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

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4 **1 Fusarium head blight and associated mycotoxin occurrence on**
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6 **2 winter wheat in Luxembourg in 2007/2008**
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1 **Abstract**

2 Fusarium head blight (FHB) is among the major causes for reduced quality of
3 winter wheat and its products. In addition, the causal fungi produce a variety of
4 toxins. A relatively high FHB infection rate of winter wheat was observed in
5 2007 and 2008 in Luxembourg. A fusariotoxin survey was carried out in 17
6 different geographical locations. Three groups of *Fusarium* mycotoxins
7 (trichothecenes A and B and zearalenone) were then analysed by a multi-
8 detection HPLC-MS/MS method. In addition, *Fusarium* strains were investigated
9 by morphological and molecular methods. In addition to this survey,
10 questionnaires related to cultural practices were sent to the farmers managing
11 the 17 fields investigated. FHB prevalence ranged from 0.3 to 65.8 % (mean:
12 8.5%) in 2007 and from 0 to 24.5 % (mean: 8.3%) in 2008. Results of the
13 morphological and molecular identification showed that the most common
14 species isolated from diseased wheat spikes was *F. graminearum* (33.1%),
15 followed by *F. avenaceum* (20.3%) and *F. poae* (17.8%). The chemical analysis
16 revealed that 75% of the investigated fields were contaminated by
17 deoxynivalenol (DON, range 0-8111 $\mu\text{g}/\text{kg}$). The preceding crop was highly and
18 significantly correlated to the number of grains infected and had a significant
19 impact on the disease prevalence ($P=0.025$ and 0.017 , respectively, Fisher-F-
20 test). A trend was found for maize as the preceding crop ($P=0.084$, Tukey's) to
21 predict the amount of DON in the fields. This is the first report on the natural
22 occurrence of DON and ZON in naturally infected wheat grains sampled from
23 Luxembourg.

24
25 **Keywords:** *Fusarium*; Luxembourg; mycotoxins; winter wheat

1 Introduction

2 Wheat is, after maize, the most important crop for human consumption
3 worldwide (global production ca. 653 mio. t/year, 2006; Economic Research
4 Service, 2007). In Luxembourg, winter wheat is the most important cereal crop
5 (11 947 ha) with an annual production of ca. 75 000 tons in 2006 (Portail des
6 Statistiques Luxembourgeoises, 2006). Even though a number of wheat
7 cultivars exist, the widely planted monoculture makes this cereal prone to
8 several diseases. Among the fungal diseases, FHB or scab is considered the
9 most important worldwide plant disease of the past century, most prominent on
10 small grain cereals, including oat, barley, rye, wheat, triticale, but also on maize,
11 resulting in ear rot (Parry *et al.*, 1995; McMullen *et al.*, 1997). This disease,
12 causing considerable yield and quality losses has received much attention in
13 recent years as *Fusarium* contaminates the grains with mycotoxins and
14 therefore the entire cereal food and feed chain, resulting in grains unusable for
15 further production into e.g. bakery products, breakfast cereals, pasta, snacks,
16 beer, or animal feed (Bullerman and Bianchini, 2007; Dexter *et al.*, 1997;
17 Pirgozliev *et al.*, 2003). FHB caused by *Fusarium* toxigenic species produces
18 several major mycotoxins, namely deoxynivalenol (DON), acetylated-DON (ac-
19 DON), nivalenol (NIV), HT-2 and T-2 toxin, and zearalenone (ZON), which may
20 contribute to impaired quality of food products, but also causing acute toxic
21 symptoms such as nausea or resulting in long-term adverse effects including
22 interior organ-damage, infertility, and cancer (SCF, 1999, 2000a, 2000b, 2001,
23 Gutleb *et al.*, 2002; Rotter *et al.*, 1996; Minervini *et al.*, 2004). As a result, there
24 is presently considerable pressure to recommend that the intake of some of
25 these compounds must be limited, by setting control levels. Recently, European

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2
3 1 legislation has established limits for DON and ZON (EU-regulation1881/2006).
4
5 2 *F. graminearum* Schwabe [teleomorph *Gibberella zae* (Schwein) Petch] was
6
7 3 the most frequently isolated pathogen in warm regions of the world such as in
8
9 4 parts of the USA, Australia and central Europe, whereas *F. culmorum* WG
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11 5 Smith, *F. poae* Peck and *M. nivale* (Fries) Samuel and Hallett are regarded as
12
13 6 important causal agents of FHB in the cooler regions of North-west Europe
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15 7 (Nicholson *et al.*, 2004). Even though different species can produce the same
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17 8 mycotoxins, information on the *Fusarium* species naturally present in cereal
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19 9 growing areas could aid in predicting the chances for mycotoxin production
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21 10 (Desjardin, 2006). Currently, the differentiation of the various FHB agents is
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23 11 based on physiological and morphological characteristics, such as the shape
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25 12 and size of the macroconidia, the presence or absence of microconidia and
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27 13 chlamydospores, and colony morphology (Nelson *et al.*, 1983,). Because
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29 14 classifying these observations need sample experience and good background
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31 15 knowledge, molecular approaches have been developed to facilitate the strain
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33 16 identification, including specific diagnostic PCR primers (Nicholson *et al.*, 1998),
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35 17 DNA sequencing (O'Donnell *et al.*, 1998, Yli-Mattila *et al.*, 2004) and genotyping
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37 18 methods, such as randomly amplified length polymorphism (RFLP) and
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39 19 amplified fragment length polymorphism (AFLP) (Schmidt *et al.*, 2004).
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41 20 As no accurate and current data are available in Luxembourg on neither FHB
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43 21 nor mycotoxin occurrence, the purpose of this study was to provide data on the
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45 22 presence of FHB by monitoring its occurrence, and to characterize the related
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47 23 *Fusarium* populations and the associated mycotoxin content in winter wheat.
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49 24 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.
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1 **Materials and methods**

2 ***Field survey***

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

8 ***Determination of the percentage of Fusarium infected heads***

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

18 ***Grain sample collection***

19 Wheat grains were harvested at BBCH98 (secondary dormancy induced) from
20 the field sites (Figure 1). Spikes that had reached maturity were randomly hand-
21 collected in each plot and manually threshed to recover a total of ca. 500–600 g
22 grains per sample. The samples were packed into paper bags, dried at 60 °C for
23 72 hours in an oven (Binder ED 53, Binder Inc., France) in order to standardize
24 humidity content, and then stored at 4 ± 1 °C prior to mycoflora and mycotoxin
25 analysis.

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6 2**Grain mycological analysis**

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8 3 From each sample of ca. 500g, approximately 50ml of grains were randomly
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10 4 selected; surface sterilized in 0.37% NaOCl (VWR Prolabo, Briar France) and
11
12 5 0.1% 'Tween 20'(Acros New Jersey, USA) for 10 min and dried on sterile filter
13
14 6 paper under a laminar flow hood.
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17 7 From each of these batches, 100 grains were randomly selected and plated on
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19 8 modified Dichloran Chloramphenicol Peptone Agar (DCPA) medium (Andrews
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21 9 and Pitt, 1986; Loos *et al.*, 2004).. Grains in DCPA petri dishes were incubated
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23 10 for 12 d at 22 ± 2 °C with a 12 h light period, using a plant incubator (ECPO1E,
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25 11 Snijders Tillburg, the Netherlands).

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29 12 The *Fusarium* spp. developing on DCPA medium were subsequently
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31 13 transferred to potato dextrose agar (PDA, Merck Darmstadt Germany) and
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33 14 incubated at 22 ± 2 °C for 6–10 d. Isolates were then identified according to
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35 15 Nelson *et al.* (1983) based on morphological characters.
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Molecular identification of *Fusarium* strains

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43 18 To extract DNA, strains were grown in 5 mL potato dextrose broth (PDB,
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45 19 Sigma), shaking the culture at 150 rpm at 22 °C for 6 d (Infors HT Ecotron,
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47 20 Bottmingen, Switzerland). Mycelium was freeze-dried for 20 h and DNA was
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49 21 extracted using a Qiagen Plant DNA extraction kit (Qiagen, Hilden, Germany),
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51 22 according to the manufacturer's protocol. DNA was quantified using a Nanodrop
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53 23 (Thermo, Waltham, MA, USA) and dilutions were performed for PCR
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55 24 optimization. Specific primers obtained from Eurogentec (Liège, Belgium) for
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1 species confirmation were used (Demeke *et al.*, 2005) in order to confirm
2 morphological identification.
3 In particular, Phusion Taq mix (Finzyme Espoo Finlande) and 500 μ M of each
4 primer in a total volume of 10 μ L were used to distinguish *F. poae*, *F. culmorum*,
5 *F. graminearum* and *F. avenaceum* (Pasquali *et al.*, 2009). Because of the
6 close genetic relationship to *F. poae*, we also investigated the presence of *F.*
7 *langsethiae* using specific primers defined by Wilson *et al.* (2004). The following
8 PCR program was used: the annealing temperature was 66 °C for the first 5
9 cycles and 64 °C for the next 5 cycles followed by 25 cycles at 62 °C. The
10 temperature cycle used consisted of denaturation (98 °C) for 20 s, annealing (as
11 described above) for 20 s and extension (72 °C) for 45 s with maximal ramping
12 rates between temperatures. A final extension step of 5 min was incorporated
13 followed by cooling to 4 °C until recovery of samples. All amplicons were
14 visualized on agarose gel 3%, separating PCR fragments with 100V tension
15 (Biorad Power Pac 300) for 40 min.

17 ***Detection of the primary inoculum for the 2008 season***

18 During the month of May, in order to verify whether soil and residues could
19 serve as a source for primary inoculum, impacting the development of the FHB,
20 the presence of the 4 *Fusarium* species detected on the wheat spikes was also
21 evaluated in the first 10 cm of soil and on the residues as described by
22 Maiorano *et al.* (2008).
23 Soil samples, residues (5 plastic-boxes with 80g for each field) and weeds
24 (between 10 and 20 plants per field) were randomly hand-collected in fields
25 localized at 3 sites (Burmerange, Christnach, and Reuler), representing the 3

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

6 7 ***Fusarium mycotoxins content***

8 Mycotoxin analysis was based on the procedure described by Spanjer et al.
9 (2008), but modified as follows: For mycotoxin assays, 500g of wheat grains per
10 field were dried during 48h at 30 °C for humidity standardization and aliquots
11 (200g) were obtained by milling with the Cyclotec™ 1093 (Foss,Belgium). For
12 further extraction of the mycotoxins, 5g of the obtained flour were homogenized
13 together with 15 mL of acetonitrile/water (80:20, v/v), and sonicated during 15
14 minutes (Elma Transonic TS540). After centrifugation (720g, 20 °C, SIGMA
15 3K12 Bioblock Scientific), a supernatant aliquot was filtered through a 0.20µm
16 GHP membrane filter (PAL, MI, USA) and diluted ten times in water.
17 The following mycotoxins were investigated: trichothecenes of the group A
18 (T2, HT2), group B (deoxynivalenol (DON), nivalenol (NIV) and the sum of 3-
19 and 15- acetylated deoxynivalenol (acDONs) and zearalenone (ZON).
20 Separation and detection were achieved by HPLC coupled to tandem mass
21 spectrometry (LC-MS/MS, Dionex Ultimate 3000; Applied Biosystems API 3200)
22 in the multiple reaction monitoring (MRM) positive mode for group A
23 trichothecenes and the negative mode for group B and ZON. An Altima HP RP-
24 C₁₈ column was used (150x2.1mm; 3µm, Grace Davison, IL) with a mobile
25 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.

9 **Statistical analysis.**

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

24 **Results**

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

2 The results are summarized in Table 1. No significant difference was found for
3 the prevalence between the two years. However, we observed a trend for a
4 lower severity in 2008 compared to 2007 ($P=0.053$). A significant difference
5 ($P<0.01$) for infected grains was found between 2007 ($37.7\pm 27.5\%$) and 2008
6 ($10.5\pm 11.6\%$). The location had a significant impact on both prevalence and
7 severity ($P = 0.012$ and $P = 0.020$ respectively, Fisher-F test). The eastern part
8 of Luxembourg showed the highest prevalence and severity in both years,
9 confirmed by a high level of *Fusarium* contamination, especially in Echternach:
10 prevalence and severity from the East were significantly higher compared to the
11 North ($P = 0.049$ and $P = 0.012$ respectively, Tukey's) and to the South (P
12 $=0.003$ and $P = 0.020$ respectively, Tukey's). A significant correlation was found
13 for the prevalence and the number of grains infected by *Fusarium* strains and
14 the maize as the preceding crop ($P = 0.017$ and $P = 0.025$ respectively) as
15 opposed to other crops, but no relation was found for severity.

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

18 **Luxembourg**

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

1 In 2007, 640 strains were identified and the 5 most predominant fungal
2 pathogens are shown in Figure 2. *F. graminearum*, *F. culmorum*, and *F. poae*
3 are known to be potential trichothecenes producers while *F. avenaceum* can
4 produce another mycotoxin, moniliformine. *M. nivale* is described as unable to
5 synthesize *Fusarium* mycotoxins. The distribution of the *Fusarium* species
6 varied strongly between agroclimatological zones (North, South, and East,
7 Figure 2A). In 2008, fewer *Fusarium* strains were identified (167) compared to
8 2007. However, the same predominant fungal pathogens as in 2007 were
9 identified, with the exception of *M. nivale*, which could not be detected at all
10 (Table 2). The proportion of strains isolated and identified as *F. poae*, *F.*
11 *avenaceum* and *F. culmorum* contributed to 20%, 17% and 6% of the total
12 population respectively. The distribution of these 3 strains did not appear to be
13 homogeneous in Luxembourg (Figure 2). Using specific primers for *F.*
14 *langsethiae*, this species could not be detected.

16 **Primary inoculum: plant debris, soil and weeds**

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

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

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4 **Mycotoxins**

5 Only 3 toxins were identified in winter wheat: DON, NIV and ZON with DON
6 being the predominant one (range 70 -8000 μ g/kg). Table 3 summarizes the
7 results of the mycotoxin analyses for the 2 years of survey. NIV was only found
8 in 3 locations (in 2007, 293 μ g/kg for the site 8 and 236 μ g/kg for the site 11; in
9 2008, 241 μ g/kg for the site 01, Figure 1), mostly in the East and with
10 concentrations below the EU guidelines. For ZON, we observed 2 contaminated
11 sites (in 2007, 200 μ g/kg for the site 7 and 113 μ g/kg for the site 11) with
12 concentrations higher than that specified by the European Union for
13 unprocessed wheat (100 μ g/kg, EU directive 856/2005). The major
14 contamination in Luxembourg came from DON with 75 % (25 sites out of 33) of
15 the sites contaminated by this toxin. Its distribution differed strongly and
16 significantly between the 3 regions (North, South and East, $P=0.008$) but no
17 significant difference was found between the 2 years studied. Twenty one
18 percent (7 out of 33 sites) of the samples contained more than 750 μ g/kg DON,
19 9 % (3 out of 33) showed a value higher than 1250 μ g/kg, the maximum level for
20 unprocessed wheat recommended by the EU (EU directive 856/2005). When
21 maize was the preceding crop, there is a trend ($P =0.084$, Tukey's) with high
22 DON contamination.

23 Very high DON levels were observed in the eastern parts of Luxemburg
24 (average of 1915 μ g/kg), which were significantly different compared to the
25 North (average of 340 μ g/kg, $P=0.007$, Tukey's) and to the South (average of

1 396 µg/kg, $P=0.007$, Tukey's). The highest average DON levels detected for the
2 2 years came from the same location in Echternach (Table 3).

4 **Discussion**

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

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

14 One of the major environmental factors influencing FHB occurrence is the
15 climate. Frequent rainfalls, high humidity and warm temperature that coincide
16 with flowering and early kernel filling period favour infection and the
17 development of the disease (Doohan *et al.*, 2003). For the 2 years of surveys,
18 the average winter temperature was comparatively warmer (4.6°C and 3.2°C for
19 2007 and 2008 respectively) compared to the average temperature recorded in
20 previous years (2.0°C for 2000-2007 period), presumably increasing the survival
21 of the FHB causal agents. In addition, it is likely that the « relatively warm »
22 spring temperature, combined with rain periods and high humidity promoted the
23 development of the FHB disease. It also can be speculated that the difference
24 observed for FHB severity between 2007 and 2008 (21.0%±17.8 vs 13.5%+/-

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1 16.2) may, at least in part, be explained by the warmer temperature observed in
2 2007 (11.9°C) than in 2008 (9.4°C) as stated by Doohan *et al.* (2003).

3 In addition to favorable climate conditions, soil conditions are also likely to
4 impact FHB development. Several reservoirs (seeds, residues of previous crops
5 etc.) of the FHB agents are known and have been described (Parry *et al.*, 1995;
6 McMullen *et al.*, 1997; Doohan *et al.*; 2003, Champeuil *et al.*, 2004).. However,
7 the role of weeds and grasses has recently been investigated as an alternative
8 potential reservoir (Inch and Gilbert; 2003), with contradictory results (Xu,
9 2003). The results obtained in the present study are especially interesting in the
10 case of one specific location (Reuler), where identical *Fusarium* species (*F.*
11 *graminearum*) were found in grains and in blackgrass, suggesting that
12 blackgrass could be a host and a source of *F. graminearum* pathogens in
13 wheat. Nevertheless, these preliminary results require further investigations on
14 a larger set of samples.

15 In addition to weed, debris from the previous crop and soil are considered the
16 principal inoculum sources for *F. graminearum* and *F. culmorum* (Champeuil *et*
17 *al.*, 2004, Osborne *et al.*, 2007). *F. culmorum* was detected in both the soil and
18 maize residues in one location (Christnach), and data from the cropping
19 systems (questionnaires, data not shown) of the 17 different studied sites made
20 clear that FHB prevalence was higher following maize as previous crop. This
21 finding confirms the importance of the inoculum for the spread of FHB disease
22 on wheat, in agreement with previous reports (Champeuil *et al.*, 2004; Osborne
23 *et al.*, 2007).

24 Earlier field surveys conducted elsewhere indicated that the prevalence and the
25 severity of FHB varied strongly from year to year and also from location to

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

10
11 FHB is caused by a complex of species (up to 17 species have been associated
12 with this disease), but the main causal agents found in the world are *F.*
13 *graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum* and *Microdochium nivale*
14 (Parry *et al.*, 1995; McMullen *et al.*, 1997; Doohan *et al.*; 2003, Champeuil *et al.*,
15 2004; Xu, 2003). However, the distribution of these 5 major species was not
16 homogenous and the *Fusarium* population patterns observed in Europe showed
17 large variations between countries (Adler *et al.*, 2002., Snijders and Perkowski
18 1990; Tomczak *et al.*, 2002; Waalwijk *et al.*, 2003). In the present study, a high
19 prevalence of *F. graminearum* (45 % in 2007 to 77% in 2008) and decrease of
20 *F. culmorum* (17% in 2007 to 3.4% in 2008) was found in the eastern part of
21 Luxembourg where *F. graminearum* dominated. As mentioned by Isebaert *et al.*
22 (2009), temperature plays a role in the composition of *Fusarium* sp population
23 and the close-by distance to the Moselle river valley may partly explain the
24 dominating presence of *F. graminearum*.

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3 1 *F. avenaceum* distribution in Luxembourg was homogeneous among the 3
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5 2 regions (19% to 21% for 2007) and was apparently stable over the years (below
6
7 3 20% deviation). This species appeared to be a common contaminant of winter
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9 4 wheat with a level of occurrence less important than previously described in
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11 5 northern Europe (Waalwijk *et al.*, 2003; Loos *et al.*, 2004; Stepien *et al.*, 2008).
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13 6 High frequencies of *M. nivale* were detected in 2007 in the north of Luxembourg
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15 7 (32%) but this strain could not be observed in 2008. The epidemiology of this
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17 8 fungus is quite different from the *Fusarium* species and its incidence is mainly
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19 9 influenced by climatic conditions (Loos *et al.*, 2004). In 2008, the conjunction of
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21 10 weakly favorable conditions, interaction with other *Fusarium* species and
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23 11 application of chemical treatment (e.g. triazoles are known to be effective
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25 12 against *Fusarium* sp but not against *Microdochium* sp.) may explain the large
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27 13 decrease of its incidence.
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29 14 Despite the high occurrence of FHB and the presence of diverse *Fusarium* sp.
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31 15 producing different mycotoxin profiles in Luxembourg, the winter wheat was
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33 16 mainly contaminated with DON, partly above levels recommended in the
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35 17 European directive 856/2005, but generally (for 91% of the sites) 15AcDON,
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37 18 3ACDON, T2 and HT-2 were not detected in the present samples.
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39 19 The frequency of ZON (2 sites positive) was found to be low but the
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41 20 concentrations measured were above the European guideline recommendation
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43 21 (100 µg/kg). According to Desjardin *et al.* (2006), previous data indicated that
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45 22 ZON levels in Europe in cereal grains rarely exceed this limit, a level associated
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47 23 with showing effects on swine. Based on its estrogenic properties and its'
48
49 24 negative impact on human and animal health, a dedicated survey of the eastern
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1 part of Luxembourg, showing high contamination levels, could be
2 recommended.
3 Maize as a preceding crop, the wheat cultivars used, and seasonal and local
4 weather conditions explained most of the variations observed in DON
5 concentration. However, no clear explanation could be found for the high level
6 of contamination detected in Echternach. Even though the range of DON level
7 contamination detected in Luxembourg was comparable to other European
8 situations (Belgium: Chandelier *et al.*, 2003, Poland: Tomczak *et al.*, 2002),
9 Luxembourg showed sites with extremely high contamination rates, higher than
10 other reported contaminated European sites, and closer to results found in
11 South America or Africa (Desjardin *et al.*, 2006).

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

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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3 Figure 1. Map of Luxembourg showing the location of the fields where wheat
4 was collected in 2007 and 2008.
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7 Figure 2A. Occurrence (% of contaminated samples) of the different *Fusarium*
8 spp. in Luxembourg in 2007 and 2008 based on the investigation of 17 and 16
9 sites, respectively at the time of harvest, and distribution of the *Fusarium*
10 species according to the 3 agro-meteorological regions in 2007. Figure 2B.
11 The circle diagrams represent the overall average of *Fusarium* occurrence for
12 all individual fields for both years.
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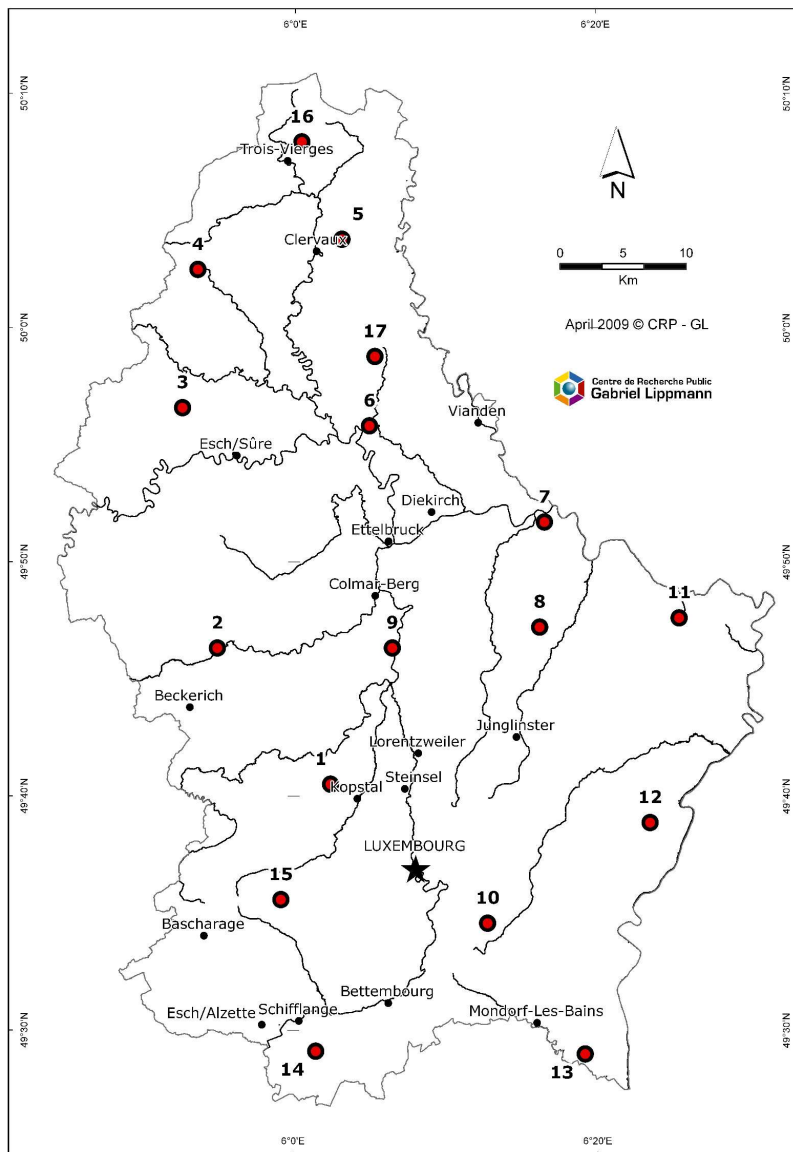


Figure 1. Map of Luxembourg showing the location of the fields where wheat was collected in 2007 and 2008.
209x297mm (600 x 600 DPI)

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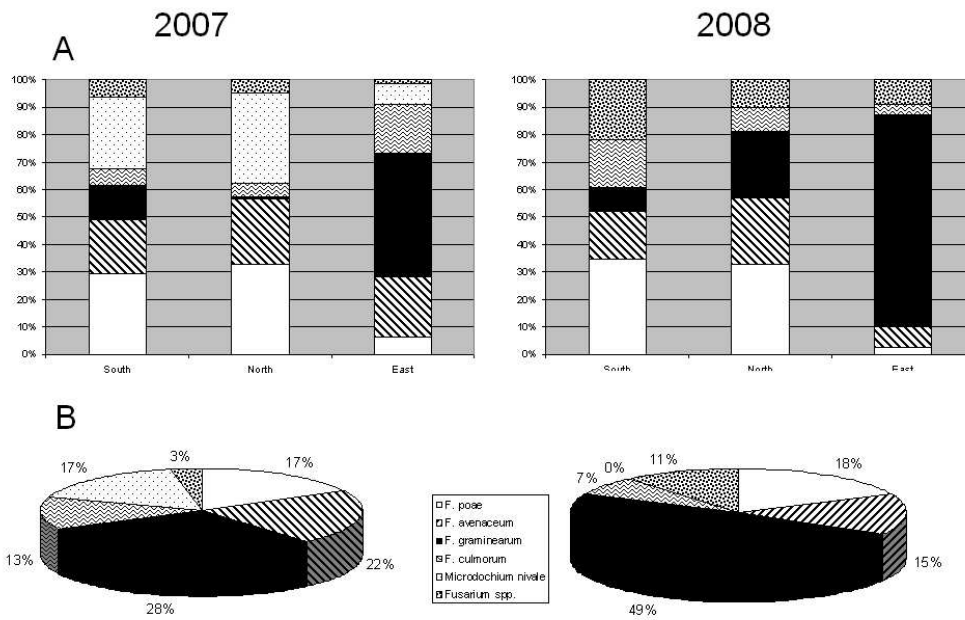


Figure 2A. Occurrence (% of contaminated samples) of the different *Fusarium* spp. in Luxembourg in 2007 and 2008 based on the investigation of 17 and 16 sites, respectively at the time of harvest, and distribution of the *Fusarium* species according to the 3 agro-meteorological regions in 2007.

Figure 2B. The circle diagrams represent the overall average of *Fusarium* occurrence for all individual fields for both years.
255x164mm (96 x 96 DPI)

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 ($\mu\text{g}/\text{kg}$)		DON ($\mu\text{g}/\text{kg}$)	
			<i>average</i>	<i>Range</i>	<i>average</i>	<i>Range</i>
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		<LOQ	-	n.d.	-
Troisvierges	16		<LOQ.	-	487	[461-512]
Hoscheid	17		147	[136-157]	681	[673-688]
Reisdorf	7	East	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 $76\mu\text{g}/\text{kg}$ (for DON) in Luxembourg (nd: no data).