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Improving risk assessment to minimise fusarium mycotoxins in harvested oats and malting barley

by

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CONTENTS

GLOSSARY

ANOVA	analysis of variance
DON	deoxynivalenol
FDG	*Fusarium* damaged grain
FEB	fusarium ear blight
FIG	*Fusarium* infected grain
FX	fusarenone X
Groat	oat grain with hull removed
HT2	HT2 toxin
HT2+T2	combined concentration of HT2 and T2 toxins
LoQ	Limit of Quantification
NEO	neosolaniol
NIV	nivalenol
Min-till	non-inversion cultivation of soil before drilling
T2	T2 toxin
ZON	zearalenone

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1. ABSTRACT

This report details HGCA-funded research to further identify the effects of agronomic practices on the concentration of fusarium mycotoxins in UK barley and oats over a number of seasons. One hundred samples both of barley and oats were collected each year at harvest, together with agronomic details, and analysed for ten fusarium mycotoxins including deoxynivalenol (DON), nivalenol, HT2, T2 and zearalenone (ZON). The European Commission (EC) set legislative limits for the fusarium mycotoxins, DON and ZON in cereals and cereal products intended for human consumption in July 2006. New investigative limits for HT2 and T2 in cereals and cereal products were proposed in May 2012. The investigative limits for unprocessed barley and oats for human consumption are 100-200 and 1000-1500 parts per billion (ppb), respectively. Further legislative measures for HT2 toxin and T2 toxin (HT2+T2) will be considered in 2015.

The incidence and concentration of most fusarium mycotoxins, including DON and ZON, have remained relatively low in both barley and oats compared to values for wheat. Concentrations of DON and ZON exceeded legislative limits in a low percentage of both barley and oats over the three years sampled. These high levels were associated with the wet summers of 2007-2009. Concentrations of HT2 and T2 in barley have only exceeded 100 ppb seven times in seven years (0.9% of samples).

HT2 and T2 levels continued to be relatively high in UK oats with an overall mean of 450 ppb for 2006-2008. From 2002-2008, between 1 and 30% of samples exceeded 1000 ppb HT2+T2 each year (annual mean was 16%). There was a negative relationship with late summer rainfall, indicating that drier conditions in July and August result in increased HT2 and T2 in UK oats. Agronomic factors that impacted upon HT2 and T2 in harvested oats were previous crop, cultivation and variety. Analysis of the previous cropping history showed there was a stepwise increase in HT2+T2 as the cereal intensity of the rotation increased. Variety was an important factor with higher levels and a wider range detected on winter compared to spring varieties. Analysis of oat samples from HGCA Recommended List trials confirmed the differences observed in the survey data and provided clear comparisons between all RL varieties under controlled field conditions.

The introduction of European legislation on HT2 and T2 mycotoxins could have serious implications for UK oat production and oat processing industries based on current levels.

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, worldwide, 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 for cereals and cereal products intended for human consumption. Guideline limits were also set for animal feed in the same year. Two other Fusarium mycotoxins related to DON are HT2 toxin and T2 toxin. There is no current legislation for these mycotoxins but investigative limits were proposed recently (May 2012) further legislative measures will be considered in 2015. Proposed investigative limits for unprocessed cereals intended for human consumption are as follows; for barley 100-200 ppb, for oats 1000-1500 ppb and for wheat 50-75 ppb HT2+T2. The EC recommendation is expected to request that member states in conjunction with food operators monitor HT2 and T2 in a wide range of cereals and cereal products and where investigative limits are exceeded then the factors resulting in the occurrence of the high concentrations and measures to avoid or reduce such high levels should be determined.

Based on a previous FSA/HGCA funded project (Edwards, 2007b), it was identified that both barley and oats tended to have low levels of fusarium mycotoxins DON and ZON compared to wheat, but oats had high concentrations of HT2 and T2 and there was an indication that levels of HT2 and T2 may be increasing in UK malting barley.

The aims of the projects detailed in this report were:

- To monitor fusarium mycotoxins in UK barley and oats over three years
- 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 barley and oats
- To monitor the HT2+T2 content of oat varieties from HGCA Recommended List trials
- To improve mycotoxin risk assessments for UK barley and oats

2.2. Materials and methods

Each year ca. 100 samples of oats (2006-2008) and malting barley (2007-2009) were collected at harvest from fields of known agronomy. Samples were collected by crop consultants (AICC, Agrovista, DARD and Scottish Agronomy). Samples were milled and then analysed for fusarium

mycotoxins; DON, ZON and another eight trichothecenes (relatives of DON including HT2 and T2) by liquid chromatography with tandem mass spectrometry (LC/MS/MS) at Campden BRI.

Summary statistics (percentage incidence and percentage above legal limits for cereals intended for human consumption, mean and median) of mycotoxin concentrations were produced and had been reported on the HGCA website Concentrations of fusarium mycotoxins were modelled against the agronomy factors to identify the importance of various agronomic factors. Where possible, data from the previous project (HGCA Project Report No. 415) were included.

Each year (2006-2011) oat variety samples from HGCA Recommended List fungicide treated trials were analysed for HT2+T2.

2.3. Results

For barley, mycotoxin levels remained low in all years except for DON and ZON. DON was above the legal limit (1250 ppb) in a single sample (0.7%) after the wet, delayed harvest of 2008. Of greater concern was ZON which was above the legal limit of 100 ppb in a low percentage of samples (ca. 5%) in all three years (2007-2009). This is markedly higher than experienced in the previous project (2002-2005) and was associated with wet weather in July and August in those years.

There were significant differences in DON and ZON concentrations for barley following different previous crops, with barley grown after maize as previous crops have been significantly more at risk from DON and ZON than other crops. There were significant differences between DON and ZON concentrations in different varieties of barley, although some of these differences may in part be due to when and where these varieties were grown.

The incidence and concentration of the HT2 and T2 continued to be high in UK oats with quantifiable concentrations in most samples and a combined mean concentration (HT2+T2) of 450 ppb for all samples from 2006-2008. This is a slight drop from the previous study which had a mean of 682 ppb for all conventional oat samples analysed from 2002 to 2005. The concentration of HT2+T2 was modelled against agronomic practices applied to each field.

There was a significant interaction between year and region, which is probably due to fluctuation in weather between years and regions. There was a strong negative relationship between harvest rainfall and HT2+T2, indicating that concentrations are higher when weather is drier in July and August. This is the opposite trend to ZON concentration in wheat and barley, which is higher after wet weather in late summer. There was no trend from North to South, as seen for DON and ZON in wheat and barley, which would indicate that the temperature difference across the UK does not

limit HT2 and T2 production in oats. Oat samples with more than 500 ppb HT2+T2 were detected in all regions of the UK.

The impact of previous crop and cultivation was studied using various datasets and categories. Analysis of previous crops showed no differences between HT2+T2 in oats after different cereal crops so the categories of cereal and non-cereal were used to look at the interaction of previous crop (cereal or non-cereal), crop debris management (baled or chopped) and cultivation (plough or min-till). Crop debris management and all interactions involving crop debris management were not significant. There was a significant interaction between cultivation and the last two previous crops with results indicating that cereal debris either left on the soil surface from the previous crop or ploughed back to the surface from two years previous, increased the risk of HT2+T2 in oats. There was a stepwise increase in HT2+T2 concentration as the cereal intensity within the rotation increased. This suggests that cereal debris is important in the epidemiology of HT2+T2 producing *Fusarium* species and the level of inoculum of these *Fusarium* species can build up over time.

There were significant differences in the HT2+T2 content of different UK varieties. HT2+T2 content of spring oat variety trial samples were consistently lower than winter oat samples. Naked oat varieties tended to have a lower HT2+T2 content compared to conventional husked oat varieties. Naked oats have a loose husk which is removed during harvesting. Short-strawed varieties tended to have a high HT2+T2 content.

2.4. Discussion

Legislative limits for fusarium mycotoxins, DON and ZON, in unprocessed cereals and cereal products intended for human consumption were set in 2006. Based on project results collated since 2002, there is a low risk of UK barley and oats exceeding these legal limits. UK barley and oats are less prone to Fusarium infection by DON and ZON-producing species, *Fusarium graminearum* and *F. culmorum*, compared to wheat. The main issue identified for barley is the impact of delayed wet harvests on ZON, resulting in 5% of samples exceeding the legal limit for ZON (100 ppb) within the current project (2007-2009).

For UK oats, DON and ZON levels were consistently low except after the delayed wet harvest of 2008 when 1 and 6% of samples exceeded the legal limits for DON (1750 ppb) and ZON (100 ppb), respectively. It is of concern, that there has been a dramatic increase in DON levels in Nordic oats in recent years, the cause of which has not yet been determined.

Investigative limits for fusarium mycotoxins, HT2 and T2 are currently under discussion within the European Commission. These mycotoxins have equivalent toxicity and any limit set will be based on a combined concentration (HT2+T2). Recently proposed investigative limits (May 2012) of

1000-1500 ppb for unprocessed oats and 100-200 ppb HT2+T2 for unprocessed barley for human consumption. The concentration of HT2 and T2 has remained consistently low in malting barley during 2007-2009; this is likely to be associated with the wet late summers experienced in these years. For UK oats, HT2 and T2 concentrations have remained relatively high although they were lower in 2008 after the delayed wet harvest. For UK oats there was a negative relationship between harvest rainfall and HT2+T2 concentration in oats. As the harvest rainfall increased, the level of HT2+T2 decreased. This is the opposite of the observed relationship for harvest rainfall and ZON concentration in wheat and barley.

Analysis of agronomic factors has again highlighted the impact of previous crop and variety on HT2 and T2 concentrations in UK oats. Analysis of HGCA RL trials over 6 years has identified consistent significant differences between winter oat varieties and that all spring varieties are consistently low.

New to this project was the identification that cereal intensity is important, with a stepwise increase in HT2+T2 as more cereals exist within a rotation.

The level of HT2 and T2 has been monitored in UK barley and oats from 2002-2008. During this time the proposed lower investigative limit for unprocessed barley (100 ppb) has only been exceeded seven times in seven years (0.9%) whilst the percentage exceeding the lower investigative limit for unprocessed oats (1000 ppb) has fluctuated between 1 and 30% with an overall average of 16%.

The introduction of European legislation on HT2 and T2 mycotoxins would have limited impact on UK barley production but could have serious implications for UK oat production and oat processing industries based on current levels in UK cereals.

To reduce the concentration of fusarium mycotoxins in UK barley growers should consider:

- Avoiding growing barley after maize
- Avoiding delays in harvest

To reduce the concentration of fusarium mycotoxins in UK oat growers should consider:

- Growing spring oats
- Growing oats in less cereal intense rotations
- Growing conventional winter varieties with consistently low HT2 and T2 levels (eg SW-Dalguise and Millennium)
- Growing naked oats for animal feed
- Avoid delays in harvest

3. TECHNICAL DETAIL

3.1. Introduction

3.1.1. **Fusarium ear blight**

Fusarium ear blight (FEB) of UK cereals may be caused by several fungal pathogens. The disease is also referred to as fusarium head blight or scab or fusarium panicle blight for oats. The vast majority of research conducted on FEB is concerned with wheat as this is the most economically important small grain cereal world-wide and is the most susceptible to FEB and mycotoxin contamination in many countries. Some FEB pathogens produce fusarium mycotoxins, whilst others do not. Fusarium ear 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 ear above the point of infection. As the whole crop ripens the symptoms are less visible. At harvest, fusarium ear blight can result in fusarium-damaged grains that may be shrivelled with a chalky white or pink appearance, although this is not always the case. The presence of fusarium-damaged grains is an indication that the 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 to splash spores from the crop debris up onto the cereal ear and warm, humid weather that allows the fusarium spores to germinate and infect the cereal ear. Once infection has occurred, further rainfall and humid conditions during the summer will allow secondary infection to occur. Cereal crops are most susceptible to FEB infection during flowering (Growth Stage 61-69); the crop is also susceptible during ripening (Growth Stage 85-92).

Most *Fusarium* species are facultative plant pathogens, i.e. 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 ear blight (FEB). FEB 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 ears during rainfall (Jenkinson & Parry, 1994; Horberg, 2002). This may occur in a stepwise manner, from leaf to leaf, and finally the ear. It was noted that during epidemic years in Idaho in 1982 and 1984, when *F. culmorum* was the dominant FEB pathogen, sprinkler irrigated fields had

severe FEB, whereas surface irrigated fields had little or no FEB (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 FEB was much more prevalent in fields with irrigation compared to fields with no irrigation (Strausbaugh & Maloy, 1986).

Wheat is most susceptible to FEB 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. FEB is assessed in the field after flowering, usually one to four weeks post-anthesis and is based on the number of ears 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 FEB and have not assessed mycotoxin concentration. In most countries where these studies have been performed, *F. graminearum* is the predominant FEB pathogen, and as this is the most potent DON producing species, there is a reasonable relationship between FEB severity, %FDG or %FIG, and DON concentration. It is, however, important to note that in the UK, *Microdochium* species can be the predominant FEB pathogen and these species do not result in FDG or any known mycotoxin. For UK data it is, therefore, advisable not to assume that a measurement of FEB is closely related to DON concentration at harvest (Edwards *et al.*, 2001). A similar situation has been reported in France (Champeil *et al.*, 2004).

Few studies have compared the FEB severity or mycotoxin contamination of wheat, barley and oats either from replicated field experiments or observational studies. In western Canada, observational data showed highest DON content was found on wheat, then barley, and lowest amounts on oats from 1991 to 1998 (Campbell *et al.*, 2002). The percentage of samples exceeding 1000 ppb DON was 31, 22 and 1.4% for wheat, barley and oats, respectively. This data was matched in a study of ear blight susceptibility of cereal species in inoculated glasshouse experiments (Langevin *et al.*, 2004). In the epidemic years of 1993 and 1994 in Minnesota commercial cereal samples were analysed for DON. Average DON concentrations in wheat, barley

and oat samples were 8.3, 10.4, and 1.4 ppm, respectively (Jones & Mirocha, 1999). There is less data on the relative concentration of other fusarium mycotoxins in wheat, barley and oats. For HT2 and T2, highest levels were detected on oats, then barley and lowest in wheat samples in Norway (Langseth & Rundberget, 1999).

There is limited data on occurrence of fusarium mycotoxins in UK cereals prior to 2001. A previous survey conducted in 1999 found highest amounts of DON on wheat, with lower levels on barley and oats. From 2002-2005, fusarium mycotoxins have been quantified in wheat, barley and oats (Edwards, 2007a; Edwards, 2007b). For DON and ZON, highest levels were detected in wheat, with much lower levels in barley and oats. In contrast to this, HT2 and T2 were high in oats with much lower levels in barley and wheat.

It should be remembered that the relative degree of mycotoxin contamination between cereals will vary between years and between regions depending on climatic conditions when each host species is in flower. This variation will also exist between winter and spring sown varieties of the same host species. In Norway, a large scale study over 6 years identified that highest DON concentrations occurred in oat samples, then wheat, and barley had the lowest DON average concentrations (Langseth & Elen, 1996). The variation in contamination levels between cereals was not observed in experimental field trials indicating that the observed differences were not solely due to inherent differences in resistance but also due to differences in agronomy.

It should be noted that the relationship between cereals and ear blight is not a static one and changes have been observed over recent years. For example, until 1993, FEB in barley was not observed in Western Canada; by 1999, barley was deemed to be as susceptible as wheat to FEB (Tekauz *et al.*, 2000). This may have been due to a fundamental shift in the pathogen population, or changes in agronomy, in particular, changes in varieties grown.

3.1.2. **Fusarium mycotoxins**

The trichothecene mycotoxins are produced by some of the Fusarium ear blight pathogens and their level within grain depends on weather conditions. High humidity during and after flowering is conducive to ear 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, which 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). Tolerable Daily Intakes (TDI) have been set for several Fusarium mycotoxins. The TDI is deemed to be the amount that is the safe limit to consume every day for a lifetime. It may, therefore, be safe to exceed this limit occasionally with no impact on health. The TDI for DON is 1 ppb body weight/day. A combined TDI for HT2 and T2 was recently set by the European Food Safety Authority (EFSA) at 0.1 ppb body weight/day (Anon, 2011b).

ZON is another mycotoxin produced predominantly by *F. culmorum* and *F. graminearum*. ZON has no known function in the fungus and is predominantly produced late in the crop growing season, near to harvest (Matthaus *et al.*, 2004). ZON has low cellular toxicity but is problematic as it has high estrogenic activity causing hyperoestrogenism 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 young females (Anon., 2000). The recent HGCA wheat mycotoxin project highlighted the impact of delayed wet harvests such as 2008, resulting in high levels of DON and ZON, but in particular more ZON (Edwards, 2011).

3.1.3. **Fusarium mycotoxin legislation**

The European Commission (EC) has set legislative limits for the fusarium mycotoxins including the trichothecene, deoxynivalenol (DON) and ZON in cereal grains and cereal-based products intended for human consumption (Table 1) (Anon, 2005; Anon., 2006b). Of the other trichothecenes, the only others currently being considered for legislation are HT2 and T2 toxins. New investigative limits for these mycotoxins were proposed in May 2012. These include a combined investigative limit of 50-75 ppb for unprocessed wheat, 100-200 ppb for unprocessed barley, 1000-1500 ppb for unprocessed oat grains for human consumption and limits of 10-75 ppb for various cereal-based retail products. The EC Recommendation, when published, is expected to request member states in collaboration with food business operators to monitor HT2 and T2 in a wide range of cereals and cereal products. Where investigative limits are exceeded factors resulting in the occurrence of the high concentrations and measures to avoid or reduce such high

levels should be determined. Further legislative measures for these mycotoxins will be considered in 2015.

Table 1. Maximum limits for DON and ZON in unprocessed cereals and finished products intended for human consumption.

Product	**Mycotoxin (ppb)**	
	DON	**Zearalenone**
Unprocessed cereals other than durum wheat and oats	1250	100
Unprocessed durum wheat and oats	1750	100
Cereal flour	750	75
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	50
Processed cereal-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.

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, a processor 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 due to their higher sensitivity to fusarium mycotoxins. The DON guidance value for complementary and complete feedingstuffs for pigs is 900 ppb. The ZON guidance value for complementary and complete feedingstuffs for sows and fattening pigs is 250 ppb and for piglets and gilts is 100 ppb.

Guideline limits for HT2+T2 in animal feed have been proposed with a limit of 1000 ppb for cereal and cereal products other than oats and 3000 ppb for oats and oat products) to be used as feed and a range of limits 250-2000 ppb HT2+T2 for compound feeds depending on the intended animal.

3.1.4. **Effects of agronomic factors**

The vast majority of previous research on the impact of agronomic factors on the mycotoxin content of cereals has been conducted on wheat. Previous studies, primarily in North America and elsewhere in Europe, have identified a number of agronomic factors which can affect the concentration of fusarium mycotoxins in wheat. Studies in the UK have primarily focussed on the use of fungicides applied to wheat during flowering to reduce fusarium ear blight; this is traditionally the third spray timing and referred to as T3. Previous studies of FEB and DON in wheat are reviewed in the wheat project reports (Edwards, 2007a; Edwards, 2011). Results from the previous wheat projects identified that the year, region, previous crop, cultivation, variety (varietal resistance to FEB) and T3 fungicide all had a significant impact on DON content of harvested wheat. Previous studies on barley and oats have primarily been restricted to varietal resistance to ear/panicle blight (Buerstmayr *et al.*, 2004; Tekauz *et al.*, 2004; Yoshida *et al.*, 2005). The previous HGCA project on fusarium mycotoxins in barley and oats (Edwards, 2007b) found little varietal difference in barley due to the low incidence and concentration of fusarium mycotoxins. There was a slightly higher HT2 and T2 incidence in spring malting barleys compared to other types. For oats there were large differences between organic and conventional oats with organic oats having ca. four times lower HT2+T2 compared to conventional samples. There was a degree of multicolinearity within the observational data in that many conventional farmers grew the variety Gerald after another cereal, usually wheat; whereas organic farmers were more likely to grow other varieties after a non-cereal. Consequently, it could be identified that organic samples had a significantly lower HT2+T2 content compared to conventional samples and that this was partly due to organic growers not growing Gerald and not following a cereal as frequently as conventional growers. Analysis indicated that one or more factors not included in the model, which differed between organic and conventional practice, also had an impact on HT2+T2 concentrations. One possible difference is rotation, with organic growers tending to use longer, less cereal-intense rotations.

3.1.5. **Effects of processing**

Oats for human consumption are de-hulled during processing. De-hulling is the removal of the outer coat, referred to as hull or husk. The de-hulled oat is referred to as a groat. The groat is further processed into various finished products for human consumption. The hulls are pelleted and used as a component in animal feeds. A recent study of industrial processing has identified a large reduction (>90%) in the mycotoxin content of oats to groats during de-hulling (Scudamore *et al*. 2007). Naked oats have a loose hull which is removed during harvesting; consequently only the groat is harvested. Naked oats are used as an animal feed.

3.1.6. Aims and objectives

- To determine the range of fusarium mycotoxin contamination within harvested UK malting barley and oat grain over a three year period (2006 – 2008 for oats, 2007-2009 for malting barley).
- To determine how agronomic factors affect the concentration of fusarium mycotoxins in harvested malting barley and oat grain in the UK. These factors included previous crop history, cultivation, variety and fungicide.
- To monitor the HT2+T2 content of oat varieties from HGCA Recommended List trials.
- To improve mycotoxin risk assessments for UK barley and oats.

3.2. Material and Methods

3.2.1. Grain sample collection

Each year 100 grain samples of oats (2006-2008) and malting barley (2007-2009), and related agronomic data were collected by crop consultants (AICC members, Agrovista, DARD and Scottish Agronomy).

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 arbitrary points around the field and combined to provide a 3 kg sample. Consultants sent these samples in cotton bags by overnight courier along with agronomic data pertaining to that field sample.

A similar number was requested from each region:

1. South
2. East
3. Midlands
4. North
5. Scotland
6. Northern Ireland

Regions were based on UK corn return regions (Fig. 1). Scottish regions were combined as a single region. North east and north west were combined, as were south east and south west.

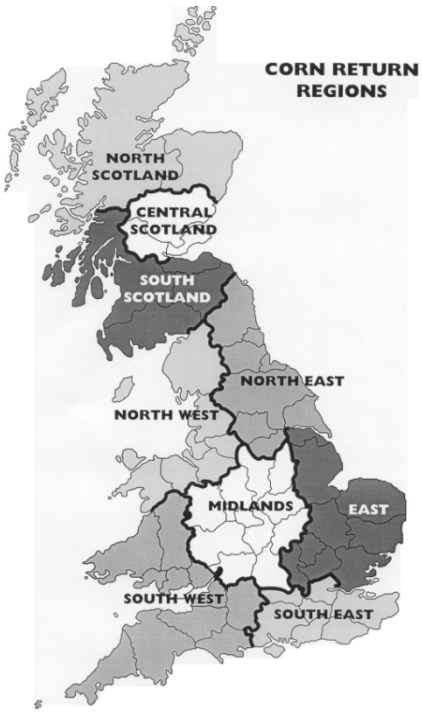


Figure 1. HGCA corn return regions.

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. A 500 g sub-sample of grain was removed using a ripple divider, dried to 12% moisture content and stored at room temperature for subsequent visual and quality assessment, if required. The remaining sample was milled with a 1 mm screen, mixed in a tumbler mixer before two 200 g sub-samples were collected. One sample was sent to Campden BRI for mycotoxin analysis, the remaining sample was held at Harper Adams as an archive sample at –20°C.

3.2.2. **Mycotoxin analysis of commercial samples**

All analysis of commercial samples was performed by Campden BRI (Chipping Campden) using UKAS accredited procedures. The trichothecenes deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-acetylDON, fusarenone X, T2 toxin, HT2 toxin, diacetoxyscirpenol (DAS) and neosolaniol and the non-trichothecene, ZON were analysed by 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 2, 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 and13% for ZON. The LoQ for the trichothecenes was 10 ppb and for ZON was 2 ppb.

3.2.3. **HGCA Recommended List oat samples**

Each year (2006-2011), single block samples (1 kg) from replicated plots were collected from each HGCA Recommended List treated (+fungicide, +PGR) oat variety trials from across the UK. On receipt of samples they were milled with a 1 mm screen, mixed in a tumbler mixer before a 200 g laboratory sample was collected. Samples were analysed using Ridascreen T2 ELISA kits (R-Biopharm, Glasgow). Based on the ratio of HT2 to T2 in UK oat samples from a previous project, the concentration of HT2+T2 was estimated.

3.2.4. **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.2010). All other statistical analysis was completed using Genstat (Lawes Agricultural Trust, v14) unless stated otherwise. Relationships between mycotoxin concentrations were determined using regression analysis. Statistical analysis to determine agronomic factors on the fusarium mycotoxin concentration of oats 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. Results are presented as bar charts of predicted means with 95% confidence limits. Upper and lower confidence limits are not symmetrical around the mean as they are calculated on $\log_{10}$ transformed values. Not all upper confidence limits are shown so differences between lower bars can be clearly seen.

For the Recommended List samples the effect of variety was tested for winter and spring oats separately using unbalanced ANOVA with trial site as a block factor and split into two datasets, 2006-2008 and 2009-2011. Individual variety predicted mean HT2+T2 concentrations were compared using LSD (p=0.05).

3.3. Results

3.3.1. Summary statistics for samples received

The target of 300 samples of oat and barley was achieved with 700 samples received and 665 samples analysed in total (Table 2).

Numbers of samples collected from all regions were reasonably balanced for oats although few samples were supplied from Scotland in 2006 (Table 3 and 4). This was corrected in later years by requesting samples from Scottish Agronomy. Low sample numbers were received from N. Ireland in all years, this resulted in very large confidence limits for mycotoxin concentrations from this region.

Table 2. Number of samples received compared to target.

Year	Barley			Oats		
	Target	**Received**	**Analysed**	**Target**	**Received**	**Analysed**
2006				100	111	111
2007	100	135	100	100	134	134
2008	100	103	103	100	117	117
2009	100	100	100			
Total	300	338	303	300	362	362

Table 3. Barley sample distribution by year and region.

Year	Region						Total
	South	**East**	**Midlands**	**North**	**Scotland**	**N. Ireland**	
2007	18	19	22	9	42	1	111
2008	17	27	26	22	39	3	134
2009	21	23	21	17	26	9	117
Total	56	69	69	48	107	13	362

Table 4. Oat sample distribution by year and region.

Year	Region						Total
	South	East	Midlands	North	Scotland	N. Ireland	
2006	22	12	28	28	3	7	100
2007	15	10	31	15	27	5	103
2008	14	10	29	17	26	4	100
Total	51	32	88	60	56	16	303

3.3.2. **Summary statistics for barley**

For comparison to previous years the four year average data for 2002-2005 is presented in Table 5. Of the ten fusarium mycotoxins analysed, five were detected in 2007-2009. Diacetoxyscirpenol, neosolaniol and acetylated versions of DON (3AcDON, 15AcDON) and NIV (fusarenone X) were not detected in any sample (LoQ = 10 ppb). DON, NIV, HT2 and ZON were occasionally detected above 100 ppb. Tables 6 ,7 and 8 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 each year. Combined values are provided for HT2 and T2 as these closely related mycotoxins have equivalent toxicity and a group Tolerable Daily Intake (0.1 µg HT2+T2/kg body weight/day) and any European limits set will be based on the combined concentration.

Table 5. Mycotoxin concentrations for all mycotoxins detected in UK barley in 2002-2005 (446 samples, 339 for ZON).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
NIV	25	<10	<10	24	45	157
DON	57	19	11	35	50	1416
15AcDON	0.5	<10	<10	<10	<10	35
3AcDON	0.2	<10	<10	<10	<10	15
FUS-X	0.7	<10	<10	<10	<10	55
HT2+T2	36	<20	<20	37	64	138
ZON	2	<3	<3	<3	6	44

Means are based on an imputation of 1.667 (0.5 for zearalenone) for all samples below the limit of quantification (10 ppb; 3 ppb for zearalenone).

Table 6. Mycotoxin concentrations for all mycotoxins detected in UK barley in 2007 (111 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90%	95%	Max
NIV	32	16	<10	33	44	365
DON	59	66	14	148	253	1002
HT2+T2	10	<20	<20	<20	30	257
ZON	18	10	<2	17	47	214

Table 7. Mycotoxin concentrations for all mycotoxins detected in UK barley in 2008 (134 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90%	95%	Max
NIV	15	10	5	17	28	206
DON	37	59	5	78	113	3599
HT2+T2	0.7	<20	<20	<20	<20	21
ZON	22	47	1	37	196	1558

Table 8. Mycotoxin concentrations for all mycotoxins detected in UK barley in 2009 (117 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
NIV	39	15	<10	38	64	122
DON	46	35	<10	65	103	707
HT2+T2	2	<20	<20	<20	<20	139
ZON	23	22	<2	14	40	1116

Means are based on an imputation of 5 (1 for zearalenone) for all samples below the limit of quantification (10 ppb; 2 ppb for zearalenone).

DON 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 (Fig. 2). HT2 was the next most common fusarium mycotoxin detected although it was never detected at a high concentration (maximum = 105 ppb). HT2 and T2 were detected in 36 and 12% of samples, respectively, the concentration was usually low with only one sample in seven years (0.1%) exceeding the proposed investigative limit of 100 ppb HT2 and T2 combined (Table 9). Zearalenone was detected in 10% of samples (LoQ = 3 ppb), only 2% of samples exceeded 10 ppb. No samples exceeded 100 ppb zearalenone. As for DON, the zearalenone distribution was also skewed (Fig. 3). Comparisons of the mean, median, 90th percentile, 95th percentile and maximum values indicates that all mycotoxin detected had a skewed distribution similar to DON and zearalenone.

Table 9. Percentage of malting barley samples exceeding 1250 ppb DON, 100 ppb HT2+T2 and 100 ppb zearalenone.

	% greater than limit		
	DON	**HT2+T2**	**ZON**
2007	0.0	0.9	3.6
2008	0.7	0.0	8.2
2009	0.0	0.9	3.4
ALL	0.2	0.6	5.1

It should be noted that the legal limits for fusarium mycotoxins include a measurement of uncertainty. Therefore, for a consignment of unprocessed barley intended for human consumption to exceed the legal limit for DON the concentration as determined by the analytical procedures employed in this project would have to exceed 1450 ppb DON (1250+16%).

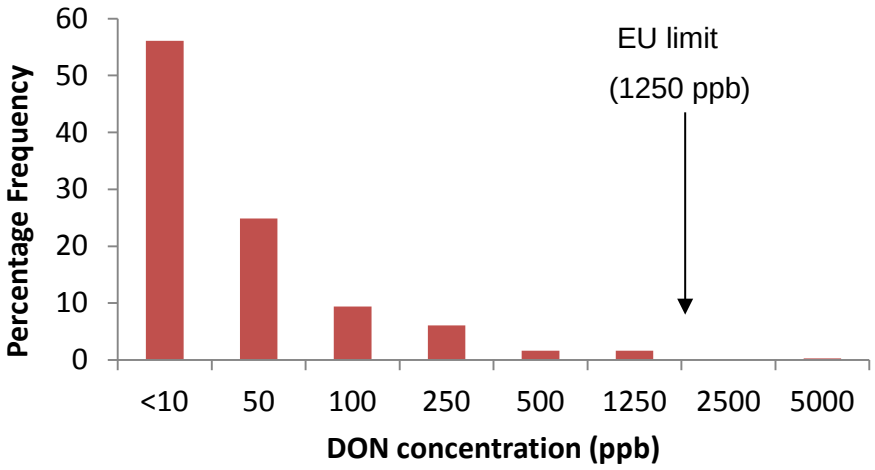


Figure 2. Percentage frequency of DON contamination in UK barley in 2002-2005 (n = 446).

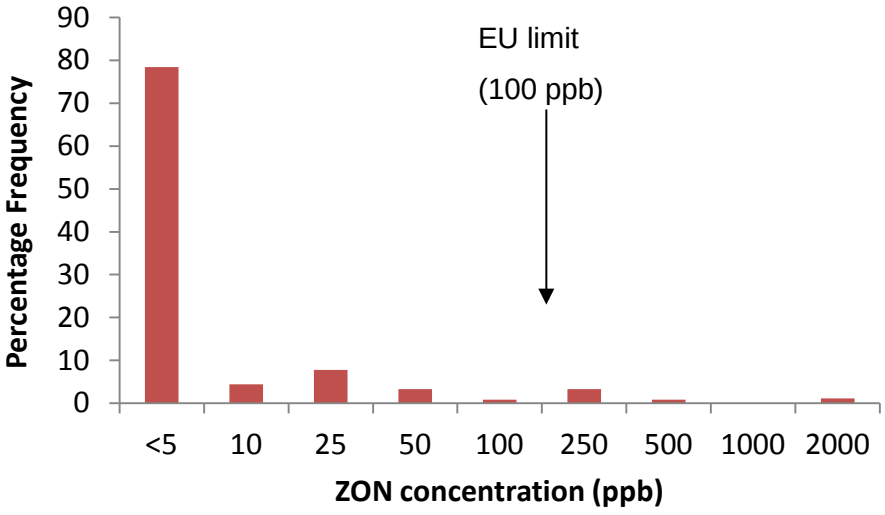


Figure 3. Percentage frequency of zearalenone contamination in UK barley in 2002-2005 (n = 339).

It should be noted that this is not a stratified survey and as such the results may not be an accurate representation of the UK situation. The selection of particular samples from specific cropping practices may bias the summary data.

3.3.3. Regression analysis for barley

Regression analysis failed to find any relationships between the concentrations of fusarium mycotoxins. This is probably due to the low incidence of many of the mycotoxins and the low concentration of the mycotoxins that were detected.

3.3.4. Statistical modelling of DON and ZON in UK malting barley

The aim of the statistical modelling was to determine the effect of agronomic factors on the fusarium mycotoxin contamination of malting barley. The methodology was as in the previous study (Edwards, 2007a), however, additional data for other agronomic factors was collected to identify the impact of these factors. Data for DON and ZON were analysed. There were too few positive samples to allow analysis of other mycotoxins.

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 (v13, 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 more beneficial for some crops).

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 and variety were all significant. As these factors were also used in the previous dataset, the two datasets were combined to give 434 malting barley samples from seven years (2002-2005 and 2007-2009).

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

For DON, the model accounted for 46% of the observed variance; 34% of variance was accounted for by year and region and their interaction. For ZON, the model accounted for 48% of the observed variance; 31% 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.

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 cereal rotation, other than as the previous crop, does not increase the DON or ZON concentration significantly and that a maize crop adjacent to a barley crop does not significantly increase the DON or ZON content of the barley crop, at the field scale.

Crop debris management, ie the baling and removal of straw, compared to incorporation had no significant effect on DON in the subsequent barley 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.

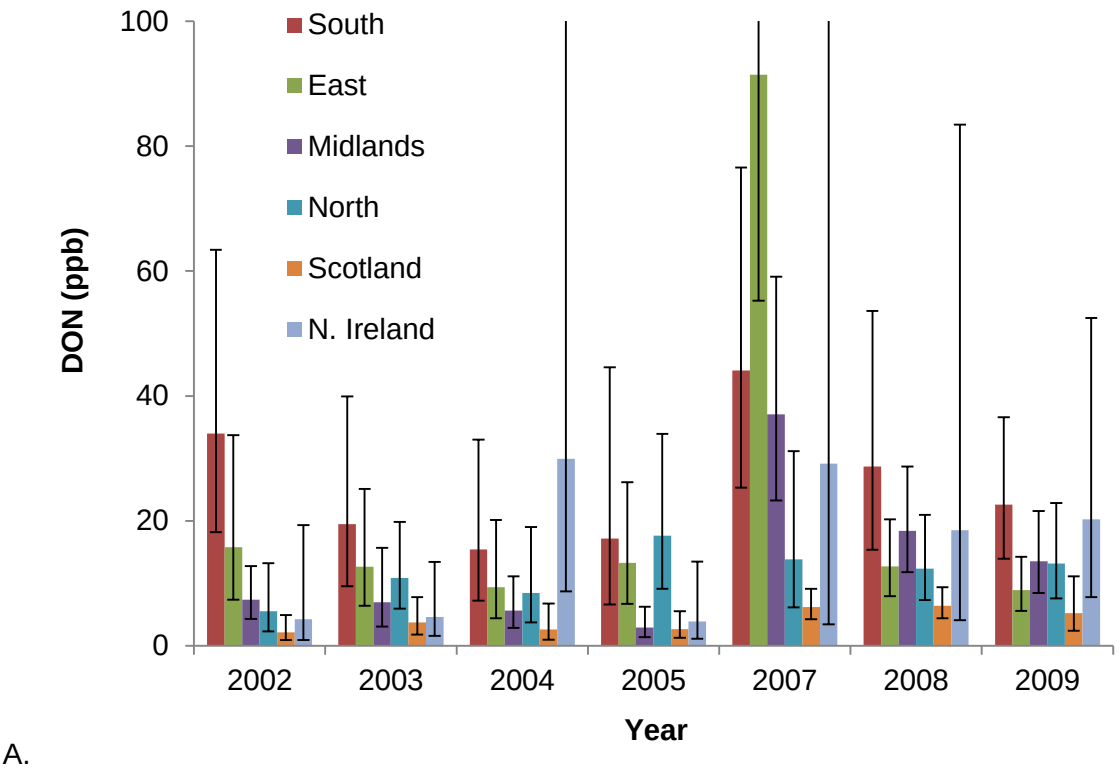
There was a significant interaction between year and region, there was a trend of DON and ZON contamination decreasing northwards (Fig. 4). The high DON and ZON levels that occurred in wheat in 2008 (Edwards, 2011) after the severely delayed harvest was less pronounced in barley as the majority of barley was harvested before the delays occurred. 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 cereal growing regions of the UK.

It was not possible to produce a satisfactory linear regression model of barley mycotoxin values and national rainfall data as the previous four year dataset had low or no detected values for all

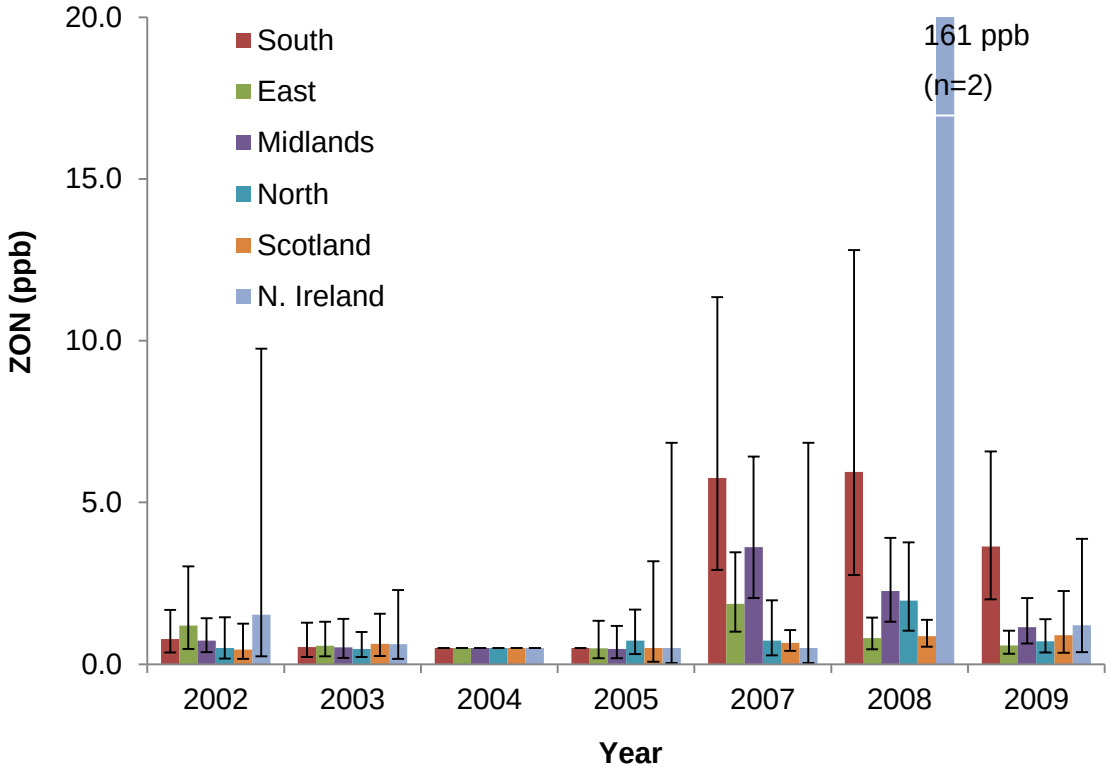
fusarium mycotoxins. The best fit non-linear regression obtained was a plot of UK mean ZON concentration against UK harvest rainfall (Fig. 5). Harvest rainfall was calculated as rainfall for July plus a contribution from August rainfall weighted by July rainfall divided by one hundred (July + [July/100]*August). This weighting increases the importance of August rainfall as more July rainfall occurs. The figure shows that ZON in barley remains low until harvest rainfall exceeds 200 mm after which there is an exponential increase in ZON concentration as rainfall increases further.

Previous crop was highly significant within both the DON ($p=0.003$) and ZON ($p<0.001$) model. Barley grown after maize had significantly higher DON and ZON contamination compared to barley grown after other previous crops (Fig. 6).

There were significant differences in the DON and ZON concentrations between varieties ($p<0.001$ and $p=0.005$ respectively; Fig. 7). These differences indicate differences in resistance to *Fusarium* do exist but care should be taken when comparing varieties using observational data, as varieties were not compared under controlled field experiment conditions with uniform disease pressure. 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; varieties Quench, Tipple and Westminster, which all had higher mean DON and ZON concentrations, only occurred in the 2007-2009 dataset which had higher overall mean DON and ZON concentrations than the previous year dataset (2002-2005), whereas Regina, which had a low mean DON and ZON concentration only occurred in 2002 and 2003. Oxbridge and Flagon, had low DON and ZON concentrations although they only occurred in the second dataset (2007-2009), which would suggest these two varieties are less susceptible to *Fusarium* compared to Quench, Tipple and Westminster.



A.



B.

Figure 4. A. DON and B. ZON contamination of barley by region for each year. Bars represent 95% confidence limits for predictions

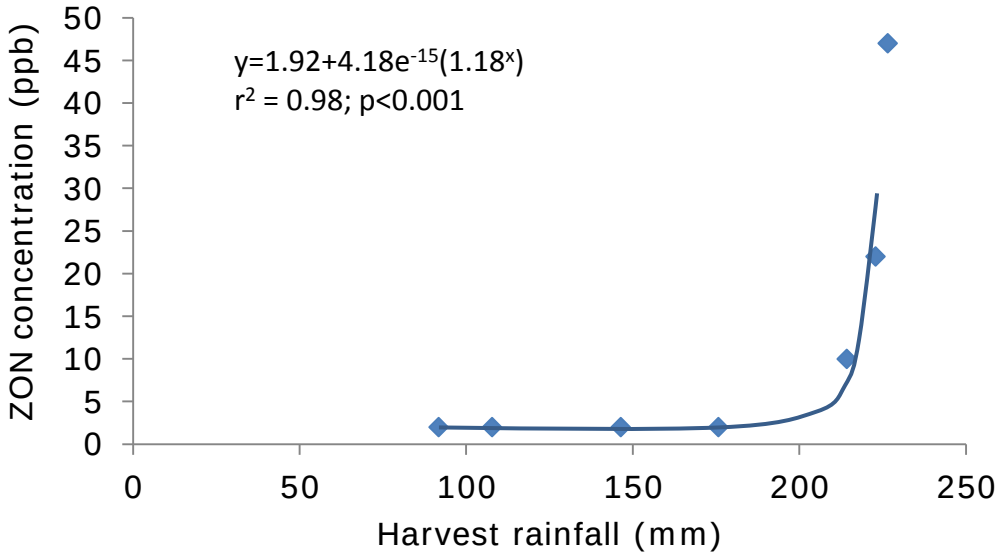
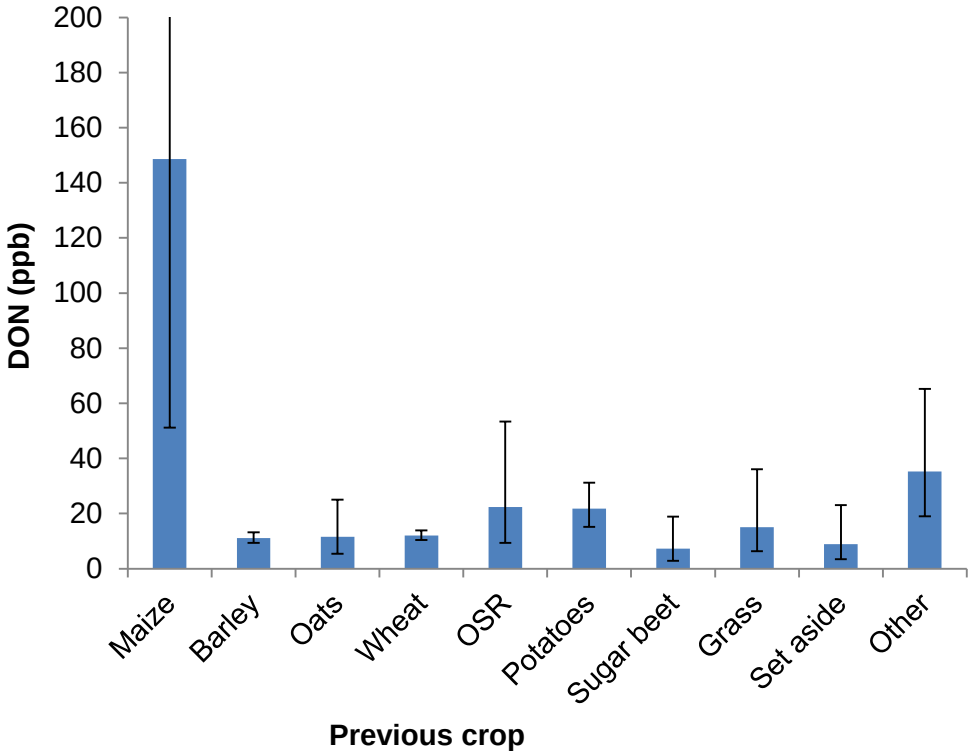
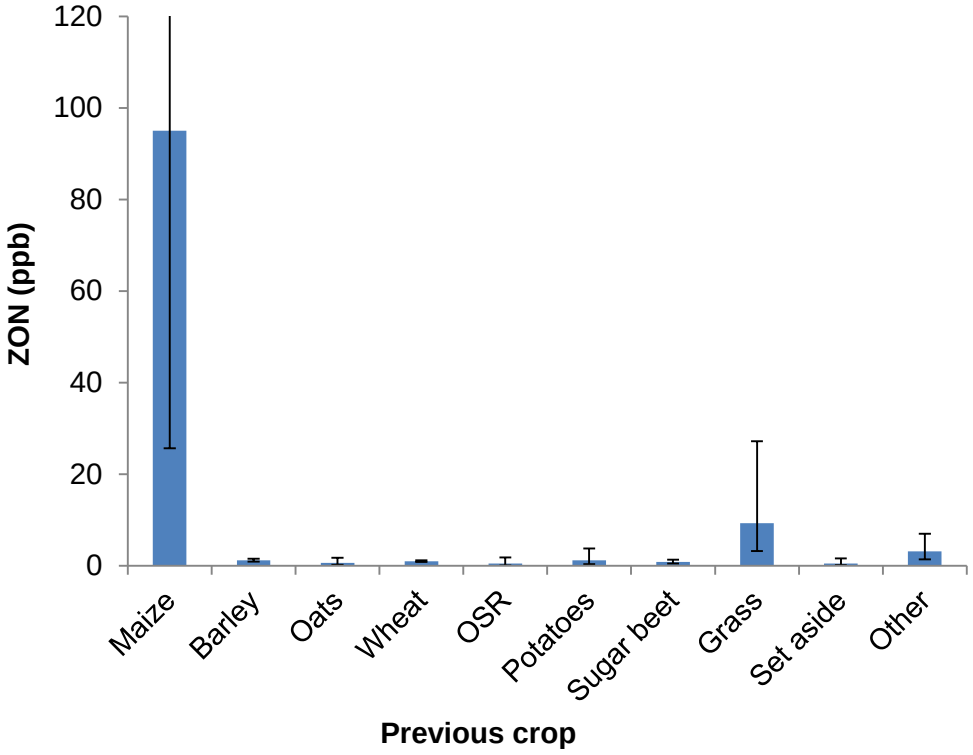


Figure 5. Mean ZON concentration against harvest rainfall for UK barley (2002-2009).



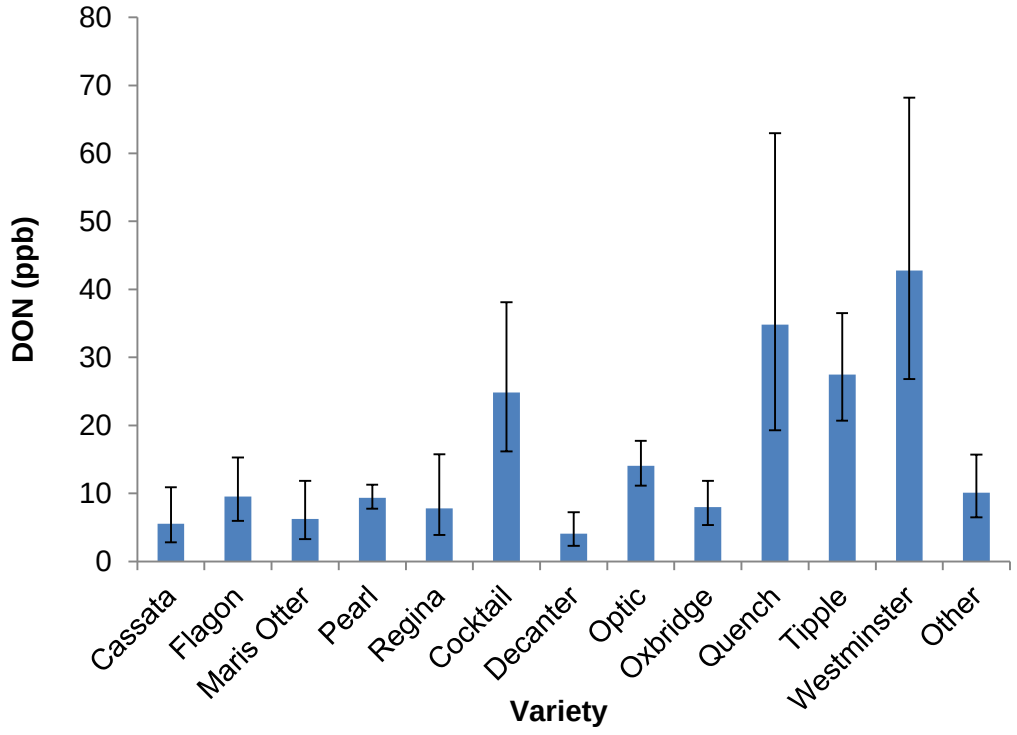
A.



B.

Figure 6. Effect of previous crop on A. DON and B. ZON contamination of barley. Bars represent 95% confidence limits for predictions.

A.



B.

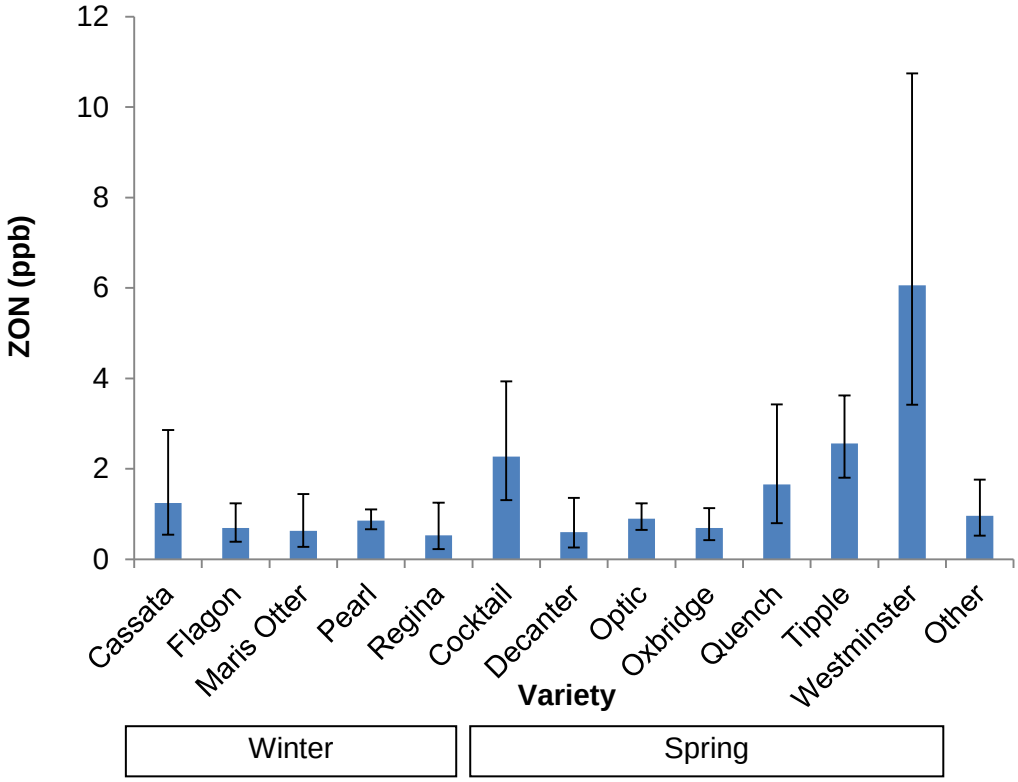


Figure 7. A. DON and B. ZON content of barley samples grouped by variety. Bars represent 95% confidence limits for predictions.

3.3.5. **Summary statistics for oats**

For comparison to previous years the four year average data for 2002-2005 is presented in Table 10. Organic samples were removed from the dataset as they represent less than 10% of commercial oat production. Of the ten fusarium mycotoxins analysed eight were detected, of these, DON, NIV, HT2, T2, T2 triol and NEO were detected above 100 ppb. Tables 11, 12 and 13 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 each year. Combined values are provided for HT2 and T2 as these closely related mycotoxins have equivalent toxicity and a group Tolerable Daily Intake (0.1 ug (HT2+T2)/kg body weight/day) and any European legislation will be set on a combined concentration.

Table 10. Concentrations for all mycotoxins detected in UK conventional oats in 2002-2005 (343 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	**Mean**	**Median**	**90th%**	**95th%**	**Max**
NIV	74	55	26	128	192	847
DON	34	15	<10	28	51	282
NEO	50	18	<10	43	57	189
T2	93	168	77	443	554	2406
HT2	97	514	208	1234	1892	7584
HT2+T2	97	682	288	1702	2438	9990
ZON	5	<3	<3	<3	<3	29

Means are based on an imputation of 1.67 (0.5 for ZON) for all samples below the limit of quantification (10 ppb; 3 ppb for ZON).

Table 11. Concentrations for all mycotoxins detected in UK oats in 2006 (100 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	**Mean**	**Median**	**90%**	**95%**	**Max**
NIV	55	42	19	100	154	427
DON	30	13	5	26	40	161
NEO	42	15	5	34	41	105
T2	95	251	135	552	865	2321
HT2	97	544	272	1271	2361	3940
HT2+T2	97	795	404	1772	3190	6261
ZON	0	<2	<2	<2	<2	6

Table 12. Concentrations for all mycotoxins detected in UK oats in 2007 (103 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90%	95%	Max
NIV	78	82	34	224	294	741
DON	26	11	5	25	42	113
FX	3	<10	<10	<10	<10	18
NEO	12	11	<10	16	31	225
T2	77	105	38	183	346	1919
HT2	88	332	123	532	1275	6480
HT2+T2	88	438	169	808	1875	8399
ZON	3	<2	<2	<2	3	22

Table 13. Concentrations for all mycotoxins detected in UK oats in 2008 (100 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
NIV	83	46	27	108	125	572
DON	39	61	<10	136	232	1866
NEO	50	12	13	21	29	47
T2	65	41	21	120	189	257
HT2	68	79	25	198	371	1005
HT2+T2	97	121	49	275	506	1213
ZON	16	24	<2	33	160	727

Means are based on an imputation of 5 (1 for ZON) for all samples below the limit of quantification (10 ppb; 2 ppb for ZON).

HT2 was the most frequently detected fusarium mycotoxin and was usually present at the highest concentration (Tables 10-13). There was a good regression relationship between this and other type A trichothecenes; T2, T2 triol and NEO (Section 3.3.6). The distribution of all mycotoxins was skewed as can be seen by the large difference between the mean and median values and as demonstrated in the frequency distribution graph of HT2+T2 (Fig. 8). Nivalenol was detected in a high percentage of samples (72% above 10 ppb) but was never present at a high concentration (>1000 ppb). DON was only an occasional contaminant of oats (32% above 10 ppb). The acetyl derivative of NIV, fusarenone X (FX), was detected at low concentrations in three samples in 2007. Acetyl derivatives of DON were not detected in any sample (LoQ = 10 ppb). ZON was rarely detected (14% of samples above 2 ppb), 6% of samples exceeded 10 ppb. The legal limit for DON (1750 ppb) and ZON (100 ppb) was only exceeded in 2008 (1 and 6%, respectively) as a result of the delayed, wet harvest that year.

The European Commission recently proposed investigative limits for HT2 and T2. The European Commission will consider further legislative measures for HT2 and T2 in cereals and cereal product in 2015. The proposed investigative limit for unprocessed oats is 1000-1500 ppb. The number of samples exceeding 1000 ppb ranged from 1-30% (Table 14). It should be noted that this was not a stratified survey and as such the results may not be an accurate representation of the UK situation.

Table 14. Percentage of oat samples exceeding 500, 1000 and 2000 ppb HT2+T2 (n=646).

	HT2+T2 (%> ppb)		
	> 500	> 1000	> 2000
2002	23	10	3
2003	41	29	8
2004	27	15	6
2005	51	30	10
2006	43	21	9
2007	18	8	5
2008	6	1	0
Overall	30	16	6

3.3.6. **Regression analysis for oats**

There was a strong positive relationship between HT2 and T2 (r^2=0.80) and between HT2 and neosolaniol (r^2=0.73) (Fig. 9 and 10). The equation for each line was similar to those for the data from the 2002-2005 dataset. These positive relationships are to be expected as these mycotoxins are all type A trichothecenes which are produced by the same species, namely *F. langsethiae, F. sporotrichioides* and *F. armeniacum*.

To determine the effect of year on the relationship between HT2 and T2, values were $\log_{10}$ transformed and grouped by year. The regression was highly significant ($p<0.001$) and was significantly different between years ($p=0.006$). Log_{10}HT2 accounted for 82% of the variance in $\log_{10}$T2 concentration whilst year accounted for only a further 2.5% of the variance, indicating that although there were significant differences in the regression between years, these differences were small; consequently the relationship between HT2 and T2 was fairly consistent during the three years of the project.

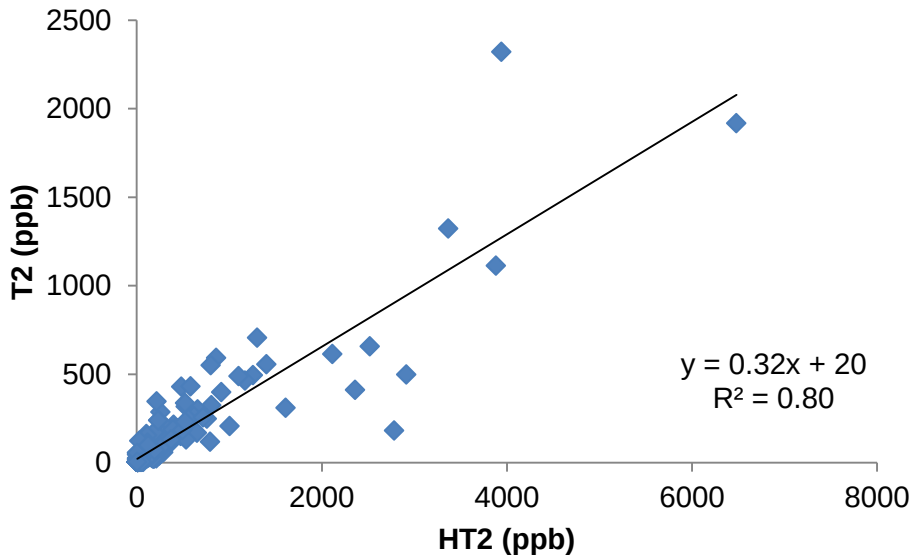


Figure 9. T2 against HT2 for oat samples from 2006-2008 (n=241). Samples with no quantifiable T2 were removed from the dataset.

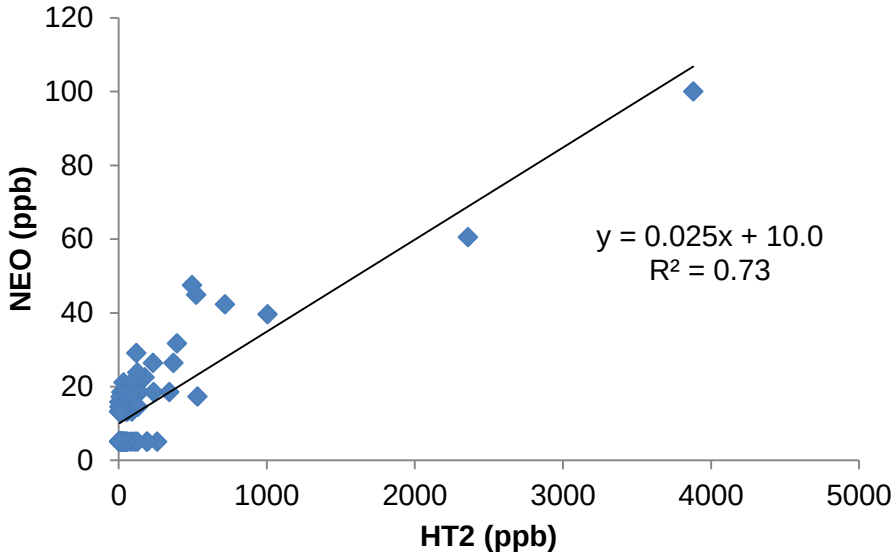


Figure 10. Neosolaniol against HT2 for oat samples from 2006-2008 (n=106). Samples with no quantifiable neosolaniol were removed from the dataset.

There are no other positive relationships between the concentrations of other commonly detected fusarium mycotoxins detected in UK oats. As with the previous dataset for 2002-2005, both NIV and DON showed signs of mutual exclusion towards HT2+T2 and towards one another, ie when one mycotoxin was present at high concentration then the other was low (Fig. 11, 12 and 13). This would suggest that DON, NIV and HT2+T2 are produced by different *Fusarium* species which have different environmental requirements or actively compete against one another within the same environmental niche.

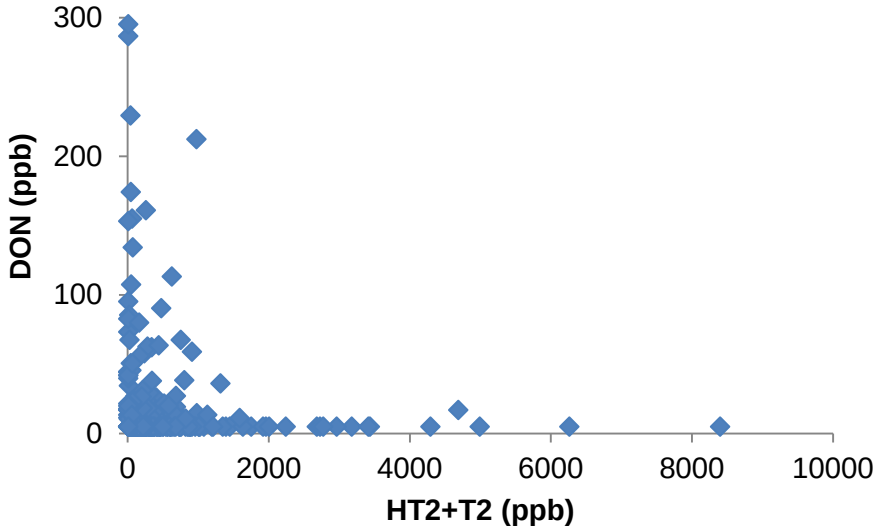


Figure 11. DON against HT2+T2 concentration for oat samples from 2006-2008 (n=303).

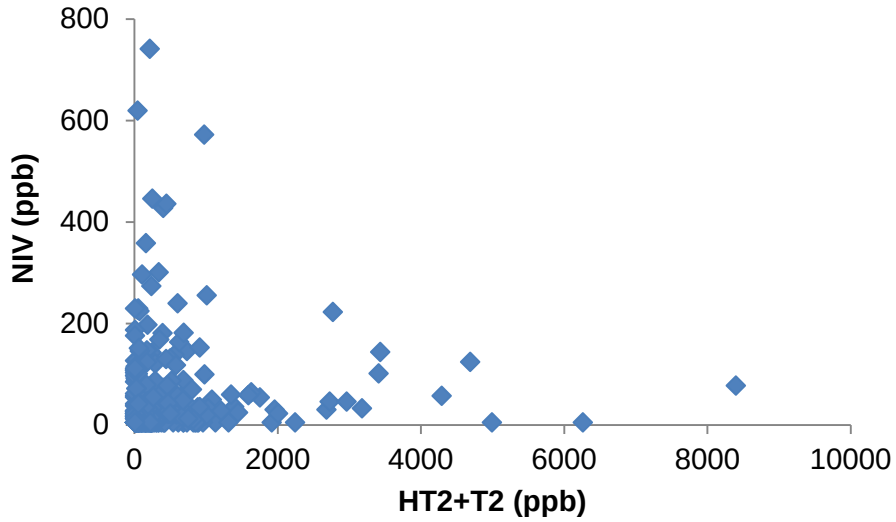


Figure 12. NIV against HT2+T2 concentration for oat samples from 2006-2008 (n=303).

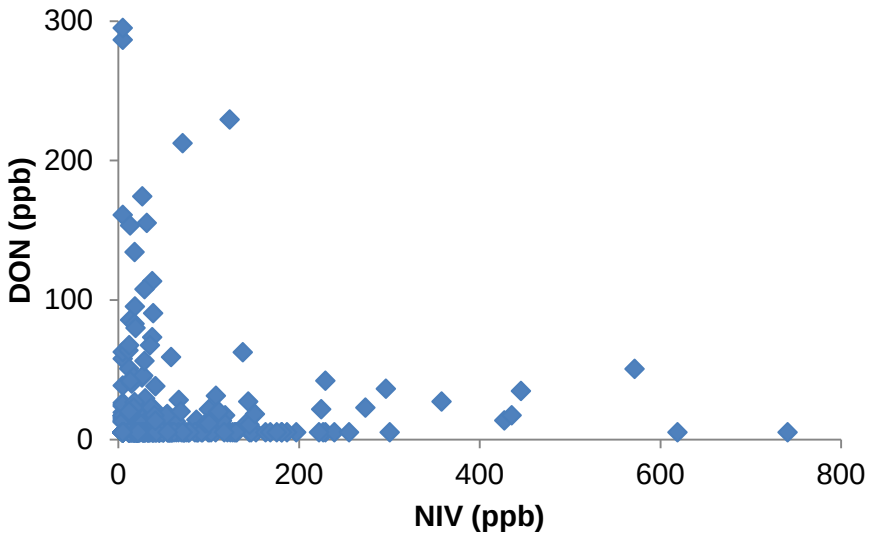


Figure 13. DON against NIV concentration for oat samples from 2006-2008 (n=303).

3.3.7. **Statistical modelling for HT2+T2 in oats**

The aim of the statistical modelling was to determine the effect of agronomic factors on the fusarium mycotoxin contamination of oats. The methodology was as in the previous study (Edwards, 2007b), 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 ppb and all samples $\log_{10}$ transformed (loga = Log10 [HT2+T2]) to stabilise the variance. Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v14, 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 ear blight epidemiology, one could expect a temporal (year) and spatial (region) interaction. As crop debris is an important parameter of fusarium ear blight epidemiology, as in the type and amount of crop debris, then one could expect an interaction between previous crop, crop debris management and the method of cultivation. For cultivation, min-till and direct drilled were combined as min-till as only five samples were supplied after direct drilling. All varieties and previous crops with less than 10 samples were entered as "Other".

Three datasets were analysed:

1. 2002-2005 and 2006-2008 combined where agronomic factors were present for both datasets.
2. 2006-2008 for new agronomic factors
3. 2006-2008 restricted to samples with full cropping history for previous four years analysed for cereal intensity and cereal sequence only.

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

Dataset 1 2002-2008

The agronomic factors entered for selection were:

- Year*region
- Intended use
- Previous crop*cultivation
- Variety
- T3 fungicide

(* indicates an interaction)

Of the factors tested, intended use and T3 fungicide were not significant (p=0.56, and 0.17, respectively). This shows that:

i) differences in agronomy in the production of oats for feed, milling or seed has no impact on the HT2+T2 content of UK oats.

ii) T3 fungicides have no measurable effect on the HT2+T2 content of UK oats. However, only 54 samples (9%) received a T3 fungicide and, therefore, there is limited ability to identify any impact.

There were significant interactions between year and region and between previous crop and cultivation. The model accounted for 42% of the observed variance. The figures below (Fig. 14, 16 and 17) 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, this is usually indicated by the large confidence limits (eg samples from N. Ireland).

There was a highly significant ($p<0.001$) interaction between year and region with no consistent trend for differences between regions (Fig. 14). Therefore, high levels could occur in any region across the UK. Year was the main factor accounting for 21% of the observed variance. There was a decline in levels from 2006 to 2008 (see Section 3.7.2). This is opposite to what occurred to DON levels in UK wheat in the same years, with lowest DON levels in 2006 and highest in 2008 due to the high summer rainfall. Annual average HT2+T2 concentrations were correlated to UK total monthly rainfall for June, July and August as single months, two months and all three months, as well as by harvest rainfall. Harvest rainfall was calculated as rainfall for July plus a contribution from August rainfall weighted by July rainfall divided by one hundred (July + [July/100]*August). This weighting increases the importance of August rainfall, as more July rainfall occurs. The best regression was obtained with annual average HT2+T2 concentrations against harvest rainfall. The regression was significant ($p=0.008$) and accounted for 74% of the observed variance (Fig 15). The regression shows a strong negative relationship with higher rainfall resulting in lower HT2+T2 concentrations. As there is no data for fusarium mycotoxins in UK oats prior to 2002, it is not possible to determine if high levels of HT2+T2 is a recent occurrence.

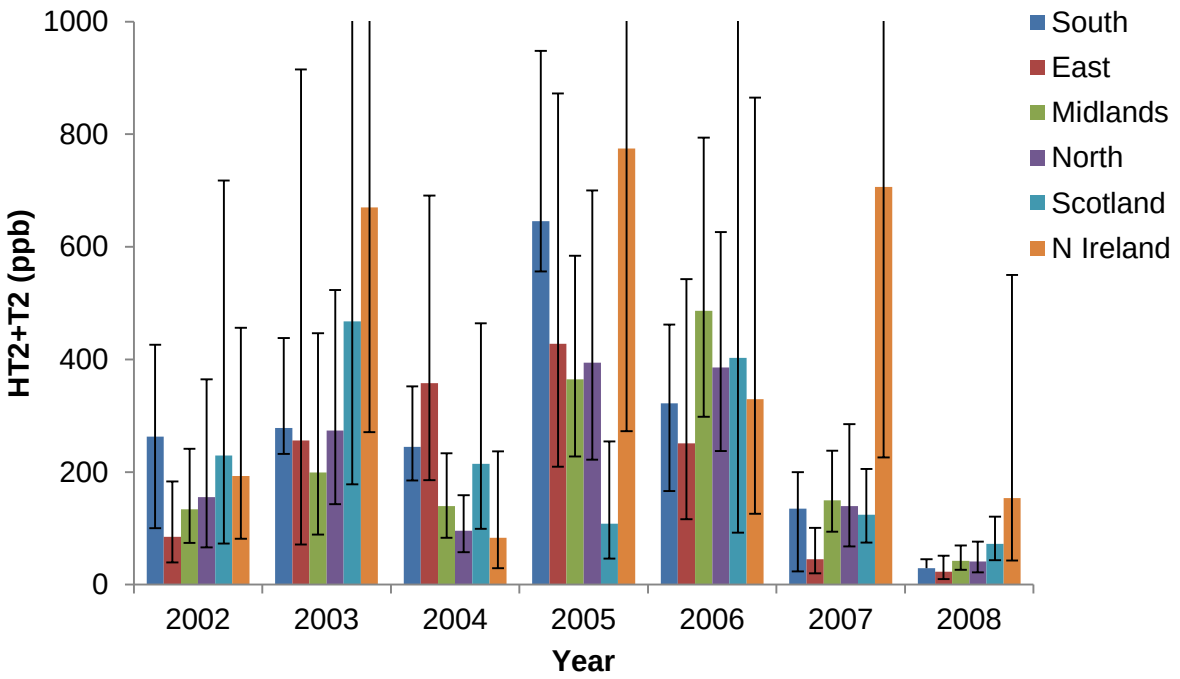


Figure 14. HT2+T2 contamination of oats by region for each year. Bars represent 95% confidence limits for predictions.

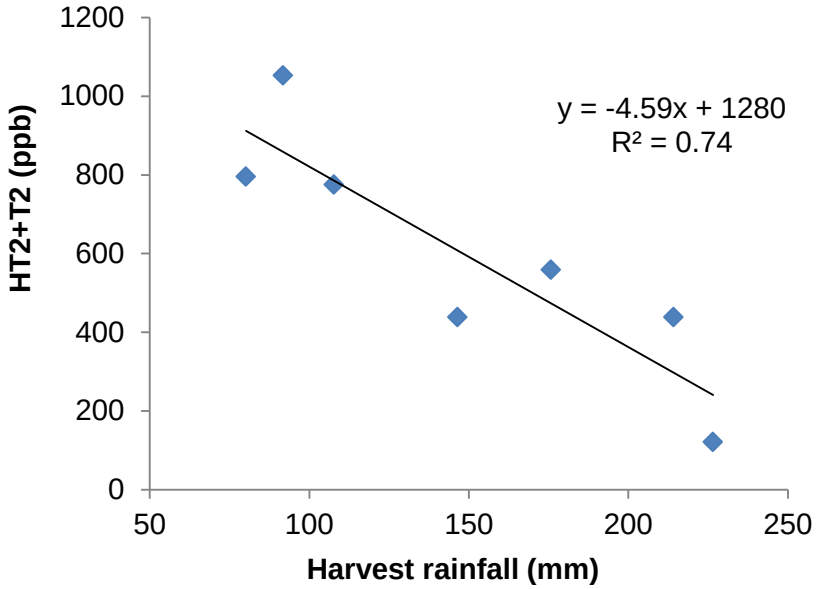


Figure 15. HT2+T2 mean concentration against harvest rainfall for UK oats (2002-2008)

Cultivation alone did not have a significant effect on HT2+T2 concentration (p=0.059). There was, however, a significant interaction between previous crop and cultivation (p=0.027) (Fig. 16). Few samples followed min-till (101 in total, 67 of which were after wheat) and as such there are large confidence limits and no significant differences between HT2+T2 concentration of oats after any previous crop and min-till. For samples after ploughing, there was no significant difference in the HT2+T2 level of oat samples after wheat, barley and oats. The HT2+T2 concentration after cereals was significantly higher than after other crops, which were significantly higher than after grass. This

data is confounded by the longer term rotation and possible interaction of cultivation with the crop two years previous. This is studied in the following section (3.7.2). For grass, it is likely that fields were in grass for several years and hence had a long break from cereals.

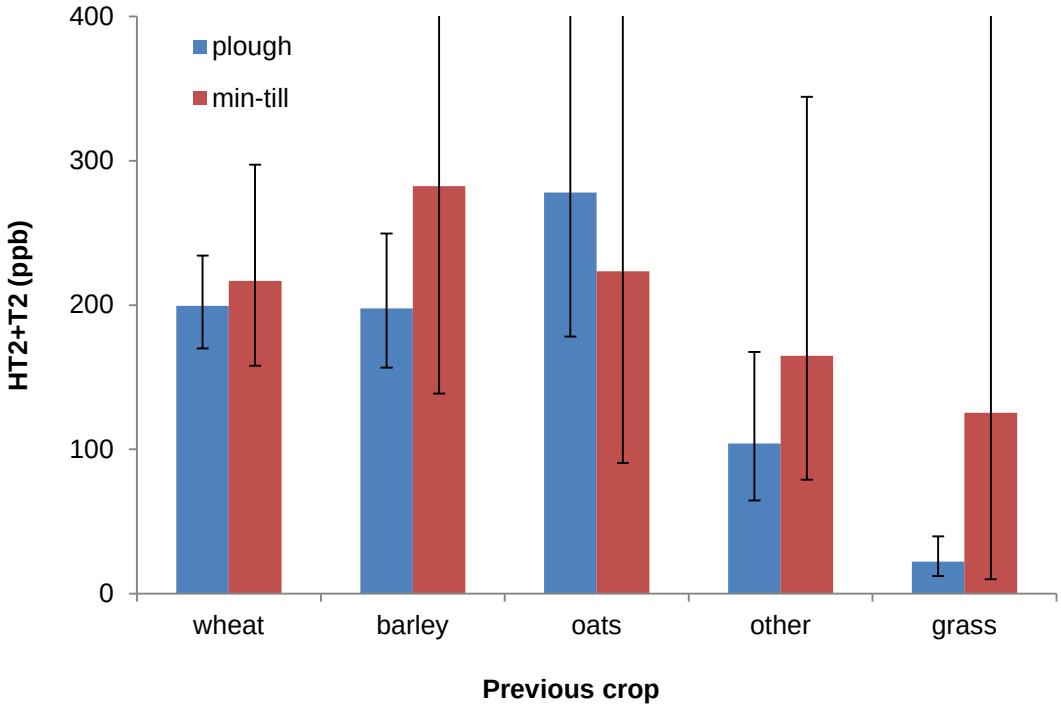


Figure 16. Effect of cultivation and previous crop on HT2+T2 contamination of oats. Bars represent 95% confidence limits for predictions.

Of the many oat varieties sampled within both projects, only eight were present in high enough numbers (>10 samples) to allow valid statistical analysis. Of these eight varieties, Gerald was the most common variety, comprising 49% of total samples, Ayr was the least common of the varieties analysed with only 13 samples. Gerald had significantly higher HT2+T2 than Jalna, SW Dalguise, Grafton, Firth and Other (Fig. 17). As this data is based on observational data, it is important to note that other factors may impact on the predicted means. In this dataset, Ayr has a high mean; this may, in part, be a consequence of when and where this variety was grown. Comparison of varieties from HGCA Recommended List trials is detailed in Section 3.8. The Recommended List trial data is more robust as varieties are compared within single fields with identical agronomy and environmental conditions and conducted over several seasons and sites.

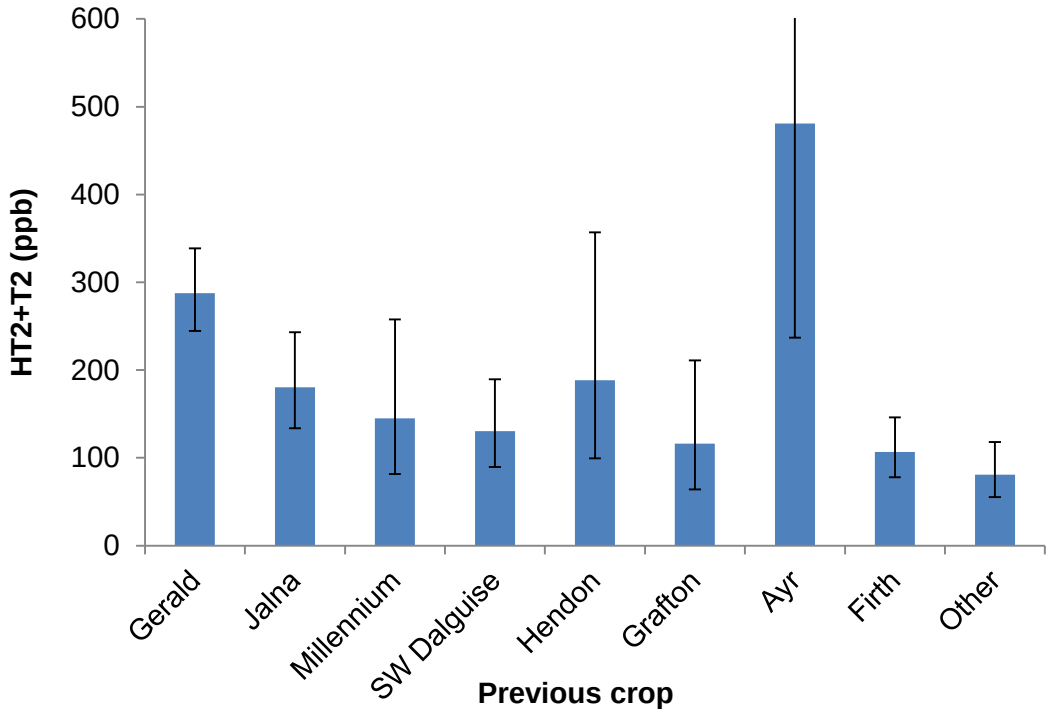


Figure 17. HT2+T2 content of oat varieties. Bars represent 95% confidence limits for predictions.

Dataset 2 2006-2008

The agronomic factors entered for selection were:

- Year*region
- Previous crop*cultivation*debris
- Previous cereal (previous year 1,2,3 and 4)
- Cereal intensity (or Cereal sequence)
- Variety
- Fungicide
- T3

(* indicates an interaction)

Various combinations of inter-related factors were entered into the model after year and region. The final model accounted for 32% of the observed variance. The figures below (Fig. 18, 19 and 20) 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, this is usually indicated by the large confidence limits.

For the three year dataset, year and region were both highly significant ($p<0.001$), while there was no year*region interaction (Fig. 18 and 19). There was decreasing levels of HT2+T2 from 2006 to 2008. 2006 was a dry summer, 2007 was a wet summer but reasonable harvest, and 2008 was a wet summer and harvest. The observed trend is the opposite experienced for DON in wheat.

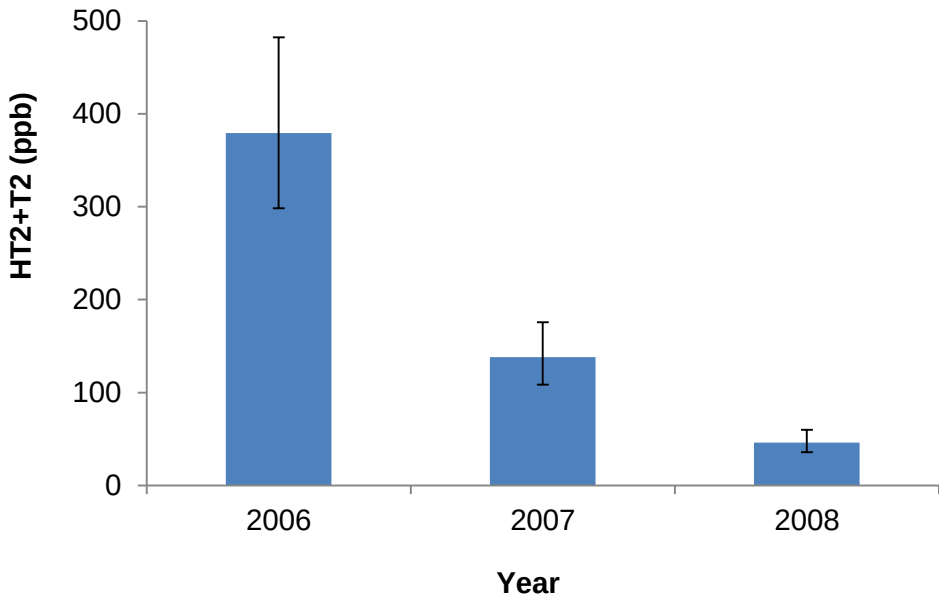


Figure 18. HT2+T2 content of oats by year. Bars represent 95% confidence limits for predictions.

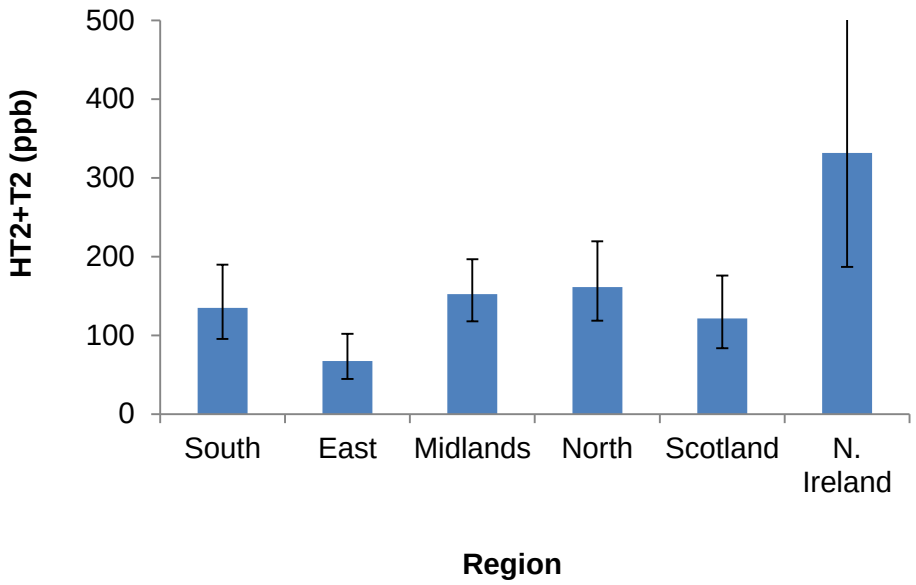


Figure 19. HT2+T2 content of oats by region. Bars represent 95% confidence limits for predictions.

The interaction of previous crop and other factors could not be analysed due to all the possible combinations with numerous previous crops, which resulted in combinations with no or very few samples. For this reason, and based on the results of the previous section, previous crops were grouped into cereal and non-cereal.

Analysis of the interaction of previous crop (cereal or non-cereal), debris management (incorporated or baled) and cultivation (ploughed or min-till) identified that debris management and all interactions with debris management were not significant. Previous crop and cultivation and the interaction of the two factors were all significant. To test previous crop history beyond one year, the

second, third and fourth year previous crops were included with all interactions. Previous crop from years three and four and all interactions containing these factors were not significant. The interaction of previous crop years one and two and cultivation was significant (p=0.005) (Fig. 20). The main significant difference identified was the consistent low levels for samples after ploughing and after two years of non-cereal. Of these 17 samples, ten were after at least four years under grass. Of more interest is comparison of the means of samples that contained cereal within at least one of the last two years, and was, therefore, within arable rotations prior to oat production. Although not significantly different for these samples, the trends would suggest important interactions exists between cultivation and the last two year's previous crops. For crops after two years of cereals, cultivation appears not to have an effect. For crops after a cereal last year and a non-cereal the year before,, the average content is lower after ploughing as the cereal debris is buried. For crops after a non-cereal last year and a cereal the year before, the average content is higher after ploughing, as the cereal debris from two years previous is returned to the surface. Again, it should be stressed that these differences are not significant and further studies would need to be conducted to prove such a relationship exists and to accurately quantify this effect.

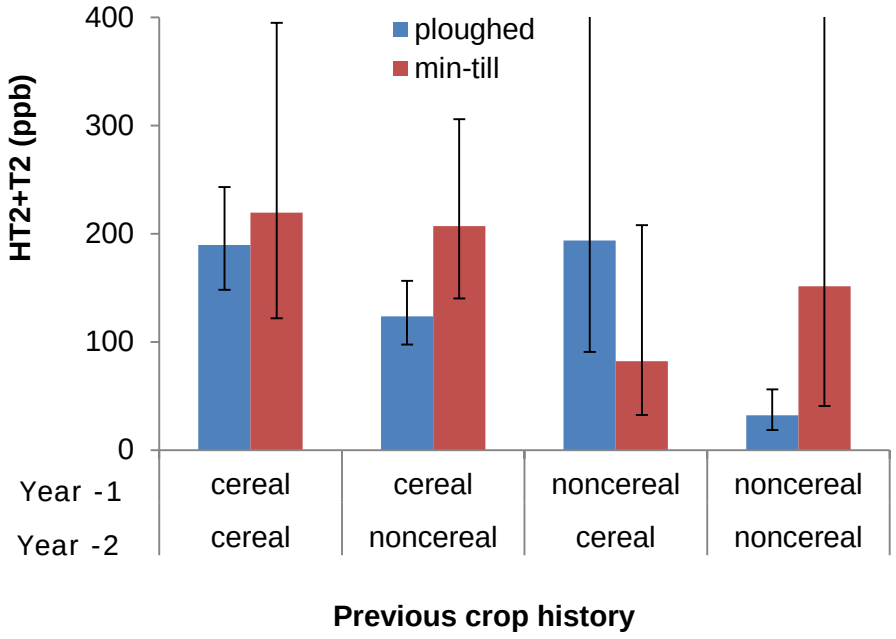


Figure 20. Effect of cultivation and previous crop on HT2+T2 contamination of oats. Bars represent 95% confidence limits for predictions.

Dataset 3 2006-2008 (restricted)

The agronomic factors entered for selection were:

- Year*region
- Cereal intensity (or Cereal sequence)

(* indicates an interaction)

Although previous crops after two years were not significant, this was tested further by including two other variations of previous crop. These were cereal intensity (number of cereal crops in the last four years) and cereal sequence (number of last four years in continuous cereal production). As several samples did not have a full cropping history for all four years, the dataset was filtered of samples lacking this information and re-analysed. Both factors were highly significant ($p<0.001$); cereal intensity accounted for an additional 6.4% of the observed variance, whilst cereal sequence accounted for an additional 4.9% (Fig. 21 and 22). For cereal intensity, a value of zero signifies a crop which was not preceded by a cereal for at least four years. For cereal sequence, a value of zero signifies a crop which was not preceded by a cereal for at least one year. For both factors, a value of greater than three indicates the crop was preceded by cereals for at least four years.

Both datasets were analysed by dose response ANOVA without blocking. Results for factor and linear were highly significant ($p<0.001$), whilst quadratic and deviation were not significant. This indicates that there is a positive relationship between cereal intensity (or cereal sequence) and HT2+T2 content of oats, and this relationship is linear; as cereal intensity (or sequence) increases then the HT2+T2 content increases.

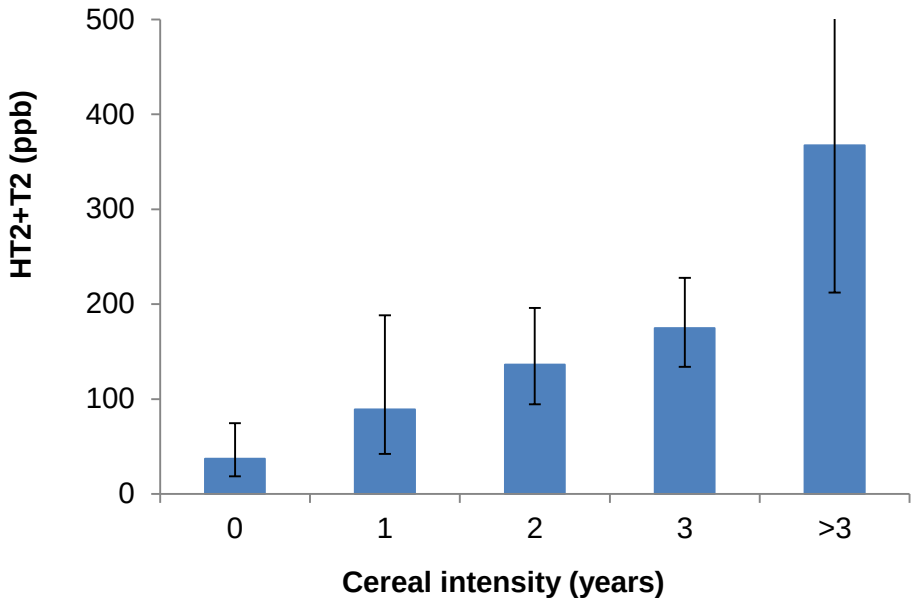


Figure 21. Effect of cereal intensity on HT2+T2 contamination of oats. Bars represent 95% confidence limits for predictions.

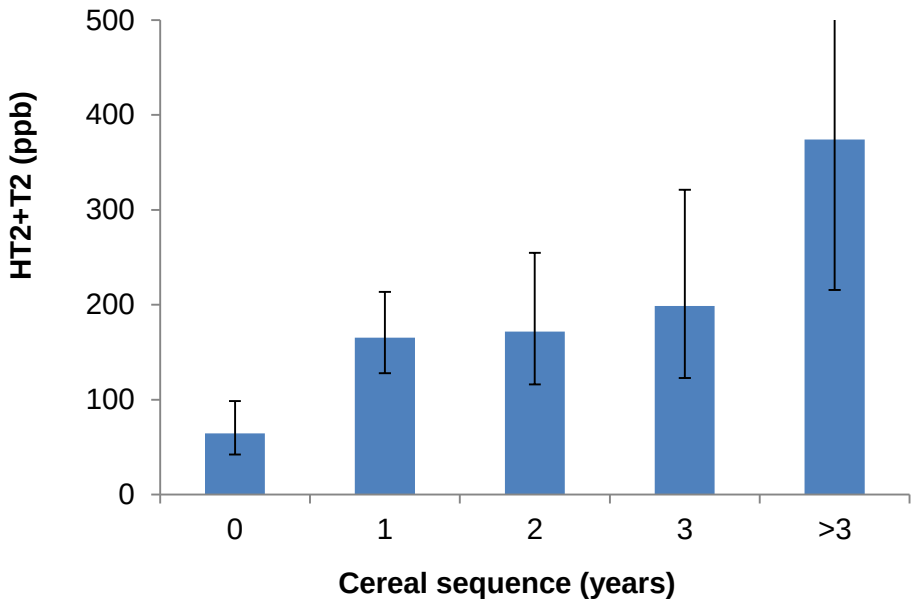


Figure 22. Effect of cereal sequence on HT2+T2 contamination of oats. Bars represent 95% confidence limits for predictions.

3.3.8. HGCA Recommended List oat variety trials

From 2006-2011, a total of 48 winter and 30 spring oat RL trials were analysed for HT2+T2 content (Table 15). The data was analysed as two datasets of three years each (2006-2008 and 209-2011). As the varieties present within RL trials change over time, the number of trials for each variety is unbalanced. As such, the difference between varieties that is statistically significant varies depending on the pair of varieties compared. For simplicity, the minimum, average and maximum least significant difference (LSD) is presented. There were highly significant differences between varieties for both the winter and spring variety trials ($p<0.001$) in the 2006-2008 dataset. For the 2009-2011 dataset, there were highly significant differences between HT2+T2 for winter varieties ($p<0.001$) but not the spring varieties ($p=0.207$). The overall results are detailed in Tables 16, 17, 18 and 19 and Figures 23-24. As can be seen. there is little difference between spring varieties with all varieties close to the overall mean HT2+T2 concentrations for both datasets (222 and 91 ppb, respectively). In comparison, there was a larger difference in HT2+T2 concentrations between winter varieties and a larger overall mean of 271 and 708 ppb, respectively.

There were consistent trends for the winter oat varieties across the two datasets, indicating that the differences in HT2+T2 concentration, which is a measure of the varieties resistance to HT2+T2 producing *Fusarium* species, is stable over time. Naked oat varieties, which lose their husk during harvesting (eg Expression and Grafton), tended to have a lower HT2+T2 content than other varieties. Hendon and Fusion, which are naked short oat varieties, had intermediate HT2+T2 content. It should be noted that Hendon is classed as a dwarf variety with a straw height of 84 cm and Fusion is a short-strawed variety with a straw height of 91 cm (HGCA Recommended Lists

2011/12 for cereals and oilseeds). Balado is a short-strawed conventional oat variety with a height similar to Fusion (91 cm). Of the current conventional height and husked varieties, SW Dalguise has had consistently low levels while Brochan and Gerald have had consistently high levels of HT2+T2 within RL trials.

Table 15. HGCA RL oat samples received.

Year	Winter oats		Spring oats	
	Trials	**Varieties**	**Trials**	**Varieties**
2006	5	11	5	8
2007	7	10	5	10
2008	3	10	3	10
2009	9	11	6	11
2010	9	10	5	8
2011	5	11	6	16

Table 16. HGCA RL Spring oat predicted HT2+T2 content based on three years data (2006-2008).

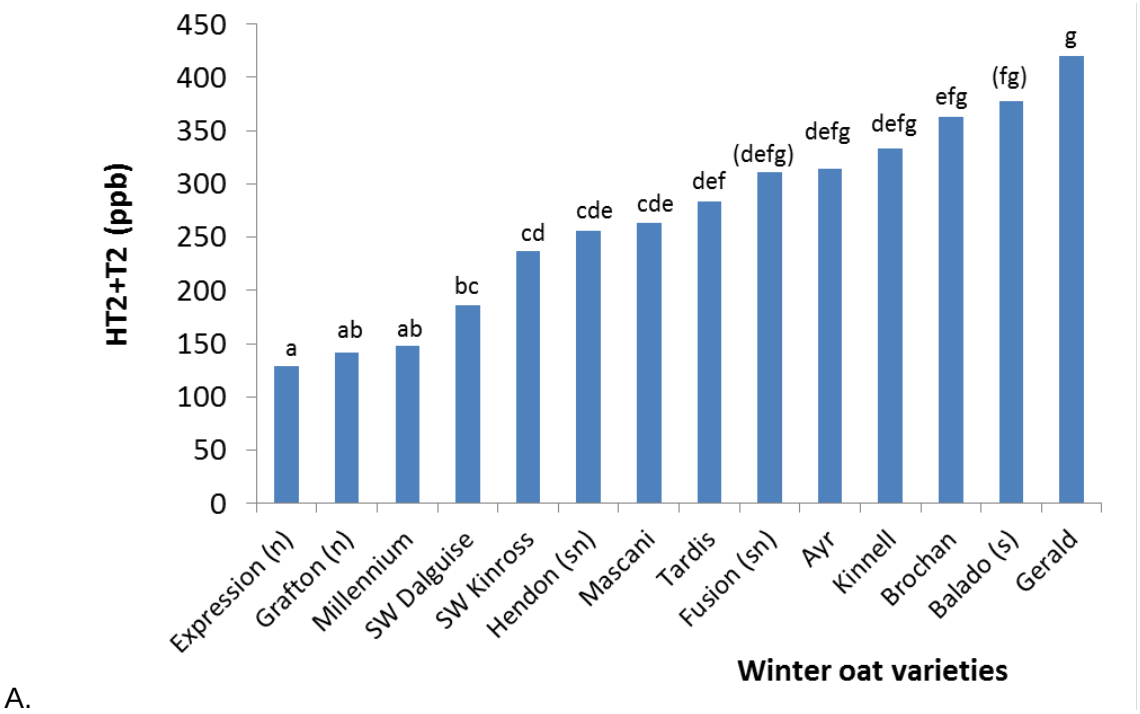
Variety	**Years**	**Trials**	**Log(HT2+T2)**	**HT2+T2 (ppb)**
SW Argyle	3	13	2.217	165
Lennon (n)	1	3	2.239	173
Zuton (n)	2	8	2.272	187
Ascot	3	13	2.283	192
Emotion	2	10	2.295	197
Carron	1	3	2.304	201
Leven	3	13	2.322	210
Husky	2	8	2.339	218
Drummer	3	13	2.392	247
Atego	3	13	2.399	251
Firth	3	13	2.462	290
Winston	2	10	2.464	291
P (DF=100)			<0.001	
Minimum LSD			0.110	
Average LSD			0.146	
Maximum LSD			0.228	

(n, naked variety)

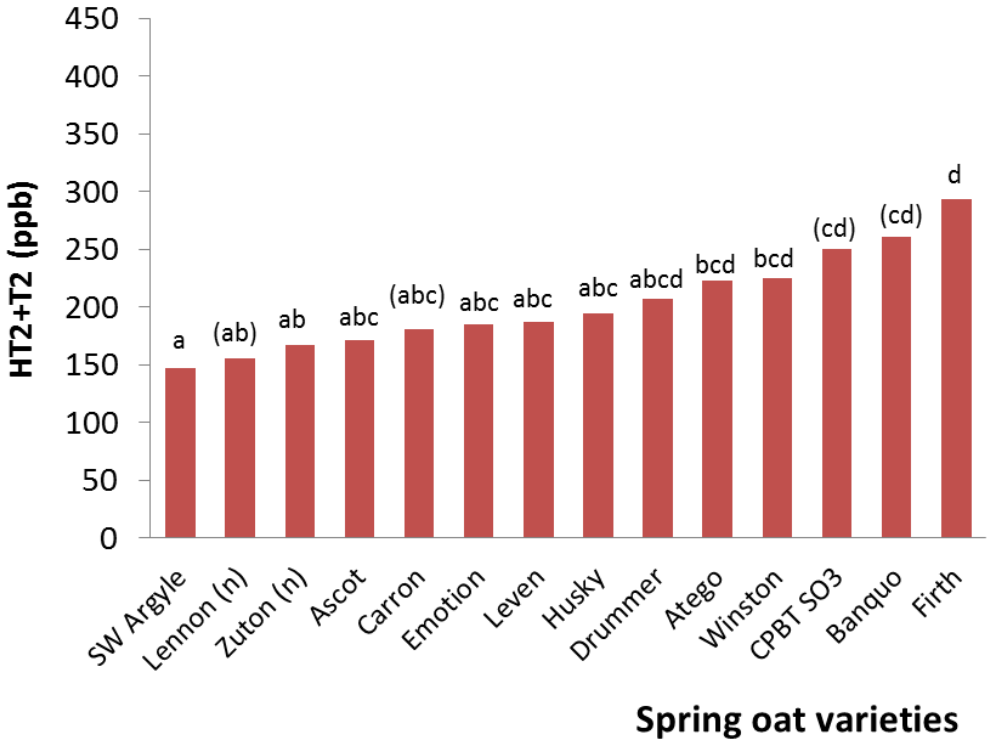
Table 17. HGCA RL Winter oat predicted HT2+T2 content based on three years data (2006-2008).

Variety	Years	Trials	Log(HT2+T2)	HT2+T2 (ppb)
Expression (n)	2	12	2.230	170
Grafton (n)	3	15	2.246	176
SW Dalguise	3	15	2.346	222
Millennium	1	5	2.347	222
SW Kinross	3	15	2.398	250
Mascani	3	15	2.415	260
Hendon (sn)	3	15	2.443	277
Tardis	3	15	2.496	313
Fusion (sn)	1	3	2.523	333
Gerald	3	15	2.557	361
Kinnell	2	12	2.569	371
Brochan	3	15	2.603	401
Balado	1	3	2.607	405
P (DF=100)			<0.001	
Minimum LSD			0.103	
Average LSD			0.137	
Maximum LSD			0.230	

(n, naked variety; s, short-strawed variety)



A.



B.

Figure 23. HT2+T2 concentration of winter and spring oat varieties from HGCA RL trials 2006-2008. After variety names: (n), naked; (s), short-strawed. Varieties with the same letter are not significantly different based on the average LSD (p=0.05). Letters in brackets from limited number of trials.

Table 18. HGCA RL Spring oat predicted HT2+T2 content based on three years data (2009-2011).

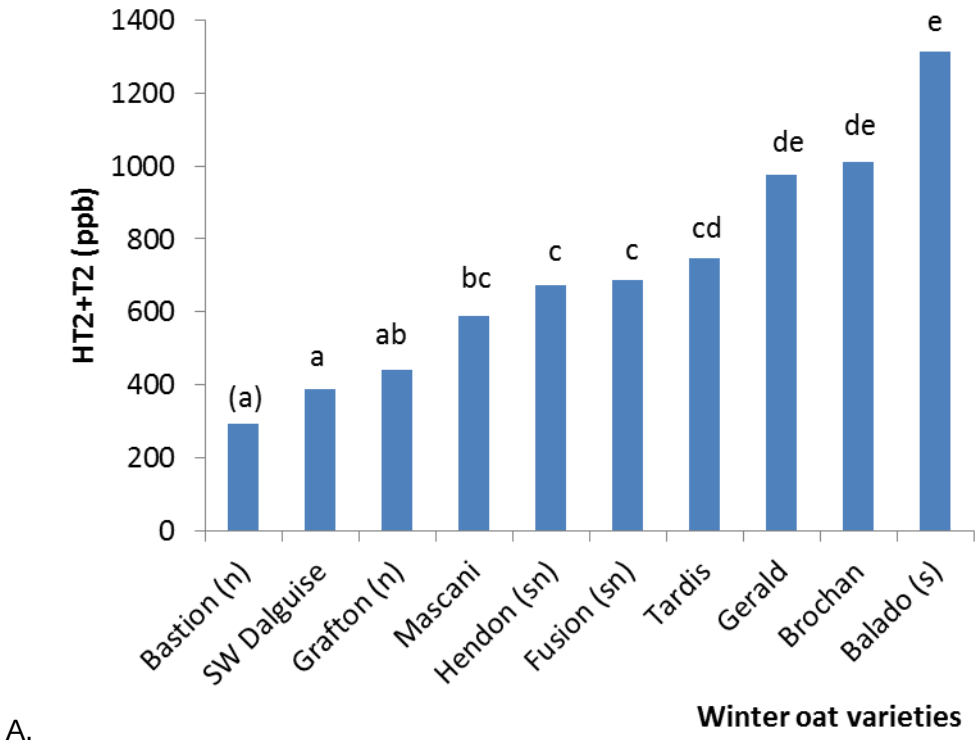
Variety	Years	Trials	log(HT2+T2)	HT2+T2 (ppb)
Circle	1	6	1.888	77
Gandalf	1	6	1.895	79
Rozmar	3	17	1.905	80
Olympic	1	6	1.913	82
Atego	3	17	1.927	85
Valene	1	6	1.929	85
Husky	3	17	1.943	88
Ascot	3	17	1.957	91
Canyon	3	17	1.969	93
Dominik	1	6	1.973	94
Leven	3	17	1.98	95
SW Argyle	3	17	1.996	99
Firth	3	17	2.018	104
P (DF=169)			0.207	
Minimum LSD			NA	
Average LSD			NA	
Maximum LSD			NA	

(n, naked variety)

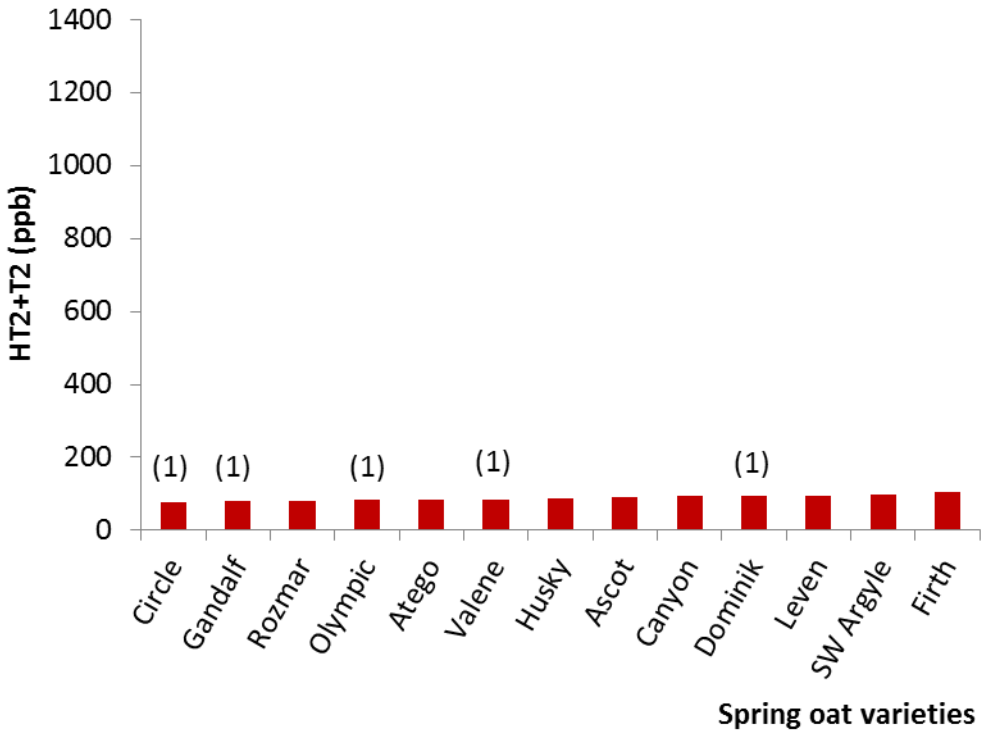
Table 19. HGCA RL Winter oat predicted HT2+T2 content based on three years data (2009-2011).

Variety	Years	Trials	log(HT2+T2)	HT2+T2 (ppb)
Mason (n)	3	23	2.416	261
Bastion (n)	1	6	2.481	303
SW Dalguise	3	23	2.587	386
Grafton (n)	3	23	2.645	442
Mascani	3	23	2.771	590
Kinross	1	9	2.788	614
Hendon (sn)	3	23	2.829	675
Fusion (sn)	3	23	2.838	689
Tardis	3	23	2.873	746
Gerald	3	23	2.989	975
Brochan	3	23	3.004	1009
Balado (s)	3	23	3.118	1312
P (DF=179)			<0.001	
Minimum LSD			0.135	
Average LSD			0.167	
Maximum LSD			0.256	

(n, naked variety; s, short-strawed variety)



A.



B.

Figure 24. HT2+T2 concentration of winter and spring oat varieties from HGCA RL trials 2009-2011. After variety names: (n), naked; (s), short-strawed. Varieties with the same letter are not significantly different based on the average LSD (p=0.05). Letters in brackets from limited number of trials. (1) data from single year only.

3.4. Discussion

Amounts of fusarium mycotoxins in UK barley samples in the previous study (2002-2005) were consistently low (Edwards, 2007b). There was an indication from previous studies that levels of HT2 and T2 may be increasing (Baxter, 2006). This, together with the fact that high levels of HT2 and T2 had been detected in French malting barley (Orlando *et al.*, 2009) warranted the continued monitoring of fusarium mycotoxins in UK malting barley. Results from this study show no indication of a rise in HT2 and T2 in UK barley. The incidence (%>10 ppb), mean and high values (%>100ppb) remained low over this three year period. Of greater concern for barley was that the wet summers of 2007-2009 all resulted in samples exceeding the legal limit of zearalenone, with an overall average of 5% for the three year period. ZON exceeded legal limits in more samples than DON in all three years. This is thought to have occurred because ZON is produced later in the season as cereals ripen (Matthaus *et al.*, 2004), and all three years within this dataset had a wet July. All three years had more than 100 mm rainfall in July compared to the 100-year-average of 73 mm.

Due to the low levels of mycotoxins detected in UK barley, there was no regression relationships between mycotoxins. Modelling of DON and ZON from the 2007-2009 dataset identified year, region, previous crop and variety as significant factors. As all these factors were present in the previous dataset (2002-2005), the dataset were combined and re-analysed. There was a significant interaction between year and region, indicating the importance of weather in the infection and subsequent mycotoxin production of *Fusarium* species. The plot of ZON concentration against harvest rainfall suggested that rainfall at this timing, primarily July, is key to ZON production. This is slightly different to what was experienced in wheat, which had a particularly high ZON in 2004 and 2008 associated with wet weather in August. This fits with the identification by Matthaus *et al*. (2004) that ZON is produced during the cereal ripening phase, which for UK barley is July and for wheat is August.

Previous crop and variety were both significant factors for DON and ZON concentration. Numerous studies have shown that maize as the previous crop increases risk for DON (Edwards, 2004). This study shows that the same is true for barley. The difference in mean DON and ZON between maize and other crops was ca. 7 and 30-fold, respectively, although it should be noted the predicted means after maize have large confidence limits as few barley samples followed maize crops (n=4).

There were significant differences between varieties but some of the differences may, in part, be due to when these varieties were in use, due to the large differences in overall mean values for the two datasets (2002-2005 and 2007-2009). However, results for five varieties that occur solely

within the later dataset do suggest that Oxbridge and Flagon have more resistance to fusarium head blight than Quench, Tipple and Westminster.

To reduce the concentration of fusarium mycotoxins in UK barley, growers should consider:

- Avoiding growing barley after maize
- Avoiding delays in harvest

Amounts of fusarium mycotoxins, DON and ZON in UK oat samples from 2002-2005 were generally low (Edwards 2009). This remained the case for 2006-2008 in this study. Legal limits for DON and ZON were only exceeded by a low percentage of samples (1 and 6%, respectively) after the delayed wet harvest of 2008. This is in stark contrast to Nordic countries, where DON has become a major problem for oat producers and processors in recent years. From 2007 to 2011, the mean DON concentration of Norwegian oats has exceeded 1000 ppb (Stokke, 2011).

There was a high incidence and high mean concentrations of fusarium mycotoxins, HT2 and T2 in UK oats identified from 2002-2005 (Edwards, 2007b). These high levels continued in 2006, which had 21% of samples above 1000 ppb. This is similar to the previous high year of 2005, which had 30% of conventional oat samples above 1000 ppb. Subsequent years experienced wet summers (2007 and 2008) and a corresponding drop in levels of HT2 and T2. These results suggest that infection and subsequent HT2 and T2 production is greater in drier summers, and as such, is opposite to what is experienced for DON and ZON in wheat and barley. This was confirmed by modelling the mean HT2 and T2 concentration against harvest rainfall. The negative relationship was significant ($p=0.008$) and accounted for 74% of the observed variance. The same relationship has been identified in several surveys (van der Fels-Klerx & Stratakou)

The positive relationships between type A trichothecenes; HT2, T2 and NEO and the exclusion relationships between HT2 and DON were reported in the last survey (Edwards, 2009) and have been reported elsewhere (Orlando *et al.*, 2009; van der Fels-Klerx & Stratakou, 2010). The good relationships between type A trichothecenes is to be expected, as they are produced by the same species on the same metabolic pathway. The exclusion relationship between HT2 and DON suggests that these mycotoxins are produced by different species, which either directly compete or prefer different environmental conditions. As the relationship with late summer rainfall is positive for DON and ZON in wheat and barley, but negative for HT2+T2 in oats, this would suggest the pathogens responsible prefer different conditions.

Modelling of HT2+T2 concentration of oat samples against agronomic factors identified a significant interaction between year and region. This is probably due to fluctuation in weather between years and regions. There was no trend from North to South, as seen for DON in wheat,

which would indicate that the temperature difference across the UK is not restrictive to HT2 and T2 production on oats. This is different to DON in wheat where there was a lower concentration in the North of Britain. Oat samples with more than 500 ppb HT2+T2 were detected in all regions of the UK.

The previous oat mycotoxin project (Edwards, 2007b) identified several agronomic factors that had an impact on the HT2 and T2 concentration of oats. However, the project included organic oat production and there were issues of multicollinearity within the dataset. This means that the dataset was clearly divided into conventional samples that tended to be after a cereal, usually wheat, and commonly the variety Gerald, whereas, organic samples tended to follow a non-cereal and was rarely Gerald. As a result, the model could identify that practice (organic or conventional), previous crop and variety were all significant factors, but it could not determine how important each factor was. Practice was still significant when placed at the end of the model which indicated that some other factor within organic production, not included in the model, was also important. It was postulated that this may be a result of differences in rotation beyond the previous crop, as organic growers tend to have long rotations with break crops between cereals. Consequently, the second dataset included information on the previous crop history for the last four years so cereal intensity within the rotation could be measured.

Agronomic factors were analysed within three datasets based on the availability of data from this and the previous oat project (Edwards, 2007b). Previous crop, cultivation and variety were all significant factors using data from 2002-2008. The trends were similar to those reported previously (Edwards, 2007b), except the mean values for non-cereals were higher, which is probably because organic samples were not included within the more recent dataset. HT2 and T2 levels were lowest in oats after grass; this is probably because such fields would have been in grass for several years and hence, had a long break from cereals. This result is also seen in the analysis of the second dataset, which was previous crop over four years in interaction with cultivation and crop management. Due to the large number of possible combinations, there were too few samples for many of these combinations so the data was analysed as previous crop (cereal or non-cereal). Only two years previous crops were significant and there was a significant interaction with previous crop and cultivations. Two years non-cereal after ploughing was lowest and was largely formed of oat samples after grass, as seen with the first dataset. Of more interest, were arable rotations with combinations including cereals. For oats following two years of cereals, the level of HT2+T2 in oats were high, irrespective of the cultivation technique used, whereas, the predicted means for HT2+T2 were higher when one of the last two years crops was a cereal, depending on when the cereal occurred and what cultivation was used. The results appear to indicate that the inoculum is largely present on cereal crop debris and the risk is increased if either the cereal debris from the last season is not ploughed in or if the cereal debris from two years previous is returned to the

surface by ploughing. The importance of cereal rotation was further analysed by cereal sequence (number of last four years in continued cereal production) and cereal intensity (number of last four years in cereal production). The results clearly show a large difference in risk for oat crops following a break crop compared to oat crops following a long succession of cereal crops. These results clearly show that the intensity of cereal crops within a rotation as well as the single previous crop are important in HT2 and T2 risk for oat crops and this factor is probably one of the main contributors to the differences identified between organic and conventional crops observed in the previous HGCA project (Edwards, 2007b). There is very limited data on the impact of rotation on fusarium mycotoxins beyond the previous crop. Schaafsma *et al*. (2005) found that the crop 2-years-previous had a significant effect on DON in wheat in 1 out of 4 years studied. Orlando *et al*. (2009) found that the crop 2-years-previous increased the variance accounted for by a mixed linear model, but was not quite significant for HT2+T2 in French malting barley.

The analysis of the impact of agronomic factors on the HT2+T2 concentration of commercial oat fields in this and in the previous HGCA project (Edwards, 2007) identified significant differences between HT2+T2 concentration in oat varieties and, where a reasonable number of samples are present, these differences have been consistent over time and consistent with results from HGCA Recommended List trials. However, HGCA Recommended List trials allow more varieties to be compared under uniform experimental conditions across several years and locations. Analysis of HT2 and T2 from HGCA Recommended List trial samples has identified that spring varieties have little difference between them. For winter varieties, the overall mean is higher than spring varieties and they have a wider range. Naked varieties had less HT2 and T2 than conventional husked varieties. This has been reported previously (Edwards, 2007b) and is thought to occur as most of the HT2 and T2 are present on the husk (Scudamore *et al.*, 2007), which is removed from naked oat varieties during harvest. Short-strawed varieties have higher levels of HT2 and T2 and naked short-strawed varieties have intermediate levels. Short-strawed varieties may have higher concentrations of HT2 and T2 as they are nearer to the source of *Fusarium* inoculum at ground level, or there may be some genetic linkage between dwarfing genes and susceptibility to HT2+T2-producing *Fusarium* species. Such linkage has been shown for some dwarfing genes in wheat (Srinivasachary *et al.*, 2008). Of the current conventional husked varieties, Gerald and Brochan have been consistently high and SW-Dalguise has been consistently low.

The opinion of the EFSA Panel was that there are currently no health concerns for HT2 and T2 (Anon, 2011b). This is based on the levels of HT2 and T2 detected in cereals and cereal products in the recent five year period (2005-2010), the estimated safe limit to consume (Tolerable Daily Intake; TDI) and food consumption data. EFSA calculated a TDI of 100 ng HT2+T2 /kg body weight/day.

There are currently no legislative limits for HT2 and T2 within the European Commission (EC). New investigative limits were proposed in May 2012. The proposed investigative limits for unprocessed barley and oats intended for human consumption is 100-200 and 1000-1500 ppb, respectively. From 2002-2008, the lower investigative limit for unprocessed barley (100 ppb) has only been rarely exceeded with an annual average of 0.9% and ranged between 0-1.6%, whilst the percentage exceeding the proposed lower investigative limit for unprocessed oats (1000 ppb) has fluctuated between 1 and 30%, with an overall average of 16%.

The proposed investigative limits for HT2+T2 in cereal products range from 10 to 75 ppb, depending on the category. The lower limit for oat-based products is 50 ppb and the lower limit for infant food is 10 ppb. During a survey of retail oat products collected in 2003, the FSA detected 5% of samples exceeded 50 ppb (Anon, 2004). In a more recent survey of food products for infant and small children collected in 2010, there was no HT2 or T2 detected (LoQ=10 ppb) in 35 oat-based products (Anon, 2011a).

The proposed guidelines for HT2 and T2 in animal feed include a limit of 1000 ppb for cereals and cereal products other than oats and 3000 ppb for oats and oat products intended for use as feed. There are also a range of proposed limits (250-2000 ppb) for compound feed dependent on the intended animal use. Based on the results of this study there are unlikely to be issues with oats used directly in animal feed. However, oatfeed (by-product from oat mills used within animal feed) has a high proportion of oat husks, and as such has a high proportion of the fusarium mycotoxins that were present in the unprocessed oat (Scudamore *et al.*, 2007; Baxter *et al.*, 2009). It is likely that some oatfeed and compound feeds which have a high proportion of oatfeed within them will exceed the proposed guidelines.

The introduction of European legislation on HT2 and T2 mycotoxins would have serious implications for UK oat production and oat processing industries based on current levels in UK cereals.

To reduce the concentration of fusarium mycotoxins in UK oats, growers should consider:

- Growing spring oats
- Growing oats in less cereal intense rotations
- Growing naked oats for animal feed
- Growing conventional winter varieties with consistently low HT2 and T2 levels (eg SW-Dalguise and Millennium)
- Avoiding delays in harvest

3.5. References

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