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Fusarium species and DON contamination associated with head blight in winter wheat over a 7-year period (2003–2009) in Belgium

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Abstract The occurrence of *Fusarium* species in winter wheat in southern Belgium (Wallonia) and the deoxynivalenol content in 692 samples collected in commercial fields in the region's main cereal growing area were investigated. The main *Fusarium* species associated with head blight in wheat were identified at levels that varied from year to year. Interactions between fungal species causing head blight in wheat were detected, most of them positive. The years 2007 and 2008 were very conducive to the disease and there was a strong correlation between mean annual deoxynivalenol content and number of days with a mean relative humidity above 80% over a period starting from 7 days before the mean flowering date and ending 16 days after this date. A two-stage approach, based on type of year (at risk or not) and agricultural practices during risk years has been developed to help cereal storage companies reduce the risk of mixing sound and deoxynivalenol-contaminated lots at harvest and to limit the number of analyses.

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Introduction

Fusarium head blight (FHB) in wheat (*Triticum aestivum* L.) is an economically important disease in wheat-growing regions around the world. In Europe, the disease is caused by a complex of fungal pathogens, including *Fusarium* species and *Microdochium nivale* (Fries) Samuels & I.C. Hallett (Xu et al. 2005). In wheat, the main *Fusarium* species are *F. graminearum sensu stricto* (teleomorph= *Gibberella zeae* [Schwein] Petch.), *F. avenaceum* (teleomorph= *Giberella avenacea*), *F. culmorum* (Wm. G. Sm.) Sacc. and *F. poae* (Peck) Wollenw. (Parry et al. 1995; Waalwijk et al. 2003). Apart from causing yield loss and low seed germination, *Fusarum* species are known to produce fungal toxins (mycotoxins), including trichothecenes and the estrogenic mycotoxin zearalenone, which make raw grains unfit for human or animal consumption (Stepień and Chelkowski 2010). Among the trichothecenes, deoxynivalenol (DON, vomitoxin) is considered to be the major molecule in naturally infected grains. It is known to cause food rejection, diarrhoea, vomiting and depressed immune function (Bennett and Klich 2003). As this mycotoxin is subject to an EU regulatory limit of 1,250 μg kg^{-1} in raw grains that could be used for human consumption (EU directive 1881/2006), DON-

containing grain exceeding the permitted levels can be downgraded at the market.

The disease is favoured by warm, moist conditions during the flowering stage of the cereal (Hooker et al. 2002; Osborne and Stein 2007). The type of previous crop, combined with soil management (occurrence of host residues on soil which can act as an inoculum reservoir) (Dill-Macky and Jones 2000; Maiorano et al. 2007) and the susceptibility of the wheat variety to the disease (Bai and Shaner 2004) could also influence the occurrence of FHB.

Forecasting models based on climatic conditions during the wheat flowering period have been proposed for predicting FHB or DON contamination in winter wheat at the field scale (Musa et al. 2007; Prandini et al. 2009). The well-known Canadian model "DONcast" (Hooker et al. 2002; Schaafsma and Hooker 2007) predicts DON level in mature grains and is mainly based on weather conditions during 3 critical periods of 4 days around wheat heading. Although of great interest, these models cannot be standardised to be applicable in any region of the world, as shown by Franz and co-workers (2009) in evaluating the DONcast model in the Netherlands. The lack of fit of predictive models could probably be explained by regional factors. In this context, regional differences in fungal populations causing head blight have been reported (Jennings et al. 2004; Isebaert et al. 2009; Parry et al. 1995; Waalwijk et al. 2003), with potential interactions (Xu et al. 2005, 2008) leading to differences in aggressiveness and mycotoxin production. In addition, the type of previous crop, especially the maize varieties available could influence the inoculum level as some maize varieties are less susceptible to *Fusarium* infection. The wheat varieties also vary greatly among countries and from 1 year to another. For these reasons, an evaluation of FHB and DON contamination must take account of factors at the regional scale.

The objective of this study was to determine the occurrence of the main *Fusarium* species and the DON content in commercial winter wheat fields over a 7-year period in Wallonia in the southern part of Belgium, and to evaluate the impact of regional meteorological conditions, wheat varieties and previous crops on DON content and *Fusarium* species in order to develop a simple decision-support system for the post-harvest management of grains.

Materials and methods

Survey strategy In the period from 2003 to 2009, data were collected from 692 wheat fields located in the cereal cropping area of Belgium mainly in Wallonia. Samples were collected by hand a few days before harvest (maximum 7 days). Each field was identified by spatial coordinates (Fig. 1). Ear samples (about 300) were collected at random along the greatest field diagonal and threshed with a stationary plot thresher that was carefully cleaned between each sample to avoid any cross-contamination. A 1.5 kg wheat sample was also obtained from each of 38 grain bins following machine harvesting in 2007. The fields sampled each year were selected to take account of the main cultural practices used in Wallonia (previous crop, tillage, wheat varieties) and were evenly distributed in the cereal cropping area.

Deoxynivalenol quantification A part of the sample (~ 600 g) was milled (particle size=0.5 mm—Cyclotec Sample Mill, Foss Tecator, USA) in order to determine the deoxynivalenol (DON) content by ELISA test using a commercial kit (Veratox 5/5 for DON, Neogen, USA). DON was extracted from a 10 g ground sample shaken in 100 ml distilled water. The limits of detection and quantification (110 μg kg^{-1} and 360 μg kg^{-1} respectively) determined according to the standard NF V 03–110, were in agreement with the performance characteristics provided by the manufacturer. An expanded uncertainty (Ue) of 35.75% determined according to the standard ISO5725 (top-down approach) was in the range of Ue values obtained in other studies (Ambrus and Soboleva 2004). The ELISA method has been accredited since 2008 in line with the standard ISO17025.

Fungal analyses of grains Grain samples were surface-disinfected in 3% ethanol/0.3% NaOCl for 5 min. They were dried in a laminar flow and put onto PDA medium (potato dextrose agar, Becton Dickinson, Le Pont de Claix, France) with 13 kernels per plate and 16 plates per sample in 2003–2007 and 5 kernels per plate and 30 plates per sample in 2008–2009 in order to reduce the total number of kernels analysed per sample (from 208 to 150 kernels). The plates were incubated at 20–22°C under a 12 h light/dark regime for 5–6 days before analysis. The *Fusarium* species were

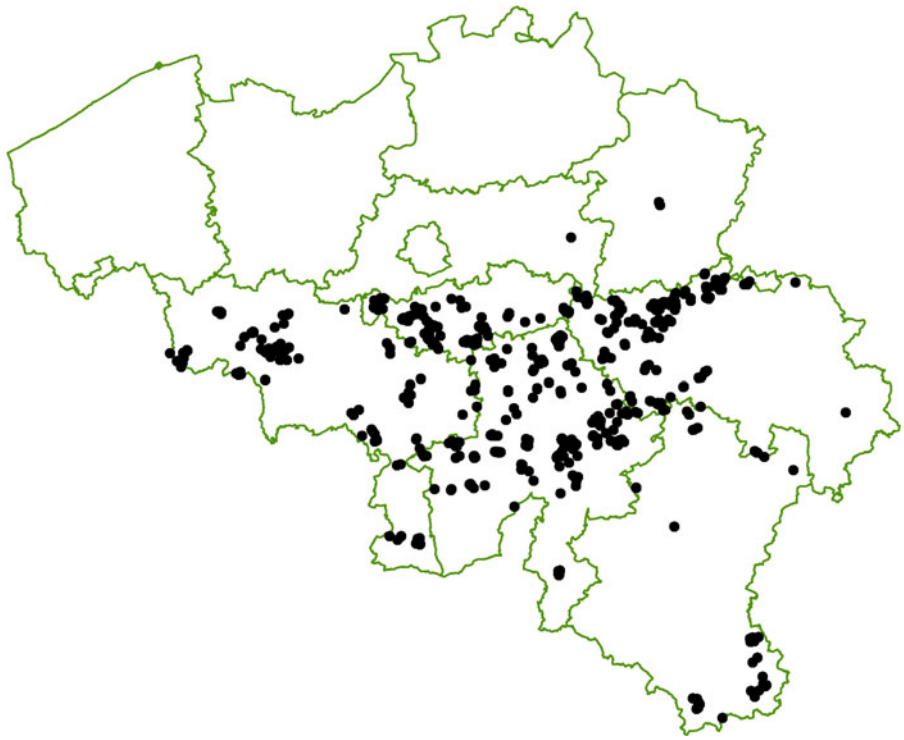

Fig. 1 Distribution of wheat samples collected in the cereal cropping area of Wallonia, Belgium from 2003 to 2009

identified following the method described by Burgess et al. (1994) and Leslie and Summerell (2006). Subcultures were carried out on SNA (synthetic low nutrient agar, Nirenberg 1990) for the *Fusarium* isolates of the section *Sporotrichiella* and for other *Fusarium* that did not sporulate on PDA. In order to validate the morphological identification of *Fusarium* isolates, PCR were carried out on some isolates from our collection using tests described in the literature for the main *Fusarium* species found in wheat kernels (Doohan et al. 1998; Nicholson et al. 1998; Parry and Nicholson 1996; Turner et al. 1998). Moreover, one isolate of the main *Fusarium* species identified in this study was deposited in the CBS Fungal Biodiversity Centre (The Netherlands) collection (*Fusarium graminearum*, accession number: CBS128539; *F. culmorum*, accession number: CBS128537; *F. avenaceum*, accession number: CBS128538; *F. poae*, accession number: CBS128536). Three parameters were calculated: the percentage of grains infected by each species (PG) for each field of the survey (692 values per fungal species), disease incidence corresponding to the percentage of sampled fields infected with a specific head blight species (IS; 1 = presence; 0 = absence) for each year of the survey (7 values per fungal species), and a disease intensity for each specific head blight species expressed as the annual mean percentage of infected grains (MIG) in the fields where infection by a particular fungus is >0 (7 values per fungal species).

Meteorological data The weather data were collected each year (from 2003 to 2009) over a period of 15 days centred around the flowering date (period 1) or a period starting from 7 days before flowering to 16 days after flowering (period 2) to take account of late infection, as noted by Del Ponte et al. (2007) and Yoshida and Nakajima (2010). Measurements of daily temperature (°C, TEMP), relative humidity (%, RH), total number of days with a mean RH above 80% (DRH80), total precipitation (log-transformed amount of rain in mm per square metre (PRE)) and number of days with rain above 0.1 mm (DPRE) were obtained from 118 weather stations that were part of the Belgian synoptic network (Fig. 2). Evaporation potential (PET) was calculated following the Penman equation (van der Voet et al. 1994). The data were interpolated to a 10×10 km grid following a protocol used by the European Crop Growth Monitoring System (van der Voet et al. 1994). For each time period, average

Fig. 2 Distribution of weather stations (N=118) which are part of the Belgian synoptic network (black dots)

values for each grid and each meteorological parameter were calculated and attributed to each field of the survey according to its location in the network of grids.

Cultural practices and flowering date Information on wheat variety, preceding crop and soil preparation before sowing (ploughing or no ploughing) were provided by the farmers participating in the survey (Table 1). The types of previous crops were classified into 4 categories using information from the literature (Dill-Macky and Jones 2000) or existing predictive tools (Myco-LIS®—Arvalis Institut du végétal 2008): type 1 (low risk) = rape, flax or pea; type 2 (medium risk) = wheat, barley, oats, sugar beet, potato, chicory; type 3 (high risk) = maize for silage with ploughing and type 4 (very high risk) = maize for silage without ploughing. No sample was taken from fields cultivated after maize for grain and very few samples were taken from fields with maize without ploughing because these situations are not very frequent in Wallonia. Wheat cultivars widely used in 2006–2009 were grouped into 4 categories, with 4 being 'most susceptible' and 1 meaning 'least susceptible'. The classification was based on visual observations of FHB symptoms in experimental fields that were not sprayed with fungicide. The data were collected in 2007 and 2008 in the framework of the wheat-breeding program carried out at the Walloon Agricultural Research Centre. Disease severity was considered to be a good evaluation of DON content because a strong relationship between these parameters had been shown previously (Brennan et al. 2007). The mean flowering date was calculated for each year of the study from the flowering date registered for each field by the farmers.

Statistical analysis Pearson correlation coefficients (r) were calculated to determine the degree of linear relationship between two variables. The interaction between head blight fungal species was tested using a log-linear model as described by Xu et al. (2005) by considering the five most important head blight species as factors and all the samples of the study (N=692). Each factor had two levels: present (1) or absent (0). In case of a significant interaction ($P<0.05$), the type of association (positive or negative) was determined. Statistical analyses were carried out using SAS software (SAS Institute, Cary, NC, version 8.2).

Table 1 Characteristics and DON content of wheat samples collected in the Wallonia region of Belgium from 2003 to 2009

Samples	2003	2004	2005	2006	2007	2008	2009
Number of samples	186	102	107	115	67	51	64
Previous crop type 1 (%)	16.7	15.7	18.7	15.7	14.9	17.6	12.5
Previous crop type 2 (%)	50.5	53.9	45.8	62.6	56.7	49.0	60.9
Previous crop type 3 (%)	30.1	30.4	33.6	19.1	22.4	29.4	26.6
Previous crop type 4 (%)	2.7	0	1.9	2.6	6	3.9	0
Wheat varieties (Number)	38	26	33	25	23	22	19
Mean flowering date[a]	06–12	06–13	06–10	06–14	05–27	06–6	06–11
DON content[b]							
Mean DON ($\mu g.kg^{-1}$)	260	180	<110	150	1350	820	120
Median DON ($\mu g.kg^{-1}$)	< 110	<110	<110	130	870	450	<110
Maximum DON ($\mu g.kg^{-1}$)	2720	2500	190	680	5610	4790	1310
> 1,250 $\mu g.kg^{-1}$ DON (%)[c]	5	1.8	0	0	36	17.6	1.6
r DON/*F. graminearum*	0.12	0.24	0.03	0.36	0.69	0.25	0.43

[a] expressed in month-day

[b] 110 $\mu g.kg^{-1}$ = limit of detection of the ELISA method

[c] EU regulation established for grains intended for use as food (UE 1881/2006)

Results

Validation of the sampling procedure

In order to evaluate the sampling strategy which involved collecting samples a few days before harvest, the level of DON contamination in 38 fields was measured in grain collected before harvest, and in grain collected from the same fields obtained from grain bins following machine harvest. The validation was conducted in 2007, the year most conducive to DON contamination. As shown in Fig. 3, DON concentrations in samples collected by hand were strongly correlated to the levels in samples collected from grain bins following harvest (Fig. 3, r=0.77, P<0.001).

Head blight fungal species and DON survey

As shown in Table 1, the DON content varied greatly during the observation period, with the years 2007 and 2008 displaying the highest mean DON content. These years were characterized by the highest values in the meteorological variables RH, DRH80, PRE and DPRE, and by the lowest value for the variable PET (Fig. 4). In 2007, 36% of the samples contained more than 1,250 $\mu g\ kg^{-1}$ DON, which is the EU regulatory limit in raw grain that can be used for human consumption (EU directive 1881/2006). In 2008, 17.6% of the samples were above this threshold. The maximum DON content never exceeded 8,000 ppb, corresponding to the guidance value proposed by the European Commission for DON in products intended for animal feed (Commission recommendation, 17 August 2006).

Five fungal species associated with head blight in wheat were most often isolated from grains: *F. graminearum, F. culmorum, F. avenaceum, F. poae* and *Microdochium nivale*. Other species, including *F.*

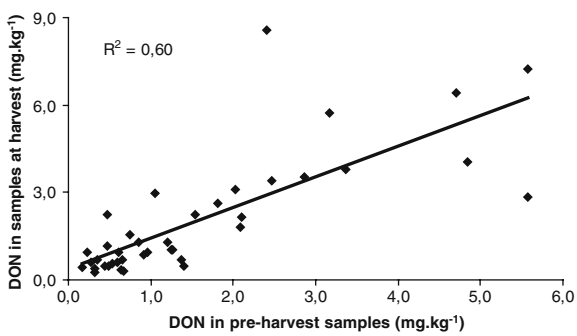

Fig. 3 Comparison between wheat samples collected a few days before harvest and samples collected from grain bins after harvest in 38 fields in the cereal growing area of Wallonia, Belgium (data from 2007)

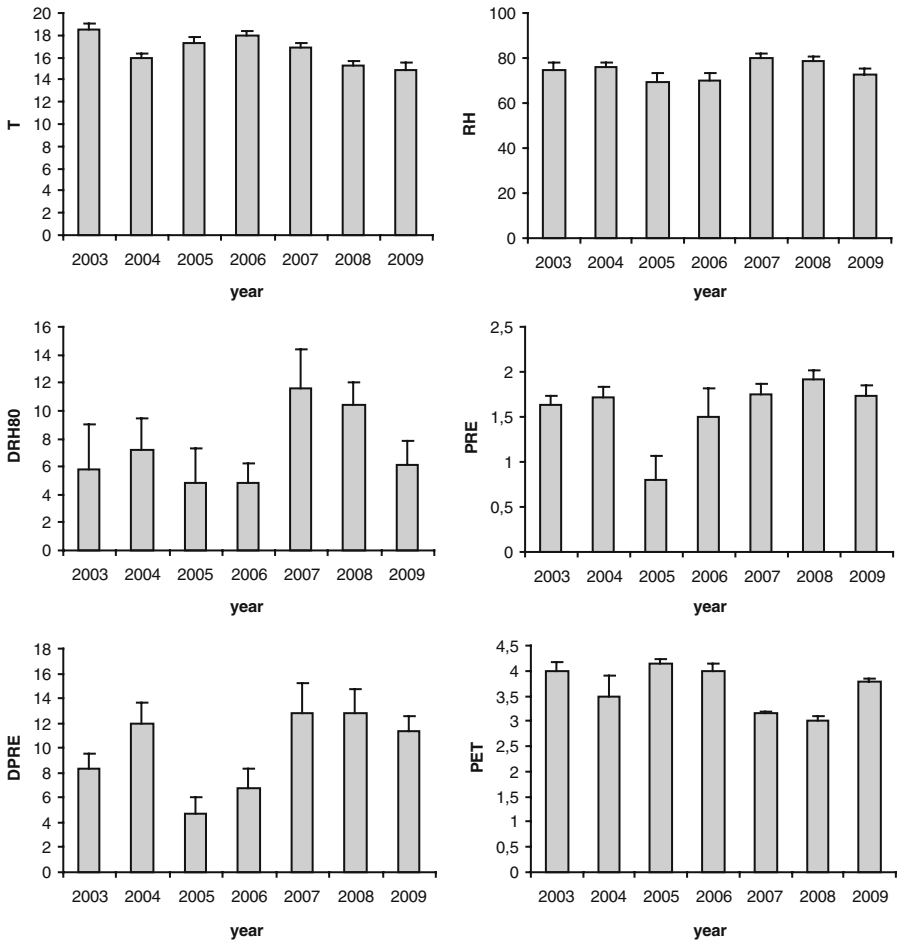

Fig. 4 Mean meteorological conditions during a period starting 7 days before the mean flowering date of the cereal and ending 16 days after it, over the 7 years of the survey in the sampled fields. T = temperature (°C), RH = relative humidity (%), DRH80 = number of days with a mean RH above 80%, PRE = total precipitation (log transformed value of $mm.m^{-2}$), DPRE = number of days with precipitation above 0.1 mm, and PET = evapotranspiration potential

sporotrichioides, *F. tricinctum* and *F. langsethiae* were also identified, but less frequently (data not shown).

In terms of overall incidence (IS value, Fig. 5a), *F. avenaceum, F. graminearum* and *M. nivale* behaved in a similar way with the highest IS values being observed in 2004 and 2007 and the lowest in 2005, 2006 and 2008. The IS value for *F. poae* was relatively constant over the years (about 70%) except for 2007 and 2008 when it was lower. The overall incidence of *F. culmorum* decreased during the course of the study, from 80% in 2003 to ~10% over the final 3 years. The interaction between *F. avenaceum*, *F. graminearum* and *M. nivale,* as shown in Fig. 5a for IS values, was confirmed by the analysis of multiple species interactions using a log-linear model. There were no significant 5- or 4-way interactions ($P>0.05$). In contrast, positive two-way interactions were detected between *F. graminearum* and *F. avenaceum*, between *F. graminearum* and *M. nivale*, and between *F. avenaceum* and *M. nivale* (Table 2). Positive interactions were also detected between *F. poae* and *F. culmorum*, between *F. avenaceum* and *F. culmorum,* and between *F. poae* and *M. nivale*. In contrast, a negative interaction was found between *F. culmorum* and *F. graminearum* (Table 2).

In terms of disease intensity (MIG value, Fig. 5b), *F. graminearum* and *M. nivale* displayed the highest values in 2007 and 2008, corresponding to the years with the highest DON content. The MIG values for the other species were generally below 5%, regardless of the year. A slight increase in the MIG value for *F. avenaceum* has been observed after 2007, with MIG values ranging from 2.2% in 2007 to 9.3% in 2009. A strong correlation was found between *F. graminearum* and *M. nivale* in terms of MIG value ($r=0.87$; $P=0.012$).

There was no clear relationship between DON content and PG value for *F. graminearum* (the most

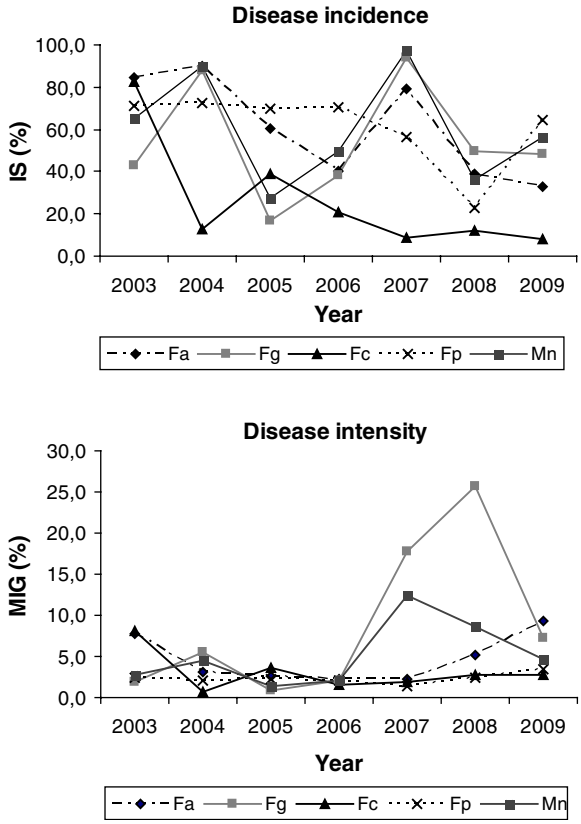

Fig. 5 Fungal analysis of grains collected from wheat between 2003 and 2009 in the Wallonia region of Belgium. **a**: Percentage of infected fields (IS values); **b**: Percentage of infected grains per infected field (MIG values). Fa = *Fusarium avenaceum*; Fg = *F. graminearum*; Fc = *F. culmorum*; Fp = *F. poae*; Mn = *Microdochium nivale*

frequent FHB species), except in 2007, the year with the highest mean DON content (r=0.69, P<0.0001, Table 1).

Relationship between weather variables, head blight populations and DON content

The relationship between weather variables, DON content and PG values for the main head blight species identified in this study was established for both time periods and for all samples (N=692). The r values were lower for time period 1 than for time period 2 (data not shown). A correlation matrix for time period 2 is presented in Table 3. Although statistically significant correlations were found, they were not very strong. The higher r values were observed between the meteorological variables RH, DRH80 and PET and the head blight variables DON, PG Fg and PG Mn. No clear relationship was found between the PG values of *F. avenaceum*, *F. culmorum* and *F. poae* and the weather variables.

Risk of DON contamination: a two-stage approach

Evaluation of the type of year according to DON content in the samples

Mean values for both DON content and meteorological variables were chosen in order to evaluate the type of year in terms of DON contamination. As shown in Table 4, there was no clear linear positive correlation between mean DON content and mean values for the meteorological variables for the 15-day period centred on the flowering date (period 1). In contrast, a highly significant positive correlation was found between mean DON content and the variable DRH80 (r= 0.946, P=0.001) for period 2. A strong relationship was also shown with the variables RH (r=0.855, P= 0.014) and PET (r=−0.843, P=0.017). As shown in Fig. 6 for the DRH80 variable, two groups of years were revealed, the first group covering the years 2003, 2004, 2005, 2006 and 2009 (not conducive to DON contamination), with less than 8 days with a mean RH above 80% in period 2, and the second group covering 2007 and 2008 (conducive to DON contamination),

Table 2 Two-way interactions between head blight pathogens using a log-linear model with 5 factors (N=692). + = positive association; –: negative association

Pathogen 1	Pathogen 2	P	Type
F. avenaceum	*F. graminearum*	<0.0001***	+
F. avenaceum	*F. culmorum*	<0.0001***	+
F. avenaceum	*F. poae*	0.2121	nr
F. avenaceum	*M. nivale*	<0.0001***	+
F. graminearum	*F. culmorum*	0.0341*	–
F. graminearum	*F. poae*	0.516	nr
F. graminearum	*M. nivale*	<0.0001***	+
F. culmorum	*F. poae*	0.0365*	+
F. culmorum	*M. nivale*	0.5246	nr
F. poae	*M. nivale*	0.0031**	+

***significant interaction (P≤0.001)

**significant interaction (P≤0.01)

*significant interaction (P≤0.05)

nr not relevant

Table 3 Pearson correlation coefficients for correlations of temperature (T), relative humidity (RH), number of days with a mean RH above 80% (DRH80), log-transformed total precipitation (PRE), number of days with precipitation above 0.1 mm (DPRE) and evapotranspiration potential (PET), with deoxynivalenol content (DON) or PG values for *Fusarium avenaceum* (Fa), *F. graminearum* (Fg), *F. culmorum* (Fc), *F. poae* (Fp) and *Microdochium nivale* (Mn). Weather variables collected during period 2, N=692 (all the sampled fields of the survey)

Variable[a]	Variable					
	DON	Fa	Fg	Fc	Fp	Mn
T	−0.114	(−0.033)	−0.338	0,232	(−0.004)	−0.194
RH	0,357	0.153	0.391	(−0.042)	(−0.030)	0.362
DRH80	0.395	(−0.089)	0.421	(−0.044)	(−0.044)	0.388
PRE	0.225	0.119	0.255	(−0.011)	(−0.017)	0.293
DPRE	0.319	(−0.079)	0.382	(−0.096)	(−0.052)	0.459
PET	−0.414	(−0.033)	−0.505	0.132	(−0.035)	−0.450

[a] Period 2 = 24 days starting 7 days before the mean flowering date and ending 16 days after it

All correlation coefficients shown are significant ($P \leq 0.01$) except where indicated by parentheses. Correlation coefficients in parentheses are not significant ($P > 0.05$)

with more than 10 days with a mean RH above 80% in period 2.

Agricultural practices

Four classes of DON were established, depending on the susceptibility of wheat varieties to FHB. The proportion of samples in each class for the four types of wheat varieties was determined for the years 2007 and 2008 (years at risk) and for the years 2006 and 2009 (years without meteorological conditions conducive to DON contamination). Samples from 2003–2005 were not integrated in the study because the data on the susceptibility of some of the wheat cultivars were lacking. As shown in Table 5, wheat variety did not influence the DON content in years non-conducive to DON contamination as most of the samples had DON contents below 500 ppb. In contrast, in 2007–2008 the proportion of samples with a DON content above 1,000 ppb ranged from 15.2% for wheat varieties less susceptible to the disease to ~43% for wheat varieties susceptible to the disease (type 3 varieties, Table 5). All the fields

Table 4 Correlation between mean annual DON content and average values for the meteorological parameters considered in this study determined for two periods around the mean flowering date of the cereal. Bold figures represent significant correlations ($P \leq 0.05$). (N=7, the 7 years of the survey)

	Period 1[a]		Period 2[b]	
	r	*P*	r	*P*
T	−0.08	0.865	0.15	0.749
RH	0.618	0.139	**0.855**	**0.014**
DRH80	0.611	0.145	**0.946**	**0.001**
PRE	0.493	0.261	0.568	0.183
DPRE	**0,764**	**0.046**	0.643	0.119
ETP	−0.615	0.142	**−0.843**	**0.017**

[a] Period 1: 15 days centred on the mean flowering date

[b] Period 2: 24 days starting 7 days before the mean flowering date and ending 16 days after it

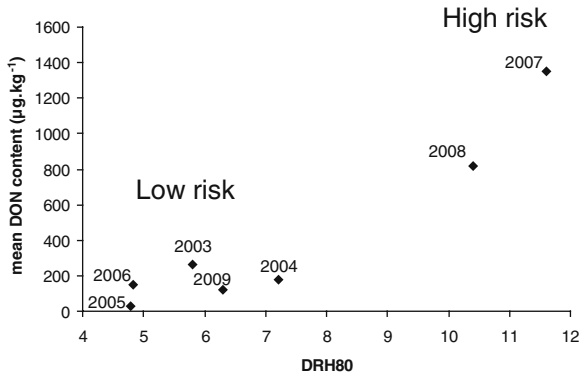

Fig. 6 Relationship between mean annual DON content and number of days with a mean relative humidity above 80% determined for a period starting 7 days before the mean flowering date of the cereal and ending 16 days after it, from 2003 to 2009

Table 5 Distribution of wheat samples in four classes of DON contamination (in μg.kg^{-1}) according to the susceptibility of the wheat variety to FHB. 1 = resistant variety; 2 = moderately resistant variety; 3 = susceptible variety, 4: highly susceptible variety. The years 2006 and 2009 were not at risk of DON contamination. The years 2007–2008 corresponded to years conducive to DON contamination. The percentages are indicated in brackets

DON	Type of wheat variety			
	1	2	3	4
2006 & 2009 (*N*=159)				
<500	57 (98.3)	53 (96.4)	24 (96)	21 (100)
500–1000	1 (1.7)	1 (1.8)	0(0)	0 (0)
1000–2000	0 (0)	1 (1.8)	1 (4)	0 (0)
>2000	0 (0)	0 (0)	0 (0)	0 (0)
Total	58 (100)	55 (100)	25 (100)	21 (100)
2007–2008 (*N*=118)				
<500	20 (60.6)	21 (52.5)	8 (22.86)	0 (0)
500–1000	8 (24.2)	7 (17.5)	12 (34.29)	0 (0)
1000–2000	4 (12.1)	7 (17.5)	9 (25.71)	2 (20)
>2000	1 (3.1)	5 (12.5)	6 (17.14)	8 (80)
Total	33 (100)	40 (100)	35 (100)	10 (100)

cultivated with highly susceptible varieties displayed DON content above 1,000 ppb.

The same analysis was conducted for four classes of risk for the previous crop in combination with the soil tillage. In 2007–2008, the percentage of wheat samples with DON content above 1,000 ppb was about 10% for fields cultivated after rape, pea or flax, about 43% for fields cultivated after small grain cereals, sugar beet or potato, about 31% for fields cultivated after maize with ploughing, and 80% for fields cultivated after maize without ploughing (Table 6). The same analysis conducted on samples in years not conducive to DON contamination confirmed the reducing effect on DON content of type 1 preceding crops (with no sample above 1,000 ppb), and the increasing effect of type 4 preceding crop (with 18.2% of samples with a DON content above 1,000 ppb) (Table 6).

Decision-support system

All the survey samples were analysed using the two-step procedure, first, on the type of year (at risk or not, based on the number of days with a mean RH above 80%) and, second, on the agricultural practices (wheat variety, previous crop, tillage) for years conducive to the disease. By fixing the DON threshold at 800 μg kg^{-1} (corresponding to the regulation limit of 1,250 μg kg^{-1} minus the expanded uncertainty of the ELISA method), and considering a previous crop of type 4 or a wheat variety of type 3 or 4 as classes at risk for 2007 and 2008 (conducive to DON accumulation), the classification of 83 samples (70.3%) was correct while false positive and false negative results were detected at the level of 12.7% and 16.9% respectively (Table 7).

Discussion

This study describes a survey carried out between 2003 and 2009 to evaluate the incidence of head blight in winter wheat in the Wallonia region of Belgium and the level of DON contamination at harvest. The survey was carried out in the region's main cereal growing area and involved collecting ear samples by hand a few days before harvest to take account of the heterogeneous distribution of patho-

Table 6 Distribution of wheat samples in four classes of DON (in μg.kg^{-1}) according to the preceding crop. 1 = flax, rape or pea; 2 = wheat, barley, oats, sugar beet, potato or chicory; 3 = maize for silage with ploughing; 4 = maize for silage without ploughing. The years 2003–2006 and 2009 were not at risk of DON contamination. The years 2007–2008 corresponded to years conducive to DON contamination. The percentages are indicated in brackets

DON (ppb)	Type of preceding crop			
	1	2	3	4
2003–2006 & 2009 (*N*=572)				
<500	92 (98.9)	292 (94.5)	139 (87.4)	6 (54.5)
500–1000	1 (1.1)	11 (3.6)	15 (9.4)	3 (27.3)
1000–2000	0 (0)	5 (1.6)	4 (2.5)	1 (9.1)
>2000	0 (0)	1 (0.3)	1 (0.6)	1 (9.1)
Total	93 (100)	309 (100)	159 (100)	11 (100)
2007–2008 (*N*=118)				
<500	11 (57.9)	21 (33.9)	16 (50.0)	0 (0)
500–1000	6 (31.6)	14 (22.6)	6 (18.8)	1 (20)
1000–2000	2 (10.5)	15 (24.2)	6 (18.8)	1 (20)
>2000	0 (0)	12 (19.4)	4 (12.5)	3 (60)
Total	19 (100)	62 (100)	32 (100)	5 (100)

Table 7 Contingency table of the observed and expected results in terms of DON contamination in 2007 and 2008 (N= 118). + = DON level above 800 μg.kg^{-1};−= DON level below 800 μg.kg^{-1}

		Observed		Total
		+	−	
Expected	+	31	15	46
	−	20	52	72
Total		51	67	118

gens in the field, to control the sample history (geographical origin, agricultural practices, and flowering date) and to avoid cross-contamination between samples at the harvest. A comparison between samples collected by hand before harvest and machine-harvested samples revealed that the method provided a good estimate of DON concentration at harvest.

Mean DON content showed considerable variation between the years as described in other European regions (Isebaert et al. 2009; Van Der Felz-Klerx et al. 2010). In order to help the industry to limit potential food or feed safety problems, a tool based on weather conditions near anthesis was developed to define the annual risk of DON contamination. The times used in this study were relative to mean flowering date instead of heading date as proposed in other studies (Hooker et al. 2002; Kriss et al. 2010). Moreover, the time frames for collecting weather data were sufficiently long (15 or 24 days) to make the tool less sensitive to the growth stage of the cereal as compared with the DONcast model which uses more narrow time frame (~4 days) (Schaafsma and Hooker 2007). An analysis of the climatic conditions over a 24-day period centred on the mean flowering date revealed a strong positive correlation between number of days with a mean RH above 80% and mean annual DON content. This finding agrees with other studies that show DON levels or FHB to be positively correlated with variables that represent atmospheric moisture or wetness conditions near flowering (Audenaert et al. 2009; Hooker et al. 2002; Kriss et al. 2010). There was no evidence of significant correlations between mean daily temperature and DON content. This result contrasts with the study by Schaafsma and Hooker (2007) who showed that average air temperatures less than 12°C in a period from 3 to 10 days after heading had a negative impact on the DON content at the harvest. However, it must be noticed that this critical value was never observed in our conditions during the period considered (data not shown). By using the number of days with a mean RH above 80% to evaluate the risk of DON contamination, one could classify the years 2003 to 2006 and 2009 as years with a low risk of DON contamination, and 2007 and 2008 as years with a high risk of contamination. Due to the low percentage of highly contaminated samples in years that were not conducive to the disease (Table 1), information on the type of year could help cereal storage companies to limit the analysis of grain samples arriving at the storage unit to years with a high risk of DON contamination.

In order to improve the accuracy of the predictive tool, the impact of wheat cultivar and previous crop, two of the main agronomic factors affecting the level of DON in wheat grains (Franz et al. 2009; Schaafsma et al. 2001) were evaluated. DON level was not significantly affected by the wheat variety in years not conducive to FHB. This reinforces the potential of using a two-stage approach to evaluate the risk of DON contamination. In years conducive to the disease, post-harvest management actions should focus on fields cultivated with susceptible (type 3) or highly susceptible varieties (type 4), because in such conditions the percentage of wheat lots with DON content above the regulated limit might be very high. Apart from the wheat variety, the type of previous crop and the occurrence of maize residues in wheat fields were also studied. Some preceding crops, such as rape, pea or flax were found to be less conducive to the disease, regardless of the climatic conditions. The reducing effect of some previous crops on DON contamination in wheat has already been described (Dill-Macky and Jones 2000). Interestingly, the use of maize for silage as a previous crop did not lead to a greater risk than wheat, sugar beet or potato if the residues were correctly incorporated by ploughing before the wheat culture. This is in agreement with the results from Maiorano et al. (2007) showing the impact of maize residues on FHB. Although ploughing may have a negative impact on soil erosion, this observation is important because maize for silage is often used in the crop sequence, especially in Belgium, as it has many advantages over other plants. A realistic approach to help farmers limit the risk of DON contamination

should therefore take account of agronomical constraints. It should also adapt the strategy to the end-purpose of the harvest (feed or food) as this will influence the acceptable level of DON in the harvested grain.

In regard to the head blight population found in wheat grains, the situation was very similar (from a qualitative point of view) to other European regions (Giraud et al. 2010; Isebaert et al. 2009; Stepień and Chelkowski 2010; Waalwijk et al. 2003) and to the results of a previous study in Wallonia in 2000–2002 (Chandelier et al. 2003), with the head blight complex in wheat mainly composed of the species *F. graminearum, F. avenaceum, F. culmorum, F. poae* and *Microdochium nivale*. Data collected for disease incidence revealed 7 out of 10 statistically significant pair-wise associations between head blight species among which 6 were positive interactions. As Xu et al. (2007) demonstrated the presence of competitive interactions between head blight species, the positive associations observed in this study could indicate common requirements in terms of environmental conditions rather than synergistic interactions. The interactions between *F. poae* and *F. graminearum* or *F. avenaceum* were not significant at harvest as already shown by Xu et al. (2008) on a 4 year-period (2001–2004). The percentage of fields where *F. culmorum* was detected showed a decreasing trend from 2003 to 2009, confirming the drift in the FHB population observed by Waalwijk et al. (2003) in the Netherlands, with *F. culmorum* being replaced by *F. graminearum* as the main FHB species in wheat.

In terms of head blight intensity, a greater proportion of variability was accounted for by weather variables with *F. graminearum* and *M. nivale* being the most abundant species in 2007 and 2008, both years being characterised by a high number of days with RH above 80%. The year 2007 was more conducive to DON contamination than 2008, but the percentage of kernels infected by *F. graminearum* (the DON-producing species) was higher in 2008 than in 2007. This suggests that fungal infection and DON accumulation are influenced by different environmental conditions during the post-flowering stage of the cereal. *Fusarium avenaceum* was the second most important *Fusarium* species in Wallonia, with proportions of infected kernels per infected sample ranging from 2.2% (in 2006 and 2007) to 9.3% (in 2009).

In terms of research priorities, a survey of other mycotoxins produced by *Fusarium* species should be undertaken, as our study showed that in some situations *Fusarium* species producing mycotoxins other than DON could be present in grains. In the context of climate change and intensive agriculture, surveys should be conducted each year to identify any drift in the regional head blight population as noted by Waalwijk et al. (2003), which could result from weather conditions or changes in agricultural practices more conducive to specific *Fusarium* species.

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