Article

Frequency of Deoxynivalenol Concentrations above the Maximum Limit in Raw Winter Wheat Grain during a 12-Year Multi-Site Survey

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Abstract: Mycotoxins such as deoxynivalenol (DON) in wheat grain pose a threat to food and feed safety. Models predicting DON levels mostly require field specific input data that in turn allow predictions for individual fields. To obtain predictions for entire regions, model results from fields commonly have to be aggregated, requiring many model runs and the integration of field specific information. Here, we present a novel approach for predicting the percentage of winter wheat samples with DON levels above the EU maximum legal limit (ML) based on freely available agricultural summary statistics and meteorological data for an entire region using case study data from Luxembourg and Switzerland. The coefficient of variation of the rainfall data recorded ± 7 days around wheat anthesis and the percentage of fields with a previous crop of maize were used to predict the countrywide percentage of winter wheat grain samples with DON levels > ML. The relationships found in the present study allow for a better assessment of the risk of obtaining winter wheat samples with DON contaminations > ML for an entire region based on predictors that are freely available in agricultural summary statistics and meteorological data.

Keywords: cereal; contaminant; food safety; *Fusarium*; mycotoxin survey; risk assessment; trichothecene

1. Introduction

Winter wheat is among the most important crops for human nutrition together with maize and rice. According to the Food and Agricultural Organization of the United Nations (FAO), the global wheat production reached 767 million tons in 2020, corresponding with 28% of the world's cereal production. Fusarium head blight (FHB) is one of the major cereal diseases. Head blight can be caused by different *Fusarium* species. *Fusarium graminearum* and *F. avenaceum* are frequently found in many European countries while *F. poae*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. sporotrichioides* and *F. tricinctum* have been reported more regionally at lower frequencies [1–3]. *Fusarium* species produce secondary metabolites called mycotoxins. The yield losses and mycotoxin contaminations make *Fusarium* one of the economically most damaging fungal genera. Due to their impact on human and animal health [4,5], but also with regard to national and international trade [6], mycotoxins are among the most important food contaminants. Wheat and maize are the crops in which *Fusarium* mycotoxins occur most frequently and where they are of greatest concern from both a health and economic perspective [7].

Trichothecenes are one of the major classes of mycotoxins including more than 200 secondary metabolites [8]. *Fusarium* species can produce type A and type B trichothecenes [9,10]. Deoxynivalenol (DON) and its acetylated forms are among the most abundant type B trichothecenes primarily produced by *F. graminearum* and *F. culmorum* [1,11,12]. DON may induce nausea, diarrhea and vomiting (hence, the common

name of vomitoxin). In terms of animal health, DON can affect the immune system as well as intestinal functions, impact growth and weight gain and induce vomiting [13–16]. DON contaminations are mainly found in wheat and maize but also in barley, rye, oat and rice. Previous reports indicated that in Europe, between approximately 50% [17] and 56% [18] of cereal-based samples contained DON in quantifiable amounts especially in wheat and maize. In order to limit the impact of mycotoxins on human and animal nutrition, many countries have established legal maximum limits (MLs) or guidance levels [19]. The European Commission (EC), the Food and Drug Administration (FDA) and the Chemical Inspection and Regulation Service in China [20] have, for example, established regulatory limits depending on the cereal concerned, the human or animal sector, the processed products and the consumer age (babies or adults) concerned. According to the European regulation EC No 1881/2006 (amended by n°1126/2007), the maximum limit for DON in unprocessed cereals is 1250 µg/kg. Several surveys were published on mycotoxin occurrence from various regions of the world [5,12,21–32]. The presence of DON in grains is strongly dependent on the year and environmental factors such as weather conditions particularly during the period of wheat anthesis. Beyer et al. [33] and Blandino et al. [34] demonstrated an important role of the previous crop and cultivar susceptibilities at the field level. Cultivar susceptibility is usually evaluated at the level of disease symptoms rather than at the level of toxin concentrations and is listed along with other cultivar traits by national institutions such as the German Federal Office for Plant Varieties (BSA) [35]. Previous models predicted DON concentrations for a specific location [36–38]. To obtain results on the risk for an entire region, many model runs requiring the full set of input variables must be done for many locations in the region under investigation with these models. So far, information on potential predictors that can be retrieved from open access agricultural summary statistics or meteorological data for the percentage of wheat samples with DON concentrations above the ML at the scale of entire regions is scarce.

The objectives of the present study were (1) to identify potential predictors for DON concentrations > 1250 µg/kg at the level of an entire region and (2) to determine the frequency of raw winter wheat grain samples with DON contents > 1250 µg/kg based on a long-term, multi-site monitoring campaign as an additional input for the risk assessment on mycotoxin contamination.

2. Materials and Methods

2.1. Sampling and Agronomic Information

Between 2007 and 2018, commercial wheat fields covering all regions of Luxembourg were selected for each year. The number of sampling locations was 172 (Table 1). For each location, at least two fields with different previous crops were selected. Agronomic data of the fields (previous crop, tillage) and maps with the position of the fields were provided by the Chambre d'Agriculture de Luxembourg (https://www.lwk.lu/, accessed on 8 May 2021) after obtaining permission from the respective farmers. Sampling locations are mapped for each year of the monitoring campaign in Figure 1.

The sampling was done between the plant growth stages 90 and 93 (Zadoks scale). From each field, 0.5 square meters of wheat heads were sampled randomly from two positions within each field. Hence, two samples were available per field except for the years 2007 and 2008 [39] where only one sample from one field per location was taken due to the sampling pattern of the previous, initial project. Wheat heads were dried at 30 °C overnight in an oven and subsequently threshed using a Minibatt grain sample harvester (Reichardt, Hungen, Germany). Cultivar susceptibility rankings for the symptom of FHB were taken from the BSA [35]. The susceptibility scale ranged from 1 (not susceptible) to 9 (very susceptible). The number of samples from cultivars with susceptibility ranks of 3, 4, 5 and 6 were 15, 382, 129 and 19, respectively. A total of 130 out of 714 samples originated from cultivars for which no susceptibility ranks were available because these cultivars were not evaluated by the BSA [35]. In addition to the data from Luxembourg, data from Switzerland previously published by Vogelgsang et al. [40] were used.

Table 1. Number of raw winter wheat samples in Luxembourg and the number of samples containing deoxynivalenol (DON) between 2007 and 2018. For the calculation of means and medians, samples with DON levels below the LoQ (limit of quantification) were set equal to the LoQ.

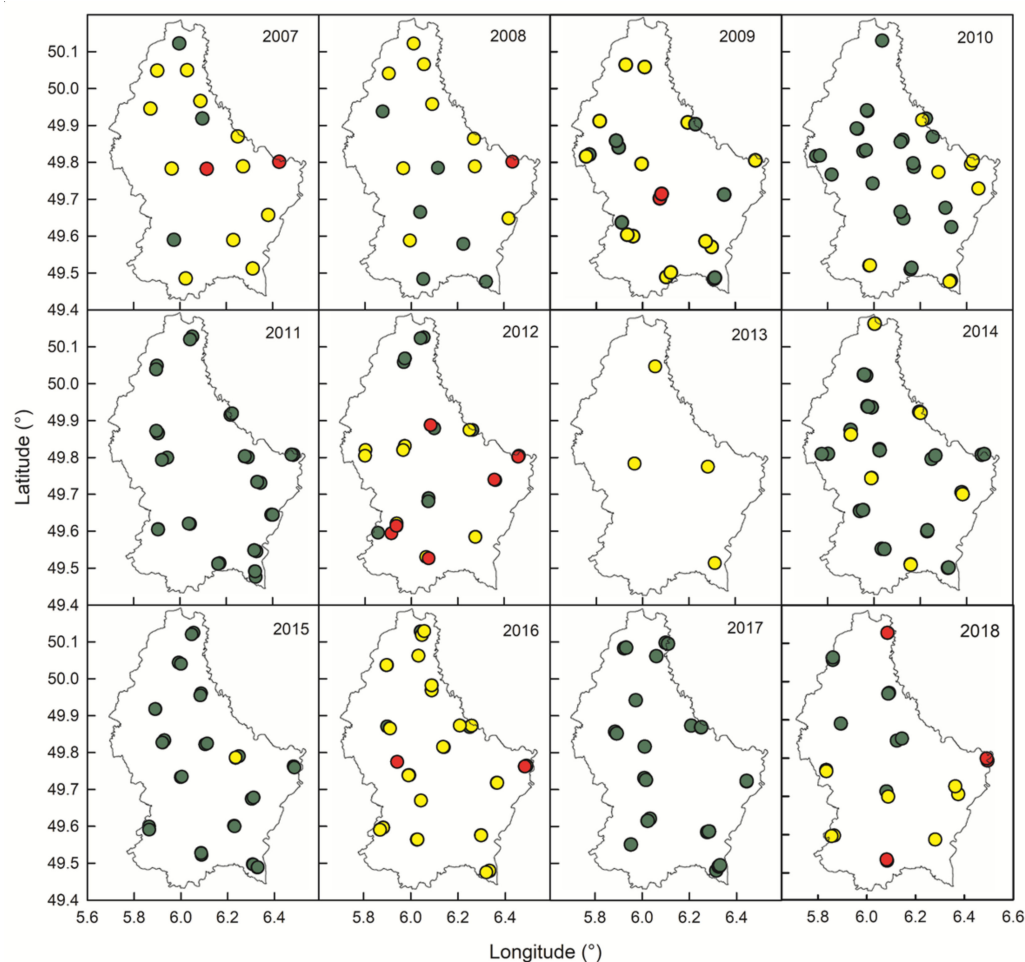|	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total

Figure 1. Sampling locations of the monitoring campaigns 2007–2018. The locations of fields where the deoxynivalenol (DON) content of the raw winter wheat grain was below the limit of quantification (LoQ) are marked in green, the locations of fields with DON concentrations > LoQ but lower than 1250 µg/kg are marked in yellow and the locations of fields with DON > 1250 µg/kg are marked in red. The size of Luxembourg from east to west is 57 km.

2.2. Mycotoxin Analysis

All reagents used for this study were of trace analytical grade (Carl Roth GmbH, Karlsruhe, Germany). DON standards were purchased from LGC Standards (Molsheim, France). Water used for the analytical part was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

After threshing the wheat heads, flour was obtained by grinding 500 g of grains in an ultra-centrifugal mill ZM 200 (Retsch GmbH, 42781 Haan, Germany) using a ring sieve with an aperture size of 1 mm.

Analyses were performed on 5 g of flour added to 15 mL of acetonitrile/water (80/20, *v/v*). Tubes were vortexed and agitated for 10 min at 10 Hz with a mixer mill MM400 (Retsch). A supernatant aliquot was filtered through a 0.20 mm GHP membrane filter (PAL) after a centrifugation step (4700 rpm, 15 min, 20 °C, Multifuge X3R, Thermo, Waltham, MA, USA). To reduce the matrix effects and to be in the appropriate solvent ratio for a chromatographic analysis, the extracts were diluted ten times in water. Prior to the analysis, extracts were stored for 1 h at 4 °C. Two analytical systems were used during this 12-year survey for DON quantification depending upon the availability of the machine.

For the samples from 2007 to 2013 and 2016 to 2018, the method described in [39] was used for DON quantification.

In 2014 and 2015, an ultra-high performance liquid chromatograph coupled to a hybrid mass spectrometer was used (UHPLC, Infinity 1260, Agilent; Q Trap 4500 AB Sciex, Santa Clara, CA, USA) incorporating electrospray ionization in multiple reaction monitoring (MRM) with a negative mode set to detect DON. A Zorbax Eclipse Plus C18 column was used (3.5 μm, 2.1 mm × 150 mm, Agilent Technologies, Santa Clara, CA, USA) at 40 °C with a mobile phase consisting of methanol and water containing 2.5 mM ammonium acetate. The quantification was based on external standards (from 1.5 to 250 ng/mL) and by measuring 295, 265 and 138 fragmentation ions from a precursor ion adduct 355. In addition, $^{13}C_{15}$ DON was used as an internal standard.

The relevance of the extraction and analytical procedure was verified using a certified reference material (LGC Standards, Molsheim, France). For each sample set, this analysis was performed resulting in an average concentration of 442 ± 67 (mean ± standard deviation, *n* = 61) for a certified concentration of 474 μg/kg.

2.3. Weather Data

Weather data were recorded by automatic weather stations. The positions of the weather stations and the weather data can be downloaded at www.agrimeteo.lu, accessed on 8 May 2021. Time series with data gaps were excluded from the analysis. The network of weather stations was continuously expanded during the period of observation such that complete data from 16 (2007) to 36 (2016) weather stations were available. Daily precipitation data starting one week before the full anthesis of winter wheat and ending one week after the peak of winter wheat anthesis were downloaded and used for a further analysis. This period is subsequently referred to as "around anthesis". The period of anthesis was determined by weekly assessments at four locations (one each in the northern, southern, western and eastern regions of Luxembourg) as previously described in Aslanov et al. [41]. The distance between the fields sampled in the present study and the fields where the growth stages were assessed was determined using the using the "near" tool of the software package ArcGIS (ArcGIS software 10.0) as previously described by Beyer et al. [42]. The plant growth stages in the sampling fields were assumed to be the same as the ones in the closest plant growth stage assessment field.

2.4. Statistical Analyses

The sum of the daily precipitation data over the period ±7 days around the day of peak anthesis was calculated for each weather station. Subsequently, the coefficient of variation (CV) from the resulting data of all available weather stations was calculated for each year. This CV thus reflected the variability among the weather stations in relation to the mean precipitation around wheat anthesis.

The relationships between the CVs, the percentage of fields with previous crop maize, the cultivar susceptibility rankings and the annual percentage of samples with DON levels > 1250 μg/kg were described using linear or sigmoidal regression models (software package SigmaPlot version 13). The equations, regression coefficients and coefficients of determination are given in the figures where the regression lines are shown.

The frequencies were compared using two-sided chi-squared tests (software package SPSS version 19, IBM Corporation, Armonk, New York, USA). The effects were considered to be significant when the *P*-values were lower than 0.05.

3. Results

Between 2007 and 2018, 172 fields were sampled for a total number of 714 samples analyzed (for details, please see Supplementary Table S1).

3.1. Spatial Distribution of Contaminated Samples

DON concentrations higher than 1250 μg/kg were found in 2007, 2008, 2009, 2012, 2016 and 2018 (Table 1) but were variable depending on the region (Figure 1). In fact, DON concentrations > 1250 μg/kg were observed in six out of twelve years in the east, in

three out of twelve years in the center, twice in the south and once in the west. The high frequency of DON levels > 1250 µg/kg in the east was not significantly related ($p > 0.05$) to the susceptibility ranks of the cultivars grown there, the previous crop alone (maize versus others) or the tillage system alone (plough versus minimum tillage). The combination of a previous crop of maize with minimum tillage was not more common within the hotspot than elsewhere ($p > 0.05$). Rainfall during anthesis was lower than the countrywide average at the weather station in the eastern DON hotspot in 2008 and 2012 but higher in 2007, 2009, 2016 and 2018 ($p < 0.041$). Other years were not included in the rainfall analysis because for those years, no DON levels > 1250 µg/kg were observed, suggesting that weather conditions were insufficient for exceeding the ML.

3.2. Inter-Annual and Annual Variability of DON Contents

A chemical analysis by HPLC-MS/MS revealed that 33% of the samples analyzed were contaminated with DON (range from 76 (LoQ) to 9247 µg/kg) (Table 1). The percentage of contaminated samples varied greatly from year to year with 0% in 2011 and 83% in 2007. A total of 37 out of 714 (=5%) raw winter wheat samples contained DON levels > 1250 µg/kg. In 6 out of 12 years (2010, 2011, 2013, 2014, 2015 and 2017), no samples with DON levels > 1250 µg/kg were found (Table 1). In the remaining years, the percentage of samples with DON levels > 1250 µg/kg ranged from 4% in 2009 to 26% in 2012 (Table 1).

A high number of contaminated samples did not necessarily result in high levels of DON. For example, in 2007, 82% of the samples were contaminated with DON but only 12% were above the maximum level of 1250 µg/kg set by the EU. In 2012, 54% of the samples contained DON and 26% contained DON at levels above the ML. DON concentrations found in commercial wheat samples collected over the 12-year period are shown in Figure 2 (maximum, mean, median). For the calculation of the means and the medians, samples with DON levels below the LoQ were set equal to the LoQ (76 µg/kg). The distribution of DON values was characterized by many small values and a few high levels and a large variability of variances was observed between years (Figure 2). Every four years, a peak in the DON values was observed (Figure 2).
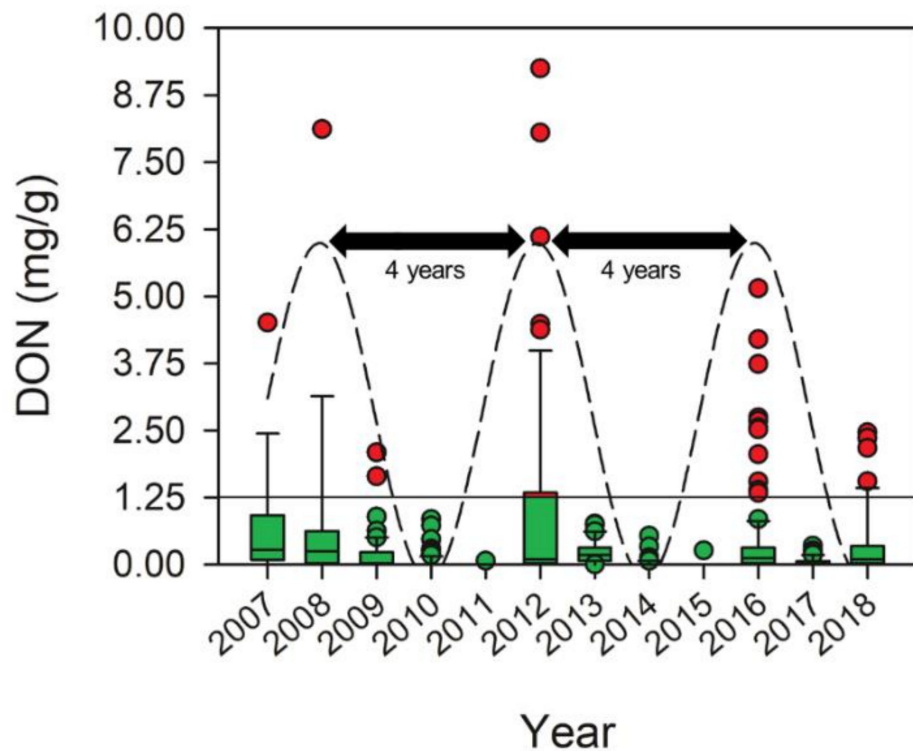
Figure 2. Boxplots of the distribution of deoxynivalenol (DON) levels detected in raw winter wheat sampled in Luxembourg. Between 2007 and 2018, 714 samples were analyzed. The samples with DON levels above the maximum limit (ML) of 1250 µg/kg based on the EU legislation are marked in red. The dashed line is a waveform sine 4 parameter regression line fitted to the annual maximum DON values.

3.3. Effect of Weather and Previous Crop on the Percentage of Samples with DON > ML

A close non-linear relationship was observed between the coefficient of variation (CV) calculated from the rainfall data during the period around wheat anthesis (Figure 3A, $r^2 = 0.74$ **). Low CVs were associated with high percentages of samples with DON levels > 1250 µg/kg (Figure 3A). The minimum average precipitation of all weather stations for a year with DON values > 1250 µg/kg was 51 mm in 2008 in Luxembourg. Hence, years with more precipitation were considered to be wet years while years with less precipitation were considered to be dry years. Another non-linear relationship ($r^2 = 0.74$ *) was found between the percentage of fields with a previous crop of maize and the percentage of samples with DON levels > 1250 µg/kg for more wet years (≥51 mm rain in the week prior plus the week after the day of maximum anthesis). However, no relationship was found for drier years (<51 mm rain in the week prior plus the week after the day of maximum anthesis, Figure 3B). Note that the latter relationships comprise data that were recorded in course of the present study as well as data previously published from Switzerland [40], suggesting that the relationship has more than a local relevance. The effect of cultivar susceptibility rankings towards FHB symptom expression on the annual percentage of samples with DON levels > 1250 µg/kg was not significant ($p > 0.05$) basically because samples from cultivars with a relatively low susceptibility rank of 3 contained high DON levels in the year with the highest disease pressure (2012).
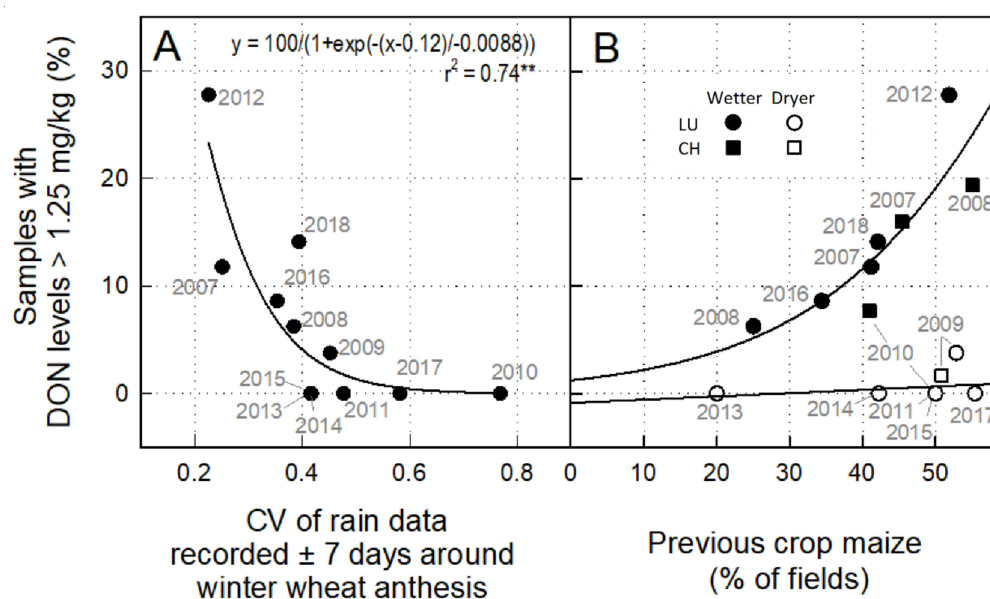
Figure 3. (A) Relationship between the percentage of raw winter wheat grain samples with deoxynivalenol (DON) > 1250 μg/kg and the coefficient of variation (CV) of precipitation data recorded ±7 days around anthesis. The samples were collected from fields scattered across Luxembourg. The network of weather stations was continuously expanded such that precipitation data from 16 (2007) to 36 (2018) weather stations were available. The total precipitation over the period ±7 days around anthesis was calculated for each weather station. Subsequently, coefficients of variation were calculated from the data of all weather stations. **(B)** Relationship between the percentage of winter wheat grain samples with DON > 1250 μg/kg in Luxembourg and the percentage of fields with a previous crop of maize for dry and wet years in the period ±7 days around wheat anthesis. The minimum average precipitation of all weather stations for a year when DON > 1250 μg/kg was observed was 51 mm in 2008 in Luxembourg in the period ±7 days around wheat anthesis. Hence, years with more precipitation were considered to be wet years while years with less precipitation were considered to be dry years. Squares represent data previously published by Vogelgsang et al. [40] for Switzerland (CH). The non-linear relationship for the wet years followed the equation $y = 100/(1 + \exp(-(x - 74.61)/16.69))$, $r^2 = 0.74$, $p = 0.0104$. The linear relationship for the dry years was non-significant ($p > 0.05$. The numbers close to plot symbols represent the years of sampling. ** = significant at the 1% level.

4. Discussion

4.1. Considerations on Temporal Risk Dynamics

DON levels above 1250 μg/kg were observed in 6 out of 12 years, indicating that there was on average a risk of relevant DON contamination almost every second year. The 12-year data available suggested that peak DON levels must be expected approximately every four years in Luxembourg. It may be speculated that this pattern could be the result of numerous farmers following the same scheme of crop rotation. However, this was not the case. The governments in the regions studied established incentives to diversify crop rotations and these programs have been largely accepted in the farming community. The reason for this pattern is currently unknown but for potential forecasts, this effect is of high interest.

4.2. Novelty

The meteorological conditions were demonstrated to have a strong impact on the DON content at the level of individual grain samples or fields [43–45]. Predictive models were developed over the years as a decision support tool for famers to deal with protection of the grains and for avoiding unnecessary fungicide applications [38]. Decision support tools often require detailed information such as the previous crop, growth stage, tillage system and cultivar susceptibility for each field [38,46–49]. They are mostly valid in the specific

country or area where they were developed and need adaptation or additional validation when being used elsewhere. To the best of our knowledge, variability parameters such as coefficients of variation of rain data have not been used before for estimating DON levels. The close relationship found in Figure 3A suggested that precipitation (62.8 ± 13.9 mm in the two weeks around anthesis in the worst year, 2012) with little variation between locations throughout a region was needed to obtain high percentages of winter wheat grain samples with DON levels > 1250 µg/kg. The data needed for taking advantage of the relationships found here are available from meteorological services and agricultural statistics in many countries (e.g., agrimeteo.lu, https://agriculture.public.lu/dam-assets/publications/ser/statistiques/landwirtschaft-in-zahlen/the-agriculture-of-luxembourg-2016.pdf, https://www.dwd.de/DE/leistungen/klimadatendeutschland/klimadatendeutschland.html, https://donneespubliques.meteofrance.fr/, accessed on 8 May 2021) and may therefore be used without a need for many model runs that predict DON levels pointwise.

4.3. Spatial Aspects

A hotspot with DON levels > 1250 µg/kg in 5 out of 12 years was found in the east of the country. The cultivar susceptibility ranks towards FHB were similar within the hotspot compared with the entire country. The combination of a previous crop of maize with minimum tillage was not more common within the hotspot than elsewhere. The rainfall during anthesis was lower than the countrywide average at the weather station in the eastern DON hotspot in 2008 and 2012 but higher in 2007, 2009, 2016 and 2018 ($p < 0.05$). Other years were not included in the rainfall analysis because for those years, no DON levels > 1250 µg/kg were observed suggesting that weather conditions were insufficient for exceeding the ML. Hence, the reason for the high frequency of samples with DON levels > 1250 µg/kg in the hotspot may be related to wetter conditions during anthesis even though this effect was not perfectly consistent among years.

In Europe, *F. graminearum*, *F. avenaceum*, *F. poae* and *F. culmorum* are the dominant species. *Fusarium graminearum* is the main FHB causing species in the southern and central parts of Europe. In the northern part, *F. graminearum* has been spreading in recent years and has now replaced *F. culmorum* as the main producer of DON [50]. For Luxembourg, it was previously demonstrated that *F. graminearum* is dominant on wheat heads in wet years while *F. culmorum* can be the most frequently isolated species in dry years [45]. Climate change is likely to affect the composition of regional *Fusarium* species as well as the frequency and severity of critical rainfall periods and, consequently, mycotoxin concentration risks [7,19]. An increase of the proportion of *F. graminearum* has been reported in northern countries or cooler climates such as the Netherlands [51] and the UK [52]. Even though Figure 3B suggests that the percentage of fields with a previous crop of maize allowed for the estimation of the percentage of winter wheat samples with DON levels > 1250 µg/kg in Luxembourg and Switzerland, this result should not be extrapolated to other regions without validation, particularly not to regions with significantly different compositions of *Fusarium* species. Major spatial *Fusarium* species and chemotype distributions for Europe can be found in Pasquali et al. [53].

4.4. Considerations on the Role of Cultivars

Cultivar susceptibility towards FHB is commonly assessed by visual symptom evaluation. However, previous studies [54,55] demonstrated that correlations between symptoms and DON are close within cultivars and years but vary not only among cultivars but also among years even more so. In the present study based on a monitoring of real farming conditions, all cultivars were selected by farmers and were of moderate resistance. Hence, differences among cultivars were rather small and non-significant. In experimental settings where more resistant and more susceptible cultivars are included than in the present study, a significance of the cultivar effect can be expected [56].

4.5. Other Factors Affecting DON Production under Field Conditions

In addition to the factors of rain during anthesis and the previous crop that have been considered in the present study, cultivar susceptibility (discussed above) and tillage had a significant impact on DON levels while reports on the effects of nitrogen fertilization and growth regulators were inconsistent [33]. Considering information on tillage would probably allow for a more precise estimation of the percentage of samples with DON levels > ML. This information is, however, not included in standard agricultural statistics published by national authorities at the moment and is therefore hard to acquire. Relative humidity (>80%) and temperature during the anthesis time have repeatedly been shown to be crucial for DON levels at the field scale [37,43]. An increase in temperature coupled with an increase in precipitation would have a positive impact on the contamination of *Fusarium* spp. and therefore on the production of DON. However, as shown by Birr et al, 2019 [37], the relationship between the precipitation parameter during anthesis alone and DON content is much more robust than the relationship between temperature and DON content. In the present study, by using precipitation data freely available in agricultural summary statistics and meteorological data, we were able to forecast the risk of obtaining winter wheat samples with DON contaminations > ML with reasonable accuracy ($r^2 = 0.74$). This prediction has a strong impact on food safety.

4.6. Comparison with Other European Surveys

Numerous studies have been published on mycotoxin occurrence from various regions of the world and on DON contamination in cereals in Europe during the past 20 years. Our study showed that 33% out of the 714 winter wheat samples analyzed contained levels of DON above the LoQ over the period 2007–2018. A high variability was detected in European countries such as 28% in Serbia [57], 23% in Albania (2014–2015) [58], 30% in samples from Finland, 21% from Sweden, 29% from Norway, 71% from the Netherlands (1990–2009) [59], 59% in Italy (2009–2010) [60], 94% in Finland (2013) [61] and 47% in Poland [62]. According to a BIOMIN survey in 2019 [18], DON prevalence was estimated to be 56% (up to 22,000 µg/kg) over 2011 cereal samples tested. Likewise, another European survey [63] on 11 countries confirmed the previous number with 61% of positive wheat samples.

All of these studies were performed on a short period with one, two or three years of sampling or it has been shown that the amount of mycotoxins is highly dependent on external factors such as environmental/meteorological conditions, fungal species and agronomical parameters [40,64]. Mycotoxin production is highly related to the weather conditions, particularly during the anthesis period. All of these conditions will lead to major changes in the mycotoxin occurrence from year to year, as shown by Uhlig et al. [65]. As it is still difficult to publish results from surveys with low mycotoxin levels, there is a bias risk that data from years with high DON levels are more likely to be published. Therefore, long-term data from the same region with sampling campaigns every year are necessary to provide a realistic picture of the overall risk and the frequency of contaminations. Only a few long-term analyses have been run in Europe. Switzerland carried out monitoring between 2007 and 2014 and highlighted that DON was detected in 80% of the samples while levels exceeding the European limit for unprocessed cereals for foodstuffs were observed in 11% (0–7%) of the samples [66]. In a joint survey between Finland, Sweden, Norway and the Netherlands during the period 1990–2009, 3% of the wheat samples were above the ML [59]. Similar results were observed in Norway between 2004 and 2009 with 9% of the samples exceeding the threshold [67]. The rate of contaminated samples can reach much higher levels as demonstrated by Chandelier et al. (2011) [68] in Belgium, where the range of samples containing more than 1250 µg/kg DON was between 0% in 2005 and 2006 campaigns and 36% in 2007. Similar results were found in our study with high disparities between years at the level of DON occurrence as well as the percentage of samples above the European ML.

5. Conclusions

In the current study, we were able to estimate the annual percentage of samples with DON levels > 1250 μg/kg with reasonable accuracy (r^2 = 0.74) from the coefficient of variation (CV) of precipitation data recorded by weather stations scattered across Luxembourg in the week before and after wheat anthesis. Furthermore, the annual percentage of samples with DON levels > 1250 μg/kg could be estimated from the percentage of fields with a previous crop of maize in Luxembourg and Switzerland (r^2 = 0.74). The latter relationship was only observed for wet years but did not hold true for dry years. The relationships found here may help estimate the percentage of winter wheat lots with critical DON levels from an entire region. This estimate may have a direct interest for authorities but also for post-harvest stakeholders in order to estimate the quality of the grains as well as possible outlets on the grain market. The present results should not be extrapolated to regions where *Fusarium* species other than *F. graminearum* sensu stricto dominate the species composition on wheat heads.

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