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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

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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.