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**Fusarium head blight and associated mycotoxin occurrence on
winter wheat in Luxembourg in 2007/2008**

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Abstract

Fusarium head blight (FHB) is among the major causes for reduced quality of winter wheat and its products. In addition, the causal fungi produce a variety of toxins. A relatively high FHB infection rate of winter wheat was observed in 2007 and 2008 in Luxembourg. A fusariotoxin survey was carried out in 17 different geographical locations. Three groups of *Fusarium* mycotoxins (trichothecenes A and B and zearalenone) were then analysed by a multi-detection HPLC-MS/MS method. In addition, *Fusarium* strains were investigated by morphological and molecular methods. In addition to this survey, questionnaires related to cultural practices were sent to the farmers managing the 17 fields investigated. FHB prevalence ranged from 0.3 to 65.8 % (mean: 8.5%) in 2007 and from 0 to 24.5 % (mean: 8.3%) in 2008. Results of the morphological and molecular identification showed that the most common species isolated from diseased wheat spikes was *F. graminearum* (33.1%), followed by *F. avenaceum* (20.3%) and *F. poae* (17.8%). The chemical analysis revealed that 75% of the investigated fields were contaminated by deoxynivalenol (DON, range 0-8111 µg/kg). The preceding crop was highly and significantly correlated to the number of grains infected and had a significant impact on the disease prevalence ($P=0.025$ and 0.017 , respectively, Fisher-F-test). A trend was found for maize as the preceding crop ($P=0.084$, Tukey's) to predict the amount of DON in the fields. This is the first report on the natural occurrence of DON and ZON in naturally infected wheat grains sampled from Luxembourg.

Keywords: *Fusarium*; Luxembourg; mycotoxins; winter wheat

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Introduction

Wheat is, after maize, the most important crop for human consumption worldwide (global production ca. 653 mio. t/year, 2006; Economic Research Service, 2007). In Luxembourg, winter wheat is the most important cereal crop (11 947 ha) with an annual production of ca. 75 000 tons in 2006 (Portail des Statistiques Luxembourgeoises, 2006). Even though a number of wheat cultivars exist, the widely planted monoculture makes this cereal prone to several diseases. Among the fungal diseases, FHB or scab is considered the most important worldwide plant disease of the past century, most prominent on small grain cereals, including oat, barley, rye, wheat, triticale, but also on maize, resulting in ear rot (Parry *et al.*, 1995; McMullen *et al.*, 1997). This disease, causing considerable yield and quality losses has received much attention in recent years as *Fusarium* contaminates the grains with mycotoxins and therefore the entire cereal food and feed chain, resulting in grains unusable for further production into e.g. bakery products, breakfast cereals, pasta, snacks, beer, or animal feed (Bullerman and Bianchini, 2007; Dexter *et al.*, 1997; Pirgozliev *et al.*, 2003). FHB caused by *Fusarium* toxigenic species produces several major mycotoxins, namely deoxynivalenol (DON), acetylated-DON (ac-DON), nivalenol (NIV), HT-2 and T-2 toxin, and zearalenone (ZON), which may contribute to impaired quality of food products, but also causing acute toxic symptoms such as nausea or resulting in long-term adverse effects including interior organ-damage, infertility, and cancer (SCF, 1999, 2000a, 2000b, 2001, Gutleb *et al.*, 2002; Rotter *et al.*, 1996; Minervini *et al.*, 2004). As a result, there is presently considerable pressure to recommend that the intake of some of these compounds must be limited, by setting control levels. Recently, European

1 legislation has established limits for DON and ZON (EU-regulation 1831/2003).
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3 *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] was
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5 the most frequently isolated pathogen in warm regions of the world such as in
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7 parts of the USA, Australia and central Europe, whereas *F. culmorum* WG
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9 Smith, *F. poae* Peck and *M. nivale* (Fries) Samuel and Hallett are regarded as
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11 important causal agents of FHB in the cooler regions of North-west Europe
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13 (Nicholson *et al.*, 2004). Even though different species can produce the same
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15 mycotoxins, information on the *Fusarium* species naturally present in cereal
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17 growing areas could aid in predicting the chances for mycotoxin production
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19 (Desjardin, 2006). Currently, the differentiation of the various FHB agents is
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21 based on physiological and morphological characteristics, such as the shape
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23 and size of the macroconidia, the presence or absence of microconidia and
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25 chlamydospores, and colony morphology (Nelson *et al.*, 1983). Because
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27 classifying these observations need sample experience and good background
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29 knowledge, molecular approaches have been developed to facilitate the strain
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31 identification, including specific diagnostic PCR primers (Nicholson *et al.*, 1998),
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33 DNA sequencing (O'Donnell *et al.*, 1998, Yli-Mattila *et al.*, 2004) and genotyping
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35 methods, such as randomly amplified length polymorphism (RFLP) and
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37 amplified fragment length polymorphism (AFLP) (Schmidt *et al.*, 2004).
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39 As no accurate and current data are available in Luxembourg on neither FHB
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41 nor mycotoxin occurrence, the purpose of this study was to provide data on the
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43 presence of FHB by monitoring its occurrence, and to characterize the related
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45 *Fusarium* populations and the associated mycotoxin content in winter wheat.
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47 For this purpose, observations were carried out at 17 different sites over the
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- 1 duration of 2 years, 2007 and 2008, covering a wide range of topoclimatological
- 2 areas in Luxembourg.
- 3

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Materials and methods

Field survey

FHB was monitored during 2 seasons (2007 and 2008) in 17 and 16 sites respectively (Figure1) in fields selected to be representative of different cereal growing areas in Luxemburg, representative of the three topoclimatological zones (North, East and South).

Determination of the percentage of Fusarium infected heads

The FHB incidence and severity were recorded for each site, carrying out visual evaluations of the disease at the anthesis half-way state (BBCH67, BASF, Bayer, Ciba-Geigy and Hoechst, Landshare *et al.*, 1991) to the late-milke state (BBCH77). The FHB incidence was calculated as the percentage of wheat plants with recognizable symptoms and was obtained by analysing 150-250 ears per field. The FHB severity was calculated as the percentage of kernels with visual symptoms per total kernels present on one ear and computing the average percentage per production field on the basis of 150-250 ears per field.

Grain sample collection

Wheat grains were harvested at BBCH98 (secondary dormancy induced) from the field sites (Figure 1). Spikes that had reached maturity were randomly hand-collected in each plot and manually threshed to recover a total of ca. 500–600 g grains per sample. The samples were packed into paper bags, dried at 60 °C for 72 hours in an oven (Binder ED 53, Binder Inc., France) in order to standardize humidity content, and then stored at 4 ± 1 °C prior to mycoflora and mycotoxin analysis.

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2 **Grain mycological analysis**

3 From each sample of ca. 500g, approximately 50ml of grains were randomly
4 selected; surface sterilized in 0.37% NaOCl (VWR Prolabo, Briar France) and
5 0.1% ‘Tween 20’(Acros New Jersey, USA) for 10 min and dried on sterile filter
6 paper under a laminar flow hood.
7 From each of these batches, 100 grains were randomly selected and plated on
8 modified Dichloran Chloramphenicol Peptone Agar (DCPA) medium (Andrews
9 and Pitt, 1986; Loos *et al.*, 2004).. Grains in DCPA petri dishes were incubated
10 for 12 d at 22 ± 2 °C with a 12 h light period, using a plant incubator (ECPO1E,
11 Snijders Tillburg, the Netherlands).

12 The *Fusarium* spp. developing on DCPA medium were subsequently
13 transferred to potato dextrose agar (PDA, Merck Darmstadt Germany) and
14 incubated at 22± 2°C for 6–10 d. Isolates were then identified according to
15 Nelson *et al.* (1983) based on morphological characters.

17 **Molecular identification of *Fusarium* strains**

18 To extract DNA, strains were grown in 5 mL potato dextrose broth (PDB,
19 Sigma), shaking the culture at 150 rpm at 22°C for 6 d (Infors HT Ecotron,
20 Bottmingen, Switzerland). Mycelium was freeze-dried for 20 h and DNA was
21 extracted using a Qiagen Plant DNA extraction kit (Qiagen, Hilden, Germany),
22 according to the manufacturer’s protocol. DNA was quantified using a Nanodrop
23 (Thermo, Waltham, MA, USA) and dilutions were performed for PCR
24 optimization. Specific primers obtained from Eurogentec (Liège, Belgium) for

species confirmation were used (Demeke *et al.*, 2005) in order to confirm morphological identification.

In particular, Phusion Taq mix (Finzyme Espoo Finlande) and 500 μ M of each primer in a total volume of 10 μ L were used to distinguish *F. poae*, *F. culmorum*, *F. graminearum* and *F. avenaceum* (Pasquali *et al.*, 2009). Because of the close genetic relationship to *F. poae*, we also investigated the presence of *F. langsethiae* using specific primers defined by Wilson *et al.* (2004). The following PCR program was used: the annealing temperature was 66°C for the first 5 cycles and 64°C for the next 5 cycles followed by 25 cycles at 62°C. The temperature cycle used consisted of denaturation (98°C) for 20 s, annealing (as described above) for 20 s and extension (72°C) for 45 s with maximal ramping rates between temperatures. A final extension step of 5 min was incorporated followed by cooling to 4°C until recovery of samples. All amplicons were visualized on agarose gel 3%, separating PCR fragments with 100V tension (Biorad Power Pac 300) for 40 min.

Detection of the primary inoculum for the 2008 season

During the month of May, in order to verify whether soil and residues could serve as a source for primary inoculum, impacting the development of the FHB, the presence of the 4 *Fusarium* species detected on the wheat spikes was also evaluated in the first 10 cm of soil and on the residues as described by Maiorano *et al.* (2008).

Soil samples, residues (5 plastic-boxes with 80g for each field) and weeds (between 10 and 20 plants per field) were randomly hand-collected in fields localized at 3 sites (Burmerange, Christnach, and Reuler), representing the 3

major agro-climatological areas (South, East and North) of Luxembourg. Weeds were also sampled from the field borders, sent to the laboratory and then dried during 24h at 60 °C in a ventilated oven. Weeds and residues were cut in 3 cm long sections and sieved to remove gravel. The samples were then analysed as described previously. Weed identification was based on Mamarot (2002).

Fusarium mycotoxins content

Mycotoxin analysis was based on the procedure described by Spanjer et al. (2008), but modified as follows: For mycotoxin assays, 500g of wheat grains per field were dried during 48h at 30 °C for humidity standardization and aliquots (200g) were obtained by milling with the Cyclotec™ 1093 (Foss,Belgium). For further extraction of the mycotoxins, 5g of the obtained flour were homogenized together with 15 mL of acetonitrile/water (80:20, v/v), and sonicated during 15 minutes (Elma Transonic TS540). After centrifugation (720g, 20 °C, SIGMA 3K12 Bioblock Scientific), a supernatant aliquot was filtered through a 0.20µm GHP membrane filter (PAL, MI, USA) and diluted ten times in water. The following mycotoxins were investigated: trichothecenes of the group A (T2, HT2), group B (deoxynivalenol (DON), nivalenol (NIV) and the sum of 3- and 15- acetylated deoxynivalenol (acDONs) and zearalenone (ZON). Separation and detection were achieved by HPLC coupled to tandem mass spectrometry (LC-MS/MS, Dionex Ultimate 3000; Applied Biosystems API 3200) in the multiple reaction monitoring (MRM) positive mode for group A trichothecenes and the negative mode for group B and ZON. An Altima HP RP-C₁₈ column was used (150x2.1mm; 3µm, Grace Davison, IL) with a mobile phase consisting of methanol/water (80:20, v/v) containing 2.5mM ammonium

1 acetate. The detection and quantification limits obtained by extracting 5g of
2 sample were 35 and 70 ng/g for the trichothecenes and 20 and 40ng/g wheat
3 for ZON, respectively, following the US-EPA approach (Zorn *et al.*, 1999).
4 Quantification was based on external standards, and by measuring the following
5 fragment ions of the parent molecules: 371→311 for NIV, 355→295 for DON,
6 397→337 for acDONs, 442→323 for HT2, 484→215 for T2 and 317→131 for
7 ZON.

8 **Statistical analysis.**

9 Statistical analyses were carried out using SPSS 16.0 (Chicago, IL, USA).
10 Normality of data was tested with Q-Q plots and Kolmogorov-Smirnoff tests,
11 equality of variance by Box-plots and Levene's test. For further data analyses,
12 data were transformed by the root mean square operation. Comparison of
13 prevalence and severity between different areas was carried out in a linear
14 mixed model using prevalence, severity, DON concentration, yield of wheat,
15 and percentage of infected grains as dependent variables, and location, field
16 site, year, and previous crop as independent variables. A post-hoc test
17 (Tukey's) was carried out given significant results by the Fisher-F test. *P*-values
18 below 0.05 (2-sided) were considered as significantly different. For correlations
19 between previous crops and FHB, Spearman correlation coefficients were
20 obtained. Unless otherwise stated, all values represent mean±SD (standard
21 deviation).

22 **Results**

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Prevalence, severity and percentage of grains infected by *Fusarium*

The results are summarized in Table 1. No significant difference was found for the prevalence between the two years. However, we observed a trend for a lower severity in 2008 compared to 2007 ($P=0.053$). A significant difference ($P<0.01$) for infected grains was found between 2007 ($37.7\pm27.5\%$) and 2008 ($10.5\pm11.6\%$). The location had a significant impact on both prevalence and severity ($P = 0.012$ and $P = 0.020$ respectively, Fisher-F test). The eastern part of Luxembourg showed the highest prevalence and severity in both years, confirmed by a high level of *Fusarium* contamination, especially in Echternach: prevalence and severity from the East were significantly higher compared to the North ($P =0.049$ and $P =0.012$ respectively, Tukey's) and to the South ($P =0.003$ and $P =0.020$ respectively, Tukey's). A significant correlation was found for the prevalence and the number of grains infected by *Fusarium* strains and the maize as the preceding crop ($P =0.017$ and $P =0.025$ respectively) as opposed to other crops, but no relation was found for severity.

Identification and distribution of the *Fusarium* species isolated in Luxembourg

During the biannual survey, more than 800 strains (Table 2) were identified by morphological characteristics and bio-molecular tools using specific primers. The most common species isolated from FHB diseased wheat spikes were *F. graminearum* (33.1%) followed by *F. avenaceum* (20.3%) and *F. poae* (17.8%). The distribution of the *Fusarium* species varied strongly from year to year and also from location to location (Figure 2).

In 2007, 640 strains were identified and the 5 most predominant fungal pathogens are shown in Figure 2. *F. graminearum*, *F. culmorum*, and *F. poae* are known to be potential trichothecenes producers while *F. avenaceum* can produce another mycotoxin, moniliformine. *M. nivale* is described as unable to synthesize *Fusarium* mycotoxins. The distribution of the *Fusarium* species varied strongly between agroclimatological zones (North, South, and East, Figure 2A). In 2008, fewer *Fusarium* strains were identified (167) compared to 2007. However, the same predominant fungal pathogens as in 2007 were identified, with the exception of *M. nivale*, which could not be detected at all (Table 2). The proportion of strains isolated and identified as *F. poae*, *F. avenaceum* and *F. culmorum* contributed to 20%, 17% and 6% of the total population respectively. The distribution of these 3 strains did not appear to be homogeneous in Luxembourg (Figure 2). Using specific primers for *F. langsethiae*, this species could not be detected.

Primary inoculum: plant debris, soil and weeds

In crop residues and soils from Burmerange and Reuler, none of the four *Fusarium* species was detected; contrarily, *F. culmorum* was identified in the soil and in the residues (maize) from East Luxembourg (Christnach). An additional strain (*F. avenaceum*) was also detected in the soil of this site. Most weeds recovered at 3 different sites (Burmerange, Reuler and Christnach) were from the plant family of *Poaceae* (*Bromus*, *Dactylis*, *Lolium*, *Festuca*, *Alopecurus myosuroides* Hudson, *Poa*) and *Asteraceae* (*Taraxacum officinale* Weber, *Achillea millefolium* L., *Centaurea cyanus* L., *Matricaria* spp.). In Reuler

F. graminearum was isolated from blackgrass (*Alopecurus myosuroides* Hudson), a common weed in cereal rotations in Europe.

Mycotoxins

Only 3 toxins were identified in winter wheat: DON, NIV and ZON with DON being the predominant one (range 70 -8000µg/kg). Table 3 summarizes the results of the mycotoxin analyses for the 2 years of survey. NIV was only found in 3 locations (in 2007, 293µg/kg for the site 8 and 236µg/kg for the site 11; in 2008, 241µg/kg for the site 01, Figure 1), mostly in the East and with concentrations below the EU guidelines. For ZON, we observed 2 contaminated sites (in 2007, 200µg/kg for the site 7 and 113µg/kg for the site 11) with concentrations higher than that specified by the European Union for unprocessed wheat (100µg/kg, EU directive 856/2005). The major contamination in Luxembourg came from DON with 75 % (25 sites out of 33) of the sites contaminated by this toxin. Its distribution differed strongly and significantly between the 3 regions (North, South and East, $P=0.008$) but no significant difference was found between the 2 years studied. Twenty one percent (7 out of 33 sites) of the samples contained more than 750µg/kg DON, 9 % (3 out of 33) showed a value higher than 1250µg/kg, the maximum level for unprocessed wheat recommended by the EU (EU directive 856/2005). When maize was the preceding crop, there is a trend ($P=0.084$, Tukey's) with high DON contamination. Very high DON levels were observed in the eastern parts of Luxemburg (average of 1915 µg/kg), which were significantly different compared to the North (average of 340 µg/kg, $P=0.007$, Tukey's) and to the South (average of

396 µg/kg, $P=0.007$, Tukey's). The highest average DON levels detected for the 2 years came from the same location in Echternach (Table 3).

Discussion

This study is the first report on the fusariotoxin contamination in Luxembourg and consisted in an integrative approach considering mycotoxin and FHB (prevalence and severity) occurrence, identification of their responsible fungi strains, together with climatological data and farming practices.

As no accurate data was available in Luxembourg on FHB occurrence, this study aimed at providing information on the presence of this disease on its territory by mapping the prevalence and the severity and to characterize the *Fusarium* populations involved in the disease.

One of the major environmental factors influencing FHB occurrence is the climate. Frequent rainfalls, high humidity and warm temperature that coincide with flowering and early kernel filling period favour infection and the development of the disease (Doohan *et al.*, 2003). For the 2 years of surveys, the average winter temperature was comparatively warmer (4.6°C and 3.2°C for 2007 and 2008 respectively) compared to the average temperature recorded in previous years (2.0°C for 2000-2007 period), presumably increasing the survival of the FHB causal agents. In addition, it is likely that the « relatively warm » spring temperature, combined with rain periods and high humidity promoted the development of the FHB disease. It also can be speculated that the difference observed for FHB severity between 2007 and 2008 (21.0%±17.8 vs 13.5%+/-

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16.2) may, at least in part, be explained by the warmer temperature observed in 2007 (11.9 °C) than in 2008 (9.4 °C) as stated by Doohan *et al.* (2003). In addition to favorable climate conditions, soil conditions are also likely to impact FHB development. Several reservoirs (seeds, residues of previous crops etc.) of the FHB agents are known and have been described (Parry *et al.*, 1995; McMullen *et al.*, 1997; Doohan *et al.*; 2003, Champeuil *et al.*, 2004).. However, the role of weeds and grasses has recently been investigated as an alternative potential reservoir (Inch and Gilbert; 2003), with contradictory results (Xu, 2003). The results obtained in the present study are especially interesting in the case of one specific location (Reuler), where identical *Fusarium* species (*F. graminearum*) were found in grains and in blackgrass, suggesting that blackgrass could be a host and a source of *F. graminearum* pathogens in wheat. Nevertheless, these preliminary results require further investigations on a larger set of samples. In addition to weed, debris from the previous crop and soil are considered the principal inoculum sources for *F. graminearum* and *F. culmorum* (Champeuil *et al.*, 2004, Osborne *et al.*, 2007). *F. culmorum* was detected in both the soil and maize residues in one location (Christnach), and data from the cropping systems (questionnaires, data not shown) of the 17 different studied sites made clear that FHB prevalence was higher following maize as previous crop. This finding confirms the importance of the inoculum for the spread of FHB disease on wheat, in agreement with previous reports (Champeuil *et al.*, 2004; Osborne *et al.*, 2007). Earlier field surveys conducted elsewhere indicated that the prevalence and the severity of FHB varied strongly from year to year and also from location to

location (Tomczak *et al.*, 2002, Isebaert *et al.*, 2009). Nevertheless, few data are available in Europe and often comparability to our situation is limited due to difference in methodology used to describe the impact of the disease. For Luxembourg, different climatological conditions around flowering stage and changes in *Fusarium* population composition may account for the variability of prevalence and severity during the observation period. No effect on the yield was recorded. Therefore, it seems difficult to forecast the consequences of the mycotoxin production on the wheat yield, without possessing further data over an extended time period.

FHB is caused by a complex of species (up to 17 species have been associated with this disease), but the main causal agents found in the world are *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum* and *Microdochium nivale* (Parry *et al.*, 1995; McMullen *et al.*, 1997; Doohan *et al.*; 2003, Champeuil *et al.*, 2004; Xu, 2003). However, the distribution of these 5 major species was not homogenous and the *Fusarium* population patterns observed in Europe showed large variations between countries (Adler *et al.*, 2002., Snijders and Perkowski 1990; Tomczak *et al.*, 2002; Waalwijk *et al.*, 2003). In the present study, a high prevalence of *F. graminearum* (45 % in 2007 to 77% in 2008) and decrease of *F. culmorum* (17% in 2007 to 3.4% in 2008) was found in the eastern part of Luxembourg where *F. graminearum* dominated. As mentioned by Isebaert *et al.* (2009), temperature plays a role in the composition of *Fusarium* sp population and the close-by distance to the Moselle river valley may partly explain the dominating presence of *F. graminearum*.

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1 *F. avenaceum* distribution in Luxembourg was homogeneous among the 3
2 regions (19% to 21% for 2007) and was apparently stable over the years (below
3 20% deviation). This species appeared to be a common contaminant of winter
4 wheat with a level of occurrence less important than previously described in
5 northern Europe (Waalwijk *et al.*, 2003; Loos *et al.*, 2004; Stepien *et al.*, 2008).
6 High frequencies of *M. nivale* were detected in 2007 in the north of Luxembourg
7 (32%) but this strain could not be observed in 2008. The epidemiology of this
8 fungus is quite different from the *Fusarium* species and its incidence is mainly
9 influenced by climatic conditions (Loos *et al.*, 2004). In 2008, the conjunction of
10 weakly favorable conditions, interaction with other *Fusarium* species and
11 application of chemical treatment (e.g. triazoles are known to be effective
12 against *Fusarium* sp but not against *Microdochium* sp.) may explain the large
13 decrease of its incidence.
14 Despite the high occurrence of FHB and the presence of diverse *Fusarium* sp.
15 producing different mycotoxin profiles in Luxembourg, the winter wheat was
16 mainly contaminated with DON, partly above levels recommended in the
17 European directive 856/2005, but generally (for 91% of the sites) 15AcDON,
18 3ACDON, T2 and HT-2 were not detected in the present samples.
19 The frequency of ZON (2 sites positive) was found to be low but the
20 concentrations measured were above the European guideline recommendation
21 (100 µg/kg). According to Desjardin *et al.* (2006), previous data indicated that
22 ZON levels in Europe in cereal grains rarely exceed this limit, a level associated
23 with showing effects on swine. Based on its estrogenic properties and its'
24 negative impact on human and animal health, a dedicated survey of the eastern

part of Luxembourg, showing high contamination levels, could be recommended.

Maize as a preceding crop, the wheat cultivars used, and seasonal and local weather conditions explained most of the variations observed in DON concentration. However, no clear explanation could be found for the high level of contamination detected in Echternach. Even though the range of DON level contamination detected in Luxembourg was comparable to other European situations (Belgium: Chandelier *et al.*, 2003, Poland: Tomczak *et al.*, 2002), Luxembourg showed sites with extremely high contamination rates, higher than other reported contaminated European sites, and closer to results found in South America or Africa (Desjardin *et al.*, 2006).

Many efforts were realized to relate measurements from the fields (disease prevalence or severity) to mycotoxin concentrations. However, results were very variable, from weak to strong positive correlations as well as negative ones (Paul *et al.*, 2005; Isebaert *et al.*, 2009). In 2008, the average DON contents were similar to the previous season despite the fact that field severities were lower. High disease pressure of *Fusarium* is not automatically related to a higher DON content (Everlange in 2008, severity: 36% and DON: 301 µg/kg). Edwards *et al.* (2001) suggested that some pathogens do not produce mycotoxins, which means that mycotoxin contamination is not related to visual symptoms of disease severity. Opinions also vary strongly to prove the existence of a relationship between DON and the amount of FHB pathogen (Isebaert *et al.*, 2009). For Luxembourg, no clear relation could be detected between DON level and the species profile of the agents causing FHB. This result is probably due to the large variability of toxin production between and

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1 within the *Fusarium* sp. and to micro-environmental factors (climate, use of
2 several chemical treatments inducing high toxin levels).

3
4 **Conclusion**

5 This large-scale sampling provided an overall picture of FHB occurrence in
6 Luxembourg. *Fusarium* populations causing FHB in naturally infected winter
7 wheat in the different sites differed considerably, indicating that the profile of the
8 *Fusarium* population is influenced by several regionally linked parameters and
9 comparison with other countries should be carried out carefully. Further
10 epidemiological surveys are required to adapt specific preventive practices such
11 as chemical treatment application to reduce the infection level. Mycotoxin-levels
12 identified at present are not likely to present a health risk to animal/human
13 consumers but deserve further investigation. Future studies are warranted to
14 follow *Fusarium* distribution and to improve climatological models able to predict
15 *Fusarium* occurrence.

16
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Figure 1. Map of Luxembourg showing the location of the fields where wheat was collected in 2007 and 2008.

Figure 2A. Occurrence (% of contaminated samples) of the different *Fusarium* spp. in Luxembourg in 2007 and 2008 based on the investigation of 17 and 16 sites, respectively at the time of harvest, and distribution of the *Fusarium* species according to the 3 agro-meteorological regions in 2007. Figure 2B. The circle diagrams represent the overall average of *Fusarium* occurrence for all individual fields for both years.

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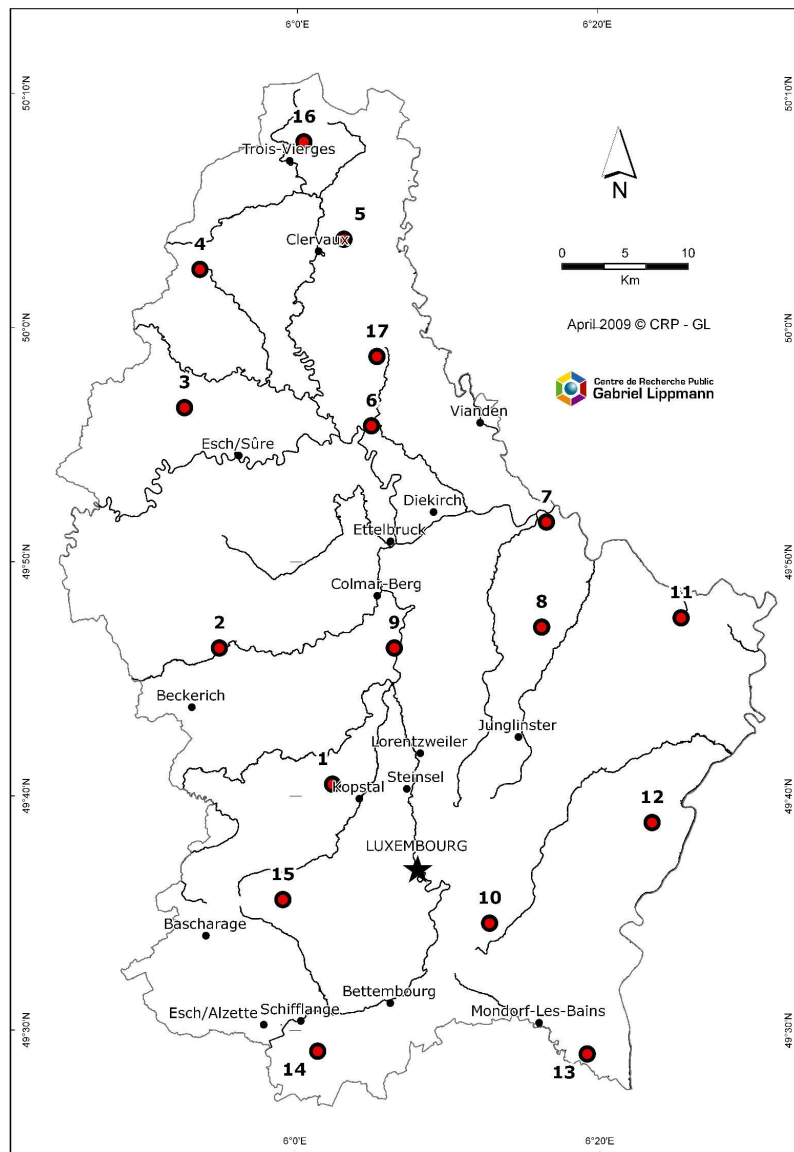


Figure 1. Map of Luxembourg showing the location of the fields where wheat was collected in 2007 and 2008.

209x297mm (600 x 600 DPI)

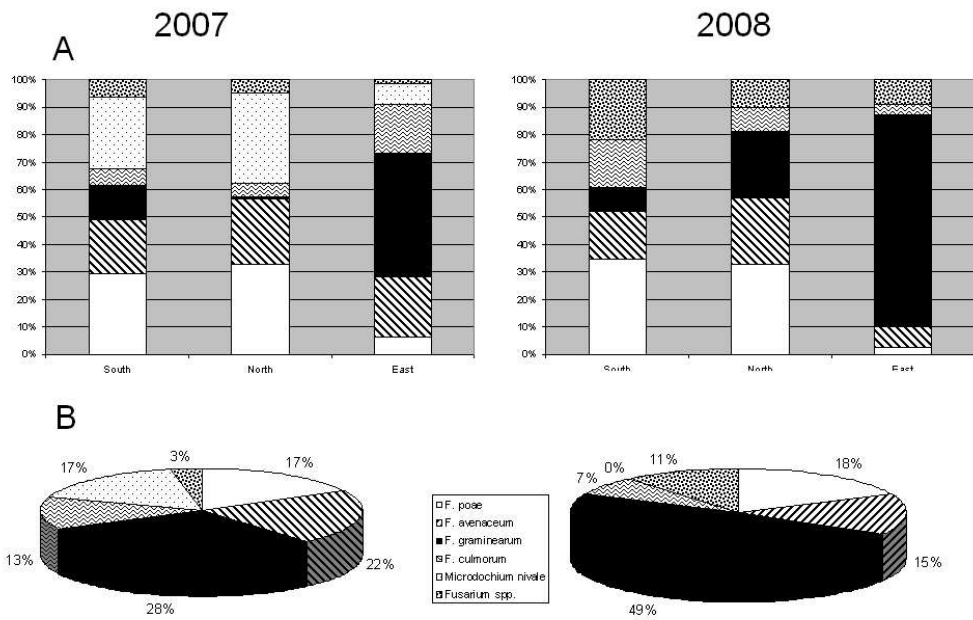


Figure 2A. Occurrence (% of contaminated samples) of the different *Fusarium* spp. in Luxembourg in 2007 and 2008 based on the investigation of 17 and 16 sites, respectively at the time of harvest, and distribution of the *Fusarium* species according to the 3 agro-meteorological regions in 2007.

Figure 2B. The circle diagrams represent the overall average of *Fusarium* occurrence for all individual fields for both years.

Table 1. Prevalence (% infected wheat spikes), severity (% infected grains/spike), occurrence of grains infected by *Fusarium* spp. and preceding crop studied in various geographical areas of Luxembourg (n=17) during 2 years.

Year	Site	Location	Preceding crop	Prevalence	Severity		% of grains infected by FHB agents
				%	Mean (%)	SD	
2007	1	Dondelange	maize	6.00	8.8	6.3	44
	2	Everlange	pea	0.3	nd	nd	8
	3	Nothum	nd	1.0	10.0	0	38
	4	Hamiville	colza	2.8	6.3	2.3	25
	5	Reuler	maize	5.8	21.6	16.7	5
	6	Lipperscheid	maize	2.6	11.5	5.6	39
	7	Reisdorf	nd	16.1	14.7	15.4	92
	8	Christnach	maize	10.2	16.1	20.2	40
	9	Essingen	maize	12.3	19.6	11.1	81
	10	Contern	wheat	1.0	6.2	0	42
	11	Echternach	maize	65.8	46.4	20.5	88
	12	Oberdonven	colza	9.6	39.5	28.1	26
	13	Burmerange	colza	0.5	61.9	38.6	43
	14	Kayl	maize	3.9	40.8	26.1	24
	15	Dippach	triticale	1.1	31.2	0	18
	16	Troisvierges	nd	1.4	5.5	0	4
	17	Hoscheid	colza	3.6	5.6	0.5	19
AVERAGE-2007				8.5	21.6	17.8	37.7
2008	1	Kehlen	maize	0.6	5.6	0	10
	2	Everlange	fallow	23.3	36.2	39.8	13
	3	Nothum	oats	0	0	0	3
	4	Hamiville	maize	12.2	7.8	3.5	18
	5	Reuler	colza	10.3	7.8	8.9	9

6	Lipperscheid	nd	nd	nd	nd	nd
7	Reisdorf	barley	9.9	52.4	37.1	17
8	Christnach	maize	24.5	16.6	11.3	4
9	Essingen	nd	1.6	24.1	19.5	6
10	Contern	nd	0	0	0	4
11	Echternach	maize	31.5	34.8	25.9	49
12	Oberdonven	wheat	0	0	0	1
13	Burmerange	colza	2.4	22.9	28.1	1
14	Kayl	wheat	0	0	0	7
15	Dippach	lucerne	0	0	0	2
16	Troisvierges	barley	16	8.1	3.1	10
17	Hoscheid	colza	0	0	0	14
AVERAGE-2008			8.3	13.5	16.2	10.5

No data are available for site 6 (Lipperscheid) in 2008 because the cereal planted was spelt wheat and not winter wheat. nd: no data.

Table 2. Occurrence of grains infected by *Fusarium* spp. studied in 17 geographical areas of Luxembourg.

Location	No	Year	<i>F. poae</i>		<i>F. avenaceum</i>		<i>F. graminearum</i>		<i>F. culmorum</i>		<i>Fusarium</i> spp.		<i>M. nivale</i>	
			<i>x/n</i>	%	<i>x/n</i>	%	<i>x/n</i>	%	<i>x/n</i>	%	<i>x/n</i>	%	<i>x/n</i>	%
Dondelange	01	2007	17/48	35.4	8/48	16.6	5/48	10.4	0/48	0	1/48	2.1	17/48	35.4
		2008	2/10	20.0	3/10	30.0	1/10	10.0	4/10	40.0	0/10	0	0/10	0
Everlange	02	2007	2/8	25.0	3/8	37.5	1/8	12.5	2/8	25.0	0/8	0	0/8	0
		2008	2/13	15.4	5/13	38.5	1/13	7.7	3/13	23.0	2/13	15.4	0/13	0
Nothum	03	2007	14/38	36.8	3/38	7.9	0/38	0	5/38	13.1	0/38	0	16/38	42.1
		2008	2/3	66.6	1/3	33.4	0/3	0	0/3	0	0/3	0	0/3	0
Hamiville	04	2007	1/25	4.0	4/25	16.0	0/25	0	0/25	0	0/25	0	20/25	80.0
		2008	4/21	19.0	4/21	19.0	9/21	42.8	2/21	9.5	2/21	9.5	0/21	0
Reuler	05	2007	1/5	20.0	3/5	60.0	0/5	0	0/5	0	1/5	20.0	0/5	0
		2008	4/9	44.4	1/9	11.2	4/9	44.4	0/9	0	0/9	0	0/9	0
Lipperscheid	06	2007	22/39	56.4	11/39	28.2	0/39	0	0/39	0	4/39	10.2	2/39	5.1
		2008	-	-	-	-	-	-	-	-	-	-	-	-
Reisdorf	07	2007	0/92	0	35/92	38.0	47/92	51.0	8/92	8.7	2/92	2.1	0/92	0
		2008	1/18	5.5	1/18	5.5	13/18	72.2	0/18	0	3/18	16.6	0/18	0
Christnach	08	2007	3/40	7.5	8/41	20.0	10/40	25.0	18/40	45.0	1/40	2.5	0/40	0
		2008	0/4	0	0/4	0	4/4	100.0	0/4	0	0/4	0	0/4	0
Essingen	09	2007	9/81	11.1	4/81	4.9	44/81	54.3	3/81	3.7	0/81	0	21/81	25.9
		2008	1/7	14.3	3/7	42.8	1/7	14.3	2/7	28.6	0/7	0	0/7	0
Contern	10	2007	3/42	7.1	13/42	30.9	7/42	16.6	7/42	16.6	1/42	2.3	11/42	26.2
		2008	2/4	50.0	1/4	25.0	1/4	25.0	0/4	0	0/4	0	0/4	0
Echternach	11	2007	0/88	0	12/88	13.7	64/88	72.7	9/88	10.2	2/88	2.2	1/88	1.1
		2008	0/57	0	2/57	3.5	50/57	87.7	0/57	0	5/57	8.8	0/57	0
Oberdonven	12	2007	12/26	46.1	7/26	27.0	1/26	3.8	0/26	0	0/26	0	6/26	23.1
		2008	0/1	0	1/1	100	0/1	0	0/1	0	0/1	0	0/1	0
Burmerange	13	2007	0/43	0	14/43	32.5	2/43	4.6	27/43	62.8	0/43	0	0/43	0
		2008	0/1	0	0/1	0	0/1	0	1/1	100.0	0/1	0	0/1	0
Kayl	14	2007	11/24	45.8	0/24	0	4/24	16.6	1/24	4.1	6/24	25.0	2/24	8.3
		2008	2/7	28.6	0/7	0	0/7	0	0/7	0	5/7	71.4	0/7	0
Dippach	15	2007	8/18	44.4	5/18	27.7	0/18	0	0/18	0	0/18	0	5/18	27.7
		2008	2/2	100	0/2	0	0/2	0	0/2	0	0/2	0	0/2	0
Troisvierges	16	2007	0/4	0	1/4	25	0/4	0	0/4	0	0/4	0	3/4	75.0
		2008	6/10	60.0	0/10	0	3/10	30.0	0/10	0	1/10	10.0	0/10	0
Hoscheid	17	2007	5/19	26.3	8/19	42.1	0/19	0	0/19	0	2/19	10.5	4/19	21.1
		2008	5/14	35.7	6/14	42.8	0/14	0	1/14	7.1	2/14	14.3	0/14	0

x = number of samples infested with the respective specific *Fusarium* species, *n* = total number of analysed samples infested with any of the 6 head blight causing species. No data are available for site 6 (Lipperscheid) in 2008 because the cereal found was spelt wheat and not winter wheat.

Table 3. DON content determined in wheat samples collected from different locations and growing seasons.

Location		Year 2007		Year 2008		
Name	No	Situation	DON (µg/kg)		DON (µg/kg)	
			average	Range	average	Range
Dondelange	1	South	278	[255-301]	220	[201-240]
Contern	10		657	[627-687]	<LOQ	-
Kayl	14		365	[358-372]	<LOQ	-
Dippach	15		<LOQ	-	463	[443-483]
Everlange	2	North	249	[238-260]	301	[286-316]
Nothum	3		139	[129-148]	<LOQ	-
Hamiville	4		88	[86-91]	928	[871-984]
Reuler	5		89	[84-94]	291	[291-291]
Lipperscheid	6	East	<LOQ	-	n.d.	-
Troisvierges	16		<LOQ.	-	487	[461-512]
Hoscheid	17		147	[136-157]	681	[673-688]
Reisdorf	7		1213	[1202-1224]	1014	[1004-1025]
Christnach	8		501	[492-510]	204	[199-210]
Essingen	9		1933	[1593-2273]	<LOQ.	-
Echternach	11		4506	[4366-4645]	8111	[7604-8945]
Oberdonven	12		382	[350-414]	101	[94-107]
Burmerange	13		1189	[1178-1200]	<LOQ	-

data based on means of duplicate analyses, the level of quantification or LoQ was 76µg/kg (for DON) in Luxembourg (nd: no data).