg were only detected in oats, no binary logistic regression analysis was done in wheat.

Table 2. The predicted probability (*P*) for a grain sample to contain deoxynivalenol (DON) ≥100 µg/kg, grouped according to occurrence of *Fusarium graminearum* or *Fusarium culmorum*.

P (DON ≥100 µg/kg)[a]	*F. graminearum* = 0[b]	*F. graminearum* = 1[b]
F. culmorum = 0	0.17 (oats); 0.28 (wheat)	0.86 (oats); 0.92 (wheat)
F. culmorum = 1	0.82 (oats); 0.90 (wheat)	0.99 (oats); 1.00 (wheat)

[a] Calculation based on Supplementary Equation S1.
[b] 0 = fungal DNA <0.1 pg/ng plant DNA; 1 = fungal DNA ≥0.1 pg/ng plant DNA.

Table 3. The predicted probability (*P*) for a grain sample to contain zearalenone (ZEA) ≥100 µg/kg, grouped according to occurrence of *Fusarium graminearum*, *Fusarium culmorum* or *Fusarium avenaceum*.

P (ZEA ≥100 µg/kg [a])	*F. graminearum* = 0[b]	*F. graminearum* = 1[b]
F. culmorum = 0	0.00 (*F. avenaceum* = 0); 0.01 (*F. avenaceum* = 1)	0.04 (*F. avenaceum* = 0); 0.18 (*F. avenaceum* = *1*)
F. culmorum = 1	n.c. (*F. avenaceum* = 0); 0.03 (*F. avenaceum* = 1)	0.1 (*F. avenaceum* = *0*); 0.39 (*F. avenaceum* = 1)

[a] Calculation based on Supplementary Equation S2.
[b] 0 = fungal DNA <0.1 pg/ng plant DNA; 1 = fungal DNA ≥0.1 pg/ng plant DNA; n.c. = not calculated.

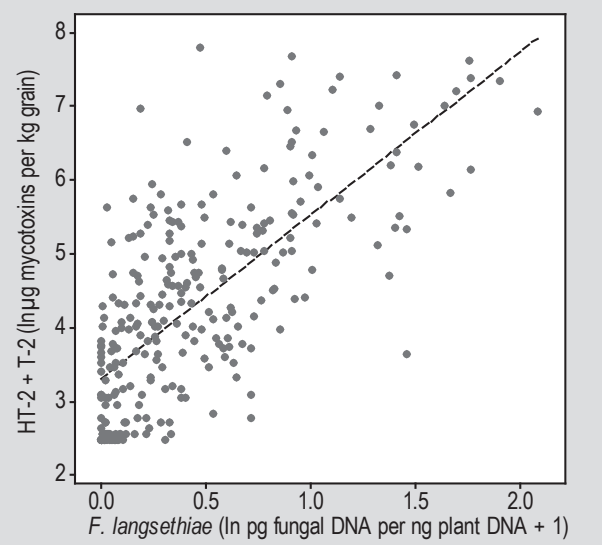

Figure 4. The association between ln(µg HT-2 + T-2 toxins/kg grain) and ln(*Fusarium langsethiae* pg DNA/ng plant DNA + 1) in cereal grains of oats (n=277) from farmers' fields in SouthEast of Norway during years 2004-2009. $R^2_{(adj)}$=51%.

The binary logistic regression analysis showed that the likelihood of detecting ENN-B at a level of 100 µg/kg or above, was significantly related to cereal species and content of *F. avenaceum* DNA (Goodman-Kruskal Gamma = 0.69, Supplementary Equation S4). According to the fitted model, the highest predicted probability (*P*=0.87 and 0.59 for wheat and oats, respectively) to contain ENN-B levels from 100 µg/kg and above was found for samples where the DNA levels of *F. avenaceum* were ≥0.1 pg/ng plant DNA (Table 5). Whereas the probability of detecting ENN-B levels from 100 µg/kg and above was reduced in samples where DNA levels of *F. avenaceum* were <0.1 pg/ng (*P*=0.46 and 0.15 for wheat and oats, respectively). Due to non-accuracy in the data obtained for ENNs at high concentrations, no regression analysis were performed for ENNs content versus fungal species.

The binary logistic regression analysis showed that the probability of detecting antibiotic Y at a level of 100 µg per kg or above, was significantly related to cereal species and content of *F. avenaceum* DNA (Goodman-Kruskal Gamma = 0.33, Supplementary Equation S5). According

Table 4. The predicted probability (*P*) for an oat grain sample to contain HT-2 + T-2 toxin ≥100 µg/kg, grouped according to the DNA content of *Fusarium langsethiae* and *Fusarium graminearum*.

***P* (HT-2 + T-2 ≥100 µg/kg)[a]**	***F. langsethiae* = 0[b]**	***F. langsethiae* = 1[b]**
F. graminearum = 0	0.06	0.65
F. graminearum = 1	0.03	0.46

[a] Calculation based on Supplementary Equation S3.
[b] 0 = fungal DNA <0.1 pg/ng plant DNA; 1 = fungal DNA ≥0.1 pg/ng plant DNA.

Table 5. The predicted probability (*P*) for a cereal grain sample to contain a concentration of enniatin B (ENN-B) ≥100 µg/kg, grouped according to cereal species and content of *Fusarium avenaceum*.

***P* (ENN-B ≥ 100 µg/kg)[a]**	***F. avenaceum* = 0[b]**	***F. avenaceum* =1[b]**
	0.15 (oats); 0.46 (wheat)	0.59 (oats); 0.87 (wheat)

[a] Calculation based on Supplementary Equation S4.
[b] 0 = fungal DNA <0.1 pg per ng plant DNA; 1 = fungal DNA ≥0.1 pg/ng plant DNA.

Table 6. The predicted probability (*P*) for a cereal grain sample to contain a concentration of antibiotic Y ≥100 µg/kg, grouped according to cereal species and content of *Fusarium avenaceum*.

***P* (antibiotic Y ≥100 µg/kg)[a]**	***F. avenaceum* = 0[b]**	***F. avenaceum* =1[b]**
	0.52 (oats); 0.65 (wheat)	0.71(oats); 0.81(wheat)

[a] Calculation based on Supplementary Equation S5.
[b] 0 = fungal DNA <0.1 pg/ng plant DNA; 1 = fungal DNA ≥0.1 pg/ng plant DNA.

to the model, the highest predicted probability (P=0.81) to contain antibiotic Y levels from 100 µg/kg and above was found for wheat samples where the DNA levels of *F. avenaceum* were ≥0.1 pg/ng plant DNA (Table 6).

When studying the relationship between different *Fusarium* species, low positive associations ($R^2_{(adj)}$=23%, P<0.001), was found between ln(*F. avenaceum* DNA + 1) and ln(*F. graminearum* DNA + 1) in oats and wheat, and between ln(*F. avenaceum* DNA + 1) and ln(*F. poae* DNA + 1) in oats ($R^2_{(adj)}$=21.6%, P<0.001).

No association was detected between the concentration of ln(*F. langsethiae* DNA +1) and ln(*F. graminearum* DNA + 1) ($R^2_{(adj)}$=0%, P<0.335) in oats (Figure 5). Likewise, no association was detected between the concentration of ln(HT-2 + T-2) and ln(DON + 3-ADON) (R^2_{adj}=0.4%, P=0.135) in oats (Figure 6). Furthermore, no significant associations were detected between DNA content of *F. langsethiae* versus *F. avenaceum* or *F. poae* in oats, but a weak association ($R^2_{(adj)}$=3.2%, P=0.001) was found between ln(*F. poae* DNA + 1) and ln(*F. graminearum* DNA + 1) in oats.

4. Discussion

Through studies of Norwegian oat and wheat grain collected in the period from 2004-2009, we identified *F. graminearum* as the main DON producer in oats and spring wheat, whereas *F. langsethiae* was identified as the main HT-2 and T-2-producer in oats. No association was detected between the concentration of *F. langsethiae* DNA and *F. graminearum* DNA or between the mycotoxins produced by these two fungal species. *F. avenaceum* and its associated mycotoxins were highly prevalent in both oats and spring wheat.

Fusarium graminearum, Fusarium culmorum and associated mycotoxins

We detected concentrations of *F. graminearum* DNA ≥0.1 pg per ng plant DNA in 69% of the oat samples and in 62% of the spring wheat samples, which corresponded well with DON concentrations ≥100 µg/kg in more than 60% of these samples. The significant positive association between DON + 3-ADON and *F. graminearum* DNA content, in contrast to no association between *F. culmorum* and DON + 3-ADON in our samples, identifies *F. graminearum* as the most important DON + 3-ADON producer in Norwegian grain. Similarly, a high correlation between DON concentration and *F. graminearum* infestation

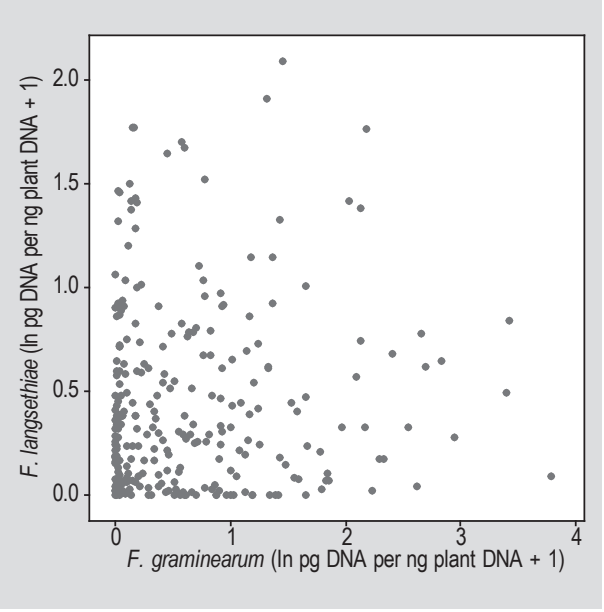

Figure 5. The association between ln(*Fusarium langsethiae* pg DNA/ng plant DNA +1) and ln(*Fusarium graminearum* pg DNA/ng plant DNA +1) in oats (n=277) from farmers' fields in South East of Norway during years 2004-2009. No significant relationship was observed.

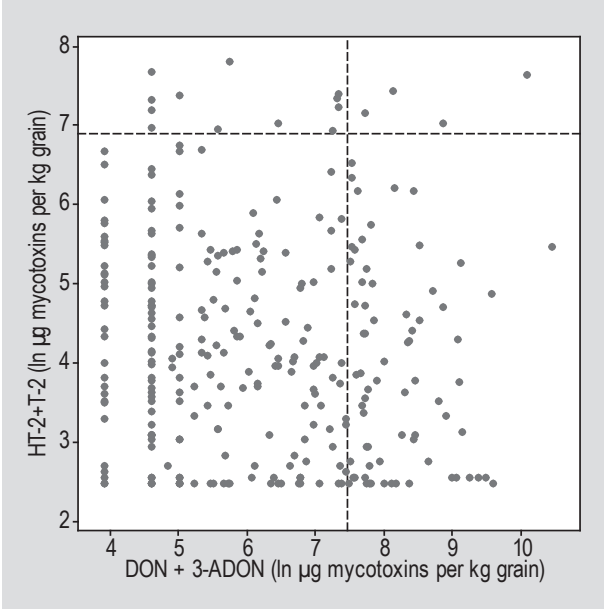

Figure 6. The association between ln(µg HT-2 + T-2 toxins/kg grain) and ln(µg deoxynivalenol (DON) + 3-acetyl deoxynivalenol (3-ADON)/kg grain) in oats (n=289) from farmers' fields in South East of Norway during years 2004-2009. The dotted lines indicate EC legislation or indicative limit for the content of DON (1,750 ug/kg) or HT-2 + T-2 (1000 ug/kg), respectively, in unprocessed oats intended for human consumption. No significant relationship was observed.

was also registered in Norwegian barley, oats and wheat harvested in years 2002-2004 (Bernhoft *et al.*, 2010).

Likewise, reports from several other European countries indicate an increased prevalence of *F. graminearum* during the most of the last twenty years (Fredlund *et al.*, 2008; Nielsen *et al.*, 2011; Waalwijk *et al.*, 2004; Xu *et al.*, 2005). This is in contrast to studies performed during the 1990's where *F. culmorum* generally was regarded as the main DON producer in Norwegian cereals, and *F. graminearum* was registered at low frequencies (Henriksen and Elen, 2005; Kosiak *et al.*, 2003). Our binary regression analysis confirmed that *F. culmorum* still can be an important DON producer in grain samples where *F. graminearum* is detected at low levels. Altogether, data from Bernhoft *et al.* (2010) and our data confirm *F. graminearum* as the main DON producer in Norwegian cereals. 15-ADON was not analysed in our study. However, isolates with the 15-ADON chemotype have recently been detected in Norway (Aamot *et al.*, 2015). Thus, it needs to be considered whether 15-ADON analysis should be included in future surveys.

20% of the oat samples and 9% of the spring wheat samples in our study had a DON concentration above legislation limits for unprocessed oats and wheat intended for human consumption. Furthermore, the calculated median DON values in oats and spring wheat for 2004-2009, agrees well with levels detected in Swedish oats during 2010 and 2011 (Fredlund *et al.*, 2013). The yearly median values of DON + 3-ADON increased during our study, and in 2008 and 2009 the median values in oats were alarmingly high. Similarly, high median DON levels in oats were also detected in a survey of Norwegian cereals during 2010-2012 (Uhlig *et al.*, 2013). This trend corresponds with data from the Norwegian Scientific Committee for Food Safety (2013). Increasing levels of seed infestation with *Fusarium* spp. have been recorded in Norwegian oats, barley and spring wheat during the last ten years (Norwegian Scientific Committee for Food Safety, 2013). This increase probably includes a considerable amount of *F. graminearum*, which may be responsible for the increased DON-levels recorded in recent studies. In contrast, median DON values were much lower (36 µg/kg in oats and 51 µg/kg in wheat) in a survey performed in Norway prior to our study, 2002-2004 (Bernhoft *et al.*, 2010). Since 2011, all oat grain lots in Norway have been analysed for DON at delivery, in addition to wheat grain lots intended for food. Our data shows that this routine analysis is needed to sort out highly contaminated grain lots.

11% of the oat samples and 10% of the wheat samples had a ZEA content above legislation limits for unprocessed cereals intended for human consumption (100 µg/kg). The highest risk of exceeding these levels was found for grain samples that contained *F. graminearum, F. culmorum* as well as *F. avenaceum*. Both *F. graminearum* and *F. culmorum* are known ZEA producers. The association between occurrence of *F. avenaceum* and ZEA content may be a result of a similar influence of environmental conditions on growth of *F. avenaceum* as for the production of ZEA (Edwards, 2011; Xu *et al.*, 2008). Alternatively, this may indicate that *F. avenaceum* somehow stimulates the mycotoxin production of ZEA producers. A maximum ZEA concentration of 2,300 µg/kg oats was detected in a sample in our study. Similar high levels of ZEA, were detected in Swedish oats in 2011 (Fredlund *et al.*, 2013), and in a Norwegian study describing samples from 2010-2012 (Uhlig *et al.*, 2013). Both the occurrence and levels of ZEA in grain samples in our and other recent studies were higher compared to those reported in earlier years (Bernhoft *et al.*, 2010). High ZEA levels seem connected to delayed wet harvests (Edwards, 2011). In the period of our survey, high precipitation was recorded prior to harvest in some years, which may explain the increased occurrence of ZEA. Despite a positive association between DON and ZEA levels, we identified some grain samples with DON levels below and ZEA levels above the limits set for unprocessed cereals intended for human consumption (1,250 µg/kg in wheat and 1,750 µg/kg in oats for DON, and 100 µg/kg grain for ZEA). Likewise, only a moderate correlation was detected between DON and ZEA in another study of Norwegian cereals during 2010-2012 (Uhlig *et al.*, 2013). The discrepancy between DON and ZEA levels in cereals might be partly explained by a leakage of DON at high rainfall (Gautam and Dill-Macky, 2012). Alternatively, it might be caused by differences in modes of expression of the genes involved in the biosynthesis of these secondary metabolites. Thus, a survey of ZEA content of Norwegian oats in years where DON levels are generally high, or in years with a wet harvest, should be considered.

Fusarium langsethiae and associated mycotoxins

F. langsethiae was indeed detected more frequently in oats than in spring wheat. A higher occurrence of *F. langsethiae* in Norwegian oats compared to spring wheat was also documented in the period 2002-2004 (Bernhoft *et al.*, 2010). Furthermore, positive associations between HT-2 + T-2 and *F. langsethiae,* as detected in our study, are reported in several studies of oats from Northern Europe (Bernhoft *et al.*, 2010; Edwards *et al.*, 2012; Fredlund *et al.*, 2010, 2013). Despite the high association detected between HT-2 + T-2 content and *F. langsethiae* DNA in oats in our study, the level of *F. langsethiae* could not account for more than 51% of the total variation in HT-2 + T-2. This is most likely influenced by the oats samples with high amounts of HT-2 and T-2 and low levels of *F. langsethiae* DNA. Mycotoxin content does not necessarily correspond to the amount of the respective mycotoxin producers present, as *Fusarium* mycotoxin production can be influenced by several factors, such as other fungal species present, fungicides used, and environmental conditions (Xu and

Nicholson, 2009). Since the grain samples were harvested in various seasons and locations, it is likely that HT-2 + T-2 levels were differently influenced by environmental factors. Furthermore, the occurrence of *F. sporotrichioides* might have influenced the HT-2 + T-2 levels. This fungus is detected in Norwegian cereals though at considerable lower levels than *F. langsethiae* (Kosiak *et al.*, 2003), but was not analysed in oats in our study. Some of the HT-2 and T-2 may have been produced by *F. poae* (Thrane *et al.*, 2004), as this fungal species was registered in more than 30% of the oat samples. However, this is less likely, as no significant effect of *F. poae* was detected in the binary logistic regression analysis of HT-2 + T-2 content in oats. The high occurrence and the association between *F. langsethiae* and HT-2 + T-2 in oats, identifies *F. langsethiae* as the main HT-2 + T-2 producer in Norwegian oats.

The median values of HT-2 and T-2 detected in our study of oats during 2004-2009 were in line with surveys performed in Norway the preceding years (Bernhoft *et al.*, 2010), and in Sweden the following years (Fredlund *et al.*, 2013). Somewhat higher median values were detected in Norwegian oats in 2010-2012 (Uhlig *et al.*, 2013). In contrast, the concentrations measured in our study were far below the median values reported for HT-2 and T-2 in UK oats (Edwards, 2009). We detected a sum of HT-2 + T-2 above the indicative level (1000 µg/kg) set by the European Commission (EC, 2013) in 6% of the oat samples, which is a higher quantity than reported for Swedish oats from the 2010 and 2011 harvest (Fredlund *et al.*, 2013). As the indicative level for HT-2 + T-2 is occasionally exceeded, more attention should be focused on these potent mycotoxins.

No positive association was detected between the concentration of HT-2 + T-2 and DON + 3-ADON, nor between its producers, in our oat samples. This is in accordance to other studies (Bernhoft *et al.*, 2010; Edwards, 2009; Fredlund *et al.*, 2010), and may indicate that the HT-2 and T-2 producers have different environmental requirements and/or epidemiology than the DON producers (Edwards, 2009; Xu *et al.*, 2014). Based on the inverse relationship detected between HT-2 and DON in UK oats, Edwards (2009) suggested a mutual exclusion between DON and HT-2 producers. We observed a similar non-linear negative association between HT-2 + T-2 and DON + 3-ADON in our data (results not shown), although this association was not evident in our regression analysis based on natural logarithm transformed data. However, in the binary logistic regression model, the occurrence of *F. graminearum* had a negative impact on the concentration of HT-2 + T-2. In contrast, the occurrence of *F. langsethiae* did not influence the DON levels. This could indicate that *F. graminearum* is a stronger competitor than *F. langsethiae*. Since there is no positive association between HT-2 + T-2 and DON + 3-ADON levels, routine analyses of HT-2 and T-2 in oats should be considered to sort out grain lots highly contaminated with these toxins.

Fusarium avenaceum, Fusarium poae and associated mycotoxins

F. avenaceum was one of the most common fungi observed in both oats and wheat in our study. A high occurrence of *F. avenaceum* in Norwegian cereals was also documented in previous studies (Bernhoft *et al.*, 2010; Henriksen and Elen, 2005; Kosiak *et al.*, 2003), as well as in surveys performed in Sweden (Fredlund *et al.*, 2013). The majority of the grain samples also contained ENNs, and the content of *F. avenaceum* was highly associated with ENN-B levels, suggesting *F. avenaceum* as the main ENN-B producer in these samples. Wheat grain samples with a *F. avenaceum* DNA content above 0.1 pg/ng had a higher probability to contain ENN-B above 100 µg/kg compared to oats grain lots with similar *F. avenaceum* content, suggesting a relatively higher content of this mycotoxin versus the producer *F. avenaceum* in wheat than in oats. Oat grains were analysed with hull. This difference in relative content of ENNs versus *F. avenaceum* in wheat and oats, may be caused by saprophytic growth, but not necessary accompanied by mycotoxin production, in the outermost parts of the oat grain (hull). Moreover, as mentioned above, mycotoxin production can be influenced by several factors such as other fungal species, fungicides and environmental conditions (Xu and Nicholson, 2009). ENNs are also produced by *F. tricinctum,* a *Fusarium* species often found in Norwegian grains (Bernhoft *et al.*, 2010; Henriksen and Elen, 2005; Kosiak *et al.*, 2003), but not included in our analysis. Anyway, our data and other Norwegian studies (Bernhoft *et al.*, 2010; Henriksen and Elen, 2005; Kosiak *et al.*, 2003) indicate *F. avenaceum* as an important ENN producer in Norwegian cereals.

BEA was detected in more than half of the grain samples, and a higher frequency was registered in grain samples of oats compared to spring wheat. This is in accordance with previous reports (Uhlig *et al.*, 2006). In our study, BEA content corresponded with the higher occurrence of the BEA producers *F. poae* and (to a lesser extent) *F. avenaceum,* registered in oats as compared to spring wheat (Figure 2). Similar to our results, the content of *F. poae* was associated with BEA content of oats in a Swedish survey (Fredlund *et al.*, 2013). The BEA in our grain samples could also have been produced by *F. equiseti,* a *Fusarium* species sometimes recorded in Norwegian grains (Bernhoft *et al.*, 2010; Kosiak *et al.*, 2003), but not included in our study. Our data indicate that BEA producers are more commonly occurring in Norwegian oats compared to spring wheat.

MON was detected in a relatively high percentage of the samples, despite the high LOD values. We detected this toxin in a higher proportion of spring wheat compared

to oat samples, and this is also supported by other studies (Uhlig *et al.*, 2004, 2013). Higher maximum levels were observed in our study compared to studies of grains in Northern Europe during the same period (Uhlig *et al.*, 2007, 2013). However, this can be an effect of differences in sampling, since our samples represent single farmers' fields compared to samples representing harvests from several fields (dilution effect). Despite the relatively higher occurrence of MON in wheat compared to oats, the MON producer *F. avenaceum* was equally present in both cereal species. Alternatively, the higher occurrence and concentrations of MON in spring wheat might be caused by the presence of *F. tricinctum*, a MON producer recorded in Norwegian grain (Bernhoft *et al.*, 2010; Henriksen and Elen, 2005; Kosiak *et al.*, 2003), but not included in our study. Anyway, ours and other Norwegian studies (Bernhoft *et al.*, 2010; Henriksen and Elen, 2005; Kosiak *et al.*, 2003; Uhlig *et al.*, 2013) indicate that *F. avenaceum* and the mycotoxins produced by this fungal species are commonly occurring in Norwegian cereals.

5. Conclusions

To conclude, our studies confirm the recently observed shift in the relative species prevalence towards more *F. graminearum,* rather than *F. culmorum* in Norwegian cereals. Based on the results from the current study and other recent studies, *F. graminearum* can now be regarded as the main DON producer in Norwegian oats and spring wheat. Furthermore, *F. langsethiae* can be regarded as the main HT-2 and T-2 producer in oats. No association was detected between the content of HT-2 + T-2 and DON + 3-ADON, nor between their producers in oats. However, the risk of elevated HT-2 + T-2 levels was reduced in *F. graminearum* contaminated samples. As high concentrations of DON or HT-2 and T-2 were detected in some grain lots, screening of DON at delivery of grain lots to the elevators should be continued, and extended to analyses of HT-2 and T-2 contents in oats. As ENNs were detected in nearly all grain samples, the implications of ENN contamination of cereals needs further attention.

Supplementary material

Supplementary material can be found online at http://dx.doi.org/10.3920/WMJ2015.2003.

Equation S1. Fitted model to predict the probability for a cereal grain sample to contain a concentration of deoxynivalenol ≥100 µg/kg.

Equation S2. Fitted model to predict the probability for a cereal grain sample to contain a concentration of zearalenone ≥100 µg/kg.

Equation S3. Fitted model to predict the probability for a cereal grain sample to contain a concentration of HT-2 + T-2 toxin ≥100 µg/kg.

Equation S4. Fitted model to predict the probability for a cereal grain sample to contain a concentration of enniatin B ≥100 µg/kg.

Equation S5. Fitted model to predict the probability for a cereal grain sample to contain a concentration of antibiotic Y ≥100 µg/kg.

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