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Improving risk assessment to minimise fusarium mycotoxins in harvested wheat grain

by

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Glossary

ANOVA	analysis of variance
DON	deoxynivalenol
FDG	*Fusarium* damaged grain
FHB	fusarium head blight
FIG	*Fusarium* infected grain
HT2	HT2 toxin
HT2+T2	combined concentration of HT2 and T2 toxins
LC/MS/MS	liquid chromatography with tandem mass spectrometry
LoQ	limit of quantification
No-till	drilling of seed directly into previous crop residue
Min-till	non-inversion cultivation of soil before drilling
NIV	nivalenol
ppb	parts per billion (= micrograms per kilogram, µg/kg)
T2	T2 toxin
ZON	zearalenone

CONTENTS

1. ABSTRACT

European legislative limits for Fusarium mycotoxins, deoxynivalenol (DON) and zearalenone (ZON) were introduced in 2006 for cereals and cereal products for human consumption. Fusarium mycotoxins were monitored in UK wheat from 2006-2008. There was a large variation in levels detected each year. Lowest levels were detected in 2006, which had a dry summer. Highest levels were detected in 2008, which had a wet summer and, in particular, a delayed wet harvest. ZON was particularly high in 2008 with 29% of wheat grain samples at harvest exceeding legal limits. This caused major issues for the cereal processing industry, particularly for the supply of bran for human consumption, as ZON concentrations were highest in this mill fraction.

Modelling the mycotoxin concentrations against the known agronomy of each sample produced similar models for DON and ZON. The majority of the variation between samples was explained by year and region, indicating that weather is the main factor affecting the concentration of these toxins. Previous crop, cultivation and variety were also significant factors within the models. Additional factors of cereal intensity within the rotation and crop debris management were found to be not significant.

The HGCA fusarium mycotoxin risk assessment was validated/amended based on each year's results. The main modification to the risk assessment was the increase in the number of risk categories for rainfall at flowering and pre-harvest, in light of the high mycotoxin levels experienced in 2008.

To improve the predictive ability of the mycotoxin models further would require more precise weather date. The variance accounted for by year and region is largely attributable to differences in climate at specific crop growth stages. The inclusion of weather parameters from pre-flowering to harvest is likely to account for much of this variance. The access to meteorological data, the necessary model development and software development and maintenance will need to be considered by HGCA and other relevant government and industry bodies.

2. SUMMARY

2.1. Introduction

Fusarium mycotoxins are toxic compounds that are produced as a result of the disease fusarium head blight, caused by *Fusarium* species. The most important head blight pathogens are *F. graminearum* and *F. culmorum*, which produce deoxynivalenol (DON) and zearalenone (ZON). The mycotoxins are present in both grain and straw at harvest and are hazardous to human and animal health at high concentrations. European Commission (EC) legislative limits for the fusarium mycotoxins, DON and ZON were introduced in 2006. Guideline limits were also set for animal feed in the same year. Other Fusarium mycotoxins, related to DON include nivalenol (NIV), HT2 toxin and T2 toxin. There is no current legislation for these mycotoxins but limits may be set in the near future.

Based on a previous FSA/HGCA funded five-year project (Project Report No.413), HGCA developed "G34: Guidelines to minimise risk of *Fusarium* mycotoxins in cereals" which included a "Fusarium mycotoxin risk assessment". For the current risk assessment, see www.hgca.com/mycotoxins.

The aims of this project were:

1) To monitor fusarium mycotoxins in UK wheat over the first three years (2006-2008) after the introduction of legislative limits.
2) To determine the impact of additional agronomic factors, such as cereal intensity within rotations and crop debris management, on the fusarium mycotoxin contamination of UK wheat.
3) To improve the HGCA fusarium mycotoxin risk assessment by inclusion of additional agronomy factors and by inclusion of weather parameters.

2.2. Materials and methods

Each year ca. 300 samples of wheat were collected at harvest from fields of known agronomy. Samples were either collected by growers involved in the Defra-funded winter wheat disease survey as part of CropMonitor (www.cropmonitor.co.uk) or by crop consultants (AICC, Agrovista, DARD and Scottish Agronomy). These additional samples allowed selected low frequency/high risk (eg crops following maize) samples to be collected and a wider geographic range (ie samples from Scotland and Northern Ireland). Samples were milled and then analysed for fusarium mycotoxins. In 2006, DON and ZON were analysed by ELISA, whereas in 2007 and 2008, DON, ZON and another eight trichothecenes (relatives of DON) were analysed by liquid chromatography with tandem mass spectrometry (LC/MS/MS).

Summary statistics (percentage incidence and percentage above legal limits for cereals intended for human consumption, mean and median) of mycotoxin concentrations were produced and reported on the HGCA website (www.hgca.com/mycotoxins). Concentrations of fusarium mycotoxins were modelled against the agronomy factors to identify the importance of various agronomic factors. A sub-set of samples was used each year to validate/modify the fusarium mycotoxin risk assessment.

2.3. Results

Of the ten mycotoxins analysed from field samples of wheat only five were detected; of these only three, DON, NIV and ZON were detected above 100 ppb. DON was the most frequently detected fusarium mycotoxin, present in 91% of samples, and was usually present at the highest concentration.

Incidence and concentrations of NIV, HT2 and T2 were consistently low in UK wheat and are unlikely to be of concern if legislative limits are introduced for these mycotoxins.

The concentrations of DON and ZON were low in 2006, moderate in 2007, and very high in 2008. This indicates the very large seasonal differences as a consequence of weather at key timings having a major impact on these mycotoxins. ZON was particularly high in 2008 and this appears to be a result of the wet August and September resulting in long delays at harvest.

The concentrations of DON and ZON were modelled against agronomic practices applied to each field. Year, region, previous crop, cultivation and variety all had statistically significant effects on DON and ZON concentration. The models for the two were quite similar. There was a significant interaction between year and region, which was probably due seasonal fluctuations in weather at specific crop growth stages. Highest concentrations were found in the south and east of England;

lowest concentrations occurred in Scotland. Year and region accounted for the vast majority of the variance within the model (ie they were the most important factors identified). This confirmed that seasonal differences in weather are critical to fusarium mycotoxin contamination.

There was also a significant interaction between previous crop and cultivation. This is probably due to the importance of crop debris in the epidemiology of head blight. Highest predicted DON concentration occurred in wheat following maize, which is a known alternate host for *Fusarium* species. Ploughing generally reduced DON concentration; this reduction was greatest for crops following maize then for crops following wheat. Other recent studies in France and Germany have shown that the risk is greater after grain maize compared to forage maize, probably due to the greater amount of crop debris remaining. The acreage of grain maize in the UK is currently very low but it is predicted to increase in the future.

Varieties of UK winter wheat are assessed for head blight resistance as part of the HGCA Recommended List trials (1-9, 9 = resistant). Results showed that varietal resistance score for head blight was not a significant factor although a consistent trend for both DON and ZON between head blight resistance scores four to seven existed; varieties with a higher resistance rating had a lower predicted DON and ZON concentration. When individual varieties were included in the analysis, some varieties had mean mycotoxin values which did not correlate with the varieties head blight resistance rating. This maybe an indication of a difference in resistance observed in Recommended List trials compared to under natural infection in commercial crops, or it may be a result of specific varieties being grown under different fusarium risk situations. Comparison of varieties over several seasons/locations is required to obtain an accurate assessment of differences in varietal resistance because varieties vary in flowering time and weather conditions at flowering are critical for *Fusarium* infection and subsequent mycotoxin contamination. The varieties on the current UK Recommended List have a limited range of resistance and would be classed as moderately susceptible compared to wheat varieties worldwide.

There was no significant difference in the DON or ZON content of wheat crops which received different fungicide regimes. Seed treatment was analysed based on product used. There was no difference identified although 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*.

T3 treatment (fungicide application at flowering, GS59 – 61) was analysed based on:

- Application of a triazole
- Application of a FHB recommended product
- Rate of application of a FHB recommended product

None of the above factors were significant. As these are observational data care must be taken as growers may apply a specific FHB recommended product, or a higher rate of such products specifically because the crop has a high Fusarium mycotoxin risk. Results from field experiments have consistently shown that application of specific fungicides at flowering reduces DON, although efficiency is variable.

Two additional factors specific to end-use, the growers' intended market, or the market specification for the wheat variety grown, were tested for significance. These factors had no statistically significant effect ($p>0.05$) indicating that they did not have an impact on DON concentration at harvest. This would indicate that apart from the factors in the model, no other agronomy that is specific to growing milling wheats has a significant impact on the mycotoxin content of grain grown specifically for human consumption.

Two additional factors pertaining to maize within the rotation or adjacent to the wheat crop were tested for significance. Neither of these factors were significant ($p>0.05$) indicating that the presence of maize in a wheat rotation other than as the previous crop does not increase the DON concentration significantly and that a maize crop located adjacent to a wheat crop does not significantly increase the DON content of the wheat crop, at the field scale. It should, however, be considered that an adjacent maize crop is likely to have an impact on wheat grown within a few metres of the maize. This would be particularly true for game cover crops which are in continuous maize, allowing a build-up of *Fusarium* inoculum.

Crop debris management, ie whether straw was baled and removed or incorporated had no significant effect on DON in the subsequent wheat crop. This was the case even when analysed as an interaction with previous crop and cultivation. 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 model.

2.3.1. Fusarium mycotoxin risk assessment

Each year the fusarium mycotoxin risk assessment was validated/modified based on results from this project. The original risk assessment was published in the “Guidelines to minimise risk of Fusarium mycotoxins”. In 2006, a low risk year, the model performed well and was not modified. In the spring of 2008, a series of HGCA/ACCS mycotoxin and storage workshops were held across the UK, attended by about 500 growers and agronomists. From these workshops a number of issues were identified regarding the need for clearer descriptions for some of the risk factors and the absence of some beneficial risk factors within the assessment, namely the lack of gradation within tillage (0 or 4) and lack of a negative score for T3 fungicide inputs. The risk assessment was modified accordingly in 2008 (Topic Sheet 102) and this reduced.the number of samples with a high score without increasing the number of false negatives (ie samples with low/moderate score but high mycotoxin concentration).

After the 2008 harvest the risk assessment was considered to have failed because an unacceptable number of samples with a low risk score on grain passports exceeded legal limits of DON at mill intake. This was thought to be for two reasons. Firstly, the risk assessment was developed based on conditions experienced from 2001 to 2007, and during this period the UK did not experience such a long extended wet harvest as in 2008. Secondly, some risk assessments may not have been completed correctly. As a larger proportion of samples had exceeded the legal limit for ZON than DON in 2008, the ability of the risk assessment to identify samples at risk from ZON was also assessed.

Increasing the pre-harvest rainfall scores allowed the scheme to account for the importance of pre-harvest rainfall. The false negatives were reduced to zero for DON and 8% for ZON at the legal limit of 1250 ppb DON or 100 ppb ZON. There was a corresponding increase in the number of false positives with 36% for DON. The consequence of this is that 50% of grain consignments would need to be tested in a high risk year to detect 14% of consignments exceeding the DON limit. In high risk years it would, therefore, be advisable for processors with low intake limits to test all consignments.

Although prolonged heavy rainfall was not experienced during flowering during 2001-2008, mist-irrigated trial experiments identified that continued rainfall during flowering will increase the risk of exceeding fusarium mycotoxin legal limits in harvested grain to a similar extent as pre-harvest rainfall. For this reason, additional risk scores were added to the flowering rainfall risk category. This had little implication on the re-validation of the risk assessment but will hopefully protect against the risk assessment scheme failing in future years due to high rainfall occurring during flowering. The current version of the risk assessment was first published in 2009 in Topic Sheet 104 and again in 2011 in Topic Sheet 108.

2.4. Discussion

Overall, the mycotoxin profile of UK wheat in 2006-2008 was similar to previous years as detailed in HGCA Project Report No. 413 (Edwards, 2007). There was greater variability between years due to the large difference in summer weather experienced in 2006 (dry) and 2008 (wet). The mycotoxin risk was also observed to spread northwards, with samples exceeding legal limits in the north east of England. The spread northwards has also been observed for *F. graminearum* incidence in the CropMonitor survey of FHB in winter wheat (Jennings & Humphries, 2009). The delayed wet harvest in 2008 resulted in near equivalent risk across England, probably because the delay in harvest was greater in the north.

ZON exceeded legal limits in more samples than DON in 2008. This is thought to have occurred because ZON is produced later in the season as the crop ripens (Matthaus et al., 2004), and consequently delayed harvests have a greater impact on ZON levels. Flour mills had fewer issues with ZON compared to DON and this can be explained by the negative correlation between ZON concentration and milling quality specifications (specific weight and Hagberg Falling Number), so samples with high ZON concentration routinely failed milling quality specifications.

Both DON and ZON are produced in higher concentrations in the outer layers of the grain, resulting in higher concentrations in the bran fractions. DON is highly water-soluble, whereas ZON is much less soluble in water. Limited data suggest that some DON can be removed from the outer layers of grain during wet harvests, whereas ZON remains in place. It was difficult for manufacturers of high fibre breakfast cereals to source bran that would allow production of products within legislative limits for ZON so the European Breakfast Cereal Association (CEEREAL) requested derogation limits for ZON in breakfast cereals of 135 ppb. CEEREAL considered that this would avoid major disruption to bran-based breakfast cereals production without compromising consumer health. The UK Food Standards Agency conducted a UK Risk Assessment of the CEEREAL request and recommended a more precautionary derogation limit of 100 ppb for high fibre breakfast cereals. This limit was agreed by the EC Standing Committee on the Food Chain and Animal Health (Anon, 2009) on 19 June 2009 and expired 31 October 2009, although there is scope for the limit to be made permanent subject to a review of the European Food Safety Authority scientific opinion on the associated consumer health risks which was published in July 2011 (Anon, 2011).

HT2 and T2 mycotoxin legislation is still under review and likely to be considered in 2012. For wheat, levels appear to have dropped in recent years, and are well below any likely limits.

The concentrations of DON and ZON were modelled against agronomic practices applied to each field. The models were similar and the vast amount of variance was accounted for by year and region, indicating that weather was a key factor in the determination of fusarium mycotoxin levels on UK wheat. Previous crop, cultivation, and variety were also significant factors.

The HGCA fusarium mycotoxin risk assessment was modified and validated each year of the project. The main change incorporated was the increased range of scores for flowering and pre-harvest rainfall. The increased weighting of rainfall events on the risk assessment increases the risk of inaccurate completion of the assessment because:

1) Growers rarely record growth stages of wheat after the last fungicide is applied at flowering (GS59-69).

2) Growers may not have access to farm weather data

It is, therefore, advised that the fusarium mycotoxin risk assessment is heavily promoted by the industry, and growers are reminded in a timely fashion of the need to monitor rainfall at flowering and pre-harvest.

To improve the predictive ability of the DON model further would require more precise weather data. The variance accounted for by year and year*region interaction, is largely attributable to differences in weather and would be unknown in a predictive model. The inclusion of weather parameters from pre-flowering to harvest is likely to account for much of this variance. There is a need to incorporate accurate weather parameters into the model to improve the predictive ability of the model. The ideal scenario would be if national weather data was collected and used within the risk assessment model. Growers would enter a geographical reference, drilling date, harvest date and variety. The model would then predict flowering and pre-harvest dates and input relevant rainfall values. The access to meteorological data, the necessary model development and software development and maintenance will need to be considered by HGCA and other relevant government and industry bodies.

3. TECHNICAL DETAIL

3.1. Introduction

3.1.1. Fusarium head blight

Fusarium head blight (FHB) of UK cereals may be caused by several fungal pathogens. The predominant species are *Fusarium graminearum, F. culmorum, F. poae, F. avenaceum, Microdochium nivale* and *M. majus*. The disease is also referred to as fusarium ear blight or scab. Some of the fungi that cause FHB produce fusarium mycotoxins while others do not. Fusarium head blight can be detected in crops around the milky ripe stage (Growth Stage 75) as premature ripening (bleaching) of individual spikelets. Orange/pink spores of *Fusarium* may be seen on infected spikelets. Infection can result in bleaching of the head above the point of infection. As the whole crop ripens the symptoms are less visible. At harvest, fusarium head blight can result in fusarium-damaged grains that may be shrivelled with a chalky white or pink appearance. The presence of fusarium-damaged grains is an indication that fusarium mycotoxins may be present.

Fusarium species can be readily isolated from seed, stem bases, soil, weeds and insects, although the main source of inoculum is crop debris. The ideal conditions for *Fusarium* infection are heavy rainfall, which splashes spores from the crop debris up onto the cereal head, and warm, humid weather to allow the *Fusarium* spores to germinate and infect the cereal head. Cereal crops are most susceptible to FHB infection during flowering (Growth Stage 61-69). Further rainfall and humid conditions in late summer as the crop ripens allow saprophytic growth of *Fusarium* on the standing crop.

Most *Fusarium* species are facultative plant pathogens; they are capable of living on dead organic material in the soil but can switch to a pathogenic mode of existence when suitable host plants appear (Parry et al., 1995). Several species, including *F. culmorum* and *F. graminearum*, can cause fusarium seedling blight, brown foot rot and fusarium head blight (FHB). FHB infection may be due to inoculum present in the soil, on crop debris or be seed-borne.

There is strong evidence that rain is important in the dispersal of *F. culmorum* and *F. graminearum*. For *F. culmorum*, macroconidia which are produced at ground level are splashed onto the wheat heads during rainfall (Jenkinson & Parry, 1994; Horberg, 2002). This may occur in a stepwise manner, from leaf to leaf, and finally onto the head. It was noted that during epidemic years in Idaho in 1982 and 1984, when *F. culmorum* was the dominant FHB pathogen, sprinkler-irrigated fields had severe FHB whereas surface-irrigated fields had little or no FHB (Mihuta-Grimm & Forster, 1989). For *F. graminearum*, ascospores are produced at ground level and are released throughout the day, spore release peaks late evening and is highest 1-3 days after rainfall events (>5 mm) (Fernando et al., 2000; Inch et al., 2005). Rainfall events also result in splash dispersal of

F. graminearum ascospores and macroconidia (Paul et al., 2004). An observational study of wheat fields in Washington State showed that FHB was much more prevalent in fields with irrigation compared to fields with no irrigation (Strausbaugh & Maloy, 1986).

Wheat is most susceptible to FHB during flowering (Obst *et al.*, 1997; Lacey *et al.*, 1999) with symptoms developing two to four weeks later. Flowering in the UK occurs from early June in the south of England to mid-July in the north of Scotland. Flowering time varies with drilling date, weather and variety. Flowering duration varies with weather and variety. FHB is assessed in the field after flowering, usually one to four weeks post-flowering and is based on the number of heads with blight symptoms (incidence) or the number of spikelets with blight symptoms (severity). The two measurements are closely correlated (Xu et al., 2004).

At harvest, grains can be visually assessed for *Fusarium*-damaged grain (FDG) or infection can be measured by culturing the *Fusarium* from grain on blotting paper or microbiological media to determine *Fusarium*-infected grain (FIG).

Many studies have been directed at the control of FHB and have not assessed mycotoxin concentration. In most countries where these studies have been performed, *F. graminearum* is the predominant FHB pathogen, and as this is the most potent deoxynivalenol (DON) producing species, there is a reasonable relationship between FHB severity, %FDG or %FIG and DON concentration. It is however important to note that in the UK, *Microdochium* species can be the predominant FHB pathogen and these species do not result in FDG or FIG or any known mycotoxin. For UK data it is therefore advisable not to assume that a measurement of FHB is closely related to DON concentration at harvest (Edwards et al., 2001). A similar situation has been reported in France (Champeil et al., 2004).

3.1.2. Fusarium mycotoxins

The trichothecene mycotoxins are produced by some of the Fusarium head blight pathogens and their levels within grain depend on weather conditions. High humidity during and after flowering is conducive to head blight epidemics and mycotoxin production. 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. *F. poae* has also been linked to high levels of NIV. HT2 and T2 are Type A trichothecenes, which are thought to be produced predominantly by *F. sporotrichioides* and *F. langsethiae*.

Surveys of cereal products have indicated that fusarium mycotoxins are a common contaminant of human and animal diets. They frequently occur at low concentrations. DON causes reduced feed intake, reduced weight gain and vomiting in farm animals (Anon, 2004a). Nausea, vomiting, diarrhoea, abdominal pain, headache, dizziness and fever have been reported when high concentrations of DON were consumed by humans (Anon, 1999). Other trichothecenes have the same cellular activity which is disruption of protein synthesis, and have a higher cellular toxicity than DON. Nivalenol and T2 are ca. 20 times more toxic than DON, although the relative differences are dependent on the target cell or animal studied (Desjardins, 2006). HT2 and T2 were implicated in Alimentary Toxic Aluekia caused by the consumption of cereals which had overwintered in fields in Russia in the 1940s (Desjardins, 2006).

Although DON is considered the predominant trichothecene mycotoxin within grain, some of the other trichothecenes have greater toxicity, so it is important that they are also monitored. Of the other trichothecenes, the only other ones currently being considered for legislation are HT2 and T2 toxins, which have a proposed combined maximum level of 100 ppb for unprocessed wheat grains.

Zearalenone is another mycotoxin produced predominantly by *F. culmorum* and *F. graminearum*. Zearalenone function in the fungus is not known and is predominantly produced late in the crop growing season, near to harvest (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 (Anon, 2004b). There are no proven cases of human exposure but the mycotoxin has been implicated in cases of premature puberty in girls (Anon, 2000).

3.1.3. Fusarium mycotoxin legislation

The European Commission (EC) set legislative limits for the fusarium mycotoxins including deoxynivalenol (DON) and zearalenone (ZON) in cereal grains and cereal-based products intended for human consumption in 2006 (Table 1) (Anon, 2006b). Limits are set in parts per billion (ppb = μg/kg = ng/g).

Any mycotoxin analysis to determine compliance with legislation must comply with pre–defined performance criteria and reported results include the known margin of error of the analytical method. This is different for each laboratory and is reported as the expanded measurement of uncertainty (2MU) and approximates to 95% confidence limits. The expanded measurement of uncertainty is typically around 20%. For a laboratory with 20% expanded measurement of uncertainty the legislative limit for ZON in unprocessed wheat would be 100+20% = 120 ppb.

Table 1. Maximum limits for deoxynivalenol (DON) and zearalenone (ZON) in unprocessed wheat and finished products intended for human consumption

Product	Mycotoxin (ppb)	
	DON	ZON
Unprocessed wheat	1250	100
Wheat flour and bran	750	75
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	50
Processed wheat-based food for infants and young children and baby food	200	20

The maximum levels set for unprocessed cereals apply to cereals placed on the market for processing. Cereal grains may have been cleaned, dried and/or sorted prior to being placed on the market; these grains are still classified as unprocessed cereals. 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.

Processing can reduce the mycotoxin content of some cereal products; limits for processed products are therefore lower. However, for some products with high wholegrain content there is little reduction during processing and products high in bran can have a higher mycotoxin content than the original unprocessed wheat. Processors may specify their own limits for unprocessed grain due to the limited ability of their process to reduce the mycotoxin content of certain products.

The European Commission also set guideline limits in 2006 for fusarium mycotoxins in animal feed (Anon, 2006a). 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 ppb. The zearalenone guidance value for complementary and complete feedingstuffs for sows and fattening pigs is 250 ppb and for piglets and gilts is 100 ppb.

In 2010, European Commission asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the risks for animal and public health related to the presence of NIV, T-2 and HT-2 toxins in food and feed. The opinion should consider any new results of toxicological studies, deliver an updated dietary exposure assessment, and determine the daily exposure levels of the different animal species. The European Commission also asked EFSA to provide a scientific opinion on the effects on consumer health risk of a possible increase of the maximum level for zearalenone in breakfast cereals. The assessment should be performed taking into account the exposure to zearalenone from other food sources and therefore data on zearalenone occurrence in food in general have to be collected. EFSA will provide opinions in early 2011 and it is expected that the European Commission will consider legislative limits for these mycotoxins later in 2011.

3.1.4. Effects of agronomic factors

Previous research, primarily in North America and elsewhere in Europe has identified a number of agronomic factors which can affect the concentration of fusarium mycotoxins in harvested cereals. These factors were analysed in a previous FSA/HGCA study during 2001-2005 (Edwards, 2007); results from that study are summarised below.

Year, region, previous crop, cultivation, variety and fungicide application all had statistically significant effects on DON concentration. Statistical tests of the predictive quality of the model indicated it may be a good predictor of new observations. There was a significant interaction between year and region, which is probably due to fluctuation in weather between years and regions. Highest concentrations were found in the south and east of England; lowest concentrations occurred in Scotland. There was also a significant interaction between previous crop and cultivation. This is probably due to the importance of crop debris in the epidemiology of ear blight. Highest predicted DON concentration occurred in wheat following maize, which is a known alternate host for *Fusarium* species. Ploughing generally reduced DON concentration; this reduction was greatest following maize, wheat and potatoes. Other recent studies in France and Germany have shown that the risk is greater after grain maize compared to forage maize, probably due to the greater amount of crop debris remaining. At the moment, the acreage of grain maize in the UK is very low but it may increase in the future.

Varieties of UK winter wheat are assessed for ear blight resistance as part of the HGCA Recommended List trials. Results showed that varieties with a higher resistance had a lower predicted DON concentration. However, varieties in the current UK Recommended List have a limited range of resistance and would be classed as moderately susceptible compared to wheat varieties worldwide (Gosman et al., 2007).

There was no significant difference in the predicted DON concentration between organic and conventional samples. Within conventional samples, those which received an azole fungicide ear spray (T3 timing) had significantly lower DON than those which received no ear spray.

The effect of agronomy on zearalenone is likely to be similar to that for DON; however, owing to the low incidence of zearalenone from 2001-2005 this could not be analysed with the same statistical robustness. One difference that was identified was the significantly higher zearalenone concentration in samples of spring wheat compared to winter wheat. This may be because spring wheat ripens slightly later in the season and zearalenone is known to be produced once the crop ripens, and therefore conditions may be more conducive to zearalenone production later in the summer.

The effect of agronomy on HT2 and T2 appeared to be different to that for DON and zearalenone. This is understandable as HT2 and T2 are produced by different *Fusarium* species than those which produce DON and zearalenone. One important difference was that high levels of HT2 and T2 occurred all over the UK with no decline towards the north, indicating that temperature is not a critical factor in HT2 and T2 production in the UK.

HGCA published “G34: Guidelines to minimise risk of fusarium mycotoxins in cereals” (Anon, 2010b) which included a “Fusarium mycotoxin risk assessment” (Anon, 2010a) based on the results of the previous study (Edwards, 2007). The risk assessment was integrated into cereal crop assurance schemes and results are required on grain passports. Results from the previous project identified the importance of weather for DON and ZON contamination of wheat, as indicated by the seasonal and regional variation. The regional variation, in combination with what is known of the lifecycle of *Fusarium* species indicated that other factors, such as cereal intensity within rotations and crop debris management, may also be important agronomic factors for DON and ZON contamination of cereals.

3.1.5. Aims and objectives

To determine the range of trichothecene and ZON contamination within harvested UK wheat grain over a three-year period (2006 – 2008).

To determine the impact of additional agronomic factors, such as cereal intensity within rotations and crop debris management, on the fusarium mycotoxin contamination of UK wheat.

To improve the HGCA fusarium mycotoxin risk assessment by inclusion of new factors and by inclusion of weather parameters.

3.2. Materials and methods

3.2.1. Grain sample collection

Each year 300 grain samples of winter wheat and related agronomic data were requested by the Food and Environment Research Agency (Fera). These samples were collected by growers involved in the Defra-funded winter wheat disease survey as part of CropMonitor (www.cropmonitor.co.uk). The winter wheat disease survey is a stratified survey of wheat within England. As such, mycotoxin results generated provide an accurate assessment of fusarium mycotoxins in England. Each year, a further 200 grain samples of winter wheat and related agronomic data were requested from crop consultants (AICC, Agrovista, DARD and Scottish Agronomy). These additional samples allowed selected low frequency/high risk samples to be collected (eg crops following maize) and a wider geographic range (ie from Scotland and Northern Ireland). The target number of samples for each year was 300 in total.

Samples were collected at harvest from specific fields either from the combine or from trailers leaving the field. Approximately 300 g sub-samples were taken from ten arbitrary points around the field and combined to provide a 3 kg sample. Growers/consultants sent these samples in cotton bags by overnight courier, along with agronomic data pertaining to that field sample.

Agronomy details requested were:
Location; Variety; Intended end use; Previous crops for last 4 years; Crop debris management; Cultivation technique; Drilling date; Maize in the rotation; Maize next to this crop; Seed treatment; Fungicides use

On receipt of samples their moisture content was determined. Any samples with a moisture content greater than 18% were dried overnight on a heated-air dryer, the moisture content was re-assessed the next day and then processed. A 500 g sub-sample of grain was removed using a ripple divider, dried to 12% moisture content and stored at room temperature as a grain archive. The remaining sample was milled with a 1 mm screen and mixed in a tumbler mixer before two 300 g sub-samples were collected. One sample was used for mycotoxin analysis, the remaining sample was held at Harper Adams University College as an archive sample at –20°C.

3.2.2. Mycotoxin analysis of grain samples

In year one (2006), all samples were analysed for deoxynivalenol (DON) and zearalenone (ZON) using Ridascreen ELISA assays (R-biopharm Rhone). The quantification limit for DON was 10 ppb and a recovery rate of 85-110%. The detection limit for ZON was 2 ppb and a recovery rate of approx. 80%. Concentrations determined were not adjusted for recovery.

In the subsequent two years (2007 and 2008) all samples were analysed by Campden BRI using UKAS accredited procedures. The trichothecenes (DON, nivalenol (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 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. The Limit of Quantification (LoQ) for the trichothecenes was 10 ppb and for ZON was 2 ppb.

3.2.3. Statistical analysis

For summary statistics, samples with a mycotoxin content below the limit of quantification (LoQ) were assigned a value of (LoQ)/2 for calculation of mean values. Summary statistics (percentage greater than 10 ppb, mean, median, 90^{th} percentile, 95^{th} percentile and maximum) were calculated using Excel (Microsoft v.2007). All other statistical analysis was completed using Genstat (Lawes Agricultural Trust, v12) unless stated otherwise.

Statistical analysis to determine agronomic factors on the fusarium mycotoxin concentration of wheat was performed using a stepwise selection ANOVA. For modelling the mycotoxin concentration of samples, samples with a mycotoxin concentration below the LoQ were assigned a value of (LoQ)/2 and $\log_{10}$ transformed and analysed using a normal distribution.

3.3. Results

3.3.1. Summary of samples received

Total number of samples was 8% greater than the target number of 900 (Table 2). The low response rate in 2007 was probably a result of the very low level of fusarium mycotoxins reported in 2006.

Table 2. Number of samples received compared to target.

	2006	2007	2008	Total	Target
CropMonitor	182	152	176	510	450
Consultants	148	147	169	464	450
Total	330	299	345	974	900

Of the total samples received, 956 samples had all the required agronomic information supplied (98%). Numbers of samples collected from Scotland and Northern Ireland were lower than for other regions, this was due to the smaller areas of wheat in these regions and possibly due to the lower concern for fusarium head blight and mycotoxins in those countries (Table 3). Northern Ireland may have a lower concern as nearly all wheat is grown for animal feed. Scotland may have a lower concern as the colder climate results in less head blight occurring (Xu et al., 2007).

Table 3. Sample distribution by year and region.

Year	Region						Total
	South	East	Midlands	North	Scotland	N.Ireland	
2006	68	105	72	59	5	6	315
2007	55	74	67	62	9	29	296
2008	63	89	85	73	11	24	345
Total	186	268	224	194	25	59	956

3.3.2. Summary statistics

Of the ten mycotoxins analysed only five were detected, of these only three, DON, NIV and ZON, were detected above 100 ppb. Tables 4 to 6 below show the percentage above 10 ppb (the limit of quantification for trichothecenes), the mean, median, the 90th percentile, the 95th percentile and the maximum concentration for each mycotoxin detected in CropMonitor samples each year. Only CropMonitor samples were used to calculate summary statistics as these samples are collected as part of a Defra-funded stratified survey of wheat within England (www.cropmonitor.co.uk). As a consequence, summary statistics for these samples are an accurate measure of fusarium mycotoxins in English wheat. Data on samples from Scotland and Northern Ireland and regional differences within England are highlighted in Section 3.4.

Table 4. Fusarium mycotoxin summary statistics for English wheat in 2006 (n=182)

	%>10ppb	Mean	Median	90th%	95th%	Max
DON	77	37	17	92	155	597
ZON	10	2	<5	<5	<5	89

Samples below limit of quantification (LoQ) allocated value of half LoQ (5 ppb for DON and 1 ppb for ZON).

Table 5. Fusarium mycotoxin summary statistics for English wheat in 2007 (n=152)

	%>10ppb	Mean	Median	90th%	95th%	Max
DON	98	305	140	723	1089	4450
NIV	33	12	<10	23	35	179
ZON	18	14	<2	26	81	446

Samples below limit of quantification (LoQ) allocated value of half LoQ (5 ppb for DON and 1 ppb for ZON). No other trichothecenes were detected in 2007 except one sample containing HT2 and T2 (HT2+T2 = 35ppb).

Table 6. Fusarium mycotoxin summary statistics for English wheat in 2008 (n=176)

	%>10ppb	Mean	Median	90th%	95th%	Max
DON	98	584	306	1310	1911	6940
NIV	59	15	11	29	44	189
ZON	87	120	47	260	403	1754

Samples below limit of quantification (LoQ) allocated value of half LoQ (5 ppb for DON and 1 ppb for ZON). No other trichothecenes were detected in 2008 except for 3-AcDON in 2 samples (1%, max = 47) and 15-AcDON in 1 sample (0.05%, 19 ppb). Both samples with AcDON present had DON concentrations greater than 2000 ppb.

Deoxynivalenol was the most frequently detected fusarium mycotoxin and was usually present at the highest concentration. The distribution was skewed as can be seen by the large difference between the mean and median values and the frequency distribution graph (Figure 1). Nivalenol was not analysed for in 2006 but was frequently detected in 2007 and 2008 although it was never detected at a high concentration (maximum = 189 ppb). ZON was quantified above 10 ppb in 10% of samples in 2006 but this increased dramatically to 87% of samples in 2008. Due to the lower legal limits for this mycotoxin, more samples exceeded the legal limit for ZON than for DON (Tables 7 and 8). As for DON, the ZON distribution was also skewed (Figure 2).

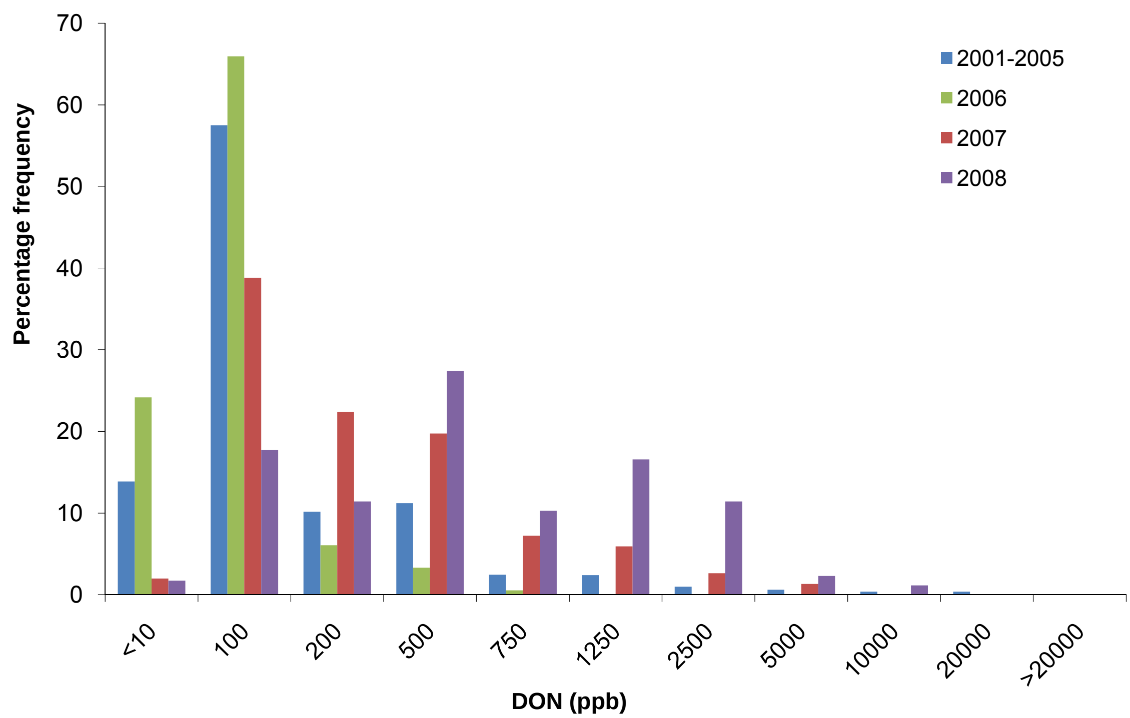


Figure 1. Distribution of DON concentration for each year. Includes previous five-year average for comparison.

Table 7. Summary statistics for DON for 2001 to 2008. Includes previous five-year dataset for comparison.

	2001	2002	2003	2004	2005	2006	2007	2008
Number	343	343	328	344	326	182	152	175
Incidence, %	80	78	89	92	92	77	99	98
Mean	80	117	218	469	242	41	305	584
Median	32	30	38	65	65	17	140	306
>1250ppb, %	0.4	1.7	2.4	5.5	1.5	0.0	3.9	13.1

Samples below limit of quantification (LoQ) allocated value of 5 ppb.

Table 8. Summary statistics for ZON for 2001 to 2008. Includes previous five-year dataset for comparison.

	2001	2002	2003	2004	2005	2006	2007	2008
Number	343	343	328	344	326	182	152	175
Incidence, %	13	43	31	63	23	8	28	87
Mean	<5	12	9	55	9	1	17	120
Median	<5	<5	<5	10	<5	<5	<5	47
>100ppb, %	0.7	0.6	1.2	11.3	1.2	0.0	2.6	28.6

Samples below 5 ppb allocated value of 2.5 ppb (standardised with earlier LoQ)

Table 9. Summary statistics for HT2 and T2 combined for 2001 to 2008. Includes previous five-year dataset for comparison.

	2001	2002	2003	2004	2005	2006	2007	2008
Number	283	343	328	344	326	0	152	175
Incidence, %	30	16	69	14	44	NA	0.67	0
Mean	<20	<20	22	<20	<20	NA	<20	<20
Median	<20	<20	<20	<20	<20	NA	<20	<20
>100ppb, %	0.7	0.0	0.6	0.9	0.3	NA	0.0	0.0

Samples below limit of quantification (LoQ) allocated value of 10 ppb. NA; not analysed

Acetylated derivatives, 3-acetylDON and 15-acetylDON were detected in very few samples and always as a low concentration secondary contaminant in the presence of a high concentration of a primary contaminant, DON. T2 and HT2 were only detected in one sample in 2007 (combined HT2+T2 = 35 ppb). Fusarenone X, diacetoxyscirpenol and neosolaniol were not detected in any samples (LoQ= 10 ppb).

It should be noted that the legal limits for DON and ZON include a measurement of uncertainty. Therefore for a consignment of unprocessed wheat intended for human consumption to exceed the legal limit for DON and ZON the concentration as determined by the analytical procedures employed in 2007 and 2008 would have to exceed 1450 ppb DON or 113 ppb ZON.

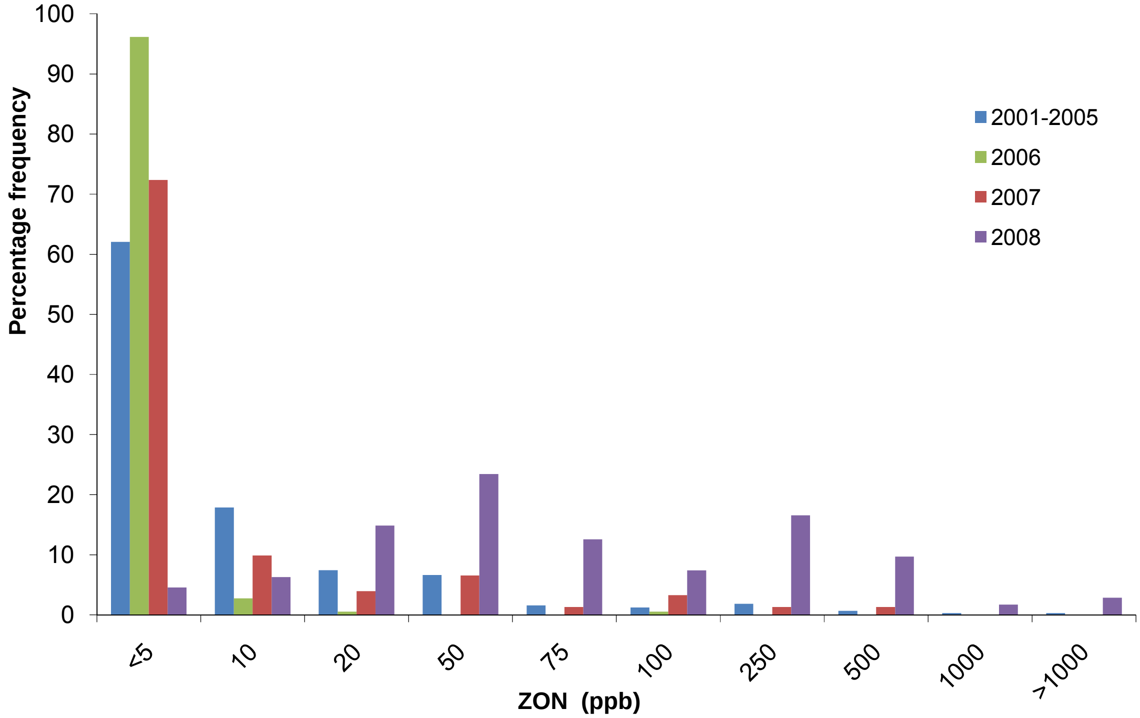


Figure 2. Distribution of ZON concentration for each year. Includes previous five-year average for comparison.

3.3.3. Regression analysis

There was a strong positive relationship ($p<0.001$; $r^2=0.49$) between $\log_{10}$ transformed DON and ZON (Figure 3). This is to be expected as DON and ZON are produced by the same species, namely *F. culmorum* and *F. graminearum*, but isolates of these species may or may not produce DON or ZON. An important point to note in Figure 3 is that samples which exceed 1250 ppb DON may or may not exceed 100 ppb ZON, and *vice versa*. The percentage of samples which exceeded 1250 ppb DON and 100 ppb ZON fluctuated each year and the relationship between the two was not stable between years (Figure 4). The relationship between DON and ZON concentration was analysed with regression analysis of $\log_{10}$ transformed values, grouped by year. The regression and year were both highly significant ($p<0.001$). The regression, when samples below the LoQ were removed, was best fitted by separate, parallel lines ($r^2 = 0.57$). It appears that the relationship alters dependent on harvest conditions. The 100-year average for rainfall in August in England is 74 mm. In 2006 there was near average rainfall in August of 88 mm. In 2007, the month of August was dry with only 54 mm, whereas in 2008 it was one of the wettest harvests for many years with a rainfall of 106 mm in August (and harvest extending into September which was also wet). This can be seen in Figure 4, where a wetter harvest appeared to result in a higher ZON to DON ratio, and *vice versa* in a dry harvest year, with some samples having much greater or lower ratios of ZON to DON as highlighted by circles in 2008 (green) and 2007 (red) respectively. There will be exceptions to these scenarios, for example, in 2008 a small proportion of fields were harvested before the wet weather caused severe delays.

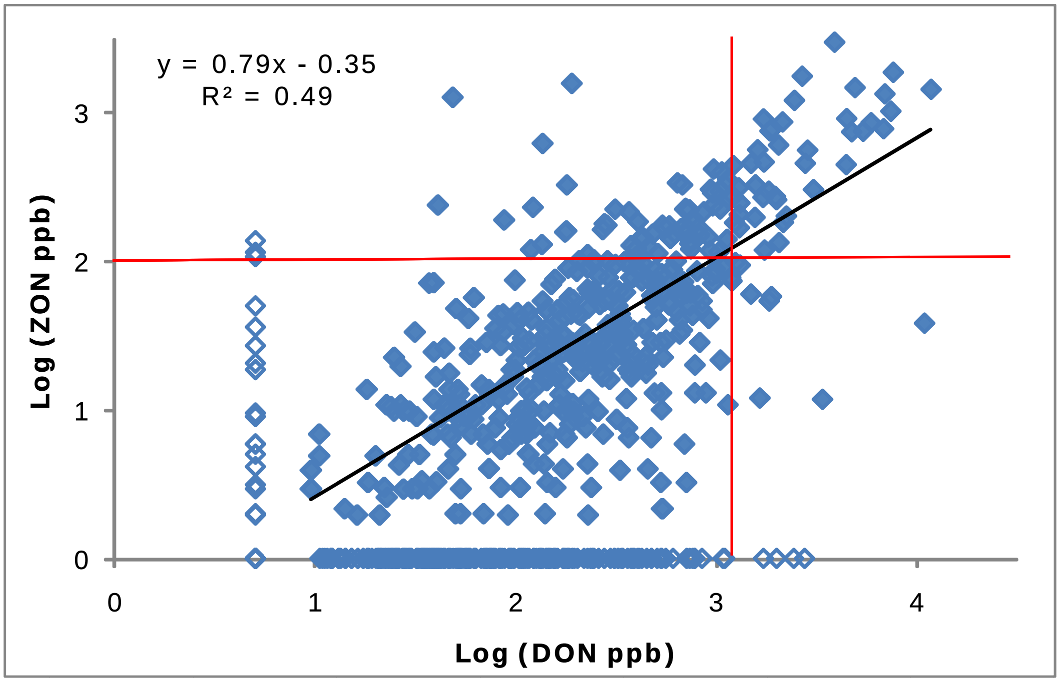


Figure 3. ZON against DON (log log plot) for wheat 2006-2008 (n=972). Lines represent 100 ppb ZON and 1250 ppb DON.

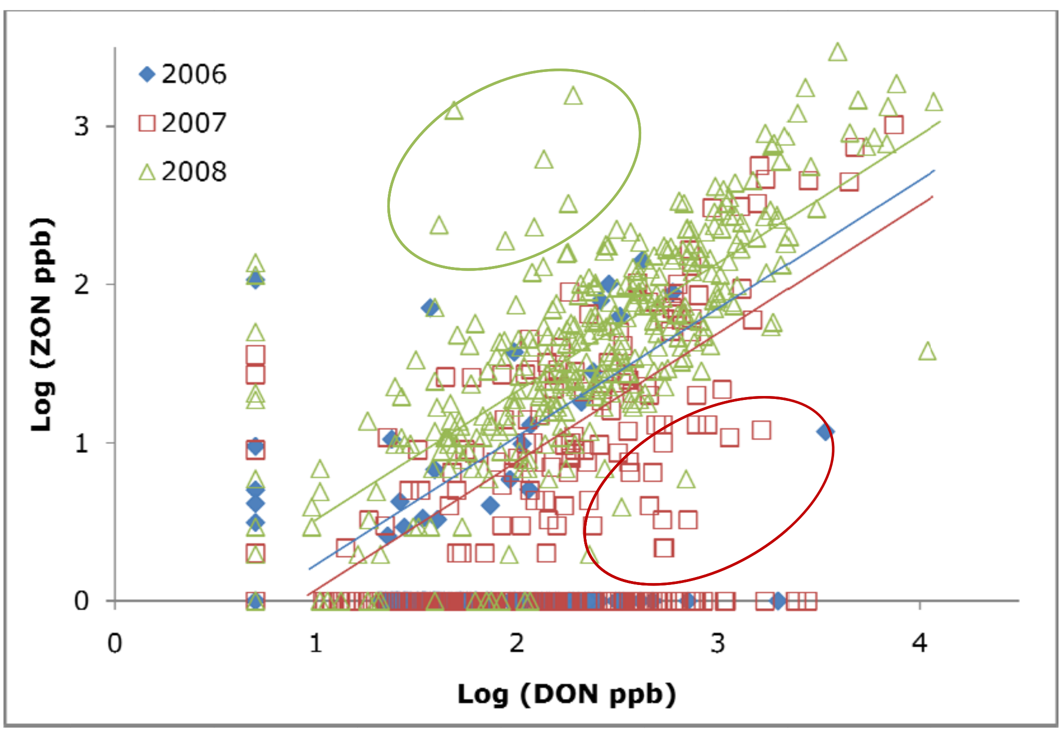


Figure 4. ZON against DON (log log plot) for wheat for each year from 2006-2008 (n = 972).

There was a very weak positive relationship ($p<0.001$; $r^2=0.17$) between the concentrations of NIV and DON (Figure 5). The relationship between DON and NIV is more complex because:
a) NIV and DON are produced by different chemotypes of the same species (*F. culmorum* and *F. graminearum*),
b) NIV is produced as a low level co-contaminant by DON chemotypes (and hence there is always some NIV present in samples with high DON concentration),
c) NIV is also produced by *F. poae*, which has different environmental requirements to *F. culmorum* and *F. graminearum*.

The acetylated versions of DON (3-acetyl and 15-acetyl DON) are co-contaminants of DON which occur at a low percentage of the DON concentration, and as such are only normally detected when DON is present at a high concentration. Acetylated DON rarely occurred in UK wheat and only at very low concentrations.

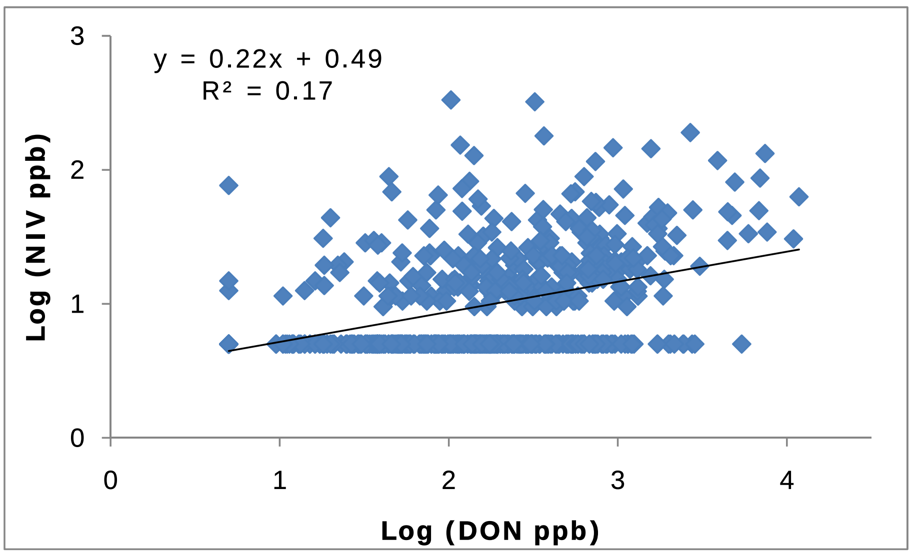


Figure 5. NIV against DON concentration for wheat 2007-2008 (n=643).

3.3.4. Statistical analysis of DON and ZON

The aim of the statistical analysis was to determine the effect of agronomic factors on the fusarium mycotoxin contamination of wheat. The methodology was as in the previous study (Edwards, 2007), however, additional agronomic data was collected to identify the impact of these factors.

Samples with less than the LoQ were given a value of ½(LoQ) i.e. 5 and 1 ppb for DON and ZON respectively, and all samples $\log_{10}$ transformed (logd = $\log_{10}$ of DON, logz = $\log_{10}$ of ZON) to stabilise the variance.

Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v12, 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 fusarium head blight epidemiology one could expect a temporal (year) and spatial (region) interaction. As crop debris is an important parameter of fusarium head blight epidemiology, as in the type and amount of crop debris, then an interaction between previous crop, crop debris management and the method of cultivation (± ploughing) could be expected (ie removal of straw and/or ploughing would be beneficial for some crops but not others).

After selection of factors to be used in the model the data file was filtered of all samples containing blanks within these factors and the data was re-analysed.

Of the factors tested, year, region, previous crop, cultivation and variety were all significant. There were significant interactions between year and region and between previous crop and cultivation.

The models generated identified the same agronomic factors were significant for DON and ZON concentrations and the trends are similar for the two mycotoxins.

For DON, the model accounted for 61% of the observed variance; 52% of variance was accounted for by year and region and their interaction. For ZON, the model accounted for 64% of the observed variance; 60% of variance was accounted for by year and region and their interaction.

The figures below show the back-transformed predicted means for each significant factor and the 95% confidence limits for the predicted means. For some agronomic factors there are low numbers of samples, these can be identified by the large confidence limits.

Two additional factors specific to end-use were tested for significance by placing in the model either after year*region or at the end of the model. In both positions the factors had no statistical significant effect ($p>0.05$) indicating that the growers' intended market, or the market specification for the wheat variety grown do not have a significant impact on the DON and ZON concentration at harvest.

Two additional factors pertaining to maize were tested for significance by placing at the end of the model. These factors were "Maize in rotation" and "Maize next to crop". Neither of these factors were significant ($p>0.05$) indicating that the presence of maize in a wheat rotation other than as the previous crop does not increase the DON or ZON concentration significantly and that a maize crop adjacent to a wheat crop does not significantly increase the DON or ZON content of the wheat crop, at the field scale. It should, however, be considered that an adjacent maize crop is likely to have an impact on wheat grown within a few metres of the maize. This would be particularly true for game cover crops where maize is grown year after year, allowing a build-up of *Fusarium* inoculum.

Crop debris management, ie the baling and removal of straw, compared to incorporation had no significant effect on DON in the subsequent wheat crop. This occurred even when analysed as an interaction with previous crop and cultivation. 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 model.

There was a significant interaction between year and region, there was a trend of DON and ZON contamination decreasing northwards but this did not occur in 2008 when the delayed harvest was worse in the North (Figure 6). The location of samples exceeding legal limits of DON and ZON have spread northwards since monitoring started in 2001. In 2008, samples above legal limits were detected as far north as Darlington. Northern Ireland had samples with high DON and ZON in all of the last three years. This region has wetter summers than the rest of the wheat growing regions of the UK and in the “dry” summer of 2006 had 33 mm of rainfall during flowering including three days of rainfall over 5 mm, one of which was 15 mm. There is some evidence that the intensity of rainfall, as well as the duration of rainfall is critical to severe head blight infections. This may be due to the involvement of rainfall in different stages of the *Fusarium* life-cycle. Heavy rainfall splashes *Fusarium* spores from ground level to the wheat heads and then long periods of rainfall/high humidity allow the spores to germinate and penetrate the wheat heads.

Ploughing after maize and wheat reduced DON and ZON contamination of wheat significantly (Figure 7). The difference was greatest for maize. The low number of samples following minimum cultivation for some crops resulted in large confidence intervals and the inability of statistical analysis to identify significant differences.

The Fusarium head blight (FHB) resistance rating was not a significant factor in this dataset, although as can be seen in Figure 8, the trend is similar to that seen previously with a fall in DON as the varietal FHB resistance rate increases. The trend for ZON is identical to that for DON. The resistance score of three does not fit the trend, this resistance score was represented by a low number (n=17) of a single variety, Ambrosia. This may indicate that this variety was assigned a lower rating than its resistance as experienced in commercial crops would justify or this variety tended to be grown under low risk agronomy. The resistance score of four is represented by only three samples of a single variety, Charger, and therefore has very large error bars.

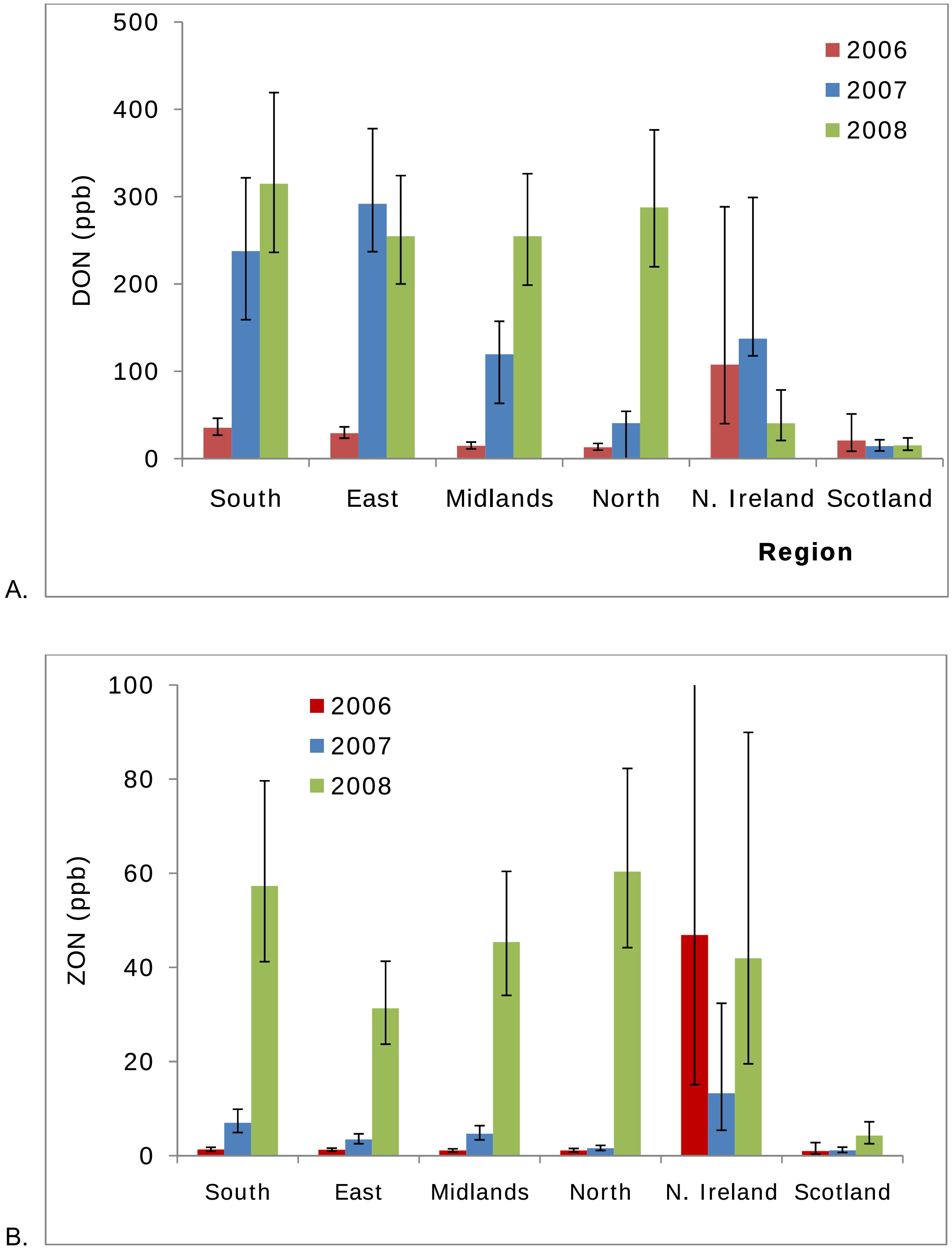
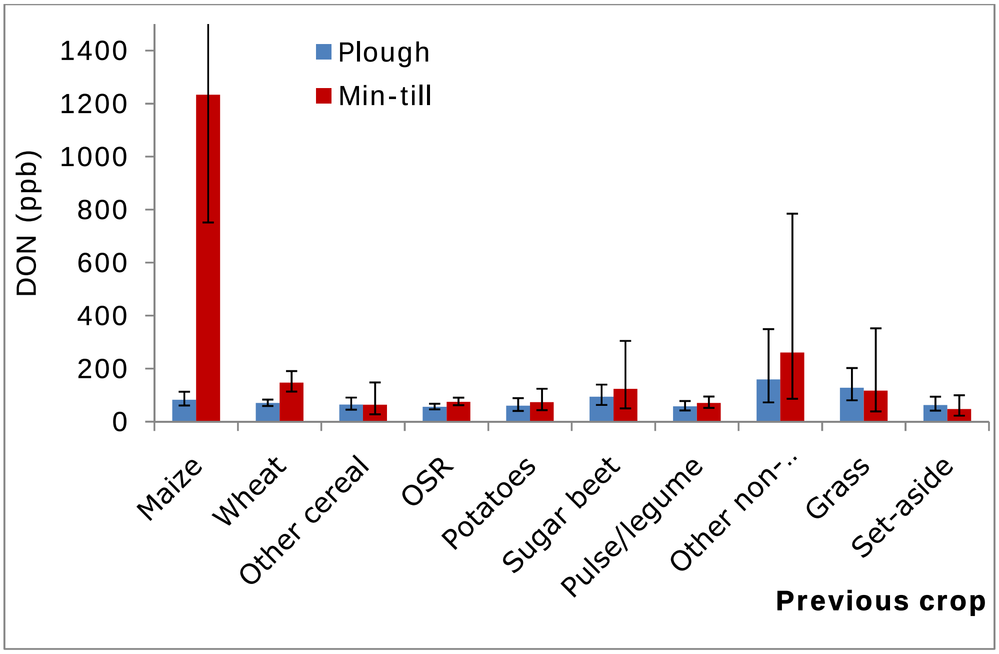
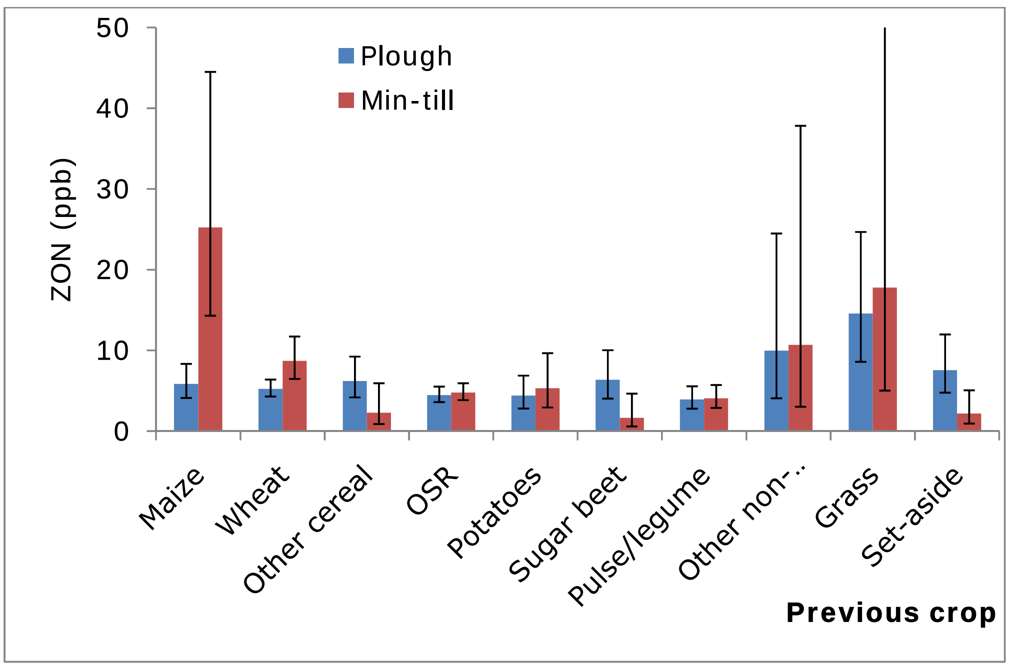


Figure 6. A. DON and B. ZON contamination of wheat by region for each year. Bars represent 95% confidence limits for predictions.



A.



B.

Figure 7. Effect of cultivation and previous crop on A. DON and B. ZON contamination of wheat. Bars represent 95% confidence limits for predictions.

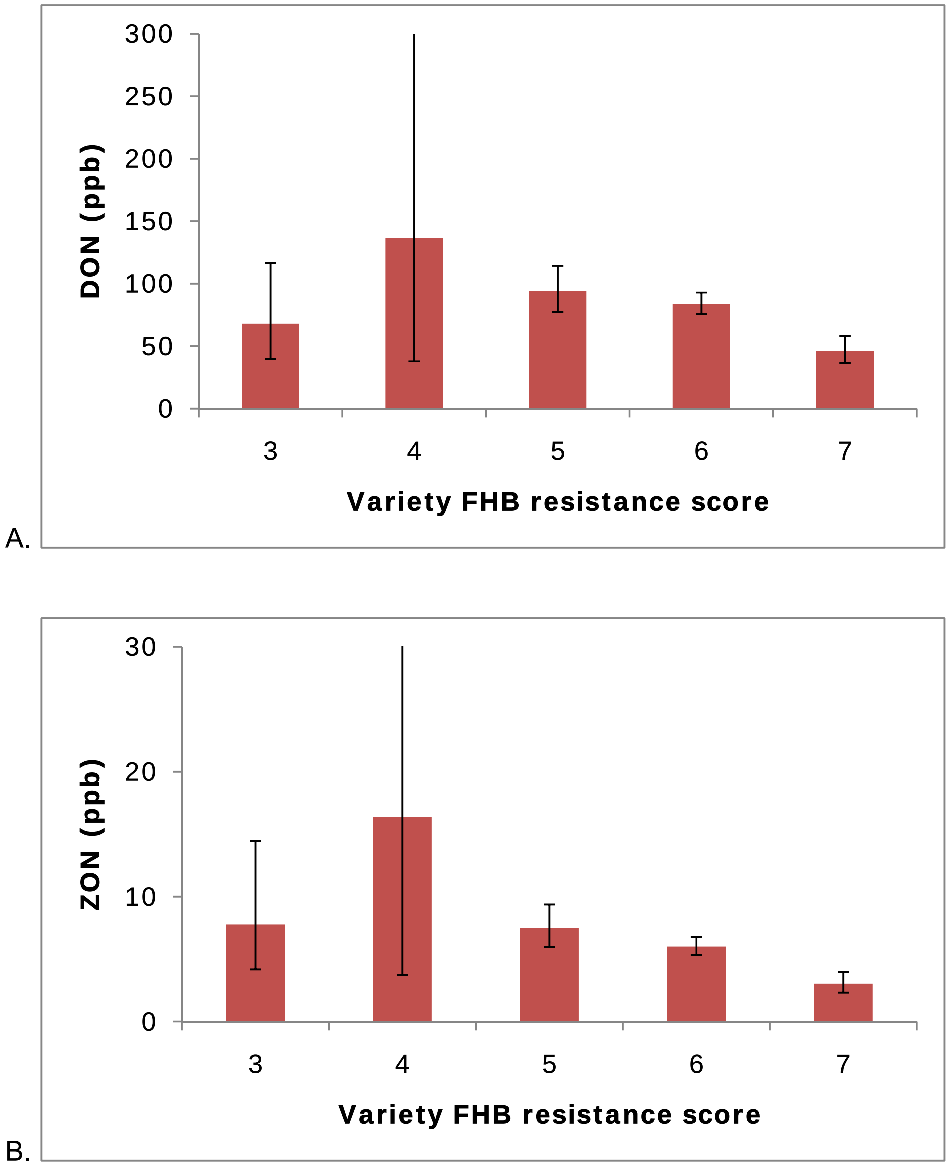
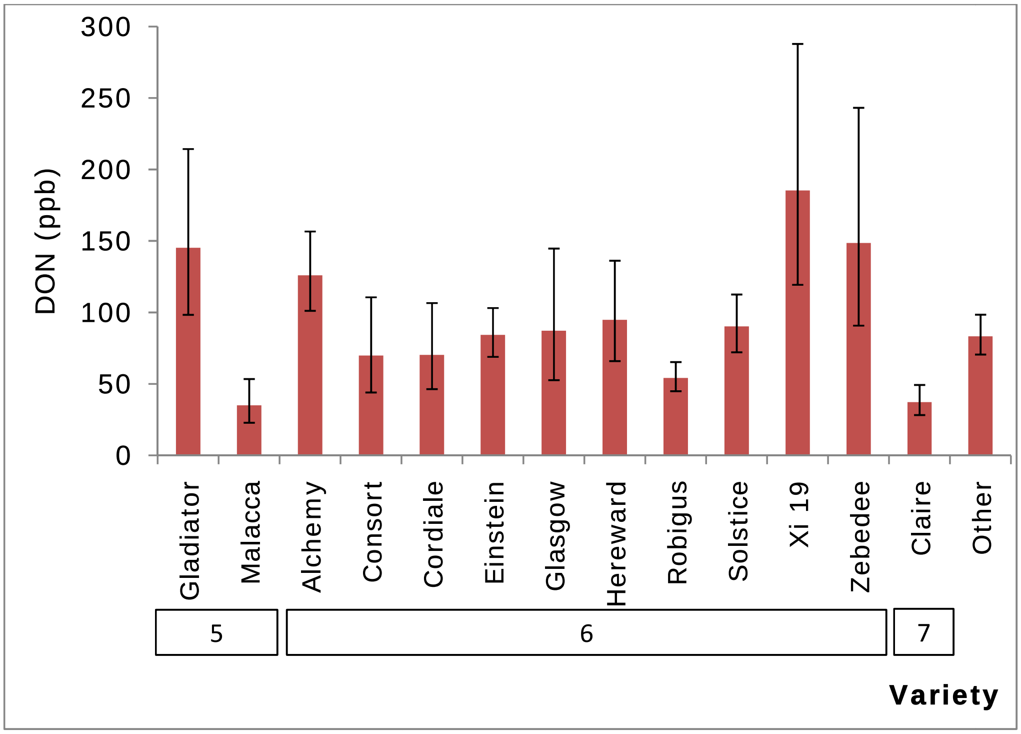
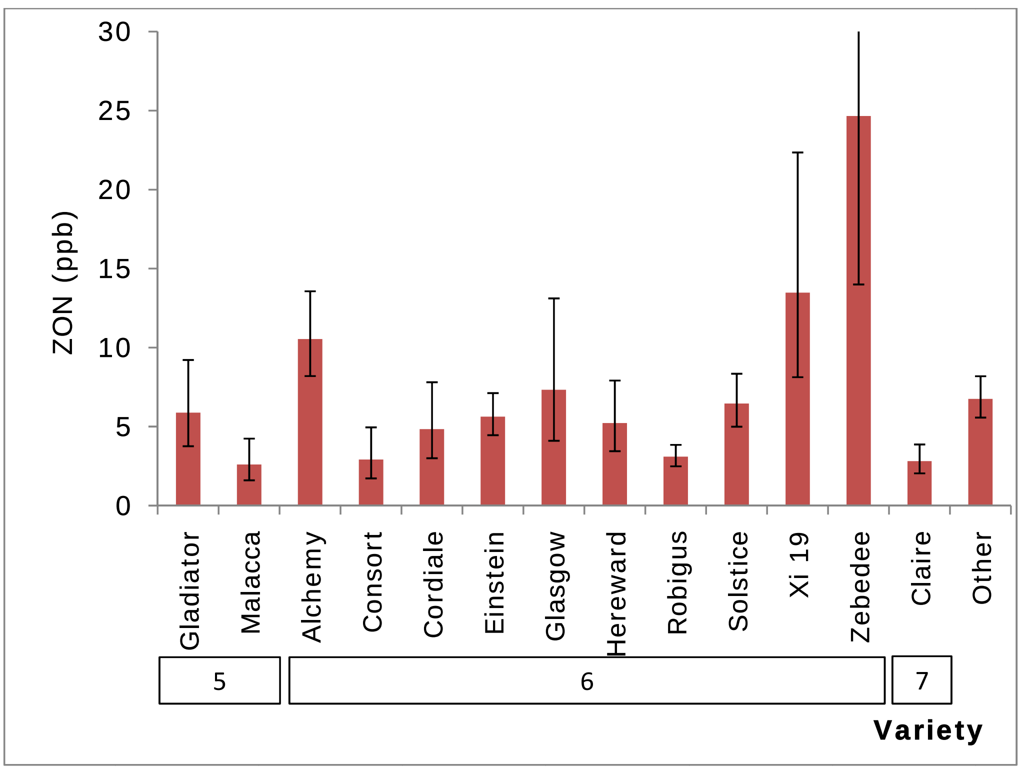


Figure 8. A. DON and B. ZON content of samples grouped by Fusarium head blight resistance score. Bars represent 95% confidence limits for predictions.



A.



B.

Figure 9. A. DON and B. ZON content of wheat samples grouped by variety. Bars represent 95% confidence limits for predictions. Numbers in boxes indicate FHB resistance scores.

Varieties present in more than 20 samples were analysed individually and this did result in significant differences for DON and ZON (p=0.002 and 0.003 respectively).

Figure 9 shows that there were large differences between varieties and that these do not always correspond to the varieties' resistance scores. This again may be an indication of a difference in resistance observed in Recommended List trials and under natural infection in commercial crops, or it may be a result of specific varieties having been grown under different fusarium risk situations. For example, Xi19 has a high DON content relative to its resistance score; this may be because it is a preferred variety for late drilling and late drilling may increase risk. Although year was included earlier in the model, and therefore differences between years should have been accounted for, some differences observed between varieties may also be partly due to their frequency in specific years. For example, Claire and Malacca were more common in 2006 than in subsequent years whereas Alchemy and Zebedee were more common in 2007 and 2008 compared to 2006.

There was no significant difference in the DON or ZON content of wheat crops which received different fungicide regimes. Seed treatment was analysed based on the product used. There was no difference identified, although 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*.

T3 treatment (fungicide application at flowering, GS59 – 61) was analysed based on:

- Application of a triazole
- Application of a FHB recommended product
- Rate of application of a FHB recommended product

None of the above were significant. As this is observational data care must be taken as growers may apply a specific FHB recommended product, or a higher rate of such products specifically because the crop has a high fusarium mycotoxin risk.

3.3.5. Improvements to HGCA Fusarium Mycotoxin Risk Assessment

The HGCA fusarium mycotoxin risk assessment was first developed as a tool for growers to allow them to assess the risk of fusarium mycotoxins, in particular DON. The risk assessment was constructed in the spring of 2006 based on data generated from the previous project (Edwards, 2007) which modelled UK wheat DON content against agronomic practices (2001-2005). The risk assessment was validated using grain samples from the 2006 harvest and was shown to perform well in a year of low risk. The risk assessment was published in the “G34: Guidelines to minimise risk of fusarium mycotoxins in cereals” in the summer of 2007. The risk assessment was adopted by the cereal crop assurance schemes, and became a requirement of these schemes in 2007, with the requirement of a risk assessment score on grain passports and a DON test for consignments identified as high risk.

Post-2007 Modifications

In the spring of 2008, a series of HGCA/ACCS mycotoxin and storage workshops were conducted across the UK, attended by about 500 growers and agronomists. From these workshops a number of issues were raised regarding the need for clearer descriptions for some of the risk factors and the absence of some beneficial risk factors within the assessment, namely the lack of gradation within tillage (0 or 4) and lack of a score for T3 fungicide inputs.

Due to the increase in the importance of the risk assessment and the issues raised by growers it was decided to complete a retrospective analysis of the performance of risk assessment from samples collected during 2007 and consider modifications to the scoring system and supply clearer descriptions for the scoring system.

The retrospective analysis was completed on 80 samples which covered the whole range of DON detected and included all samples greater than 1250 ppb. Samples were split into four categories (<50, 50-500, 500-1250, >1250 ppb). Scores were calculated based on agronomic data provided and from rainfall data collected from the nearest Met Office meterological station. Total rainfall was calculated from flowering (GS59-69) and pre-harvest (GS87-harvest).

The following scores were allocated for rainfall:

Timing	Period	Total Rainfall (mm)	Score
Flowering	GS59-69	<10	0
		10-40	2
		>40	5
Pre-harvest	GS87+	<20	0
		>20	3

In the risk assessment cultivation had two scores, ploughing 0 and min-till 4. For the modification to the scoring system the additional scores were included to show the benefit of chopping and mixing of crop debris during min-till. The benefit was based on field trial data generated by Arvalis in France (Labreuche et al., 2005).

Cultivation	Score
Direct drilled	4
Standard non-inversion tillage	3
Intensive non-inversion tillage	2
Plough (soil inversion)	0

T3 fungicides were also included in the modified score based on the known benefits of particular T3 fungicides applied at robust rates. Benefits of fungicides were based on published field experiments.

T3 fungicide	Score
Fungicide known to reduce fusarium head blight and/or mycotoxins (0.5-0.74 field rate)	-2
Fungicide known to reduce fusarium head blight and/or mycotoxins (>0.75 field rate)	-3

Both these alterations resulted in lower scores for some samples; no samples would have had a higher score as a result of these modifications. Numbers of samples in each category were weighted based on the DON concentration distribution in 2007 and converted to percentages.

Table 10. Percentage number of samples within each risk category for each concentration range of DON and the number of false positives (DON below 1250 ppb but classified as high risk) and false negatives (DON above 1250 ppb but not classified as high risk) using the 2007 HGCA risk assessment.

DON ppb	Low	Mod	High	Total	False+	False-
0-50	13	10	0	23	0	
50-500	14	31	15	60	15	
500-1250	4	6	3	13	3	
1250+	0	2	2	4		2
Totals	31	49	21	100	18	2

Table 10 shows a population shift in category score as the DON concentration increases. The percentage of samples in the high category advances from 0 to 50% from the <50 ppb range to the >1250 ppb range. Testing all high category samples results in 18% false positives and 2% false negatives. Testing all moderate and high samples would result in zero false negatives but 64% false positives.

Table 11. Percentage number of samples within each risk category for each concentration range of DON and the number of false positives (DON below 1250 ppb but high risk category) and false negatives (DON above 1250 but not high risk category) using the 2008 modified risk assessment.

DON ranges	Low	Mod	High	Total	False+	False-
0-50	13	10	0	23	0	
50-500	14	37	10	60	10	
500-1250	4	8	1	13	1	
1250+	0	2	2	4		2
Totals	31	57	13	100	11	2

Table 11 shows the same population shift in category score as the DON concentration increases. The percentage of samples in the high category advances from 0 to 50% from the <50 ppb range to the >1250 ppb range. The modified scores, by taking into account additional beneficial agronomic practices have maintained the same number of false negatives (2%) while reducing the number of false positives to 11%. These modifications would therefore reduce the number of samples to be tested without increasing the risk of samples exceeding 1250 ppb not being tested.

Post-2008 Modifications

In 2008 the risk assessment was considered to have failed as an unacceptable number of samples with a low risk score on plant passports exceeded legal limits of DON at mill intake. This was perceived to be for two reasons. Firstly, the risk assessment was developed based on conditions experienced from 2001 to 2007, and during this period the UK has not experienced such a long extended wet harvest as in 2008. Secondly, some risk assessments may not have been completed correctly.

The retrospective analysis after the 2008 harvest was completed on 60 samples which covered the whole range of DON detected and included a proportional number of samples in the three categories (<500, 500-1250 and >1250 DON) as occurred in the 2008 harvest. Scores were calculated based on agronomic data provided and from rainfall data collected from the nearest Met Office meterological station. Total rainfall was calculated for flowering (GS59-69) and pre-harvest (GS87-harvest) risk periods.

As a larger proportion of samples had exceeded the legal limit for ZON, than DON, in 2008, the ability of the risk assessment to identify samples at risk from ZON was also assessed.

The following scores were allocated for rainfall in the 2008 risk assessment:

Timing	Period	Total Rainfall (mm)	Score
Flowering	GS59-69	<10	0
		10-40	2
		>40	5
Pre-harvest	GS87+	<20	0
		>20	3

The following scores were used in the revised 2009 risk assessment:

Timing	Period	Total Rainfall (mm)	Score
Flowering	GS59-69	<10	0
		10-40	3
		40-80	6
		>80	9
Pre-harvest	GS87+	<20	0
		20-40	3
		40-80	6
		80-120	9
		>120	12

Table 12. Percentage number of samples within each risk category for each concentration range of DON and the number of false negatives (DON above 1250 ppb but not classified as high risk) and false positives (DON below 1250 ppb but classified as high risk) using the 2008 HGCA risk assessment.

DON ranges	Risk Score Low	Risk Score Mod	Risk Score High	Total	False-	False+
0-500	29	36	0	64		0
500-1250	8	14	0	22		0
1250+	5	5	3	14	10	
Totals	42	54	3	100	10	0

Table 13. Percentage number of samples within each risk category for each concentration range of ZON and the number of false negatives (ZON above 100 ppb but not classified as high risk) and false positives (ZON below 100 ppb but classified as high risk) using the 2008 HGCA risk assessment.

ZON ranges	Risk Score Low	Mod	High	Total	False-	False+
0-50	14	25	0	39		0
50-100	15	10	0	25		0
100+	14	19	3	36	32	
Totals	42	54	3	100	32	0

Tables 12 and 13 show that the 2008 risk assessment did fail to detect many samples with a high DON or ZON content, failing to classify 10% and 32% of samples which exceeded either DON or ZON legal limits respectively as high risk.

Table 14. Percentage number of samples within each risk category for each concentration range of DON and the number of false negatives (DON above 1250 ppb but not classified as high risk) and false positives (DON below 1250 ppb but classified as high risk) using the revised 2009 HGCA risk assessment.

DON ranges	Risk Score Low	Mod	High	Total	False-	False+
0-500	7	34	24	64		24
500-1250	2	8	12	22		12
1250+	0	0	14	14	0	
Totals	8	42	49	100	0	36

Table 15. Percentage number of samples within each risk category for each concentration range of ZON and the number of false negatives (ZON above 100 ppb but not classified as high risk) and false positives (ZON below 100 ppb but classified as high risk) using the revised 2009 HGCA risk assessment.

ZON ranges	Risk Score Low	Mod	High	Total	False-	False+
0-50	7	25	7	39		7
50-100	2	8	15	25		15
100+	0	8	27	36	8	
Totals	8	42	49	100	8	22

Increasing the pre-harvest rainfall scores allowed the scheme to account for the importance of pre-harvest rainfall. The false negatives were reduced to zero for DON and 8% for ZON at the legal limit of 1250 ppb DON or 100 ppb ZON. There was a corresponding increase in the number of false positives with 36% for DON. The consequence of this is that 50% of grain consignments would need to be tested in a high risk year to detect 14% of consignment exceeding the DON limit. For end-users who require lower limits at intake a lower risk score can be set for acceptance. For example, if a limit of 10 is used to minimise samples exceeding 500 ppb DON at intake then 93% of samples would need to be tested to detect 36% of samples and 2% of samples above 500 ppb would not be detected. In such high risk years it would therefore be advisable for processors with low intake limits to test all consignments.

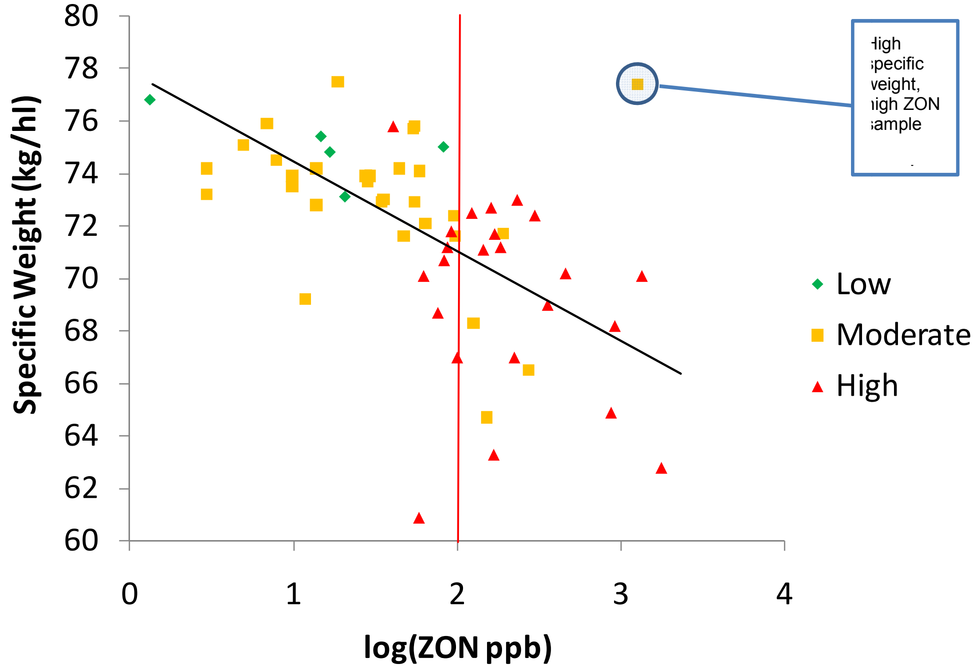
Although prolonged heavy rainfall has not been experienced during flowering during 2001-2008, mist-irrigated trial experiments have identified that continued rainfall over this period will increase the risk of exceeding fusarium mycotoxin legal limits in harvested grain to a similar extent as pre-harvest rainfall. For this reason, additional risk scores were added to the flowering rainfall risk category. This had little implication on the re-validation of the risk assessment but will hopefully protect against the risk assessment scheme failing in future years due to high rainfall occurring during flowering.

One issue of increasing the risk scores for flowering and pre-harvest rainfall was that it would be easier to obtain a high risk score in Northern England and Scotland where high DON and ZON in wheat are rarely detected (Note: Trade samples exceeding legal limits for DON and ZON were detected in Northumberland and Scottish Borders in 2009). For this reason the North of England (low risk) score was altered from 1 to -2; and the risk score for Scotland was altered from 0 to -4. This would ensure that only the most conducive conditions for fusarium head blight, namely a wet flowering and wet harvest period would be required before Scottish wheat reached a high risk score (>15).

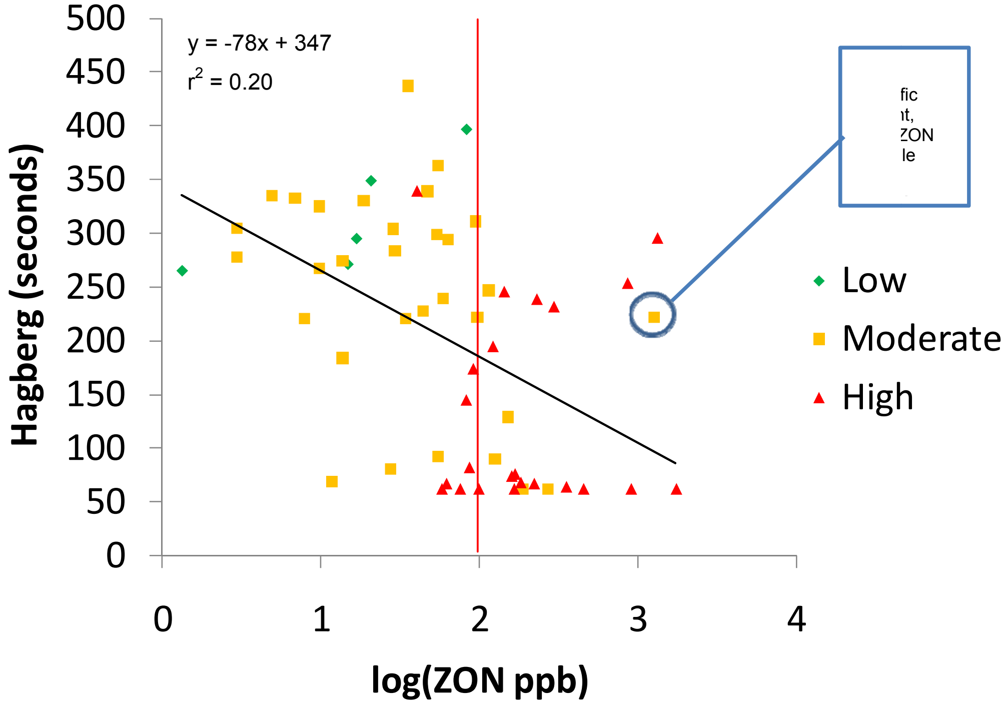
Grain quality

Selected samples from 2008 harvest were also assessed for grain quality using two standard measurements of grain quality for mill intake, namely, specific weight and Hagberg Falling Number; two grain parameters that are also affected negatively by delayed/wet harvests. There were highly significant negative regressions between ZON (log_{10} transformed) and these two quality parameters ($p<0.001$). As can be seen in Figure 10, the relationships between specific weight and ZON or Hagberg Falling Number and ZON were weak with ZON concentration accounting for 37% of the variance in specific weight and 20% of the variance of Hagberg Falling Number. All samples that had a high or moderate risk score and a ZON concentration above the legal limit of 100 ppb had a low specific weight and Hagberg Falling Number except for one sample, which had a high specific weight but a low Hagberg Falling Number (circled in Figure 10).

Although 8% of samples were false negatives for ZON at 100 ppb with the revised risk assessment these samples would have failed other milling quality parameters as they had either low Specific Weight and/or Hagberg Falling Number (Figure 10). Four samples with high ZON and moderate risk scores (due to moderate pre-harvest rainfall) had very low Hagberg Falling Numbers suggesting that they had experienced more pre-harvest rainfall than estimated during this validation exercise. The trend of high ZON samples to fail milling quality specifications is a useful screen for many processors; however, not all processors use these specifications at intake. For end-users which have fewer or less stringent intake quality parameters should consider screening more consignments with low specific weights.



A.



B.

Figure 10. Relationship of A. Specific Weight and B. Hagberg Falling Number to ZON concentration for 2008 harvest grain samples for each category of fusarium mycotoxin risk (low, moderate and high). Vertical line = 100 ppb.

3.4. Discussion

Overall the mycotoxin profile of UK wheat was similar to previous years as detailed in HGCA Project Report No. 413 (Edwards, 2007). There was greater variability between years due to the large difference in summer weather experienced between 2006 (dry) and 2008 (wet). There was also observed a spread northwards of the mycotoxin risk, with samples exceeding legal limits in the North East of England. The spread northwards has also been observed for *F. graminearum* incidence in the CropMonitor survey of FHB in winter wheat (Jennings & Humphries, 2009). The delayed wet harvest in 2008 also resulted in near equivalent risk across England, probably because the delay in harvest was greater in the north.

UK wheat harvested in 2008 had the highest fusarium mycotoxin content since monitoring started. ZON exceeded legal limits in more samples than DON in 2008. This is thought to have occurred because ZON is produced later in the season as the crop ripens (Matthaus et al., 2004), and consequently delayed harvests have a greater impact on ZON levels. Flour mills had fewer issues with ZON compared to DON and this can be explained by the negative correlation between ZON concentration and milling quality specifications (specific weight and Hagberg Falling Number); so samples with high ZON concentration routinely failed milling quality specifications. Both DON and ZON are produced in higher concentration in the outer layers of the grain resulting in higher concentrations in the bran fractions. DON is highly water-soluble, whereas ZON is much less soluble in water. Limited data suggest that some DON can be removed from the outer layers of grain during wet harvests, whereas ZON remains in place. It was therefore difficult for manufacturers of high fibre breakfast cereals to source bran that would allow production of products within legislative limits from wheat harvested in 2008. For this reason, the European Breakfast Cereal Association (CEEREAL) requested derogation limits for ZON in breakfast cereals of 135 ppb. CEEREAL considered that this would avoid major disruption to bran-based breakfast cereals production without compromising consumer health. The UK Food Standards Agency conducted a UK Risk Assessment of the CEEREAL request and recommended a more precautionary derogation limit of 100 ppb for high fibre breakfast cereals. This limit was agreed by the EC Standing Committee on the Food Chain and Animal Health (Anon, 2009) on 19 June 2009 and expired 31 October 2009, although there is scope for the limit to be made permanent subject to a review of the European Food Safety Authority scientific opinion on the associated consumer health risks which was published in July 2011 (Anon, 2011).

HT2 and T2 mycotoxin legislation is still under review and likely to be considered in 2012. For wheat, levels appear to have dropped in recent years, and are well below any likely limits. The lower levels maybe a consequence of lack of suitable conditions. It is believed the pathogen responsible, *F. langsethiae*, prefers drier summers. HT2 and T2 were not assessed in 2006, which was the only dry year experienced within this project.

The concentration of DON and ZON were modelled against agronomic practices applied to each field. The highest average DON and ZON content in harvested wheat occurred after maize. Ploughing significantly reduced DON and ZON contamination of subsequent wheat crops after maize and wheat. The greatest difference in DON and ZON concentration was between ploughing and not ploughing after maize (15 and five-fold respectively). This agrees with data from previous years. Studies in France have determined that crop debris management can have a large impact on DON concentration at harvest, particularly after maize. Highest DON concentration was found after no-till, followed by min-till and then lowest levels after ploughing. The reduction in DON has been linked to the reduction in crop residue on the soil surface. However, the reduction in DON with min-till, compared to no-till is usually greater than the reduction of crop residue on the soil surface (Labreuche et al., 2005; Maumene, 2005). This is probably due to the fact that min-till increases the colonisation of crop debris with soil saprophytic microorganisms, which compete with *Fusarium* species. Chopping of maize debris before minimum tillage also caused a marked decrease in DON concentration in the following wheat crop (Maumene, 2005), again this is likely to increase the mixing of crop debris with soil. In this study, samples were split by ploughed and not ploughed as too few samples were collected from no-till fields to allow analysis of min-till versus no-till.

Winter wheat varieties in the UK are assessed for FHB resistance as part of the HGCA Recommended List trials. Resistance is scored from one to nine with nine equalling high resistance. From 2006-2008 the range of resistance available on the Recommended List was from three to seven, although most varieties had a score of five or six. Results for DON and ZON showed an inverse relationship between the FHB resistance rating and the DON and ZON content of grain samples for winter wheat cultivars although this relationship was not significant for this dataset. Analysis of individual varieties was significant and indicated that some had DON and ZON contents which did not correspond to their resistance score. This may be due to these varieties having been favoured in high or low risk situations rather than an incorrect evaluation of their varieties resistance to FHB. Comparison of varieties over several seasons/locations are required to obtain an accurate assessment of differences in varietal resistance as varieties vary in flowering time and weather conditions at flowering are critical for *Fusarium* infection and subsequent mycotoxin contamination. It is important to note that all UK varieties would be classed as susceptible to FHB compared to other varieties worldwide (Gosman et al., 2007).

There was no significant difference in DON and ZON concentration between fungicide regimes. Previous field experiments have shown that a good reduction in DON can be achieved in artificially inoculated field trials when inoculation of *Fusarium* and treatments are closely synchronised (ca. 90% reduction) (Nicholson et al., 2003). Reduction achieved is generally less in field trials with natural infection, which occurs over a longer period of time (ca. 50% reduction) (Simpson *et al.*, 2001; Ioos *et al.*, 2005).

In order to modify and validate the fusarium risk assessment a sub-sample of samples were selected to give an equivalent distribution of DON concentrations as the whole dataset for each year. The rainfall at flowering and pre-harvest were estimated and used to complete risk assessment scores. The percentage of false negative (ie low score but high DON) and false positive (ie high score but low DON) samples were calculated. The validation indicated that by increasing the risk categories for rainfall at flowering and pre-harvest reduced the number of false negatives to zero for DON and 8% for ZON in 2008. This was deemed to be acceptable for flour mills, which can screen out high ZON samples based on standard quality specifications, but unsuitable for processors with fewer or less stringent quality specifications or with lower intake limits due to production of high-fibre/wholewheat products.

The increased weighting of rainfall events on the risk assessment increases the risk of inaccurate completion of the assessment for the following reasons:
1) Growers rarely record growth stages of wheat after the last fungicide is applied at flowering (GS59-69).
2) Growers may not have access to farm weather data

It is therefore advised that the fusarium risk assessment is heavily promoted by the industry, and growers are reminded in a timely fashion of the need to monitor rainfall at flowering and pre-harvest.

To improve the predictive ability of the DON model further would require more precise weather date. The variance accounted for by year and year*region interaction, is largely attributable to differences in weather and would be unknown in a predictive model. The inclusion of weather parameters from pre-flowering to harvest is likely to account for much of this variance. There is a need to incorporate accurate weather parameters into the model to improve the predictive ability of the model. The ideal scenario is that national weather data is collected and used within the risk assessment model. Growers would enter a geographical reference, drilling date, harvest date and variety. The model would then predict flowering and pre-harvest dates and input relevant rainfall values. The access to meteorological data, the necessary model development and software development and maintenance will need to be considered by HGCA and other relevant government and industry bodies.

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