

Influence of agronomic and climatic factors on *Fusarium* infestation and mycotoxin contamination of cereals in Norway

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A total of 602 samples of organically and conventionally grown barley, oats and wheat was collected at grain harvest during 2002-2004 in Norway. Organic and conventional samples were comparable pairs regarding cereal species, growing site and harvest time, and were analysed for Fusarium mould and mycotoxins. Agronomic and climatic factors explained 10–30% of the variation in Fusarium species and mycotoxins. Significantly lower Fusarium infestation and concentrations of important mycotoxins were found in the organic cereals. The mycotoxins deoxynivalenol (DON) and HT-2 toxin (HT-2) constitute the main risk for human and animal health in Norwegian cereals. The impacts of various agronomic and climatic factors on DON and HT-2 as well as on their main producers F. graminearum and F. langsethiae and on total Fusarium were tested by multivariate statistics. Crop rotation with non-cereals was found to reduce all investigated characteristics significantly – mycotoxin concentrations as well as various Fusarium infestations. No use of mineral fertilisers and herbicides was also found to decrease F. graminearum, whereas lodged fields increased the occurrence of this species. No use of herbicides was also found to decrease F. langsethiae, but for this species the occurrence was lower in lodged fields. Total Fusarium infestation was decreased with no use of fungicides or mineral fertilisers, and with crop rotation, as well as by using herbicides and increased by lodged fields. Clay and to some extent silty soils seemed to reduce F. graminearum in comparison with sandy soils. Concerning climate factors, low temperature before grain harvest was found to increase DON; and high air humidity before harvest to increase HT-2. F. graminearum was negatively correlated with precipitation in July but correlated with air humidity before harvest. F. langsethiae was correlated with temperature in July. Total Fusarium increased with increasing precipitation in July. Organic cereal farmers have fewer cereal intense rotations than conventional farmers. Further, organic farmers do not apply mineral fertiliser or pesticides (fungicides, herbicides or insecticides), and have less problem with lodged fields. The study showed that these agronomic factors were related to the infestation of Fusarium species and the concentration of mycotoxins. Hence, it is reasonable to conclude that farming system (organic versus conventional) impacts Fusarium infestation, and that organic management tends to reduce Fusarium and mycotoxins. However, Fusarium infestation and mycotoxin concentrations may be influenced by a range of factors not studied here, such as local topography and more local climate, as well as cereal species and variety.

Keywords: mycology; GC/MS; mycotoxins – trichothecenes; cereals

Introduction

Fusarium is a common mould in cereal fields. The infestation (superficial contamination) and infection of Fusarium in cereals are of great concern worldwide – as plant pathogens and producers of mycotoxins. Several factors influence the occurrence of Fusarium in the soil and the infestation and infection it generates in cereal plants. Geographical factors including climate are of superior importance for the occurrence of Fusarium and for the pattern of infestation by various Fusarium species (Placinta et al. 1999; Miller 2008). Under Norwegian conditions, mycotoxins have been a problem, especially in oats (Avena sativa L.), but also in spring wheat (Triticum aestivum L.) and barley

(Hordeum vulgare L.). By less favourable weather conditions at grain harvest, significant amounts of cereals may become unsuited for animal fodder, especially for pigs which are particularly sensitive. The problem has increased over time, and several trends in agriculture such as more humid growing seasons, soil compaction (heavier machinery), specialised cash cropping (lack of crop rotation), and reduced soil tillage combined with herbicide spraying may contribute to explain this increase. The concentrations and patterns of mycotoxins produced are dependent on the Fusarium species as well as the cereal species (Foroud and Eudes 2009; Bernhoft et al. 2010; Hofgaard et al. 2010).

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Several studies have found no significant influence of farming system on the mycotoxin levels in cereals. However there is also a range of papers reporting lower Fusarium mycotoxins in organically than in conventionally produced cereals (reviewed in Köpke et al. 2007; Bernhoft et al. 2010). Bernhoft et al. (2010) reported significantly lower Fusarium infestation and levels of the mycotoxins deoxynivalenol (DON), HT-2 toxin and T-2 toxin in samples of organic cereals compared with paired samples of conventional cereals (n = 602). These mycotoxins are regarded as those of major concern for human and animal health in Northern Europe (Edwards et al. 2009). In the present study, the differences between organic and conventional cereal samples reported by Bernhoft et al. (2010) are further elaborated, based on multivariate statistical analysis. The aim was to reveal which agronomic and climatic factors are most closely related to the Fusarium infestation and mycotoxin concentrations, and thereby explain the reasons for the significant impact of the farming system.

Materials and methods

Sampling of cereals and information at the farms

Samples of organically and conventionally grown barley, oats and spring wheat were collected shortly after grain harvest (within 4 days) in the autumns of 2002, 2003 and 2004 at farms in the main cereal districts in Norway. Organic and conventional cereals were sampled as pairs of the same species harvested at about the same date (within 2 days) from farms localised close to each other (a maximum of a few kilometres distance). Thus, for the organic and conventional paired samples, the climate and soil conditions should be comparable. Samples of barley and oats were collected in four regions: (1) Akershus and Østfold; (2) Buskerud, Vestfold and Telemark; (3) Hedmark and Oppland, all counties in eastern Norway; and (4) South and North Trøndelag counties in central Norway. Samples of wheat were not collected in region 4, as wheat is rarely produced so far north. Region 3 was not sampled in 2002. Apart from these exceptions, the samples were evenly collected in the various regions. The numbers of paired samples of barley, oats and wheat were 108, 101 and 92, respectively, in total 301 pairs.

The sample collectors were skilled persons from the Norwegian Agriculture Control Authority, the Norwegian Veterinary Institute, and in 2004 also from the Norwegian Agricultural Extension Service. More details on the cereal sampling are given in Bernhoft et al. (2010).

In connection with the sampling, the farmers were interviewed on topics related to their cereal production following an interview guide prepared by the authors

of this paper. Information was recorded each year on the areas grown of the sampled cereal species, cereal variety, main soil type (clay, silt or sand), preceding crop (same cereal species, other cereal species or other crop), fertilisers (no fertiliser, mineral fertiliser, animal manure or other fertilisers), fungicides, herbicides and insecticides (use or no use), use of catch crop (none, grass-clover or other), and estimations about yield level and the percentage of lodged field were collected. Moreover, information on the use of growth regulators (use or no use) was collected in 2003 and 2004, and on soil cultivation (ploughed, harrowed or reduced tillage) was collected in 2004. The moisture of the cereal samples was measured with Wile-55 moisture meter (Farmcomp OY, Vantaa, Finland) in 2003 and 2004.

These data were used as explanatory variables in multivariate tests to reveal the most important factors influencing the *Fusarium* infestation and the mycotoxin contamination of the cereals.

Climate registration

For registration of climate parameters, the weather database of Bioforsk (2011) was used. Data for 2002–2004 were collected from one climate registration station situated centrally in each cereal sampling region. Rakkestad, Sande, Moelv and Frosta were chosen for regions 1, 2, 3 and 4, respectively. The collected data were mean air temperature, mean precipitation and mean relative humidity for July each year and for the last 2-week period before harvest of each cereal sample. These data were selected to reveal if the flowering and grain maturing seasons were dry or humid, as this has a large impact on the Fusarium infestation.

Fusarium and trichothecene analyses

To study the frequency of isolation of *Fusarium* spp. from kernels, 49 kernels were plated out on agar, incubated and identified as described in Bernhoft et al. (2010). No surface treatment was given to the kernels before the plating. The results are given as the percentage of cereal kernels infested with total *Fusarium* and with the identified *Fusarium* species.

Details of grain milling, extraction, purification, derivatisation and quantitatively determination with gas chromatography-mass spectrometry (GC-MS) of the trichothecene mycotoxins are also described in Bernhoft et al. (2010).

Statistical analyses

Based on the conclusion of Bernhoft et al. (2010), total *Fusarium* infestation, infestation of *F. graminearum*

(the main DON producer), infestation of *F. langsethiae* (the main HT-2/T-2 producer), and the concentrations of the toxins DON and HT-2 were included as dependent variables in the statistical multivariate analyses. T-2 was omitted as it is heavily correlated with HT-2 and found at lower concentrations.

For mycological and chemical results below detection limits, the half-value of the detection limits was used in the statistical tests. To examine differences between farming systems, the collected continuous data about area grown, yield level, percentage lodged field, cereal moisture at sampling, as well as the percentage of Fusarium infestation, and the mycotoxin concentrations were statistically analysed for each cereal species by use of the Wilcoxon method. The Kruskal-Wallis method was applied to test significant differences in Fusarium infestation and mycotoxin concentrations between cereals produced in fields that were subject to reduced tillage, harrowing or ploughing irrespective of the farming system. These non-parametric statistical methods were selected due to lack of normal distributions of the data. The statistical significance level was set at p < 0.05.

To illustrate differences between faming systems, collected categorical data about the preceding crop, fertilisers, fungicides, herbicides, insecticides, catch crop and growth regulators are presented without a statistical treatment for comparison between organic and conventional practice. Soil type is included in this presentation to exclude a systematic variation related to farming system for this categorical factor.

For multivariate analysis the percentage infestations of total *Fusarium*, *F. graminearum* and *F. langsethiae* were used directly as respond characteristics, whereas the continuous data on DON and HT-2 concentrations were transformed into groups each of six even intervals and then used as respond characteristics. The explanatory factors used were the categorical variables soil type, preceding crop, fertiliser, fungicide, herbicide, insecticide, catch crop, and the continuous variables share of lodged field and climate data (see above).

A mixed-effects Poisson regression model was used to fit the respond characteristics to the explanatory factors. Region and cereal species were both used as random effects to account for the fact that the means of the respond characteristics differ both between regions as well as between cereal species. The analysis was conducted using library lme4 function glmer (Bates et al. 2011) in R version 2.5.1 (R Development Core Team 2007). Model selection was done based on backward selection and ANOVA tests. Only covariates with p < 0.001 were regarded as significant and included in the final model. The concluded models for each of the respond characteristics are reported with and without climate data as explanatory factors. When two covariates had a correlation coefficient

higher than 0.50, only the covariates explaining most of the variation were included. To evaluate the effect of the concluded models, differences in deviance explained was compared with a zero model, where cereal species and region were used as random effects were calculated.

Results

Cereal production

The area of the organic cereal fields was generally smaller, with medians about half the size of conventional fields (Table 1). Organic cereal yields were also lower, with medians about 70% of the conventional. The percentages of lodged field were lower in organic barley and oats, whereas the share of lodged fields was not significantly different between organic and conventional wheat fields. The moisture content in organic barley and oats at cereal harvest was slightly, but statistically significantly higher than in the conventional cereal samples. In wheat, a corresponding difference in moisture content was not statistically significant (Table 1).

As expected, the soil type was mostly identical, with some deviations (Table 1). This may be due to different interpretations among the farmers of what is the soil type, but also to real differences because the distance between the paired sampled fields was up to 2–3 km.

The organic farmers practised crop rotation much more actively than the conventional ones. The frequencies of the preceding crop of the same cereal species or another cereal species were lower in organic fields (Table 1). For the type of soil cultivation (only registered 2004) there was no difference; most fields were ploughed in both farming systems and very few farmers used reduced soil tillage (Table 1). The most striking difference between farming systems were found for fertilisation and pesticide treatments. Close to all conventional fields were fertilised. Most of these fields received mineral fertilisers, only a few received animal manure. More organic fields received no manure, possibly because the preceding crop was a green manure or clover ley. Usually, organic cereal fields had received animal manure, and in some cases other soil conditioners (e.g. meat and bone meal). A few organic fields had received some mineral fertiliser; this is probably potassium (K) fertiliser that may be applied when soil concentrations of K are low.

Fungicides were not used in organic fields. In conventional fields fungicides were largely used on wheat, to a lower extent on barley and hardly on oats. Most commonly used fungicides were azoxsystrobin combined with fenpropimorf or propiconazol combined with trifloxystrobin. Herbicides were not used in organic fields but used in most conventional fields irrespective of cereal species. A long list of herbicide

Table 1. (Panel a) Agronomic continuous data for paired samples of cereals given as medians with minimum–maximum values. For comparison of organic and conventional data a Wilcoxon statistical method was used. (Panel b) Agronomic numeric data for paired samples of cereals.

	Ba		Oats		Wheat	
	Organic Co	nventional	Organic Co	onventional	Organic C	onventional
Panel a Area						
(ha)	3.0	6.7	3.1	5.0	3.4	6.9
(IIII)	(0.1–24)	(0.1–37)	(0.1-20)	(0.3–27)	(0.1–24)	(1.0–80)
	$p < \ell$			0.002		0.001
N	107	108	100	100	92	90
Cereal yield						
(tons/ha)	3.0	4.2	3.4	4.8	3.0	4.5
	(0.4-5.5)	(2.5-6.8)	(0.5-6.0)	(2.0-7.5)	(1.0-5.4)	(1.7-7.5)
	$p < \ell$		<i>p</i> <	0.001		0.001
N	104	107	98	99	89	91
Lodged field						
(%)	0	2	0	5	0	0
	(0-85)	(0–90)	(0-100)	(0–100)	(0-20)	(0-50)
3.7	p = 0			0.010		0.132
N	106	106	101	100	91	91
Moisture content						
(2003–2004)	17.0	15.5	15.5	14.7	16.5	15.0
(%)	(10.1.20.4)	(11.2.21.4)	(10.0.27.5)	(10.7.00.5)	(11 6 20 0)	(11.0.22.7)
	(10.1-30.4) p = 0	(11.2–31.4)	(10.0-27.5)	(10.7–22.5) 0.009	(11.6–30.0)	(11.0–23.7) 0.068
N	p = 0	.003 75	p = 0	75	<i>p</i> = 6	57
14	74	75	73	73	30	37
Panel b						
Soil						
Sand	19	18	14	7	9	5
Silt	26	15	18	25	17	21
Clay	53	58	59	60	62	60
Not registered	10	17	10	9	4	6
Preceding crop						
Same cereal species	20	48	28	42	12	32
Other cereal species	27	41	35	56	14	35
Other crops	48	15 4	31	2 1	33	7 3
Not registered	13	4	7	1	7	3
Soil cultivation						
(2004)	_	_				
Reduced tillage	3	2	1	1	0	3
Harrowed Ploughed	0 32	5 28	1 32	4 29	0 27	5 19
_	32	26	32	29	21	19
Fertiliser	4.7	^				
None Minaral	17	0	22	1	14	0
Mineral Animal manure	0 86	76 3	1 71	80	2 62	78 0
Other	5	0	6	0	10	0
Not registered	0	29	1	17	4	14
_						
Fungicide Not used	108	63	101	93	92	20
Used	0	43	0	93 7	0	72
Not registered	Ö	2	Ö	1	Ö	0
Herbicide						
Not used	108	25	101	24	92	10
Used	0	82	0	77	0	82
Not registered	Ö	1	Ö	0	Ö	0

(continued)

Table 1. Continued.

	Barley		Oats		Wheat	
	Organic C	onventional	Organic C	onventional	Organic C	Conventional
Insecticide						
Not used	108	90	101	89	92	62
Used	0	14	0	12	0	26
Not registered	0	4	0	0	0	4
Plant growth regulator (2003–2004)						
Not used	75	63	75	64	57	50
Used	0	12	0	11	0	6
Not registered	0	0	0	0	0	1
Catch crop						
None	31	95	37	93	22	77
Grass/clover	70	11	62	7	65	12
Other	5	2	1	1	4	2
Not registered	2	0	1	0	1	1

Note: N, number of sampled fields where the respective data were recorded.

compounds was reported. The most common compounds were tribenuron-methyl or MCPA (2-methyl-4-chlorophenoxyacetic acid) but also glyphosate was used to a certain extent. Insecticides were not used in organic fields, but to some extent they were in conventional fields, particularly in wheat. The most commonly used compounds were alphacypermetrin or esfenvalerat. Chemical plant growth regulators were used to some extent in conventional cereal production. The most common compounds were chloromequate chloride or etefon. Most organic producers used catch crops in the cereal fields, usually grass/clover, while only few conventional producers used catch crops.

Explanation of Fusarium infestation and mycotoxin contamination

The selected respond characteristics, total *Fusarium* infestation, the main DON producer *F. graminearum*, the main HT-2/T-2 producer *F. langsethiae*, and the toxins DON and HT-2 are present in Table 2.

For increased infestation of total *Fusarium*, the use of fungicide showed a significant positive correlation (Table 3). Use of mineral fertiliser, lodged field and preceding crop being a cereal species also increased total *Fusarium*, whereas herbicide use was found to reduce the total *Fusarium* infestation. When climate factors were included in the model, the amount of precipitation in July was positively correlated with total *Fusarium*.

For *F. graminearum*, lack of crop rotation (preceding crop being a cereal) use of mineral fertiliser, lodged field and herbicide use increased the infestation. Furthermore, *F. graminearum* was also increased by use of green and animal manure as fertilisers. Soil type

seemed to have an impact, and *F. graminearum* was less frequently found on heavy soils, especially clay soil. The use of insecticides showed a negative correlation with the infestation of *F. graminearum*. Opposite to the result for total *Fusarium*, *F. graminearum* seemed to be depressed by precipitation in July, whereas increased air humidity before harvest increased this mould species.

As for *F. graminearum*, its mycotoxin DON increased by lack of crop rotation. This was the only significantly explanatory agronomic factor for this characteristic. The only climate factor with a significant influence was temperature before harvest. The DON content decreased by increasing temperature.

The main HT-2 and T-2 producer *F. langsethiae*, primarily found in oat samples, was increased by lack of crop rotation and by use of herbicides. *F. langsethiae* was less frequent in lodged fields and increased with increasing temperature in July. Its related mycotoxin HT-2 was also reduced by crop rotation. Increased air humidity before harvest increased HT-2.

Cereal varieties

The cereal varieties that were commonly used (grown on five or more fields from each farming system) are presented as Table 4. Altogether 21 varieties were grown of barley, 15 of oats and eight of wheat. Most varieties were grown in organic as well as in conventional agriculture but some (e.g. barley Gaute and Sunita) were clearly more popular among organic growers. For common varieties, the percentage infestation of total *Fusarium* and concentrations of main mycotoxins (DON and HT-2 in barley and oats; DON in wheat) were compared among farming systems for

Table 2. Percentage of cereal kernels infested with total *Fusarium*, *F. graminearum* and *F. langsethiae* and concentrations $(\mu g k g^{-1})$ of deoxynivalenol (DON) and HT-2 toxin in paired samples of organically and conventionally produced barley, oats and wheat. The Wilcoxon statistical method was used.

		Organic		Conventional				
		Mean	Median	95%	Mean	Median	95%	<i>p</i> -value
Barley	Total Fusarium	81	87	100	85	92	100	0.020
N = 108	F. graminearum	8	2	28	10	4	43	0.028
	F. langsethiae	< 2	< 2	2	< 2	< 2	2	0.774
	DON	44	< 20	154	44	< 20	207	0.167
	HT-2	< 20	< 20	36	21	< 20	57	< 0.001
Oats	Total Fusarium	81	84	100	86	92	100	0.029
N = 101	F. graminearum	11	4	44	19	6	90	0.027
	F. langsethiae	2	< 2	6	3	< 2	12	0.028
	DON	114	24	447	426	36	2056	0.056
	HT-2	80	< 20	271	117	62	427	0.001
Wheat	Total Fusarium	64	65	98	75	80	98	0.001
N = 92	F. graminearum	7	4	23	10	2	32	0.177
	F. langsethiae	< 2	< 2	< 2	< 2	< 2	2	0.033
	DON	86	29	358	170	51	797	0.016
	HT-2	n.d.			n.d.			

Note: n.d., Not detected. Source: Bernhoft et al. (2010).

Table 3. Agronomic factors and climate factors influencing the infestation of total Fusarium, F. graminearum and its major mycotoxin deoxynivalenol (DON), and F. langsethiae and its major mycotoxin HT-2 toxin in cereals.

	Estimate	SD	<i>p</i> -value
Total Fusarium explained 12%			
Use of fungicide	0.116	0.015	4.6×10^{-14}
Use of herbicide	-0.097	0.016	5.3×10^{-10}
Use of mineral fertiliser	0.113	0.022	3.5×10^{-7}
Lodged field	0.001	0.0002	3.4×10^{-6}
Preceding crop other cereal species	0.039	0.012	8.5×10^{-4}
With climate factors included, explained 14%			
Precipitation in July	0.064	0.007	$< 10^{-16}$
Use of fungicide	0.114	0.015	1.2×10^{-14}
Lodged field	0.001	0.0002	3.7×10^{-8}
Use of herbicide	-0.077	0.016	8.6×10^{-7}
Use of mineral fertiliser	0.106	0.022	2.1×10^{-6}
Preceding crop other cereal species	0.041	0.012	4.2×10^{-4}
Fusarium graminearum explained 11%			
Preceding crop other cereal species	0.506	0.033	$< 10^{-16}$
Clay soil	-0.524	0.040	$< 10^{-16}$
Use of mineral fertiliser	0.542	0.077	1.7×10^{-12}
Lodged field	0.004	0.0006	1.6×10^{-11}
Silty soil	-0.291	0.045	6.8×10^{-11}
Use of herbicide	0.282	0.049	6.2×10^{-9}
Use of green manure	0.513	0.089	8.6×10^{-9}
Use of animal manure	0.252	0.063	5.9×10^{-5}
Use of insecticide	-0.167	0.046	3.2×10^{-4}
With climate factors included, explained 30%			
Precipitation in July	-0.581	0.024	$< 10^{-16}$
Air humidity at harvest	0.131	0.004	$< 10^{-16}$
Preceding crop other cereal species	0.522	0.033	$< 10^{-16}$
Clay soil	-0.423	0.041	$< 10^{-16}$

(continued)

Table 3. Continued.

	Estimate	SD	<i>p</i> -value
Use of mineral fertiliser	0.631	0.074	< 10 ⁻¹⁶
Use of animal manure	0.352	0.063	2.6×10^{-8}
Lodged field	-0.004	0.001	2.9×10^{-8}
Use of green manure	0.440	0.090	9.4×10^{-7}
Use of herbicide	0.209	0.048	1.6×10^{-5}
DON explained 5%			
Preceding crop other cereal species With climate factors included, explained 10%	0.471	0.116	5.0×10^{-5}
Preceding crop other cereal species	0.473	0.115	4.0×10^{-5}
Temperature at harvest	-0.072	0.019	1.2×10^{-4}
Fusarium langsethiae explained 11%			
Preceding crop non-cereal species	-1.199	0.021	5.6×10^{-9}
Lodged field	-0.019	0.004	4.7×10^{-8}
Preceding crop other cereal species	0.546	0.116	2.7×10^{-6}
Use of herbicide	0.389	0.114	6.4×10^{-4}
With climate factors included, explained 15%			
Temperature in July	0.310	0.091	2.8×10^{-10}
Preceding crop non-cereal species	-1.218	0.208	4.6×10^{-9}
Preceding crop other cereal species	0.604	0.118	2.9×10^{-7}
Lodged field	-0.015	0.004	3.9×10^{-5}
HT-2 toxin explained 16%			
Preceding crop non-cereal species	-1.080	0.215	5.3×10^{-7}
With climate factors included, explained 18%			
Air humidity at harvest	0.116	0.017	2.6×10^{-11}
Preceding crop non-cereal species	-1.139	0.215	1.2×10^{-7}

Notes: Variances are related to cereal species and regions are corrected for. Factor estimates with standard deviations for p < 0.001 are shown.

each single variety. In three out of 12 analyses of *Fusarium*, and in five out of 21 analyses of mycotoxins statistically significant differences were found. All significant differences were in favour of organic production.

Soil cultivation

The data on soil cultivation, only collected from the farmers in 2004, were not included in the comprehensive multivariate analyses. The impact of soil cultivation on the infestation of total *Fusarium* and concentrations of main mycotoxins (DON and HT-2 in barley and oats; DON in wheat) in cereals are presented in Table 5. Most farmers had ploughed their fields (Table 1), and hence the number of fields with reduced tillage or only harrowing is low. However, in barley higher levels of total *Fusarium* were found by reduced soil tillage.

Discussion

Organic versus conventional production

The descriptive agronomic data present in Table 1 are the basic information of this paper. Smaller fields, more crop rotation, no use of pesticides or mineral fertilisers, and extensive use of catch crops and lower yields are common characteristics of organic cereal fields (Köpke et al. 2007). However, there is not a complete distinction between conventional and organic cereal fields; also conventional farmers may execute crop rotation, grow catch crops and avoid pesticides. For example, the use of pesticides was lower (Table 1) than might have been expected in conventional fields. The use of mineral versus organic (animal or green manure) fertilisers was the most evident difference among farming systems in this study. Soil cultivation was not particularly different by agronomic practice; most fields were ploughed in both farming systems.

More commonly found lodged fields in conventional production may be due to higher yields with heavier ears, making the straw more vulnerable for rainfall and wind. The higher moisture in organic cereals at harvest was unexpected, as humidity is often linked to the incidence of lodged fields. However, the explanation may be the more extended use of catch crops in organic fields, increasing the total plant biomass and thereby the humidity of the cereal canopy. Another factor could be less evaporation of water from plants grown without easily dissolvable nitrogen, due to a more robust cell wall of such plants (van Arendonk et al. 1997).

Table 4. Names and numbers of cereal varieties representing at least five samples from each farming system used in the sampled fields.

	Organic	Conventional	<i>p</i> -value
Barley			
Arve	5	5	
Fusarium	84	84	0.675
DON	< 20	< 20	0.317
HT-2	< 20	< 20	1.000
Gaute	14	5	
Fusarium	91	100	0.211
DON	< 20	87	0.548
HT-2	< 20	< 20	0.550
Kinnan	8	16	
Fusarium	83	76	0.877
DON	< 20	< 20	0.343
HT-2	< 20	27	0.027
Sunita	30	11	
Fusarium	87	84	0.605
DON	< 20	< 20	0.085
HT-2	< 20	21	0.002
Thule N	8	9	
Fusarium	88	100	0.024
DON	< 20	43	0.007
HT-2	< 20	< 20	0.822
Ven N	21	11	
Fusarium	82 82	82	0.632
DON	< 20	< 20	0.632
HT-2	<20 <20	< 20	0.030
	<20	<20	0.240
Oats	•	40	
Belinda	20	43	0.645
Fusarium	83	94	0.645
DON	< 20	26	0.179
HT-2	59	99	0.194
Biri	37	19	
Fusarium	88	94	0.781
DON	< 20	27	0.566
HT-2	< 20	31	0.412
Lena	22	16	
Fusarium	86	96	0.162
DON	114	468	0.043
HT-2	< 20	49	0.001
Wheat			
Avle	50	40	
Fusarium	69	75	0.034
DON	22	25	0.883
Bastian			
Bastian Fusarium	22 65	18 87	0.200
DON	63 62	87 82	0.288 0.391
			0.331
Zebra	9	21	
Fusarium	66	82	0.012
DON	164	254	0.556

Note: The median percentage of total *Fusarium* infestation and median concentrations (µg kg⁻¹) of major mycotoxins with results of Wilcoxon statistics are presented.

Agronomic explanations of Fusarium and mycotoxins in the cereals

Less infestation of *Fusarium* species and lower levels of related mycotoxins in grain samples from organic

Table 5. Median percentage of total *Fusarium* infestation and median concentrations ($\mu g kg^{-1}$) of major mycotoxins in each cereal species in 2004 in fields after reduced soil tillage, only harrowing or traditional cultivation with ploughing.

	Reduced	Harrowed	Ploughed	
Barley				
N	5	5	60	
Fusarium	100	94	80	p = 0.032
DON	< 20	60	< 20	p = 0.110
HT-2	< 20	< 20	< 20	p = 0.468
Oats				
N	2	5	61	
Fusarium	92	100	86	p = 0.134
DON	< 20	< 20	42	p = 0.256
HT-2	144	41	< 20	p = 0.186
Wheat				
N	3	5	46	
Fusarium	72	64	70	p = 0.772
DON	95	254	138	p = 0.969

Note: p-values are from the Kruskal-Wallis statistical method.

farming were presented in detail by Bernhoft et al. (2010). An extract of these data is given in Table 2. The present paper reveals some explanations for these differences.

Lack of crop rotation was significantly connected to increases of measured characteristics: total Fusarium, F. graminearum, F. langsethiae, DON and HT-2. The results are in accordance with a range of reports discussing Fusarium and DON content in cereals (Aldred and Magan 2004; Edwards 2004; Oldenburg 2004; Beyer et al. 2006; Köpke et al. 2007; Pageau et al. 2008). Most authors also indicate that maize before cereals is particularly risky, but maize is not a common crop in Norway. The factor 'Other cereal species last year' had a larger importance than 'Same cereal species last year'. In addition, the factor 'Non-cereal crop last year' significantly decreased F. langsethiae and HT-2. These results indicate that continuous cropping of cereal species is a disadvantage with respect to Fusarium infestation and mycotoxins. The finding of largely the same Fusarium species on barley, oats and wheat (Bernhoft et al. 2010) may indicate that any of these cereal species may make the Fusarium inoculum correspondingly available for the crop produced next year.

In the present study the use of mineral fertilisers was significantly connected to an increase of total *Fusarium* and *F. graminearum*. The results are in accordance with those of a range of previous studies on the effect of nitrogen fertilisers on *Fusarium*, particularly on *F. graminearum* and DON, in cereal grains (Martin et al. 1991; Elen et al. 2000; Yi et al. 2001; Lemmens et al. 2004; Heier et al. 2005). Organic

fertilisers seem to support *Fusarium* to a lower extent. In the present study both manure and other organic fertilisers were significantly connected to increased *F. graminearum* infestation, but to a lesser degree than mineral fertilisers. There may be several explanations for the finding of particularly more *Fusarium* in the cereals with easily soluble nitrogen fertilisers. The nitrogen supply influences the cell wall structure and chemical composition of the plants (van Arendonk et al. 1997), which may make them more susceptible to mould attack. Nitrogen fertilisation makes the plants bushier and the fields more crowded and humid, which may be optimal for the *Fusarium* spreading. Furthermore, nitrogen fertilisation implies taller plants with heavier ears more vulnerable for lodging.

Just like use of mineral fertilisers, lodged field was connected to an increase of total *Fusarium* and *F. graminearum*. There was also a significant correlation between lodged fields and use of mineral fertilisers in the dataset. As lodged fields were less connected to total *Fusarium* and *F. graminearum* than the use of mineral fertilisers, an indirect connection between *Fusarium* and lodged fields via the use of mineral fertilisers producing tall and heavy plants is plausible. Furthermore, the close contact between soil *Fusarium* and the cereal ears of lodged fields which have a lesser possibility of drying up after rainfall and morning dew may also play a role.

On the other hand, lodged fields were connected to decreased infestation of *F. langsethiae*. Cereal ears close to the soil may receive less radiation from the sun. Accordingly, *F. langsethiae* infestation was connected to elevated temperature in July.

Thus, *Fusarium* species may behave differently to environmental conditions. Some kind of competition between *Fusarium* species is plausible.

The use of fungicides was strongly connected to the increase of total Fusarium. Furthermore, the use of herbicides was connected to increases of F. graminearum and F. langsethiae. On the other hand, herbicide use was connected to a decrease of total Fusarium, and insecticide use was connected to decreased F. graminearum. Thus, the present study confirms results from several studies that the impact of different chemicals on Fusarium species is not straightforward. Different Fusarium species may respond differently to the chemicals used. For the present study, the net effect was a negative impact of pesticides. As a range of chemical compounds of pesticides was used, it was not possible to test a relation between Fusarium and individual fungicides, herbicides or insecticides. The effects of fungicides on Fusarium head blight have been extensively studied. So far, elimination of Fusarium by fungicides seems impossible (Martin et al. 1991; Simpson et al. 2001; Aldred and Magan 2004; Edwards 2004; Oldenburg 2004; Heier et al. 2005; Henriksen and Elen 2005; Beyer et al. 2006; Klix et al.

2007; Paul et al. 2007; Pirgozliev et al. 2008; Xue et al. 2009; Edwards and Godley 2010; Lehoczki-Krsjak et al. 2010).

Relations between Fusarium infestation and the use of herbicides in cereals are far less reported. In spite of that, the herbicide glyphosate was shown to increase the incidence of Fusarium and other soil-borne pathogens more than two decades ago (Altman and Rovira 1989), Henriksen and Elen (2005) did not find an effect of glyphosate spraying on total Fusarium in wheat, barley or oats. However, Fernandez et al. (2009) report a range of experiments of the effect of glyphosate on Fusarium in wheat and barley fields. Glyphosate treatment was consistently associated with higher Fusarium head blight, particularly due to F. graminearum and F. avenaceum. The authors suggest that the herbicide might cause changes in fungal communities via various mechanisms implying stimulation of Fusarium and impairment of other fungi as well as potentially impacting plant resistance.

Also insecticides influence the ecosystem of the cereal fields in complicated ways. In the present study rather few farmers had used insecticides and the authors find it to be speculative to discuss further the single, rather weak negative impact of insecticide use on *F. graminearum*.

In addition to the huge range of biotic soil factors influencing the Fusarium loads, physico-chemical factors of the soil may also play a role. Particularly clay soil but also silty soil were connected to reduced infestation of F. graminearum. A range of reports indicate that soils rich in clay are more suppressive to Fusarium than coarser silty and particularly sandy soils (Amir and Alabouvette 1993; Huang and Wong 1998; Alabouvette 1999: Knudsen et al. Shakhnazarova et al. 2000; Kurek and Jaroszuk-Scisel 2003). An improved environment for Fusarium antagonistic microorganisms in clay soils is the proposed explanation.

As most of the sampled fields were ploughed, soil cultivation was not found to be particularly different by agronomic practice. However, only one year of registration implies a restricted amount of these data so it was not possible to include soil cultivation in the multivariate statistical testing. An attempt to perform a descriptive analysis of total *Fusarium*, DON and HT-2 in groups of fields with different soil cultivation could not verify increased mycotoxin level in cereals after reduced tillage. However, total *Fusarium* was increased in barley after reduced tillage. Increased *Fusarium* and mycotoxin levels by reduced soil tillage have been observed in several studies (Köpke et al. 2007).

The difference in the susceptibility of cereal varieties to *Fusarium* is well known. In the present study a range of varieties of each cereal species was used. For most varieties, the number of registered fields was too

small to reveal any impact of variety. Most varieties were represented in organic as well as in conventional agriculture, and hence it is not likely that different varieties have contributed significantly to the effect of farming system found in the present study. The differences found between *Fusarium* and mycotoxin in commonly used varieties were in favour of organic production. However, cereal varieties are probably responsible for some of the large unexplained and unassessed variation in *Fusarium* and mycotoxin levels in this study.

Climate explanations

Precipitation during cereal flowering will increase Fusarium infestation in the mature grain (Köpke et al. 2007). In Norway grain flowering takes place in July and increased cereal Fusarium as well as DON were found in years with a rainy July during 1988–1996 (Langseth and Elen 1997). Accordingly, a strong correlation between precipitation in July and total Fusarium was found in the present study. However, F. graminearum was negatively correlated with July precipitation. F. graminearum infestation is generally favoured by warm weather (Miller 1994). In temperate areas as in Norway, warm weather seldom occurs with precipitation. Thus, the previous finding of elevated cereal DON levels after a rainy July may have been more caused by F. culmorum. This species may grow under cooler conditions than F. graminearum (Miller 1994), and was a decade ago a more dominant source of DON in Norwegian cereals (Langseth and Elen 1997; Kosiak et al. 2003). A corresponding recent trend of decreased F. culmorum and increased F. grami*nearum* is also reported from other European countries (Xu et al. 2005).

In the present study, *F. langsethiae* was positively correlated with mean July temperature. Accordingly, Medina and Magan (2010) found a temperature optimum for *F. langsethiae* growth at 25°C, similarly as for *F. graminearum*. This is much higher than the Norwegian mean July temperature. By increasing temperatures in Norway, related to climatic change, both *F. graminearum* and *F. langsethiae* may become more common, increasing the problem with DON and HT-2/T-2, respectively.

However, in the present paper, DON and HT-2 were not increased by the same climate factors as their producers. *Fusarium* mycotoxins seem generally to be stimulated by a narrower window of climatic factors than the *Fusarium* infestation (Hope et al. 2005; Medina and Magan 2011). Both toxins increased with cold and humid weather before harvest. Low temperature was the factor found to increase DON, and high air humidity the factor found to increase HT-2. For DON this result was unexpected as the optimal

temperature for DON production may be around 25°C (Hope et al. 2005). However, low temperature is often connected to humid weather, and high water activity is more critical for toxin production than temperature (Hope et al. 2005). Furthermore, increased DON by low temperatures before harvest may also be related to a late harvest. Mean temperature and humidity during a 2-week period before harvest are also rough measures, as also temperature changes may increase DON production (Ryu and Bullerman 1999). Medina and Magan (2011) suggest that high water availability appears to be particularly important for toxin production from F. langsethiae, and far more critical than temperature. The present results are consistent with that finding. Thus, wet weather before harvest seems to be particularly bad for cereal contamination of HT-2/ T-2 as well as of DON.

Conclusion

Cereals from organic farming systems in Norway were found to be less heavily infested by *Fusarium* species than cereals from conventional farming systems. The statistical analyses revealed that this was mainly due to lack of crop rotation, use of mineral fertilisers and, to some extent, use of pesticides in the conventional systems. Climate registrations and statistical analyses indicate that the important DON and HT-2/T-2 producers *F. graminearum* and *F. langsethiae*, respectively, may become more common with warmer Julys. Furthermore, wet weather before harvest seems to be particularly bad for cereal contamination of HT-2/T-2 as well as of DON.

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References

Alabouvette C. 1999. Fusarium wilt suppressive soils: an example of disease-suppressive soils. Aust Plant Pathol. 28(1):57–64.

- Aldred D, Magan N. 2004. Prevention strategies for trichothecenes. Toxicol Lett. 153:165–171.
- Altman J, Rovira AD. 1989. Herbicide–pathogen interactions in soil-borne root diseases. Can J Plant Pathol. 11:166–172.
- Amir H, Alabouvette C. 1993. Involvement of soil abiotic factors in the mechanisms of soil suppressiveness to Fusarium wilts. Soil Bio Biochem. 25(2):157–164.
- Bates D, Maechler M, Bolker B. 2011. lme4: Linear mixedeffects models using S4 classes. R package version 0.999375-39. Available from: http://CRAN.R-project.org/ package=lme4/
- Bernhoft A, Clasen P-E, Kristoffersen AB, Torp M. 2010. Less Fusarium infestation and mycotoxin contamination in organic than in conventional cereals. Food Addit Contam. 27(6):842–852.
- Beyer M, Klix MB, Klink H, Verreet J-A. 2006. Quantifying the effects of previous crop, tillage, cultivar and triazole fungicides on the deoxynivalenol content of wheat grain a review. J Plant Dis Protect. 113(6):241–246.
- Bioforsk. 2011. Landbruksmeteorologisk tjeneste [Agronomy weather data service]; [cited 2011 Apr 27]. Available from: http://lmt.bioforsk.no/lmt/index.php? weatherstation = 5&loginterval = 1&tid = 1303897298/
- Edwards SG. 2004. Influence of agricultural practices on Fusarium infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Tox Lett. 153:29–35.
- Edwards SG, Barrier-Guillot B, Clasen P-E, Hietaniemi V, Pettersson H. 2009. Emerging issues of HT-2 and T-2 toxins in European cereal production. World Mycotox J. 2(2):173–179.
- Edwards SG, Godley NP. 2010. Reduction of Fusarium head blight and deoxynivalenol in wheat with early fungicide applications of prothioconazole. Food Addit Contam A. 27(5):629–635.
- Elen O, Abrahamsen U, Øverli A, Razzaghian MJ. 2000. Effects of agricultural measures on the occurrence of Fusarium spp. in cereals in Norway. 6. European Fusarium seminar in Berlin 11–16. September 2000. Mitt Biol Bundesanst Land-Fortwirtsch. 377:105–106.
- Fernandez MR, Zentner RP, Basnyat P, Gehl D, Selles F, Huber D. 2009. Glyphosate associations with cereal diseases caused by Fusarium spp. in the Canadian Prairies. Eur J Agr. 31:133–143.
- Foroud NA, Eudes F. 2009. Trichothecenes in cereal grains. Int J Mol Sci. 10:147–173.
- Heier T, Jain SK, Kogel KH, Pons-Kuhnemann J. 2005. Influence of N-fertilization and fungicide strategies on Fusarium head blight severity and mycotoxin content in winter wheat. J Phytopathol. 153(9):551–557.
- Henriksen B, Elen O. 2005. Natural Fusarium grain infection level in wheat, barley and oat after early application of fungicides and herbicides. J Phytopathol. 153(4):214–220.
- Hofgaard I, Aamot H, Klemsdal S, Elen O, Jestoi M, Brodal G. 2010. Occurrence of *Fusarium* spp. and mycotoxins in Norwegian wheat and oats. In: Hofgaard I, Fløistad E, editors. Nordic Baltic Fusarium Seminar (NBFS), Ski, Norway, 23–25 November 2010. Bioforsk Fokus 5(7):9.
- Hope R, Aldred D, Magan N. 2005. Comparison of environmental profiles for growth and deoxynivalenol

- production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett Appl Microbiol. 40:295–300.
- Huang Y, Wong PTW. 1998. Effect of Burkholderia (Pseudomonas) cepacia and soil type on the control of crown rot in wheat. Plant Soil. 203:103–108.
- Klix MB, Verreet J-A, Beyer M. 2007. Comparison of the declining triazole sensitivity of Gibberella zeae and increased sensitivity achieved by advances in triazole fungicide development. Crop Protect. 26:683–690.
- Knudsen IMB, Debosz K, Hockenhull J, Jensen DF, Elmholt S. 1999. Suppressiveness of organically and conventionally managed soils towards brown foot rot of barley. Appl Soil Ecol. 12:61–72.
- Köpke U, Thiel B, Elmholt S. 2007. Strategies to reduce mycotoxin and fungal alkaloid contamination in organic and conventional cereal production systems. In: Cooper J, Niggli U, Leifert C, editors. Handbook of organic food safety and quality. Boca Raton (FL): CRC Press. p. 353–391.
- Kosiak B, Torp M, Skjerve E, Thrane U. 2003. The prevalence and distribution of Fusarium species in Norwegian cereals: a survey. Acta Agric Scand B Soil Plant Sci. 53(4):168–176.
- Kurek E, Jaroszuk-Scisel J. 2003. Rye (Secale cereale) growth promotion by Pseudomonas fluorescens strains and their interactions with Fusarium culmorum under various soil conditions. Biol Contr. 26:48–56.
- Langseth W, Elen O. 1997. The occurrence of deoxynivalenol in Norwegian cereals – differences between years and districts, 1988–1996. Acta Agric Scand B Soil Plant Sci. 47:176–184.
- Lehoczki-Krsjak S, Szabo-Hever A, Toth B, Kotai C, Bartok T, Varga M, Farady L, Mesterhazy A. 2010. Prevention of Fusarium mycotoxin contamination by breading and fungicide application to wheat. Food Addit Contam A. 27(5): 616–628.
- Lemmens M, Haim K, Lew H, Ruckenbauer P. 2004. The effect of nitrogen fertilization on Fusarium head blight development and deoxynivaleol contamination in wheat. J Phytopathol. 152:1–8.
- Martin RA, MacLeod JA, Caldwell C. 1991. Influences of production inputs on incidence of infection by Fusarium species on cereal seed. Plant Dis. 75(8):784–788.
- Medina A, Magan N. 2010. Comparisons of water activity and temperature impacts on growth of Fusarium lang-sethiae strains from northern Europe on oat-based media. Int J Food Microbiol. 142:365–369.
- Medina A, Magan N. 2011. Temperature and water activity effects on production of T-2 and HT-2 by Fusarium langsethiae strains from north European countries. Food Microbiol. 28:392–398.
- Miller JD. 1994. Epidemiology of Fusarium ear diseases of cereals. In: Miller JD, Trenholm HL, editors. Mycotoxins in grain. Compounds other than aflatoxin. St. Paul (MN): Eagan. p. 19–36.
- Miller JD. 2008. Mycotoxins in small grains and maize: old problems, new challenges. Food Addit Contam. 25(2):219–230.
- Oldenburg E. 2004. Crop cultivation measures to reduce mycotoxin contamination in cereals. J Appl Bot Food Qual. 78:174–177.

- Pageau D, Lafond J, Lajeunesse J, Savard ME. 2008. Impact du précédent culural et de la fertilisation azotée sur la teneur en désoxynivalénol chez l'orge. Can J Plant Pathol. 30:397–403.
- Paul PA, Lipps PE, Hershman DE, McMullen MP, Draper MA, Madden LV. 2007. A quantitative review of tebuconazole effect on Fusarium head blight and deoxynivalenol content in wheat. Phytopathology. 97(2):211–220.
- Pirgozliev SR, Ray RV, Edwards SG, Hare MC, Jenkinson P. 2008. Effects of timing of fungicide application on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in winter grain. Cereal Res Commun. 36(2):289–299.
- Placinta CM, D'Mello JPF, Macdonald AMC. 1999. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Anim Feed Sci Technol. 78:21–37.
- R Development Core Team. 2007. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: http://www.R-project.org/
- Ryu D, Bullerman LB. 1999. Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. J Food Prot. 62:1451–1455.
- Shakhnazarova VY, Strunnikova OK, Vishnevskaya NA, Stefanova NA, Muromtsev GS. 2000. Structure and

- dynamics of Fusarium culmorum population in soils of different textures. Eurasian Soil Sci. 33(1):76–80.
- Simpson DR, Weston GE, Turner JA, Jennings P, Nicholson P. 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur J Plant Pathol. 107(4):421–431.
- van Arendonk JJCM, Niemann GJ, Boon JJ, Lambers H. 1997. Effects of nitrogen supply on the anatomy and chemical composition of leaves of four grass species belonging to the genus Poa, as determined by image-processing analysis and pyrolysis-mass spectrometry. Plant Cell Environ. 20:881–897.
- Xu X-M, Parry DW, Nicholson P, Thomsett MA, Simpson D, Edwards SG, Cooke BM, Doohan FM, Brennan JM, Moretti A, et al. 2005. Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. Eur J Plant Pathol. 112:143–154.
- Xue AG, Voldeng HD, Savard ME, Fedak G. 2009. Biological management of Fusarium head blight and mycotoxin contamination in wheat. World Mycotox J. 2(2):193–201.
- Yi C, Kaul HP, Kubler E, Schwadorf K, Aufhammer W. 2001. Head blight (*Fusarium graminearum*) and deoxynivalenol concentration in winter wheat as affected by precrop, soil tillage and nitrogen fertilization. J Plant Dis Prot. 108(3):217–230.