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FULL RESEARCH PAPER

Within-field variability of Fusarium head blight pathogens and their associated mycotoxins

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Abstract Within-field variability in the Fusarium head blight (FHB) and its associated mycotoxins was studied in four European countries. At each of 14 sites, each FHB pathogen and associated mycotoxins were quantified in 16 quadrat samples at harvest. Overall, the incidence of quadrat samples with detectable and quantifiable pathogen DNA was significantly lower in the grain than in the corresponding chaff. Deoxynivalenol (DON) was the most frequently detected toxin in the samples and its accumulation was most strongly associated with the presence of *Fusarium graminearum*. Nivalenol (NIV) accumulation was significantly associated only with the presence of *F. culmorum*. Zearalenone (ZON) accumulation was strongly associated with the presence of all three pathogens (*F. graminearum*, *F. culmorum* and *F. poae*). The levels of both DON and ZON concentrations were positively related to the amount of *F. graminearum* DNA in the grain or in the chaff. The presence/absence of FHB pathogens within a single quadrat appeared to be independent of each other. The presence of a particular FHB pathogen and the amount of its DNA, as well as the associated mycotoxin(s), varied greatly among samples at each site. This study demonstrated the large extent of within-field variability of FHB and its associated

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mycotoxins, and the importance of representative sampling in FHB studies.

Keywords Wheat head scab · DON · NIV
Fungal DNA · Aggregation · Chaff · Flour

Introduction

Fusarium head blight (FHB) of wheat has been extensively researched worldwide (Bai and Shaner 1994; Dubin et al. 1997; Mcmullen et al. 1997; Parry et al. 1995; Xu and Berrie 2005). In addition to causing significant yield loss, FHB is of greater significance because of associated mycotoxin accumulation. Contaminated grain is unsuitable for animal and human consumption because of adverse effects of such toxins on health (Bennett and Klich 2003; IARC 1993; Li et al. 1999). The most important FHB pathogens are *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*, which can produce a range of mycotoxins (Bottalico and Perrone 2002), and *Microdochium nivale* and *M. majus* (formerly *M. nivale* var. *nivale* and var. *majus*) (Glynn et al. 2005), which do not produce any known mycotoxins. The differential response of FHB pathogens to fungicides was one of the causes of the inconsistency of chemical control of FHB under field conditions in terms of the reduction in both disease and mycotoxins (Pirgozliev et al. 2003). Modelling of field data suggested that FHB development is greatly affected by temperature and moisture (De Wolf et al. 2003; Moschini and Fortugno 1996). The importance of temperature and moisture on the infection of wheat ears by FHB pathogens was confirmed using detached spikes (Rossi et al. 2001) and whole plants (Xu et al. 2007) in controlled environments.

While *F. graminearum* is the predominant species causing FHB on wheat in the USA, it is known that in Europe FHB can be caused by a complex of head blight pathogens (Doohan et al. 1998) as shown by the number of FHB pathogens detected in field samples (Xu et al. 2005). Field monitoring, based on a single bulk sample for each sampling field area, size of ca. 1 ha, revealed frequent positive associations of FHB pathogens (Xu et al. 2005), i.e. the presence of one FHB pathogen within a field was often significantly positively related to the presence of other FHB pathogens within the same field. However, it is not known whether such positive associations are also significant on a smaller spatial scale. It is important to understand the nature and the extent of FHB pathogen interactions for predicting disease development and mycotoxin production since different FHB pathogens may differ in their requirements for infection (Rossi et al. 2001).

Recently, there has been a growing interest in understanding the spatial as well as temporal dynamics of FHB epidemics. In North America, the spatial pattern of disease incidence was shown to be completely random in most sampled fields (Shah and Bergstrom 2001), indicating that the primary inocula for infection of ears were distributed randomly and were thus likely to be composed of external airborne ascospores. Only in one field was there some degree of clustering of diseased ears within the sampling quadrat, which the authors suggested was from clustered corn debris in soil. In another study, the proportion of *Fusarium*-infected seeds varied greatly between seed lots, and this variability in seed infection was significantly greater than would be expected for a binomial (i.e. random) distribution in 72% of the data sets (Shah et al. 2002). The within-field variability is important for formulating rational sampling strategies for monitoring and regulatory purposes of predicting disease severity or mycotoxin content. However, there is very little information on the within-field variability of FHB pathogens and production of associated mycotoxins; indeed there are no published studies on this aspect in Europe where several FHB pathogens can be present at the same site.

This paper reports results of a 2-year field study in Europe aimed at establishing within-field variability in the presence/absence of FHB pathogens and their associated mycotoxins. Specific objectives were to determine (1) the extent of within-field variability of fungal presence (and DNA quantity) and the associated mycotoxins, (2) whether FHB pathogens are associated at a smaller spatial scale, and (3) the relationship between mycotoxin production and pathogen presence (and DNA quantity).

Materials and methods

Field sampling

Sampling was conducted in 2003 and 2004 in four European countries (UK, Hungary, Ireland and Italy). Two sites were selected in each country based on the criterion that at least two FHB pathogens were detected at that site in the monitoring done the year before (Xu et al. 2005). No sampling was done in 2004 in Ireland. A total of 14 sites were sampled over the two years. Fungicides effective against FHB were not applied to test plots at the sites.

At each site, a total of 16 quadrats were randomly selected along a W-shaped walk across the whole sampling area (ca. 1 ha). The quadrat size was 0.5× 0.5 m (0.25 m^2). Sampling in the quadrats was done at harvest: all ears within a single quadrat were harvested and threshed; all grain and chaff from each quadrat was then milled separately (16 samples per site). Fungal DNA was extracted from the flour and milled chaff separately and analysed. Mycotoxins were only quantified for the grain samples; 50 g of flour was used for mycotoxin analysis from each quadrat sample.

Detecting and quantifying fungal DNA

Fungal DNA was extracted from milled flour and chaff using the CTAB (hexadecyltrimethyl–ammonium bromide) buffer method described by Nicholson et al. (1996), amplified in a diagnostic PCR as detailed by Nicholson et al. (2004) and quantified using the competitive-PCR method described by Simpson et al. (2000).

Following determination of the DNA concentration, the samples were adjusted to a common concentration, 20 ng μl^{-1}, for use in PCR. The species-specific PCR primer pairs used to detect *M. nivale*, *M. majus*, *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae* in the diagnostic PCR reactions were those described in Nicholson et al. (2003, 1996). The reaction components and amplification conditions for competitive PCR were the same as those for the conventional specific PCR except for the addition of the relevant competitor template for each species. Following amplification, the PCR products of each reaction were separated by electrophoresis through 2% agarose gel. Gels were stained with ethidium bromide, viewed under UV light on a 'Gel Doc 1000' system (Bio-Rad) and analysed using Molecular Analyst software (Bio-Rad) to estimate the relative degree of amplification of the fungal and competitor PCR product in each sample. The ratio of the PCR products from the target and competitor DNA components was then estimated. The amount of fungal DNA, of the relevant species, in the plant sample was estimated by reference to a standard curve that was generated using purified DNA of the relevant target species. Standard negative, positive and competitor control reactions were included in each PCR run.

The estimated limit of detection (LOD) for the diagnostic PCR from a series of dilution experiments using the pure fugal DNA was 0.00003, 0.00083, 0.00345, 0.00004, 0.00009 and 0.00017 ng.μl^{-1} for *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae*, *M. majus* and *M. nivale*, respectively; the corresponding limit of quantification (LOQ) for the competitive PCR method was estimated to be 0.0028, 0.0013, 0.0051, 0.0006, 0.0046 and 0.0014 ng μl^{-1} respectively.

Detection and quantification of mycotoxins

Deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZON) standards were purchased from Sigma Chemical Company (St. Louis, MO). Water for the high-performance liquid chromatography (HPLC) mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA). All other chemicals and solvents were HPLC-grade and were purchased from Merck (Darmstadt, Germany). Clean-up columns used to pre-purify samples were purchased from Rhone Diagnostic Technologies Ltd. (UK). Trichothecene standard stock and working solutions were prepared according to Krska et al. (2005). The vials were sealed and stored in the dark at 4°C; fresh working solution was prepared each week.

Trichothecenes were extracted according to Krska et al. (2005) using 10 g of sample and 50 ml of an acetonitrile–methanol as extraction mixture (84:16,

v/*v*). The final solution was filtered with cellulose filter through a 0.22 μl syringe filter (Millipore, Bedford, MA, USA) prior to injection (20 μl) onto the LC MS. The HPLC column was a Prodigy 5 (m ODS-3 V; Phenomenex, 250 × 4.6 μm) at constant temperature of 50°C. Mobile phases used were: A, water; and B, acetonitrile/methanol (50:50 *v*/*v*). Eluent was freshly prepared and filtered (0.22 μm) before use. Flow rate was fixed at 0.8 ml min^{-1} and the analysis required the following eluent conditions:

Time (min)	% Water	% CH_3CN/CH_3OH (70:30)
0	90	10
7	65	35
9	20	80
13	20	80
15	0	100
19	0	100
21	90	10

Spectrometric conditions utilised API 3000 triple quadrupole mass spectrometry (Ontario, CA) equipped with turbo ion-spray as interface and MRM experiment (multiple reaction monitoring), and negative ions for NIV, DON and ZON. Turbogas (nitrogen) temperature was fixed to 350°C and ion spray voltage (IS) was −4,500 V (negative ions). Spectrometric parameters were optimised for each mycotoxin by the infusion technique using standard solution at 10 μg ml^{-1} and flow of 8 μl min^{-1}. The recovery rates for NIV, DON and ZON were 81, 95 and 73%, respectively. The LOD for NIV, DON and ZON were 2.5, 1 and 0.5 ppb, respectively; the respective LOQ for NIV, DON and ZON were 5, 5 and 1 ppb.

Statistical analysis

Statistical analysis was performed to study: (1) within-field variability in pathogen and mycotoxin presence between quadrats, (2) presence and association of FHB pathogens in chaff and grain, and (3) mycotoxin production in relation to fungal presence and its DNA quantity.

Pathogen association Whether the six FHB pathogens were aggregated within a single quadrat given the incidence of each individual pathogen at the site was tested by comparing the observed variance in the number of FHB pathogens within a quadrat at each site with the variance expected under the assumption of mutual independence in pathogen presence. Under the assumption of independence, the expected variance of the number of pathogens within a quadrat is $\sum_{i=1}^{6} p_i(1-p_i)$ where the p_i is the observed incidence of FHB pathogen i at a given site (the summation is over the six FHB pathogens). The following model was used to relate the observed (V_{obs}) to the expected (V_{exp}) variance:

$$\ln(V_{obs}) = a + b\ln(V_{exp}).$$

If either a is significantly >0 or b is significantly >1, then aggregation of FHB pathogens within a single quadrat is indicated. This model was fitted to observed data from both chaff and grain samples. To investigate differences in the two parameters between chaff and grain, a dummy factor representing the comparison was fitted to the model.

Presence of fungal species in chaff and grain Logistic regression analysis (Collett 1991) was used to determine whether the incidence of each FHB pathogen or a number of FHB pathogens differed significantly between the grain and the chaff samples from the same quadrat, and between the diagnostic and competitive PCR methods, assuming a binomial distribution for the response variable. Dummy factors representing these two comparisons were used in the logistic regression.

Mycotoxin production in relation to fungal species Three statistical methods were used to determine the effect of each toxigenic FHB species on toxin accumulation. Two *Microdochium* species and *F. avenaceum* were not included in the analysis as they are not known to produce DON, NIV or ZON.

The first two methods aim to determine the extent to which the presence of an individual toxigenic species increased the odds of toxin occurrence (yes or no, i.e., not treated quantitatively). First, the Fisher's exact test was used to obtain an exact probability value for the observed data of fungal presence and toxin accumulation. Next, the magnitude of each fungus–toxin association was estimated in terms of

the odds ratio (Ψ) in the toxin accumulation. A Ψ value was estimated as $\frac{ad}{bc}$; a and d are the number of quadrat samples where the relevant pathogen and toxin were both present or absent, respectively; b and c are the respective number of quadrat samples where only the relevant pathogen or the toxin was present. The standard error of $\ln(\Psi)$ was approximated as: $se(\ln(\Psi)) = \sqrt{1/a + 1/b + 1/c + 1/d}$ (Collett 1991). If there is no association between fungal presence and toxin accumulation, ψ is equal to 1 (i.e. $\ln(\Psi)=0$). Thus, the significance of the observed association was assessed by comparing $\ln(\Psi)/se(\ln(\Psi))$ against the standard normal distribution.

Finally, regression analysis was used to determine whether mycotoxin production is quantitatively related to the amount of fungal DNA; in this analysis toxin data were logarithmically (on a natural base) transformed to reduce the heterogeneity in variance. For those samples with no quantifiable DNA but with a positive diagnostic PCR result, pathogen DNA was set to 0.001% of total DNA; this is because the minimum quantified DNA value just exceeded 0.001% for all the three toxigenic FHB pathogens. All statistical analyses were carried out using Genstat™ version 6.1 (Payne 2002).

Results

Overall incidence of FHB pathogens

The overall incidence of the six FHB pathogens within each quadrat, as determined by the diagnostic PCR method, is given in Table 1. In chaff samples, the incidence was similar, about 50%, for the FHB pathogens except *F. culmorum* for which the incidence was much lower (20%). In grain samples, *F. graminearum* and *F. culmorum* had greatest (47%) and lowest (about 13%) incidence, respectively (Table 1).

Overall, the incidence of FHB pathogens in both chaff and grain samples as determined by the diagnostic PCR was much greater than that by the competitive PCR (Table 1), indicating that many samples contained a very low amount of fungal DNA (greater than the LOD but less than the LOQ). Logistic regression showed that the overall incidence of all FHB pathogens, except *F. graminearum*, as determined by the diagnostic PCR method, was significantly ($P < 0.05$ for *F. culmorum* and $P<0.01$ for the other four pathogens) greater in the chaff than in the grain (Table 1). The incidence of FHB pathogens with quantifiable amounts of DNA was significantly ($P < 0.05$) less in the grain than in the chaff for all FHB pathogens except *F. culmorum* for which there were no significant differences – both close to zero (Table 1). As an example, at one UK site (UK/3/1 in Table 2) *F. graminearum* DNA was quantified in 15 out of 16 chaff samples but none of the grain samples. The amount of quantifiable DNA in the chaff was as much as ten times greater than in the grain.

Table 1 Overall % incidence of each FHB pathogen detected in each chaff or grain sample using the PCR-based diagnostic (dPCR) and quantification (qPCR) methods, respectively

	dPCR		qPCR	
	Chaff	Grain	Chaff	Grain
F. avenaceum	46	29	19	4
F. culmorum	20	13	2	0
F. graminearum	43	47	18	8
F. poae	54	36	16	4
M. majus	50	28	25	9
M. nivale	56	22	17	1

In total, there were 224 quadrat samples.

Variation in the incidence of FHB pathogens within each site

Pathogen occurrence, as determined by the diagnostic PCR method, varied considerably between quadrats within each site, and ranged from 0% (not present) to 100% (present in all 16 quadrats; Table 2). For example, in the UK, *F. avenaceum*, *F. graminearum* and two *Microdochium* species were present in almost every chaff sample, whereas none of the Hungarian samples contained *F. avenaceum*, *F. graminearum*, *M. majus* or *M. nivale* in 2003. In many sites, intermediate levels of fungal incidence were obtained. For example, eight and ten Hungarian chaff samples at one site in 2004 contained *F. avenaceum* and *F. graminearum*, respectively (HU/4/1 in Table 2).

There was large variation in the amount of quantified pathogen DNA between the quadrats where the amount of pathogen DNA exceeded the LOQ (Table 2); this variability can be clearly seen in Fig. 1 where quantified *F. graminearum* DNA

Table 2 Summary of the quadrat sampling data on the presence and range of quantified fungal DNA (%) of each FHB pathogen at each sampling site

Site[a]	*F. avenaceum*				*F. poae*				*F. graminearum*				*F. culmorum*				*M. majus*				*M. nivale*			
	dPCR	qPCR			dPCR	qPCR			dPCR	qPCR			dPCR	qPCR			dPCR	qPCR			dPCR	qPCR		
	No[b]	No[b]	Min	Max	No	No	Min	Max	No	No	Min	Max	No	No	Min	Max	No	No	Min	Max	No	No	Min	Max
Chaff																								
HU/3/1	0	0		0	16	0		0	1	0		0	3	0		0	0	0		0	0	0		0
HU/3/2	0	0		0	12	0		0	3	0		0	0	0		0	0	0		0	0	0		0
HU/4/1	8	0		0	11	0		0	10	0		0	9	0		0	2	0		0	16	0		0
HU/4/2	1	0		0	3	0		0	7	0		0	0	0		0	1	1	0.07	0.07	15	0		0
IE/3/1	16	10	0.32	3.31	0	0		0	6	1	2.59	2.59	6	0		0	16	4	1.08	3.49	8	0		0
IE/3/2	15	0		0	1	0		0	4	1	1.01	1.01	2	0		0	16	7	2.5	36.7	3	0		0
IT/3/1	0	0		0	5	5	0.43	2.01	0	0		0	0	0		0	0	0		0	4	0		0
IT/3/2	1	1	0.03	0.03	1	1	0.16	0.16	1	0		0	1	0		0	0	0		0	1	0		0
IT/4/1	2	0		0	6	0		0	0	0		0	0	0		0	0	0		0	0	0		0
IT/4/2	4	0		0	6	1	0.67	0.67	1	0		0	0	0		0	15	15	1.1	10.3	16	16	0.11	1.43
UK/3/1	12	0		0	16	13	0.16	2.7	16	15	0.15	2.45	8	1	3.12	3.12	13	0		0	15	10	0.02	0.32
UK/3/2	12	0		0	16	16	0.73	2.39	16	6	0.21	0.65	1	1	1.03	1.03	16	4	0.13	0.91	16	12	0.05	0.16
UK/4/1	16	16	0.72	3.96	15	0		0	16	15	0.05	7.83	14	3	0.24	0.77	16	12	0.10	0.26	16	0		0
UK/4/2	16	16	0.27	2.53	13	0		0	16	2	0.12	0.63	1	0		0	16	14	0.14	0.62	16	0		0
Grain																								
HU/3/1	0	0		0	15	0		0	8	0		0	4	0		0	0	0		0	0	0		0
HU/3/2	0	0		0	10	0		0	15	0		0	3	0		0	0	0		0	0	0		0
HU/4/1	1	0		0	0	0		0	5	0		0	0	0		0	0	0		0	2	0		0
HU/4/2	0	0		0	0	0		0	2	0		0	0	0		0	2	0		0	8	0		0
IE/3/1	16	4	0.03	0.09	0	0		0	6	1	0.06	0.06	2	0		0	16	2	0.12	0.23	6	0		0
IE/3/2	15	0		0	2	0		0	7	1	0.04	0.04	5	0		0	16	2	0.12	0.16	2	0		0
IT/3/1	0	0		0	4	4	0.11	0.98	0	0		0	0	0		0	0	0		0	0	0		0
IT/3/2	0	0		0	0	0		0	1	0		0	0	0		0	0	0		0	0	0		0
IT/4/1	2	0		0	3	0		0	0	0		0	0	0		0	5	0		0	2	0		0
IT/4/2	15	0		0	5	0		0	3	0		0	0	0		0	16	16	0.06	0.47	16	2	0.04	0.04
UK/3/1	0	0		0	16	1	0.46	0.46	16	0		0	1	0		0	0	0		0	3	0		0
UK/3/2	0	0		0	16	5	0.12	0.31	16	0		0	0	0		0	0	0		0	10	0		0
UK/4/1	4	2	0.02	0.05	10	0		0	16	12	0.03	0.43	3	0		0	0	0		0	0	0		0
UK/4/2	12	2	0.06	0.17	0	0		0	11	3	0.05	0.13	12	0		0	7	1	0.05	0.05	1	0		0

[a] Site code XX/M/N: *XX* country (*HU* Hungary, *IE* Ireland, *IT* Italy, *UK* United Kingdom), M–year (3–2003, 4–2004) and *N* site number;

[b] Number of quadrats out of the total 16 at each site with positive diagnostic PCR and quantifiable amount of fungal DNA for each FHB pathogen. The limit of quantification for *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae*, *M. majus* and *M. nivale* was 0.0028, 0.0013, 0.0051, 0.0006, 0.0046 and 0.0014 ng μl^{-1}, respectively.

There were 16 quadrats (size 0.5 × 0.5 m) at each site. The presence and the amount of fungal DNA of each FHB pathogen were determined by PCR-based diagnostic (dPCR) and quantification (qPCR) methods (Nicholson et al. 2004), respectively.

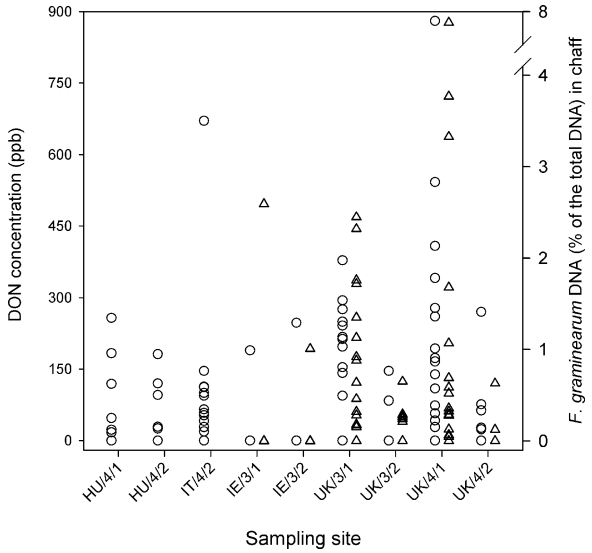

Fig. 1 Quantified *F. graminearum* DNA (*open circle*) in chaff, as percent of total DNA, and deoxynivalenol (DON, *open triangle*) levels (ppb) in each of the 16 quadrat samples of the nine sites where DON was detected: site code XX/M/N: *XX* country (*HU* Hungary, *IE* Ireland, *IT* Italy, *UK* United Kingdom), *M*-year (3–2003, 4–2004) and *N*–site number. Numbers of quadrat samples at these sites with *F. graminearum* DNA quantified in chaff and DON detected are given in Tables 2 and 4, respectively

is plotted for several selected sites. For example, at one UK site (UK/4/1 in Table 2) the amount of *F. graminearum* DNA ranged from 0.03 to 0.43% of the total DNA in the grain and from 0.05 to 7.83% of the total DNA in the chaff. At an Italian site (IT/4/2 in Table 2), the amount of *M. majus* DNA ranged from 1.1 to 10.3% of the total DNA in the grain.

Pathogen association

Logistic regression indicated that the average number of FHB pathogens detected on the chaff (2.7) was significantly ($P < 0.01$) greater than in the grain (1.9). This difference was mainly due to the greater number of FHB pathogens detected in the UK chaff samples (Table 3). In the other three countries, the number of pathogens detected was similar between the chaff and grain samples.

Table 3 Average number of FHB pathogens detected per quadrat at eight sites in four countries using the diagnostic PCR method

	Hungary	Ireland	Italy	UK
Chaff	1.8	2.9	1.0	5.1
Grain	1.7	2.9	1.1	2.4

The observed variance (V_{obs}) in the number of FHB pathogens present in each quadrat appeared to be similar to the expected variance (V_{exp}) under the assumption of independence, except for one site with $V_{obs} > V_{exp}$ (Fig. 2). The following regression model satisfactorily described the observed V_{obs}–V_{exp} relationship for the data from both chaff and grain samples:

$$\ln(V_{obs}) = 0.242 + 1.066 \ln(V_{exp}),$$

where the standard errors of the two parameter estimates were 0.138 and 0.123. This model explained about 71% of the total variation in $\ln(V_{obs})$ (Fig. 2). There were no significant differences in this relationship (i.e. the estimates of these two parameters) between grain and chaff samples. Since the parameter estimates of 0.242 and 1.066 were not significantly greater than 0 and 1.0 ($P > 0.05$), respectively, any aggregation of FHB pathogens within a quadrat was not statistically significant.

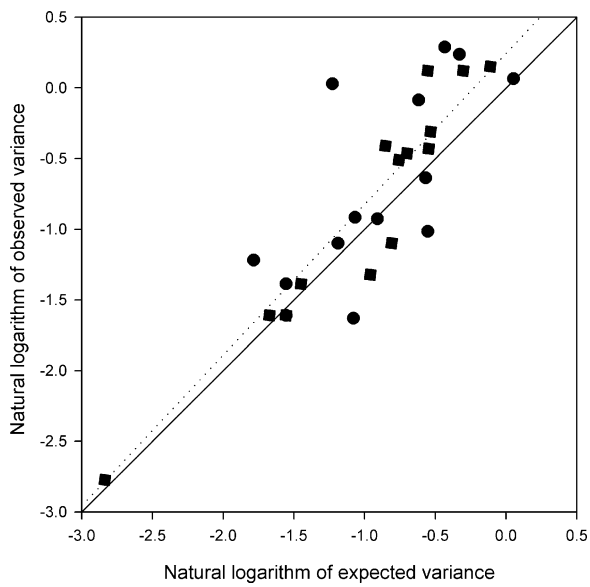

Fig. 2 The observed variance for the number of FHB pathogens within a quadrat at each site plotted against the variance expected under the assumption of independence (*filled circle* chaff samples, *filled square* grain samples) among the presence of FEB pathogens. The solid and *dotted lines* are the 1:1 (equal variance) ratio and the fitted model, respectively

Mycotoxin concentration within a single quadrat

Of the three toxins (DON, NIV and ZON), DON was most frequently detected in grain, and found in nine of 14 sites and in 58 of 224 quadrat samples, ranging from 17 to 880 ppb (median = 130 ppb; Table 4). NIV and ZON were also detected in a number of quadrat samples but their levels were very low compared to DON (Table 4). NIV was detected in 14 quadrat samples, ranging from 22 to 70 ppb (median = 37 ppb), whereas ZON was detected in 17 quadrat samples, ranging from 5 to 311 ppb (median = 35 ppb; Table 4). These toxins were most frequently detected in the UK (Table 4), e.g., DON was detected in 33 of 64 quadrat samples in the UK. The level of mycotoxin concentration varied greatly between sites, as well as between quadrat samples within a site. At one site (UK/4/1), the DON concentration ranged from <1 to 880 ppb (Fig. 1).

Relationship between FHB species and mycotoxin production

In most cases where toxins were detected, at least one of the three toxigenic fungal species also was detected by the diagnostic PCR method either in the chaff or in the grain (Table 5). However in many cases the amount of the DNA of these toxigenic FHB species was below the LOQ. In seven quadrat samples, DON or NIV were detected but none of the three toxigenic species were present; six out of these seven samples, where DON was present, came from one Italian site in 2004 (IT/4/2, Table 4). In the majority of quadrat samples, toxins were not detected even though there was one or more toxigenic species present (Table 5), in some cases with high levels of quantified DNA. For example, in 156 samples which contained at least one of the three pathogens (*F. graminearum*, *F. poae*, *F. culmorum*) in the chaff or grain, no NIV was detected (Table 5).

The probability of toxin being detected was strongly related to the presence of individual toxigenic species (Table 5). The probability that NIV was present was significantly ($P < 0.05$) associated only with *F. culmorum*; the odds ratio was estimated as 3.4, indicating that the probability of detecting NIV in the presence of *F. culmorum* was 3.4 times as much as in the absence of *F. culmorum*. The presence of DON was mainly associated with *F. graminearum* ($P < 0.001$); the estimated odds ratio was 6.4, compared to the respective value of 2.2 and 2.1 for *F. culmorum* and *F. poae*, which were both significant ($P < 0.05$). Of the three toxins, ZON was most strongly associated with the presence of individual toxigenic species; the

Table 4 Number of quadrats (out of 16, size 0.5 × 0.5 m) at each site where each of the three mycotoxins (*DON* deoxynivalenol, *NIV* nivalenol, *ZON* zearalenone) was quantified in the grain and the range of mycotoxin concentrations (ppb)

Site[a]	DON			NIV			ZON		
	No	Min	Max	No	Min	Max	No	Min	Max
HU/3/1	0			0			0		
HU/3/2	0			0			0		
HU/4/1	6	17	257	3	27	42	1	5	5
HU/4/2	5	25	181	0			0		
IE/3/1	1	189	189	0			0		
IE/3/2	1	247	247	0			0		
IT/3/1	0			0			0		
IT/3/2	0			0			0		
IT/4/1	0			0			0		
IT/4/2	12	19	671	4	22	39	0		
UK/3/1	11	94	378	0			0		
UK/3/2	2	83	146	0			0		
UK/4/1	15	28	880	7	33	70	14	9	311
UK/4/2	5	23	270	0			2	9	10

[a] Site code XX/M/N: *XX* country (*HU* Hungary, *IE* Ireland, *IT* Italy, *UK* United Kingdom), M–year (3–2003, 4–2004) and *N* site number.

Table 5 Number of quadrats with trichothecene-producing *Fusarium* species absent (−) or present (+) in the grain or chaff based on the dPCR results against the number of quadrats with toxins (*DON* deoxynivalenol, *NIV* nivalenol, *ZON* zearalenone) undetected (−) or detected (+), together with the estimated odds ratio (ψ on the natural logarithm scale) of detecting mycotoxins in the presence and absence of relevant pathogens and the probability of Fisher's exact test (FET) for the association between fungal presence and mycotoxin production

		F. culmorum		*F. graminearum*		*F. poae*		At least one of the three pathogens	
		−	+	−	+	−	+	−	+
NIV	−	151	59	95	115	87	123	54	156
	+	6	8	4	10	3	11	2	12
	ln(ψ)	1.227±0.561[a]		0.725±0.607		0.953±0.666		0.731±0.780	
FET Prob.		0.030		0.270		0.170		0.530	
DON	−	124	42	90	76	74	92	50	116
	+	33	25	9	49	16	42	6	52
	ln(ψ)	0.805±0.320		1.864±0.395		0.747±0.333		1.318±0.463	
FET Prob.		0.013		<0.001		0.029		0.003	
ZON	−	155	52	99	108	90	117	56	151
	+	2	15	0	17	0	17	0	17
	ln(ψ)	3.108±0.770		–[b]		–		–	
FET Prob.		<0.001		<0.001		<0.001		0.008	

[a] The number of after ± is the standard error of ln(ψ);

[b] Odds ratio cannot be estimated because the denominator is zero.

presence of all three species was significantly ($P < 0.001$) associated with ZON accumulation. The estimated odds ratio of ZON accumulation was 22.4 for *F. culmorum*. However, it was not possible to estimate the odds ratio for *F. graminearum* and *F. poae* because the denominator is zero, indicating a much greater odds ratio than for *F. culmorum.*

Of the three trichothecene-producing species (*F. graminearum*, *F. poae*, *F. culmorum*) detected, only the amount of *F. graminearum* DNA, either in the grain or in the chaff, was significantly ($P < 0.001$) correlated with the logarithm of DON (ln(DON)), ZON (ln(ZON)) and, to a lesser degree, NIV (ln(NIV)) concentrations (Table 6, Fig. 3), with the exception of the weak correlation ($r = 0.20$; $P < 0.05$) between the amount of *F. culmorum* DNA in chaff samples and ln(DON). *Fusarium graminearum* DNA was only quantified in a few grain samples in 2003; thus it was not possible to compare the relationship of ln(DON) or ln(ZON) with *F. graminearum* DNA in grain between the two years (Fig. 3a,c). The relationship of ln(DON) with *F. graminearum* DNA in chaff (Fig. 3b) appeared to be similar between 2003 and 2004. There was also not enough number of samples with quantifiable amounts of *F. graminearum* DNA in chaff and ZON in grain in 2003 to compare the ln(ZON)–*F. graminearum* DNA relationship between 2003 and 2004 (Fig. 3d). For this reason (only few samples with *F. graminearum* DNA in 2003), regression analysis was only applied to the 2004 data. Irrespective of whether the *F. graminearum* DNA in the grain or chaff was used, the relationship of *F. graminearum* DNA

Table 6 Correlations between the natural logarithms of deoxynivalenol (DON), nivalenol, (NIV) and zearalenone (ZON) (ppb) in quadrat flour (grain) samples and the amount of quantified *F. culmorum*, *F. graminearum* and *F. poae* DNA in chaff and flour samples ($P = 0.05$ when $r = 0.145$ with $n = 224$)

	F. culmorum		*F. poae*		*F. graminearum*	
	Chaff	Flour	Chaff	Flour	Chaff	Flour
DON	0.2	−0.09	0.13	−0.05	0.51	0.45
NIV	0.1	−0.04	−0.1	−0.05	0.34	0.34
ZON	0.12	0.09	−0.1	−0.05	0.56	0.77

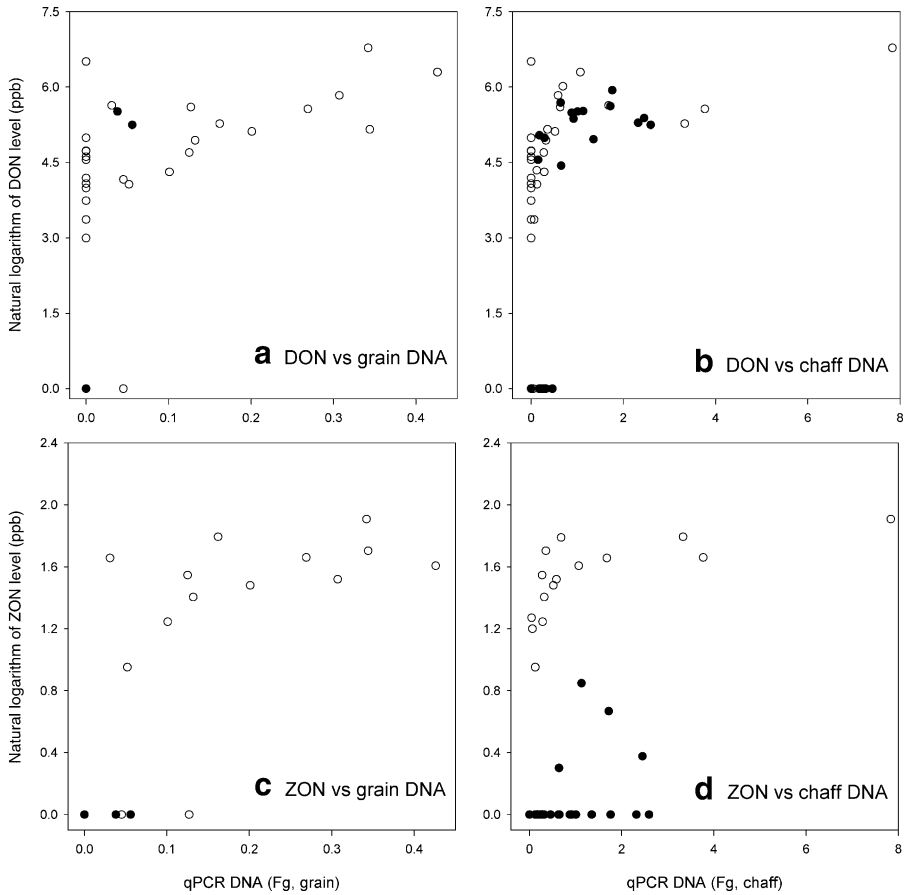

Fig. 3 Quantifiable *F. graminearum* DNA (as % of total DNA) in the grain (**a**, **c**) and chaff (**b**, **d**) plotted against the amount of deoxynivalenol (*DON*) and zearalenone (*ZON*; ppb, natural-logarithm transformed) in each quadrat sample in 2003 (*filled symbols*) and 2004 (*open symbols*)

with both ln(DON) and ln(ZON) was best described by a linear model. The fitted linear model explained a greater proportion of variation in ln(ZON) than in ln (DON). About 44 and 57% of variation in ln(ZON) was accounted for by *F. graminearum* DNA in the chaff and grain, respectively, compared to the corresponding values of 14 and 25% for ln(DON). However, in many quadrat samples toxins were not detected, even though these samples contained quantifiable amounts of *F. graminearum* DNA (Fig. 3).

Discussion

This study has demonstrated the high variability of mycotoxins associated with the FHB complex within a single field. In one field, the DON concentration ranged from < 1 to 880 ppb. Such large variability may have resulted from the phenomenon of disease aggregation at some spatial scales. Recent studies in North America suggested that there was some degree of aggregation among infected ears in one out of four fields studied (Shah and Bergstrom 2001) or among seed lots (Shah et al. 2002). The aggregation of diseased ears/plants probably results from aggregated initial inoculum of single FHB pathogens and its subsequent local dispersal via conidia (Horberg 2002) or ascospores (Schmale III et al. 2005). Superimposed on this potential non-random distribution of inoculum is the microclimatic variation, which may lead to further differences in subsequent infection, FHB development and toxin accumulation. An additional complicating factor could be the variation in the presence of one or more FHB pathogens across a site, as demonstrated in this study. As such, DON levels may still vary greatly even when the visual FHB severity is similar.

DON was the most frequently detected mycotoxin. In almost all cases, occurrence of toxins was associated with the detection of one or more of the FHB toxigenic species. The odds of mycotoxin occurrence increased significantly when one or more toxigenic

FHB species was present. DON accumulation was strongly associated with *F. graminearum*, NIV with *F. culmorum*, and ZON with all three pathogens. However, DON was detected in six grain quadrat samples from a single Italian site in 2004 but none of three species (*F. graminearum*, *F. culmorum*, and *F. poae*) was detected by PCR. The PCR assay to detect *F. graminearum* detects *F. austroamericanum*, *F. meridionale*, *F. asiaticum* and *F. graminearum* (O'Donnell et al. 2004; lineages 1, 2, 6 and 7, respectively sensu O'Donnell et al. 2000), which are most commonly associated with wheat, but does not detect *F. boothii*, *F. mesoamericanum* or *F. acaciae–mearnsii* (lineages 3, 4 and 5 respectively) (Waalwijk et al. 2003). It is conceivable that isolates of one of the species, *F. boothi*, *F. mesoamericanum* or *F. acaciae–mearnsii* were responsible for the DON detected in the grain of these samples although these species are thought to have originated in Central America, Africa and Australia. It is also possible that other DON-producing species, such as *F. pseudograminearum* (not screened for) were responsible, although *F. pseudograminearum* is more generally associated with crown-rot of cereals.

Another possible reason is fungal competition. Inoculations with mixed FHB pathogens under controlled conditions have shown that mycotoxin accumulation appeared to increase considerably compared to inoculations with single pathogens, even though the fungal biomass seemed to be reduced in the mixed inoculations (Xu et al. 2007). For example, NIV production in inoculations with combined *F. poae* and *F. avenaceum* was increased > 20 times, compared to inoculations with *F. poae* alone. *Fusarium avenaceum* was detected in five of the six grain samples where DON was detected but neither *F. graminearum*, *F. poae* or *F. culmorum* were detected. Significant interactions between isolates of the same toxigenic species have also been observed. Competition between isolates of *F. culmorum*, originating from four countries, led to increased mycotoxin productivity on winter rye in some cases (Miedaner et al. 2004). DON productivity appeared not to have differed between corn ears inoculated with *F. graminearum* only and with both *F. graminearum* and *F. moniliforme* (Reid et al. 1999). In contrast, fumonisin B-1 productivity appeared to have considerably increased in one year in the mixed inoculation, compared to the inoculation with *F. moniliforme* only. In addition, FHB pathogens may have to compete with other resident/non-resident micro-organisms, including other pathogens. It is thus possible that competition between *Fusarium* pathogens leads to increased mycotoxin production even though the biomass of one or more of the toxigenic pathogens might be below the detection threshold.

Previous studies have shown that visual disease and DON content of grain may (Boyacioglu et al. 1992; Lemmens et al. 1997) or may not (Edwards et al. 2001; Liu et al. 1997) be correlated. This is not surprising given the fact that some of the head blight pathogens identified at the sampling sites do not produce known mycotoxins. Furthermore, for those FHB pathogens that are mycotoxigenic, the relationship between disease symptoms and mycotoxin production may vary between species as well as between isolates within a single species. Recently, a meta-analysis of previously published and unpublished data suggested that there is an overall significant positive correlation between FHB incidence/severity and DON accumulation but that such a relationship is significantly influenced by other factors such as wheat type (spring versus winter wheat) and location (Paul et al. 2005). However, another recent survey based on a single bulk sample for each site sampled found no relationship between mycotoxins (DON and NIV) and the amount of FHB pathogen DNA (Xu et al. 2003). In contrast, the present study suggests that there is a significant relationship between DON and *F. graminearum* DNA. This difference is probably due to the variability in toxin production capability between and within *Fusarium* spp. In the previous survey (Xu et al. 2003), there was only a single bulk sample for each site, and as such the within and between species variability in toxin-producing ability can be expected to be greater for the single bulk sample than for the much smaller quadrat sample(s). Thus any DON-fungal relationship in the small bulk samples may have been masked by the large degree of within/between species variability. It should be noted that the positive relationship between DON and *F. graminearum* DNA found in the present study was only moderate, and that the level of DON varied greatly between the two years. Based on field samples in a single year, the concentration of DON was found to correlate equally well with the incidence of the DON-producing species *F. culmorum* and *F. graminearum* in the grain

as well as with total DNA of both these species (Waalwijk et al. 2004).

The incidence of FHB pathogens, except for *F. graminearum*, as determined by the diagnostic PCR method, was significantly greater in the chaff than in the grain. Similarly the incidence of FHB pathogens, as determined by the competitive PCR method, was significantly greater in the chaff than in the grain, except for *F. culmorum*. These differences in the incidences of FHB pathogen between the chaff and the corresponding grain samples may be due to timing of infection in relation to grain development, which is primarily determined by the environmental conditions. *Fusarium* fungi, among others, can land on the chaff at any time after emergence of the ear. They may colonise chaff tissue first and proceed to infect and colonise grain. Wheat plants differ very significantly over time in their susceptibility to FHB pathogens but the consensus is that mid-anthesis is the most susceptible period (Lacey et al. 1999; Parry et al. 1995). When conditions are not conducive to infection and colonisation, grain infection is less likely to occur than chaff infection. Furthermore, the relative incidence of chaff and grain infection also depends on fungal aggressiveness. Hence the reduction in chaff-grain incidence is likely to be less for *F. graminearum* as this species is considered the most aggressive FHB pathogen (Xue et al. 2004). Infection or saprophytic colonisation of chaff may also take place post-anthesis.

There was considerable variation in the presence/absence of individual *Fusarium* pathogens at a given sampling site. Our previous study indicated frequent positive associations among FHB pathogens in their presence when based on a single bulk sample for each field, i.e. the presence of one FHB pathogen within a field often was related positively to the presence of other FHB pathogens within the same field (Xu et al. 2005). Present quadrat data strongly suggest that the six FHB pathogens are not likely to cluster within a single quadrat, and that occurrence of one FHB pathogen is not positively related to the occurrence of other FHB pathogens. Thus, FHB pathogens are not likely to exhibit true synergy when co-infecting the same host tissues. Previously observed associations (Xu et al. 2005) probably arose from indirect interactions due to microclimatic conditions, host susceptibility and secondary colonisation by the pathogens. As all ears within a quadrat were sampled in the present study, a direct interaction between two species on the same ear tissue is far less likely than indirect interactions mediated by other factors (climatic factors, etc.), as the main cause for the observed interactions. In general, FHB pathogens need free moisture (water) for infection with the time period required dependent on temperature and *Fusarium* species (Rossi et al. 2001). Provided inoculum is present, conditions conducive to one FHB species are also likely to favour other FHB species. Additionally, current varieties may have similar resistance or susceptibility to a given pair of species, and similarly, fungicides used may also have similar efficacy on the same pair of species. In addition, saprophytic colonisation by FHB pathogens might be enhanced on ears (spikelets) already infected by another FHB pathogen.

As expected, the diagnostic PCR method was more sensitive in detecting the presence of each FHB pathogen than the competitive PCR method. Except for *F. avenaceum*, the diagnostic PCR method is at least 10 times more sensitive than the competitive PCR method in detecting the FHB pathogen DNA. DNA quantification based on the TaqMan system has recently been developed for *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. majus* (Waalwijk et al. 2004), which is likely to be more sensitive than the competitive PCR method used in the present study. The use of quantitative PCR assays for the fungal species within the FHB disease complex provide valuable tools for the development of control measures to reduce FHB severity and corresponding mycotoxin contamination of infected cereal grains.

This study has clearly demonstrated large within-field variability in the presence of FHB pathogens, in the amount of their DNA and in the accumulation of their associated mycotoxins. To obtain an accurate estimate of *Fusarium* mycotoxins within a field for the purpose of risk assessment, an adequate sample is therefore essential. Furthermore, analysis of a substantial number of small samples taken at various sites within a field can be expected to be more reliable than analysis of a single large composite sample in estimating the amount of mycotoxin present.

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References

Bai, G., & Shaner, G. (1994). Scab of wheat: Prospects for control. *Plant Disease, 78*, 760–766.

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiological Reviews, 16*, 497–516.

Bottalico, A., & Perrone, G. (2002). Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology, 108*, 611–624.

Boyacioglu, D., Hettiarachchy, N. S., & Stack, R. W. (1992). Effect of three systemic fungicides on deoxynivalenol (vomitoxin) production by *Fusarium graminearum* in wheat. *Canadian Journal of Plant Science, 72*, 93–101.

Collett, D. (1991). *Modelling binary data*. London: Chapman & Hall.

De Wolf, E. D., Madden, L. V., & Lipps, P. E. (2003). Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. *Phytopathology, 93*, 428–435.

Doohan, F. M., Parry, D. W., Jenkinson, P., & Nicholson, P. (1998). The use of species specific PCR based assays to analyse Fusarium ear blight of wheat. *Plant Pathology, 47*, 197–205.

Dubin, H. J., Gilchrist, L., Reeves J., & McNab, A. (eds.) (1997). *Fusarium head scab: Global status and prospects* (p. 130). Mexico, DF: CIMMYT.

Edwards, S. G., Pirgozliev, S. R., Hare, M. C., & Jenkinson, P. (2001). Quantification of trichothecene-producing Fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. *Applied and Environmental Microbiology, 67*, 1575–1580.

Glynn, N. C., Hare, M. C., Parry, D. W., & Edwards, S. G. (2005). Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties majus and nivale to species status. *Mycological Research, 109,* 872–880.

Horberg, H. (2002). Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum*. *European Journal of Plant Pathology, 108*, 73–80.

IARC (1993). *Monographs on the evaluation of carcinogenetic risks to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins*, vol. 56. Lyon: International Agency for Research on Cancer.

Krska, R., Schothorst, R. C., van Egmond, H. P., Josephs, R. D., Lepschy, J., Pettersson, H. et al. (2005). Processing and purity assessment of standards for the analysis of type-B trichothecene mycotoxins. *Analytical and Bioanalytical Chemistry, 382*, 1848–1858.

Lacey, J., Bateman, G. L., & Mirocha, C. J. (1999). Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. *Annals of Applied Biology, 134*, 277–283.

Lemmens, M., Josephs, R., Schumacher, R., Grausgruber, H., Buerstmayr, H., Ruckenbauer, P. et al. (1997). Head blight (*Fusarium* spp.) on wheat: Investigations on the relationship between disease symptoms and mycotoxin content. *Cereal Research Communications, 25*, 459–465.

Li, F., Luo, X., & Yoshizawa, T. (1999). Mycotoxins (trichothecenes, zearalenone and fumonisins) in cereals associated with human red intoxications stored since 1989 and 1991 in China. *Natural Toxins, 7*, 93–97.

Liu, W. Z., Elen, O. N., Sundheim, L., Langseth, W., & Skinnes, H. (1997). Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. *European Journal of Plant Pathology, 103*, 589–595.

McMullen, M. P., Jones, R., & Gallenberg, D. (1997). Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease, 81*, 1340–1348.

Miedaner, T., Schilling, A. G., & Geiger, H. H. (2004). Competition effects among isolates of *Fusarium culmorum* differing in aggressiveness and mycotoxin production on heads of winter rye. *European Journal of Plant Pathology, 110*, 63–70.

Moschini, R., & Fortugno, C. (1996). Predicting wheat head blight incidence using models based in meteorological factors in Pergamino, Argentina. *European Journal of Plant Pathology, 102*, 211–218.

Nicholson, P., Chandler, E., Draeger, R. C., Gosman, N. E., Simpson, D. R., Thomsett, M. et al. (2003). Molecular tools to study epidemiology and toxicology of Fusarium head blight of cereals. *European Journal of Plant Pathology, 109,* 691–703.

Nicholson, P., Lees, A. K., Maurin, N., Parry, D. W., & Rezanoor, H. N. (1996). Development of a PCR assay to identify and quantify *Microdochium nivale* var *nivale* and *Microdochium nivale* var majus in wheat. *Physiological and Molecular Plant Pathology, 48*, 257–271.

Nicholson, P., Simpson, D. R., Wilson, A. H., Chandler, E., & Thomsett, M. (2004). Detection and differentiation of trichothecene and enniatin-producing Fusarium species on small-grain cereals. *European Journal of Plant Pathology, 110,* 503–514.

O'Donnell, K., Kistler, H., Tacke, B., & Casper, H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proceedings of The National Academy of Science USA, 97*, 7905–7910.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., & Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics and Biology, 41*, 600–623.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grains – a review. *Plant Pathology, 44,* 207–238.

Paul, P. A., Lipps, P. E., & Madden, L. V. (2005). Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: A meta-analysis. *Phytopathology, 95,* 1225–1336.

Payne, R. (ed.) (2002). *The guide to GenStat® release 6.1–part 2: Statistics*. Oxford: VSN International.

Pirgozliev, S. R., Edwards, S. G., Hare, M. C., & Jenkinson, P. (2003). Strategies for the control of Fusarium head blight in cereals. *European Journal of Plant Pathology, 109,* 731–742.

Reid, L. M., Nicol, R. W., Ouellet, T., Savard, M., Miller, J. D., Young, J. C. et al. (1999). Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: Disease progress, fungal biomass, and mycotoxin accumulation. *Phytopathology, 89,* 1028–1037.

Rossi, V., Ravanetti, A., Pattori, E., & Giosue, S. (2001). Influence of temperature and humidity on the infection of wheat spikes by some fungi causing Fusarium head blight. *Journal of Plant Pathology, 83,* 189–198.

Schmale, D. G. III, Shah, D. A., & Bergstrom, G. C. (2005). Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. *Phytopathology, 95*, 472–479.

Shah, D., & Bergstrom, G. (2001). Spatial patterns of Fusarium head blight in New York wheat fields in 2000 and 2001. In K. Y. Erlanger (Ed.), (pp. 154–155). USA: 2001 National Fusarium Head Blight Forum.

Shah, D., Clear, R., Madden, L., & Bergstrom, G. (2002). Summarizing the regional incidence of seed-borne fungi with the b-binomial distribution. *Canadian Journal of Plant Pathology, 24,* 168–175.

Simpson, D. R., Rezanoor, H. N., Parry, D. W., & Nicholson, P. (2000). Evidence for differential host preference in *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale*. *Plant Pathology, 49,* 261–268.

Waalwijk, C., Kastelein, P., de Vries, I., Kerényi, Z., van der Lee, T., Hesselink, T. et al. (2003). Major changes in *Fusarium* spp. in wheat in the Netherlands. *European Journal of Plant Pathology, 109,* 743–754.

Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-de Corainville, G. et al. (2004). Quantitative detection of Fusarium species in wheat using TaqMan. *European Journal of Plant Pathology, 110,* 481–494.

Xue, A. G., Armstrong, K. C., Voldeng, H. D., Fedak, G., & Babcock, C. (2004). Comparative aggressiveness of isolates of *Fusarium* spp. causing head blight on wheat in Canada. *Canadian Journal of Plant Pathology, 26*, 81–88.

Xu, X.-M., & Berrie, A. (2005). Epidemiology of mycotoxigenic fungi associated with Fusarium ear blight and apple blue mould: A review. *Food Additives and Contaminants, 22,* 290–301.

Xu, X.-M., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G. et al. (2003). Is the amount of mycotoxins in cereal grains related to the quantity of Fusarium DNA? *Aspects of Applied Biology, 68,* 101–108.

Xu, X.-M., Parry, W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G. et al. (2005). Predominance and association of pathogenic species causing Fusarium ear blight in wheat. *European Journal of Plant Pathology, 112,* 143–154.

Xu, X.-M., Monger, W., Ritieni, A., & Nicholson, P. (2007). Disease development, fungal growth and accumulation of mycotoxins on wheat ears inoculated with combinations of toxigenic Fusarium head blight species. *Plant Pathology* (in press) DOI 10.1111/j.1365-3059.2007.01650.x.