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The aim of this study was to model fusarium mycotoxins against agronomic factors in order to identify those that have the greatest impact on mycotoxin levels in harvested wheat. To achieve this fusarium mycotoxins levels were monitored, and associated agronomic data collected, in approximately 150 English wheat fields/year between 2006 and 2013. Results showed large seasonal variation in fusarium mycotoxin levels, with high levels in 2008 (13% and 29% exceeding legal limit for unprocessed soft wheat intended for human consumption for DON and ZON, respectively) and 2012 (10% and 15% exceeding legal limit for unprocessed soft wheat intended for human consumption for DON and ZON, respectively) and low levels in 2006 and 2011 (no samples exceeding legal limits for unprocessed soft wheat intended for human consumption for DON or ZON). Analysis of agronomic factors identified previous crop, cultivation and variety as the greatest risk factors. The greatest risk of mycotoxin development in grain was following maize as a previous crop and minimum tillage. The combined effect of these factors gave respective average DON and ZON levels 20 and 14 times higher than other previous crop and cultivation combinations. A newly quantified risk factor was harvest date. A one month delay in harvest resulted in a 10 and 25 times greater mean DON and ZON concentration respectively, when compared to crops harvested around the long-term regional average harvest date. These results highlight the highly seasonal variation in fusarium mycotoxins in wheat and the agronomic factors that should be avoided to minimise fusarium mycotoxin levels in harvested wheat.

Keywords: fusarium, agronomy, previous crop, cultivation, variety, harvest delay, mycotoxin, trichothecene, deoxynivalenol, zearalenone, HT-2 toxin, T-2 toxin

Introduction

Fusarium head blight (FHB) of small grain cereals may be caused by several fungal pathogens. The predominant species in the UK are *Fusarium graminearum, F. culmorum, F. poae, F. avenaceum, Microdochium nivale* and *M. majus*. The *Fusarium* species within this disease complex produce a range of mycotoxins. The trichothecene mycotoxins produced by *Fusarium* species are divided into Type A and B. Deoxynivalenol (DON) and nivalenol (NIV) are Type B trichothecenes produced predominantly by *F. culmorum* and *F. graminearum*. Isolates of both these species are either DON or NIV producers. DON producers are referred to as Type 1 chemotype, this chemotype is further divided into 1A and 1B depending on the acetylated DON that is produced as a co-contaminant, 3- or 15-acetyl DON, respectively. Both DON and NIV chemotypes of *F. culmorum* (Jennings et al., 2004a) and *F. graminearum* (Jennings et al., 2004b) are present in the UK. *F. poae* has also been linked to high levels of NIV (Fredlund et al. 2013). HT-2 and T-2 are Type A trichothecenes produced predominantly by *F. langsethiae* in the UK (Edwards et al. 2012).

The occurrence, exposure and toxicity of DON was recently reviewed in an EFSA Scientific Opinion (EFSA Panel on Contaminants in the Food Chain 2017), where they assessed the risk to animal and human health of DON, the acetylated forms of DON and the DON metabolite, DON-3-glucoside. Other trichothecenes have the same cellular activity (disruption of protein synthesis) but have a higher cellular toxicity than DON. Nivalenol and T-2 are generally more toxic than DON *in vitro*, although the relative differences are dependent on the target cell studied (Escriva et al. 2015).

In addition to DON or NIV, *F. culmorum* and *F. graminearum* also produce zearalenone (ZON). The function of ZON in the fungus is not known and is predominantly produced late in the crop growing season, near to harvest (Kharbikar et

al. 2015; Matthaus et al. 2004). Zearalenone has low cellular toxicity but is problematic as it has high estrogenic activity causing hyperestrogenism in animals and humans. In animals, the mycotoxin causes a range of fertility problems, with young female pigs being particularly susceptible. There is no direct evidence of health implications in humans, although elevated ZON concentrations have been detected in urine samples in cases of premature thelarche central idiopathic precocious puberty (Asci et al. 2014).

The European Commission (EC) set legislative limits for the fusarium mycotoxins DON and ZON in cereal grains and cereal-based products intended for human consumption in 2006 (EC 2006b). The limits for unprocessed cereals other than durum wheat, oats and maize intended for human consumption is 1250 μg kg^{-1} of DON and 100 μg kg^{-1} of ZON. The maximum levels set for unprocessed cereals apply to cereals placed on the market for processing. The European Commission states that maximum levels are set on unprocessed cereals to avoid highly contaminated cereals entering the food chain and to encourage all measures to minimise fusarium mycotoxin contamination to be taken in the field and storage stages of the production chain. In 2006, The European Commission also set guideline limits for fusarium mycotoxins in animal feed (EC 2006a). General guidance limits for animal feed are 8000 μg kg^{-1} DON and 2000 μg kg^{-1} ZON. The lowest guidance limits have been set for pigs owing to their higher sensitivity to fusarium mycotoxins. The DON guidance value for complementary and complete feedingstuffs for pigs is 900 μg kg^{-1}. The ZON guidance value for complementary and complete feedingstuffs for sows and fattening pigs is 250 μg kg^{-1} and for piglets and gilts is 100 μg kg^{-1}. Currently there is no legislation for NIV; it is reported to be a co-contaminant of deoxynivalenol (Escriva, et al. 2015) and as such, levels are thought to be controlled via the limits for DON (Anon 2006).

In 2013 the European Commission published a Recommendation on the mycotoxins HT2 and T2 (EC 2013). The Recommendation included indicative levels for the combined concentration of HT-2 and T-2 toxins (HT2+T2) in unprocessed cereals and cereal products. The indicative level for unprocessed wheat is 100 μg kg^{-1}. The Recommendation states that Member States, in collaboration with industry, should continue to monitor the occurrence of the mycotoxins HT-2 and T-2. Where levels exceed the indicative level an investigation should be conducted to determine why the exceedances occurred and what mitigation could be implemented to avoid future exceedances.

Previous research has identified a number of agronomic factors which can affect the concentration of fusarium mycotoxins in harvested cereals, these include previous crop, cultivation, host cultivar and fungicide application (Wegulo 2012). The effect of agronomy on ZON contamination of grain is likely to be similar to that for DON; however, no previous studies have modelled ZON concentrations against agronomic factors.

The overall aim of this study was to identify the level of fusarium mycotoxins in English wheat at harvest over an 8-year period and to determine the impact of agronomy on these levels.

Materials and methods

Grain sample collection

Between 2006 and 2013 requests for grain samples were sent to the 300 farms involved in the Defra-funded winter wheat disease survey. The target number of samples for each year was 150 (50% return rate).

The Defra survey was stratified based on regional wheat growing area, which meant that samples were not selected based on intended end use. Samples were collected at harvest, from the field specified by the grower for use in the disease survey, this ensured that full agronomic data would be available for the sample. Grain samples were either collected direct from the combine or taken from trailers as they left the field. Sub-samples (approximately 300 g) were taken from ten arbitrary points around the field and combined to provide a 3 kg bulk sample. Samples were sent to the laboratory by next day courier service. On arrival at the laboratory the moisture content of the sample was determined, samples with a moisture content greater than 18% were dried overnight, using a heated-air dryer, and the moisture content re-assessed. For each grain sample a 500 g sub-sample was removed from the bulk sample, using a ripple divider, dried to 12% moisture content and stored at room temperature as a grain archive. The remaining sample was milled (ZM200, Retsch) using a 1 mm screen and mixed in a tumbler mixer. Once thoroughly mixed, two 300 g sub-samples were taken; the first sample was used for mycotoxin analysis and the second held as an archive sample at –20°C.

Agronomic data was supplied by growers through completion of a questionnaire. The data collected included field location, variety sown, intended use, drilling date, seed treatment, previous cropping (last four years), crop debris treatment (removal or incorporation), cultivation, presence of maize in rotation or as a neighbouring crop, fungicide applications (what applied, dose, date and growth stage) and harvest date.

Mycotoxin analysis of grain samples

In 2006, all samples were analysed for DON and ZON using Ridascreen ELISA assays (R-biopharm Rhone). The reported limit of quantification (LoQ) for DON was 17.5 μg kg^{-1} and a recovery rate of 85-110%. The LoQ for ZON was 1.75 μg kg^{-1} and a recovery

rate of approx. 80%. To allow comparable data to the subsequent years the LoQ were set at 10 and 2 μg kg^{-1} for DON and ZON respectively. Concentrations determined were not adjusted for recovery.

In subsequent years, all samples were analysed by Campden BRI using UKAS accredited procedures. The trichothecenes (DON, NIV, 3-acetylDON, 15-acetylDON, fusarenone X, T2 toxin, HT2 toxin, diacetoxyscirpenol and neosolaniol) and the non-trichothecene, ZON, were analysed by liquid chromatography with tandem mass spectrometry (LC/MS/MS). Spiked samples were included in each batch to determine extraction recovery. The method had acceptable recovery range for each trichothecene of 60-120%. Results were corrected for recovery. The Limit of Quantification (LoQ) for the trichothecenes was 10 μg kg^{-1} and for ZON was 2 μg kg^{-1}. The expanded measurement of uncertainty was calculated using a standard coverage factor of two, equivalent to a confidence of approximately 95%, that the actual level of the mycotoxin being measured lies within the quoted range. The expanded measurement of uncertainty was calculated to be 16% for DON and 13% for ZON for samples from 2007-2008 and to be 23% for DON and 24% for ZON for samples from 2009-2013.

Summary statistics

Samples with a mycotoxin concentration below the limit of quantification (LoQ) were assigned a value of (LoQ)/2 for calculation of mean values. Summary statistics (percentage greater than 10 μg kg^{-1}, mean, median and percentage greater than legal limits) were calculated using Excel (Microsoft v.2013).

Modelling fusarium mycotoxin risk – agronomy data

Mycotoxin concentrations were $\log_{10}$ transformed to normalise the residuals before analysis. Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v16, Lawes Agricultural Trust). Temporal (year) and spatial (region) factors were forced into the model. Other agronomic factors were ordered based on the order in which they occur within a growing season. Interactions between factors were entered into the model where there was a biological reason to expect one to occur. As weather is an important parameter of FHB epidemiology one could expect a temporal (year) and spatial (region) interaction. As crop debris is an important parameter of FHB epidemiology, as in the type and amount of crop debris, then an interaction between previous crop, crop debris management and the method of cultivation could be expected (i.e. benefit of removal of straw and/or ploughing would vary depending on the previous crop). Quantitative data were entered as quadratic polynomial sub-models. Any categorical factor level represented by less than 10 samples was entered as "Other". There were only a small number of samples where direct drilling was recorded so these were entered into the category "minimum tillage". Once factors for the model had been selected the data file was filtered of all samples containing blanks within these factors and the data was re-analysed. Results are presented as the predicted means and 95% confidence limits for each factor level. The predicted means are the means calculated from the fitted model as if the dataset was balanced, ie had an equal number of samples from each year and region, etc.

Results

Between 2006 and 2013, a total of 1276 harvested wheat grain samples were sent for fusarium mycotoxin analysis by growers participating in the Defra-funded winter wheat disease survey. This equated to approximately 150 samples per year. Approximately 60% of samples were intended for feed, 35% for human consumption and 5% for other uses, primarily seed.

Mycotoxin summary statistics

The winter wheat disease survey was a stratified survey within England, and as such mycotoxin results generated from these samples provide an accurate assessment of fusarium mycotoxins in England. Of the ten mycotoxins analysed eight were detected (DON, NIV, ZON, HT2, T2, 3-acetylDON, 15-AcetylDON and fusarenone X). Mycotoxin distributions were highly skewed with a left-hand truncation at the LoQ and an extended right-handed tail.

Deoxynivalenol was the most frequently detected fusarium mycotoxin with an average annual incidence (>10 μg kg^{-1}) of 79% (Table 1) and was usually present at the highest concentration, with an overall mean of 261 μg kg^{-1} and a maximum of 8106 μg kg^{-1}. An average of 4% of samples exceeded the legal limit for wheat intended for human consumption with a seasonal range of zero to 13% (Table 1).

ZON was quantified above 10 μg kg^{-1} in 38% of samples (Table 1) and had a maximum of 1754 μg kg^{-1}. Due to the lower legal limits for ZON, more samples exceeded the legal limit when compared to DON; this despite ZON occurring at lower levels than DON (Table 1). On average 7% of samples exceeded the ZON limit of 100 μg kg^{-1} with a seasonal range of zero to 29% of samples.

Nivalenol was not analysed in 2006 but was detected at varying frequencies in other years with a range of incidence (>10 μg kg^{-1}) from zero in 2010 and 2011 to 59% in 2008, with a mean of 28% and a range of annual means of less than 10 to 18 μg kg^{-1}. NIV never occurred at high concentrations with a maximum level of 189 μg kg^{-1} detected in 2008.

HT2 and T2 toxins were rarely detected in samples. They had an overall incidence of 1.4% and a combined (HT2+T2) maximum of 73 μg kg^{-1}. Acetylated derivatives, 3-acetylDON and 15-acetylDON were detected above the LoQ (10 μg kg^{-1}) in a very few samples and always as low concentration secondary contaminants in the presence of a high concentration of DON. The highest concentrations for 3-acetyl DON and 15-acetyl DON were 72 and 142 μg kg^{-1}, respectively. Fusarenone X is an acetylated version of NIV and was detected in 2012 and 2013 in a total of six samples with a maximum concentration of 32 μg kg^{-1}.

Modelling fusarium mycotoxin risk – agronomy

Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v16, Lawes Agricultural Trust). After selection of factors to be used in the model, the data file was filtered to remove all samples containing blanks within these factors and the data was re-analysed (n=1154). The models generated identified that the same agronomic factors were significant for both DON and ZON concentrations and that their impact on risk were similar for the two mycotoxins. Of the factors tested, year, region, previous crop, cultivation, variety and harvest timing were all significant. There were significant interactions between year and region and between previous crop and cultivation.

For DON, the model accounted for 74% of the observed variance; 59% of the variance was accounted for by year ($p<0.001$) and an additional 10% of the variance accounted for by region ($p<0.001$) and the year.region interaction ($p<0.001$). For ZON, the model accounted for 69% of the observed variance; 51% of the variance was accounted for by year ($p<0.001$) and an additional 10% of the variance accounted for by region ($p<0.001$) and the year.region interaction ($p<0.001$). Consequently, the agronomic factors only accounted for an additional 5 and 8% of the variance in the DON and ZON models, respectively.

Model outputs for predicted DON and ZON concentrations (Figure 1a and b) showed the large seasonal variation present in the observed data; high DON means in 2008 and 2012, and high ZON in 2008. Regional differences were also observed, these fluctuated between seasons, however there was a consistent trend of higher DON risk in the East Midlands.

The Figures presented for each agronomic factor show the back-transformed predicted means for each significant factor and the 95% confidence limits for the predicted means. For some agronomic factors, the dataset was highly unbalanced with low numbers of samples for some factor levels, these can be identified by the large confidence limits. Cultivation alone was not a significant factor for DON ($p=0.071$) or ZON ($p=0.333$), however previous crop and the interaction of previous crop and cultivation were both highly significant for DON and ZON ($p<0.001$ and $p<0.005$ respectively). The clear difference in the predicted means for the interaction of previous crop and cultivation (Figure 2) shows that growing wheat after maize and minimum tillage was a major risk factor. The predicted mean for this agronomy was nearly twice the legal limit for DON and close to the legal limit for ZON.

Varieties present in more than ten samples were analysed individually and there were significant differences for both DON and ZON ($p<0.001$ and $p=0.001$, respectively). The predicted means for each variety appeared to bear little relation to the resistance rating for FHB recorded for the varieties based on national variety trials (AHDB RL website https://cereals.ahdb.org.uk/varieties/ahdb-recommended-lists.aspx) and there was a poor correlation between the concentration of DON and ZON for each variety (Figure 3).

Figure 4 shows the impact of harvest week on the mycotoxin content of harvested wheat. The average harvest day was calculated based on the six-year average for each county. The harvest day of each sample was then calculated relative to the long-term average, this was then categorised into weeks with a minus score for early harvests and positive score for late harvests. The risk for both DON and ZON (Figure 4 a and b) increased slightly as harvest moved from early (negative values) to average timing (0). The risk of contamination increased exponentially as the delay in harvest increased from the average. The increase in risk was greatest for ZON with a 25-fold increase in the predicted mean when the harvest was delayed by 4 weeks compared to no delay. This compared to a 10-fold increase in the predicted mean for DON the same time period.

The intended use of harvested wheat was not significant when placed at the front of the models ($p=0.079$ and 0.751 DON and ZON respectively). This indicated that any variation in agronomy applied to wheat crops with a specific end-use had no significant effect on the level of DON and ZON in harvested grains. The presence of maize as a neighbouring crop or within the rotation had no significant effect on DON or ZON ($p>0.05$). Previous crop history beyond the most recent crop was tested by looking at each previous crop for the last four years, or by looking at cereal intensity (number of

cereal crops in last 4 years) or cereal sequence (number of previous crops since a non-cereal was grown). None of these factors were significant ($p>0.05$) within the DON or ZON models. Crop debris management, i.e. removal of straw from the previous crop compared with incorporation had no significant ($p>0.05$) effect on DON or ZON concentration in the subsequent wheat crop. This occurred even when analysed as an interaction with previous crop and cultivation.

There were no significant differences in the DON or ZON content of wheat crops which received different fungicide regimes. Seed treatment was analysed based on the product used, with no significant ($p>0.05$) differences identified. FHB-targeted fungicide treatment (fungicide application at flowering, GS59 – 61) was analysed based on:

- Application of a triazole (Yes/No)
- Application of a FHB recommended product based on UK guidelines (Anon. 2017) (Yes/No)
- Rate of application of a FHB recommended product (0-0.49, 0.50-0.74, 0.75-1.0 field rate)

None of the above factors were significant ($p>0.05$).

Discussion

The levels of DON and ZON found here were similar to those reported by Edwards (2009), although in the current study there were years with higher exceedances of the legislative limits for both DON and ZON (2008 and 2012). These years were reported as having delayed, wet harvests. High levels of DON and ZON were reported recently in Switzerland during a survey covering a similar timescale with 11% and 7% exceedance of the DON and ZON legislative limits respectively (Vogelgsang et al. 2017). High DON and ZON levels were also reported recently in Norway (6.5% and 10% exceedance of the legislative limits (Hofgaard et al. 2016).

In this study, year accounted for most of the variance within the DON and ZON models. Temporal and spatial variation is routinely observed in fusarium mycotoxin occurrence data, with temporal variation having the greater importance (Vogelgsang, et al. 2017). This is due to the high seasonality of FHB as a consequence of the requirement of moisture/rainfall during a narrow infection window shortly before and during anthesis (Wegulo 2012). A previous study in the UK (Edwards 2009) identified a significant interaction between year and region, which was probably due to fluctuation in weather between years and regions. Highest concentrations of DON were found in the South and East of England with lower levels in the North. By comparing data from the previous study with this study a shift northward from the East of the country was observed with higher DON levels detected in the East Midlands and Yorkshire/ Humberside in recent years. Why the East Midlands would have higher DON compared to the East region is unclear as they have similar agronomic practices and weather. This suggests that other factors were also having an impact. There was a large year.region interaction for ZON concentration and this was believed to be due to delays in harvest. In 2008, there were long delays to the harvest due to wet weather, with 20% of the wheat harvest delayed by more than one month; more crops affected by a rain delay in the South West and North West than in other regions.

Few studies have analysed the impact of agronomic factors on ZON. Krnjaja et al. (2015) showed differences in ZON in one of two years' worth of field trials, with significant difference between the two varieties tested. The current study was the first observational study to model ZON against agronomic factors. Results showed similar trends for both DON and ZON, this might be expected as they are both produced by the same two *Fusarium* species which commonly cause FHB in the UK, namely *F. graminearum* and *F. culmorum*. However, there were differences in the year and region

interaction which probably resulted from differences in the time when DON and ZON are produced; with DON produced from infection onwards and ZON only produced during periods of high moisture in the ripening phase (Kharbikar, et al. 2015). Consequently, wet weather at different times in the wheat growing season (anthesis and ripening) impacts on DON and ZON production differently.

Many studies have shown maize as a previous crop, and particularly in combination with reduced tillage/direct drilling, as a major risk factor for DON contamination (Blandino et al. 2010, Qiu et al. 2016). However, one study has shown this is not always the case (Spolti et al. 2015). Studies in Europe have shown that the risk is greater after grain maize compared to forage maize (Bottalico and Perrone 2002, Eiblmeier and von Gleissenthall 2007, Obst et al. 1997), probably due to the greater amount of crop debris remaining, but may also be in part due to differences in host susceptibility and later harvests. Landschoot et al. (2013) saw no significant difference between DON in plots of wheat after the previous crop of maize was harvested at the same time for either forage or grain maize. This may indicate harvest method is not a factor, however plots were adjacent to one another and there may have been movement of inoculum between plots. Currently, the acreage of grain maize in the UK is very low, limited to the South West, but is predicted to increase with climate change (Kenny and Harrison 1992).

Based on the known importance of crop debris within the *Fusarium* lifecycle one could expect that straw removal for some previous crops could result in a reduction in inoculum, and this would interact with method of cultivation. However, this was not identified as significant within the DON or ZON model. Blandino, et al. (2010) showed that by the manual removal of maize debris from plots they could reduce the DON content of a following wheat crop. In the current study the majority of crop

debris removed was likely to be small grain cereal straw. For this type of debris, the impact on *Fusarium* inoculum maybe minimal.

There was no significant effect of seed treatment identified within the DON and ZON models. This may be because very few wheat samples came from crops with no seed treatment applied and most single purpose dressings have good activity towards *Fusarium*. There is one publication from a limited observational study (n=13) which showed a reduction in FHB when a seed treatment was used (Teich and Hamilton 1985) however other studies have shown no reduction of DON by seed treatments (Birzele et al. 2002, Jørgensen et al. 2012). There was also no significant effect of fungicide sprays identified in either model. As this is observational data, care must be taken as growers may apply a specific FHB recommended product or apply a high rate of specifically because the crop is a high fusarium mycotoxin risk. Many previous experimental studies have shown a significant reduction of DON when triazole fungicides; prothioconazole, metconazole or tebuconazole are applied alone or in combination in field experiments at anthesis (Paul et al. 2008). Blandino et al. (2017) also showed significant reductions for several other Fusarium mycotoxins using prothioconazole but only in one out of three years for ZON. In the Blandino study ZON concentrations were low in all years (<1 $\mu g\ kg^{-1}$) which is likely to have impacted on the ability to detect significant reductions.

Varieties of UK winter wheat are assessed for head blight resistance as part of the AHDB Recommended List trials through the use of inoculated and naturally infected trial sites. Results from this survey for DON and ZON concentrations showed no clear relationship to the FHB resistance scores for the varieties generated from fully

replicated field trials and there was no clear correlation between the mean DON and ZON concentrations for each variety. As this study was an observational study, the dataset was unbalanced with a number of varieties only represented in some years or regions of the survey. For example, KWS Santiago was only present in 2012 and 2013. Also, other varieties such as Xi19 were preferred as a variety for late drilling which in itself may increase mycotoxin risk in some years due to the greater potential for harvest delays or the fact that late drilled crops follow the later harvests of the previous crops maize and sugar beet. The unbalanced nature of the distribution of varieties within the survey and the confounding impact of the favouring some varieties in particular agronomic scenarios may explain the inconsistency in correlation between the varieties reported FHB resistance ratings within replicated field trials and the observed DON and ZON concentrations within this observational study. It should also be noted that varieties in the UK have a limited range of resistance and would all be classed as moderately susceptible compared to wheat varieties worldwide (Gosman, et al. 2007)

One new significant factor identified in the model was harvest timing with a calculation for a field harvest date in respect of a regions six-year average harvest date. The data were categorised into harvest weeks with week 0 been harvested within +/- 3 days of the long-term average and a minus week harvested earlier and a positive week later. This new factor was highly significant for both DON and ZON ($p<0.001$) and accounted for 0.6 and 5.4% additional variance within the models, respectively. The impact of a one month delay in harvest was a 10-fold increase for DON and 25-fold increase for ZON. This observational data fits with recent experimental data that showed that delayed harvests and wet conditions during crop ripening increased both DON and ZON, but have a greater effect on ZON (Kharbikar, et al. 2015). In the experimental study, the importance of rainfall during the ripening phase was shown to

be critical for ZON production. When plots with high levels of FHB and DON were protected from rainfall during the ripening phase then ZON levels remained very low. This can be explained by the timing of production of DON and ZON. DON is produced during infection and can be detected to increase from flowering onwards, whereas ZON remains at low levels until the crop ripens, after this time the level of ZON increases rapidly (Matthaus, et al. 2004). Based on this information, a better prediction of ZON would require an accurate recording or prediction of the start of crop ripening, collection of rainfall data from ripening to harvest and modelling of ZON against the rainfall data. (Pageau et al. 2009) looked at the effect of delayed harvest on DON in barley and found no significant effect in a 4 year study; although a delay of only 1 and 2 weeks was used, where a longer delay may have identified a significant increase. Similarly, Eiblmeier and von Gleissenthall (2007) did not show a significant difference in DON concentration between early and late harvested wheat samples in five out of six years surveyed although the factor was only split into two categories based on if harvested was before or after 60 days from flowering. The duration of the delay is important as seen in this study but there is also a need for conditions conducive for DON and ZON production (i.e. high moisture content) (Kharbikar, et al. 2015), a late harvest without conducive conditions will not increase risk. As such a more accurate predictor of risk would be a measure of high moisture/rainfall during the ripening phase rather than days to harvest. Several studies have shown that delayed harvests can also result in increased DON in maize (Blandino et al. 2009, Lauren et al. 2007).

Results from this study clearly identify the high seasonality of fusarium mycotoxins in English wheat production with a variation in exceedances of legal limits for wheat intended for human consumption of 0-30%. The study also identified the agronomic factors associated with increased risk for both DON and ZON with the large

risk associated with wheat following maize and minimum tillage highlighted. A new factor, not previously quantified in observational models for mycotoxins in wheat is harvest timing with delayed harvests resulting in dramatic increases in DON and ZON. Growers can reduce mycotoxin risk by optimising harvest operations by sowing/harvesting wheat earlier, increasing harvest machinery capacity and harvesting at a higher moisture content followed by post-harvest drying.

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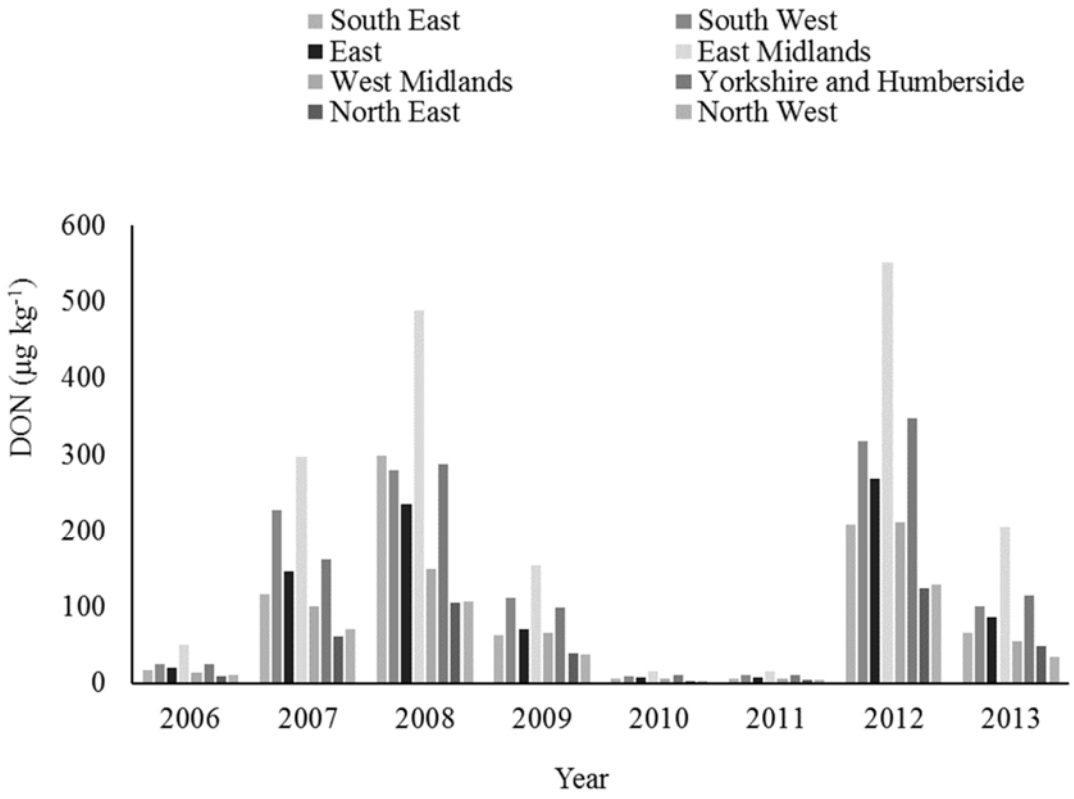
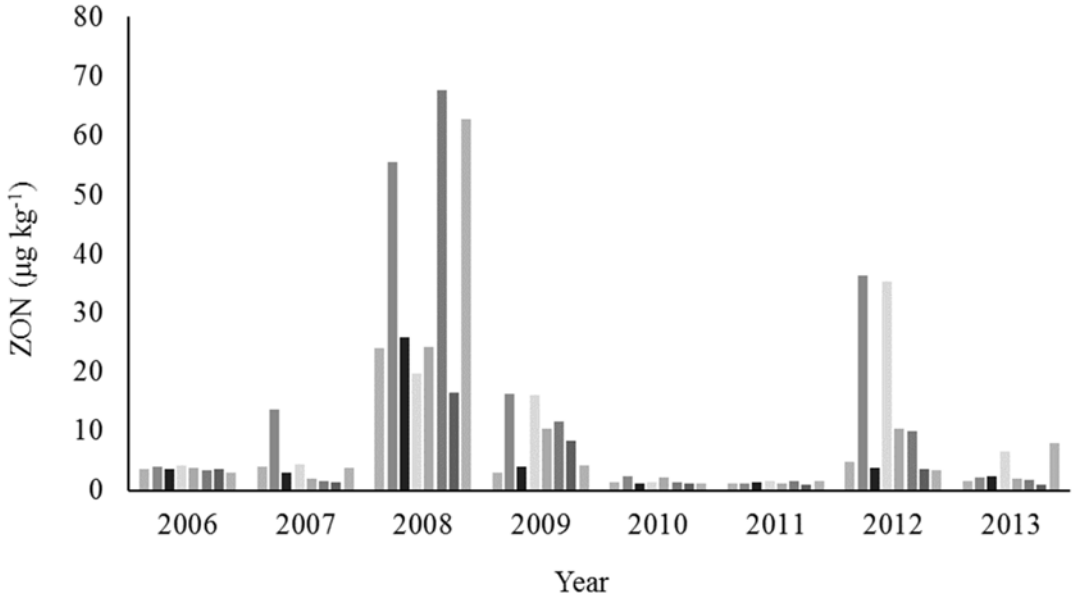
Table 1. Deoxynivalenol and zearalenone summary statistics for English wheat samples analysed between 2006 and 2013.

Year	Number	DON µg kg^{-1}				ZON µg kg^{-1}			
		% >10	Mean*	Median	** % >1250	% >10	Mean*	Median	** % >100
2006	182	77	37	17	0.0	10	2	<2	0.0
2007	152	98	305	140	3.9	18	14	<2	2.6
2008	175	98	584	306	13.1	87	120	47	28.6
2009	152	95	202	77	1.9	41	22	7	5.3
2010	177	41	14	<10	0.0	3	4	<2	0.6
2011	150	27	18	<10	0.0	0	<2	<2	0.0
2012	158	100	615	333	10.1	59	56	16	13.3
2013	130	99	309	95	3.8	15	10	<2	2.3
Average		79	261		4	38	29		7

* For calculation of the mean values, samples below the limit of quantification (LoQ) were allocated a value of half the LoQ (5 µg kg^{-1} for DON and 1 µg kg^{-1} for ZON); ** EU legal limits for unprocessed wheat intended for human consumption are 1250 and 100 µg kg^{-1} for DON and ZON respectively.

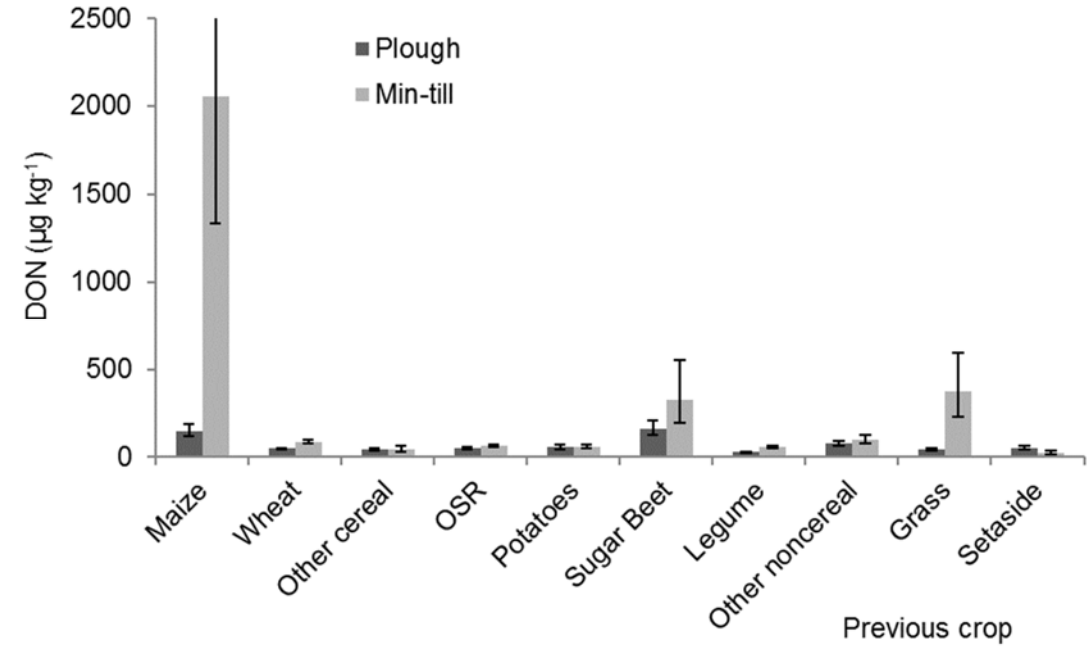


a)

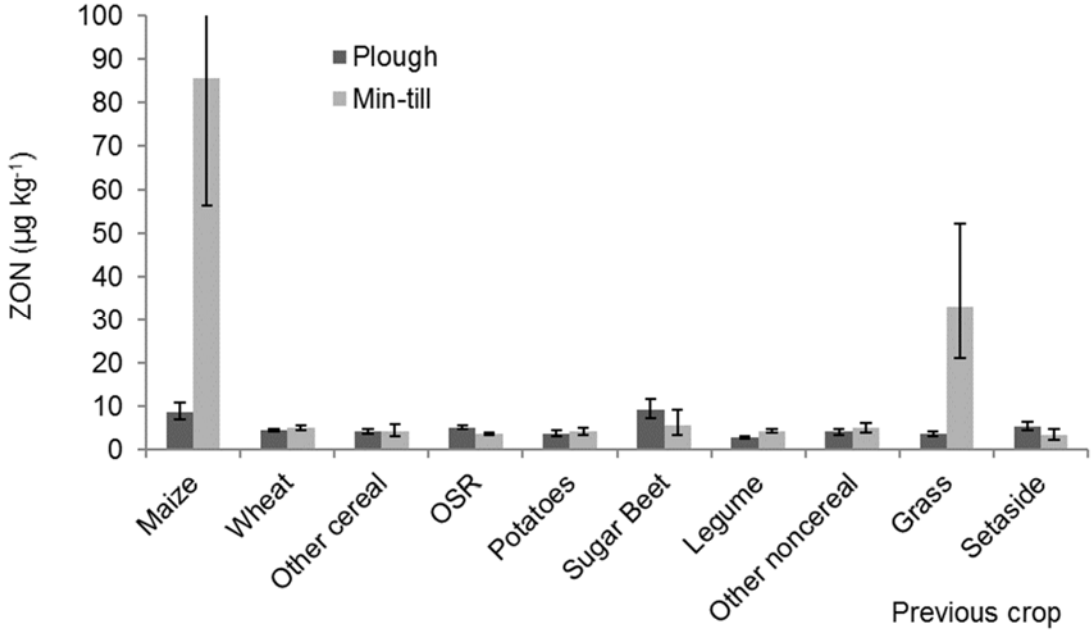


b)

Figure 1. The predicted mean concentration (µg kg^{-1}) of (a) deoxynivalenol (DON) and (b) zearalenone (ZON) in wheat grain by region for each year between 2006 and 2013.

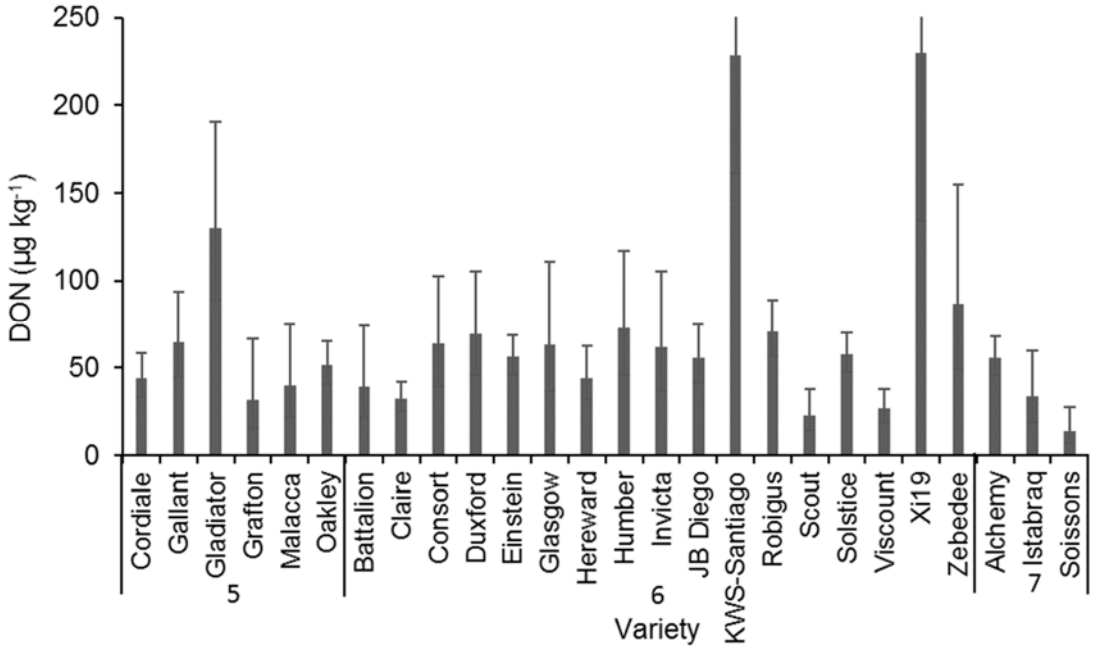


a)

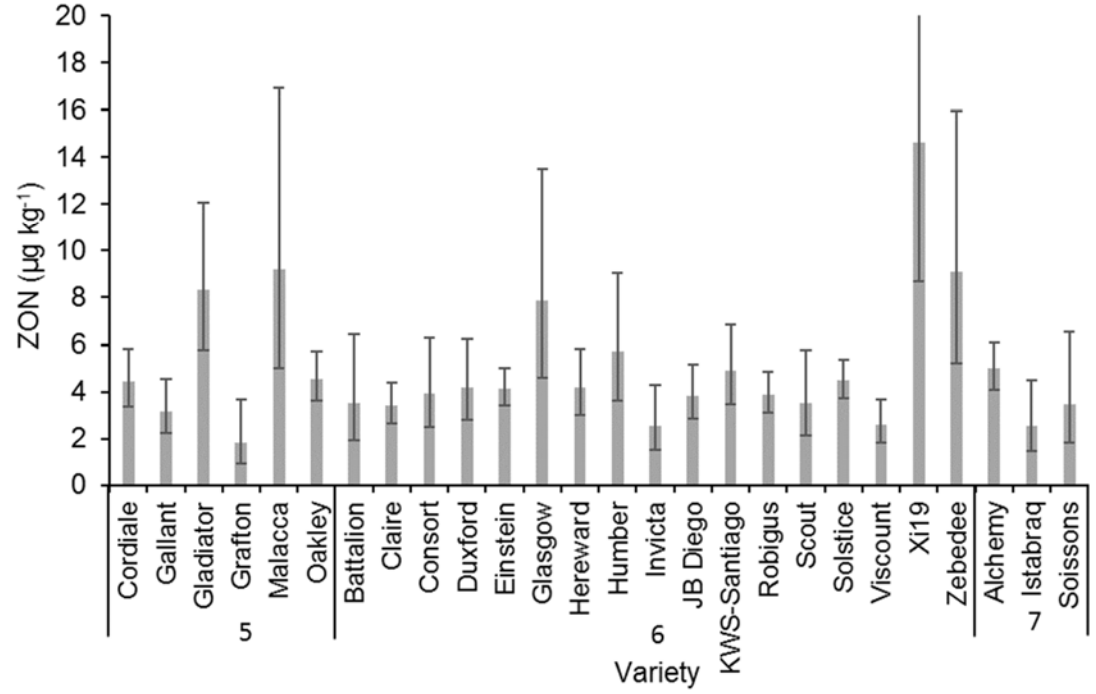


b)

Figure 2. The predicted mean concentration (µg kg^{-1}) of (a) deoxynivalenol (DON) and b) zearalenone (ZON) of wheat for the interaction between previous crop and cultivation. Bars represent 95% confidence limits for predictions.

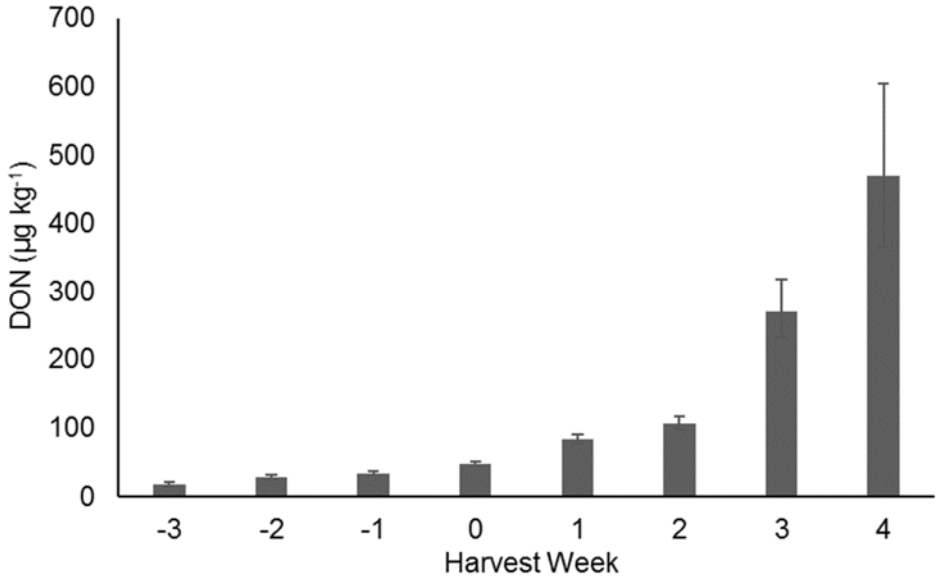


a)

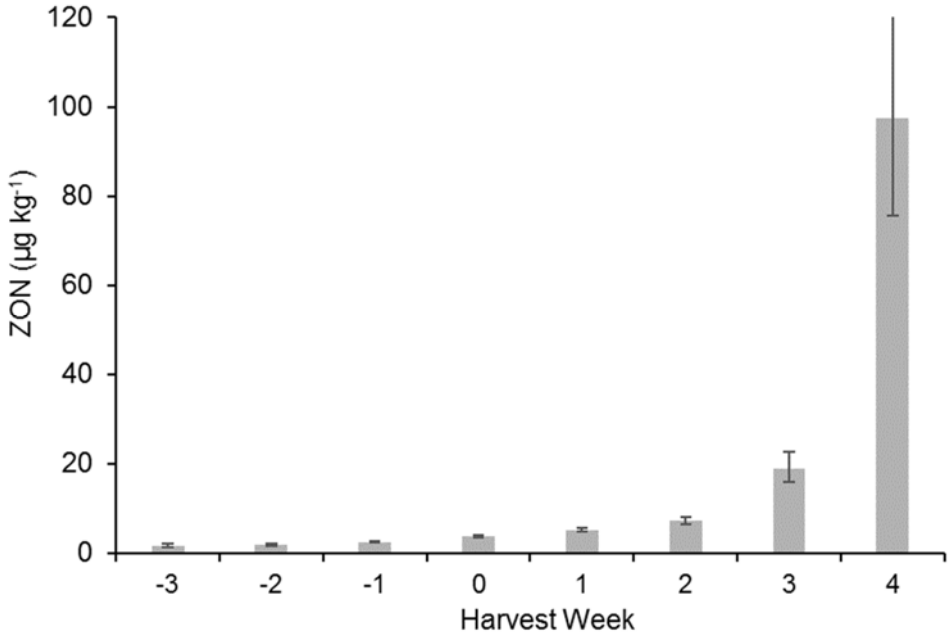


b)

Figure 3. The predicted mean concentration (µg kg^{-1}) of (a) deoxynivalenol (DON) and b) zearalenone (ZON) for wheat varieties grouped by AHDB FHB resistance ratings (1-9; 9=resistant). Bars represent 95% confidence limits for predictions.



a)



b)

Figure 4. The predicted mean concentration (µg kg^{-1}) of (a) deoxynivalenol (DON) and b) zearalenone (ZON) of wheat for each harvest week. Week zero represent the long term county average harvest date +/-3 days. Minus weeks are early harvests, plus weeks are late harvests. Bars represent 95% confidence limits for predictions.