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Investigation of Fusarium mycotoxins in UK wheat production

by

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Glossary

DON	deoxynivalenol
FDG	*Fusarium* damaged grain
FEB	fusarium ear blight
FIG	*Fusarium* infected grain
HT2	HT2 toxin
HT2+T2	combined concentration of HT2 and T2 toxins
LoQ	Limit of Quantification
No-till	Drilling of seed directly into previous crop residue
Min-till	Non-inversion cultivation of soil before drilling
NIV	nivalenol
PGR	plant growth regulator
T2	T2 toxin
ZEAR	zearalenone

1.1 Executive summary

This five-year project started in 2001 to ascertain the effects of agronomic practices on concentrations of fusarium mycotoxins in UK wheat. It involved the collection of three hundred samples of wheat per year from fields of known agronomy over a number of seasons, which were analysed for ten trichothecenes, including deoxynivalenol (DON), and zearalenone. The mycotoxin content was modelled against the agronomic practices applied to each field to identify the impact of each agronomic factor (eg variety, cultivation and previous crop). The project anticipated the introduction of European Commission (EC) legislative limits for the fusarium mycotoxins, DON and zearalenone in cereals and cereal products. Legislative limits were introduced in 2006 for DON and zearalenone; a combined limit for HT2 toxin and T2 toxin (HT2+T2) will be introduced in the near future.

Fusarium mycotoxins are produced as a result of the disease fusarium ear blight caused by *Fusarium*. The most important ear blight pathogens are *F. graminearum* and *F. culmorum* which produce DON and zearalenone. It is known that weather conditions in the summer, particularly when the wheat crop is in flower in early summer, are critical for disease occurrence and severity.

Of the eleven mycotoxins analysed from field samples of wheat only seven were detected, of these only four, DON, nivalenol, HT2 and zearalenone were detected above 100 ppb. DON was the most frequently detected fusarium mycotoxin, present in 86% of samples, and was usually present at the highest concentration. The concentration of DON and the incidence and concentration of positive samples of HT2+T2 and zearalenone were modelled against agronomic practices applied to each field.

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

Varieties of UK winter wheat are assessed for ear blight resistance as part of the HGCA Recommended List trials. Results showed that varieties with a higher resistance had a lower predicted DON concentration. However, the current UK Recommended List has a limited range of resistance and would be classed as moderately susceptible compared to wheat varieties worldwide. There was no significant difference in the predicted DON concentration between organic and conventional samples. Within conventional samples, those which received an azole

fungicide ear spray (T3 timing) had significantly lower DON than those which received no ear spray.

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

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

The percentage of samples which would have exceeded the newly-introduced legal limits varied between 0.4% and 11.3% over the five-year period. There was a good correlation between DON and zearalenone concentrations although the relative concentration of DON and zearalenone fluctuated between years, consequently more samples would have exceeded the zearalenone legal limit than the DON limit in some years but not in others. This is probably due to the fact that DON is primarily produced in early summer whereas zearalenone is produced in late summer. The wet weather in late summer of 2004 resulted in the highest relative zearalenone-to-DON ratio and the highest percentage of samples which would have exceeded both the DON and zearalenone limits.

Overall, the risk of UK wheat intended for human consumption exceeding the newly introduced legal limits is low, but the percentage of samples above these limits will fluctuate each season depending on the weather conditions during the summer months.

Results from this and other relevant studies have been used to inform the UK Code of Good Agricultural Practice to reduce fusarium mycotoxin in cereals issued by the Food Standards Agency (Anon, 2007).

The agronomic advice is summarised below:

a) Avoid maize as previous crop
b) Minimise previous crop residue on soil surface
c) Select resistant varieties
d) Consider an ear spray to control ear blight
e) Timely harvest

1.2 Introduction

1.2.1 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. Some of these fungi that cause FEB 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; warm, humid weather then 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 once ripe (Growth Stage 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 and 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 and 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 and 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 FIG 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).

1.2.2 Fusarium mycotoxins

The trichothecene mycotoxins are produced by some of the Fusarium ear blight pathogens and their level within grain depend on weather conditions. High humidity during and after flowering is conducive to ear blight epidemics and mycotoxin production. The main method to control Fusarium ear blight in the UK is a fungicide application. A recent Home-Grown Cereals Authority (HGCA) project report (Nicholson *et al.* 2003) has shown that the azole and strobilurin fungicides have different activities towards the dominant UK ear blight pathogens, *Fusarium culmorum* (a mycotoxin producer) and *Microdochium nivale* (not a mycotoxin producer).

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 nivalenol producers. DON producers are referred to as Type 1 chemotype, this chemotype is further divided into 1A and 1B depending on the acetylated DON that is produce as a co-contaminant, 3- or 15-acetyl DON respectively. *F. poae* has also been linked to high levels of nivalenol. HT2 and T2 are Type A trichothecenes, which are thought to be produced predominantly by *F. sporotrichioides* and *F. langsethiae*.

The predominant fusarium mycotoxin found in UK wheat grain is DON. An HGCA survey of mycotoxins in UK wheat grain harvested in 1999 (Prickett *et al.* 2000; MacDonald *et al.* 2004) showed levels of DON were below the previous EC monitoring level of 750 ppb although 58% were above 50 ppb in what was classed as a year with low ear blight. In 1998, which was a severe ear blight year, grain was sampled from 53 fields of winter wheat showing severe symptoms. Although *M. nivale* (not a mycotoxin producer) was shown to be the predominant pathogen present, 50% of these samples were above 100 ppb DON and 4% were above 1000 ppb DON (Turner *et al.* 1999). Neither of these two earlier HGCA surveys analysed DON levels with respect to agronomic factors such as region, rotation, cultivation or fungicide program.

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

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

Zearalenone is another mycotoxin produced predominantly by *F. culmorum* and *F. graminearum*. Zearalenone has no known function in the fungus and is predominantly produced late in the crop growing season, near to harvest (Matthaus *et al.* 2004). Zearalenone has low cellular toxicity but is problematic as it has high oestrogenic 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).

1.2.3 Fusarium mycotoxin legislation

The European Commission (EC) has set legislative limits for the fusarium mycotoxins including the trichothecene, deoxynivalenol (DON) and zearalenone in cereal grains and cereal-based products intended for human consumption except maize (Table 1.2.1) (Anon 2005). Limits will also be introduced for the trichothecenes, HT2 and T2 combined and fumonisins in maize in the near future.

Table 1.2.1 Maximum limits for DON and zearalenone 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. 2006). The lowest guidance limits have been set for pigs owing to their higher sensitivity to fusarium mycotoxins. The DON guidance value for complementary and complete feedingstuffs for pigs is 900 ppb. The zearalenone guidance value for complementary and complete feedingstuffs for sows and fattening pigs is 250 ppb and for piglets and gilts is 100 ppb.

1.2.4 Effects of agronomic factors

Previous research, primarily in North America and elsewhere in Europe has identified a number of agronomic factors which can affect the concentration of fusarium mycotoxins in harvested cereals. 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.

Results from previous studies are summarised below into categories of various agronomic factors.

Crop rotation

Numerous studies have shown that FEB and/or DON in wheat are affected by the previous crop. Maize is a major host of *F. graminearum*, which is the most potent producer of deoxynivalenol and zearalenone. An early observational study of wheat fields (n=28) in Illinois showed that a higher incidence of FEB occurred in wheat after maize and in particular wheat after a succession of two maize crops, and in wheat following grain maize compared to silage maize (Holbert *et al.* 1919).

There is also anecdotal evidence from the epidemic years of FEB in the USA from 1991-1996 when high levels were attributed to a high proportion of cultivated land under min-till and planted to susceptible host crops and short rotation intervals between susceptible crops (McMullen *et al.* 1997).

In Ontario, Canada in 1983, fields where maize was the previous crop (n=5, FEB incidence = 0.036%) had a significantly higher incidence of FEB than fields following small grain cereal (n=4, FEB incidence = 0.007%) or soybean (n=13, FEB incidence = 0.005%) (Teich and Nelson 1984). In a repeated study the following year, fields where maize was the previous crop (n=7, DON = 657 ppb) had significantly higher DON than fields following a crop other than corn (n=14, DON = 54 ppb) (Teich and Hamilton 1985). Sturz and Johnston (1985) found higher levels of FEB in wheat following wheat rather than wheat following fallow.

In replicated field experiments in Minnesota, previous crop and tillage were compared in a three-year factorial experiment (Dill-Macky and Jones 2000). On average, the DON concentration was 25% lower in wheat following soybean compared to wheat following wheat, and 50% lower in wheat following soybean compared to wheat following maize. *Fusarium* species were isolated from all crop residues. *F. graminearum* was the predominant species present on maize and wheat residues whereas other *Fusarium* species, in particular *F. sporotrichioides* predominated on soybean residues.

In the 1990s a large observational study of FEB and DON was conducted in Bavaria, Germany (n=1600). On average, wheat following grain maize had the highest DON concentrations (mean ca. 500 ppb), followed by wheat following silage maize (mean ca. 300 ppb). It is proposed that this difference was due to the higher quantity of crop residue present after harvest of grain maize (Obst *et al.* 1997). However some of this difference maybe due to differences in maize variety susceptibility or harvest dates. This study also showed that DON concentration was lower in wheat following wheat, barley or OSR (means ca. 100 ppb) compared to wheat following potatoes or

sugar beet (means ca. 200 ppb). The authors postulated that this may be due to the later sowing of wheat following potatoes or sugar beet. A large replicated field experiment in Germany identified that wheat following wheat had a higher FEB incidence and DON concentration compared to wheat following sugar beet. There was also a significant interaction with cultivation technique (Koch *et al.* 2006). The difference between the two German studies above may be due to differences in agronomy, such as sowing date (as postulated in the observational study), which were standardised in the field experiment.

In an observational study performed using commercial fields (n=233) in Canada from 1996 to 1999 (Schaafsma *et al.* 2001) identified significant lower DON in wheat following soybeans or wheat compared to wheat following maize. Three percent of the variance (P=0.05) was accounted for by the crop two years previous. In New Zealand, an observational study (n=53) determined that higher levels of DON occurred in wheat after maize (mean = 600 ppb) and after grass (mean = 250 ppb) compared to small grain cereals (mean = 90 ppb) and other crops (mean = 70 ppb). Highest levels were recorded in wheat-maize rotations (Cromey *et al.* 2002).

Cultivation

In the 1990s a large observational study of FEB and DON was conducted in Bavaria, Germany (n=1600). On average, DON concentration of wheat crops after maize was ten-times higher if the field was min-tilled compared to ploughed (Obst *et al.* 1997). An observational study performed using commercial fields (n=233) in Canada from 1996 to 1999 (Schaafsma *et al.* 2001) determined that tillage was only a significant factor (P=0.004) in one year, 1997, when it accounted for 16% of the variation observed. In 1997, wheat DON concentration after min-till was 1300 ppb, after no-till was 700 ppb and after ploughing was 500 ppb.

Large replicated field trials in Germany identified that there was a significant interaction between previous crop and cultivation technique (Koch *et al.* 2006). Following sugar beet there was no significant difference in DON concentration between wheat plots receiving different methods of cultivation, however, following a wheat crop without straw removal, direct drilled wheat had a significantly higher DON compared to wheat from plots which were either ploughed or min-tilled (Koch *et al.* 2006)

Studies in France have determined that crop debris management can have a large impact on DON concentration at harvest, particularly after maize. Highest DON concentration was found after no-till, followed by min-till and then lowest levels after ploughing. The reduction in DON has been linked to the reduction in crop residue on the soil surface. However, the reduction in DON with min-till, compared to no-till is usually greater than the reduction of crop residue on the soil surface (Labreuche *et al.* 2005; Maumene 2005). This is probably due to the fact that min-till increases the colonisation of crop debris with soil saprophytic microorganisms, which compete with *Fusarium* species. Chopping of maize debris before minimum tillage also caused a marked decrease in DON concentration in the following wheat crop (Maumene 2005), again this is likely to increase the mixing of crop debris with soil.

Crop nutrition

No significant differences in FEB incidence, resulting from either different rates of application or the form of nutrient application were reported (Teich and Hamilton 1985). In a previous study they had identified that soils high in phosphorus and nitrogen had a lower incidence of FEB (Teich and Nelson 1984). In both studies, urea was associated with lower FEB incidence compared to ammonium nitrate, but this was not significant in either year. Results from split field experiments performed in 1985 and 1986 identified significantly lower FEB incidence in wheat receiving urea rather than ammonium nitrate as a source of nitrogen. The incidence of FEB was about 30% lower with urea treatment (Teich 1987). In a large survey conducted in Saskatchewan, Canada from 1999 to 2002 (n=659), nitrogen fertiliser had no impact on FEB infection.

A replicated factorial experiment of nitrogen source and rate identified higher FEB with natural infection occurred with increasing rate of nitrogen applied from 0 to 160 kg ha^{-1} for all forms of nitrogen used. The form of nitrogen, both inorganic (ammonium nitrate urea and ammonium nitrate) and organic had no significant effect (Lemmens *et al.* 2004). A repeated study with artificial inoculation provided similar results with a ca. two-fold increase in DON after an application of 160 kg ha^{-1} ammonium nitrate urea (Lemmens *et al.* 2004). The fact that an artificially inoculated trial gave similar results indicates that nitrogen rate does not affect inoculum production or dispersal to the ear. The authors postulated that nitrogen can modify crop canopy, and thus alter the microclimate or can lead to extension of the flowering period, during which the crop is most susceptible to infection. The fact that high nitrogen is required to produce agronomically viable yields and quality (ie protein content) means modification of nitrogen inputs is not a valid method of reducing DON.

It should be considered that nitrogen inputs above the optimum increases the risk of lodging, which will result in an increased risk of high DON in harvested grain (see Section on PGR and Lodging).

Fungicides

Fungicide seed treatment

Few experiments have shown the ability of a fungicide seed treatment to reduce FEB or fusarium mycotoxins at harvest. This is probably because most experiments are performed on small plots and spread of inoculum between plots over the growing season results in no significant differences later in the season (eg (Sturz and Johnston 1985; Schaafsma and Tamburic-Ilincic 2005). One observational study by Teich and Hamilton in Ontario, Canada in 1984 (Teich and Hamilton 1985) showed a significant reduction in ear blight incidence after seed treatment (n=10, mean %FEB incidence = 0.091) compared to fields with no seed treatment (n=3; mean %FEB incidence = 0.144) in fields of wheat following maize.

As seed treatments reduce the amount of fusarium present on the stem base of cereals during early growth stages this could reduce the amount of inoculum present. However, there is much evidence to suggest that crop debris is the main source of

inoculum and therefore fungicide seed treatment is likely to be of only occasional benefit; ie where seed borne infection is the main source of infection within a field. In a series of experiments over five years it was determined that severe FEB only occurred at a UK site when local inoculum was present (Bateman 2005). Infected seed did not result in increased FEB incidence when tested under conditions that resulted in increased FEB after application of infected crop debris (two of two years). In a study of organic and conventional production at three sites over three years, there was no correlation between the incidence of *Fusarium* species on seed and in the resultant grain at harvest (Birzele *et al.* 2002).

Foliar fungicides

No published evidence found regarding the benefit of foliar fungicides either at stem extension (T1 timing) or flag leaf (T2) growth stages have any benefit at reducing FEB or DON in harvested grain. Very little *Fusarium* was found on the stem-base of wheat at stem extension in the UK during a three year, three site experiment (Nicholson *et al.* 2002) indicating that reducing fusarium on the stem base is likely to have little, if any effect on reducing FEB later in the season.

Ear fungicides (T3 application)

Numerous studies have been conducted to identify the extent to which fungicides applied during flowering can reduce FEB and subsequent DON in harvested grains. The factors determined to be important are the fungicide used, the rate and the timing of application. Most experiments are conducted with inoculation of the crop with *Fusarium* spores and mist irrigation to ensure severe FEB occurs. The most recent, independent studies performed in the UK were performed by (Nicholson *et al.* 2003) over three sites and three years. Results from this study identified that the azoles, tebuconazole, metconazole and prothioconazole significantly reduced FEB symptoms and fusarium mycotoxin concentrations. At full rate, the greatest reduction in DON concentration occurred with prothioconazole (10-fold). Efficacy was reduced as dose was reduced. Azoxystrobin had little impact on mycotoxin concentration in harvested grain when *Fusarium* species dominated the site but could result in an increase in mycotoxin concentration in grain when *M. nivale* was the predominant species present. The ability of azoxystrobin to result in an increase in FEB and DON concentration in harvested grain has been reported on a number of occasions (Mesterhazy *et al.* 2003; Ioos *et al.* 2005). Fungicide mixtures of azoxystrobin and an azole resulted in a lower reduction of DON compared to an azole alone (Edwards *et al.* 2001; Nicholson *et al.* 2003). A number of trials in Germany have indicated that some strobilurin fungicides applied before anthesis can also result in increased DON compared to unsprayed plots (Ellner 2006).

Reductions in DON observed in field experiments using fungicides against natural infections of *Fusarium* are lower and inconsistent (Simpson *et al.* 2001; Ioos *et al.* 2005) . On average, a two-fold reduction was observed in large-scale field experiments in Germany from a full rate of tebuconazole (Koch *et al.* 2006). This is probably because during natural infection, infection occurs over a greater period of time. In trials with spray inoculation the application of pathogen and fungicide are synchronised. Studies have shown that fungicide application must be close to inoculation time (± 2 days) for optimum control (Nicholson *et al.* 2003).

Several studies have been conducted on the application of fungicides to ears of wheat. Application can be modified by choice of spray volume, spray pressure, nozzle selection, sprayer type and tractor speed. All of these factors can interact with the spray conditions at the time of application (wind speed and direction) to affect the disposition of fungicide on the ear. Standard spray applications tend to result in an uneven disposition of fungicide on either side of a wheat ear (Nicholson *et al.* 2003). Double fan nozzles appeared to provide better coverage than single nozzles, and consequently less FEB, although this was not statistically analysed.

Insecticide use and insect transmission

Few studies have identified a role of insects in the transmission or infection of *Fusarium* species. *Fusarium* species were found on a wide range of insects, indicating that they can act as a vector (Miller *et al.* 1998; Mongrain *et al.* 2000). It has been determined that *F. graminearum* could be found at low incidence on wheat blossom midge and that under laboratory conditions could be transmited to wheat plants resulting in FEB infection. The low incidence of *F. graminearum* on midges would suggest this is not a major route of infection.

Herbicide use and weed density

There are conflicting results as to the impact of herbicide use and weed density on FEB and DON concentration in harvested grain.

Fusarium species were isolated from 14 of 15 broad leaf weeds surveyed on three fields in fallow in the UK. *F. culmorum* was the second most common species whereas *F. graminearum* was the least common of the species isolated (Jenkinson and Parry 1994). *F. graminearum* was the predominant *Fusarium* species isolated from 34 species of wild grasses in Canada (Inch and Gilbert 2003)

In 1983, Teich and Nelson did not identify any difference in FEB incidence in fields with and without an herbicide treatment. However, they did identify a higher incidence of FEB in fields with a high weed density (n=13, 0.064%) compared to fields with a low weed density (n=4, 0.024%) (Teich and Nelson 1984). The authors later reported that the predominant weed was quack grass (*Agropyron repens*) (Teich and Hamilton 1985). In the following year, Teich and Hamilton did not find any difference in FEB incidence with herbicide use or with weed density. They reported that weeds were mainly dicotyledons in fields studied that year (Teich and Hamilton 1985).

In a large survey conducted in Saskatchewan, Canada from 1999 to 2002 (n=659), the application of glyphosate within 18 months previous to sowing significantly increased FEB in min-tilled fields (Fernandez *et al.* 2005). As this was an observational study then a “cause and effect” relationship is not proven, however there is experimental data to show that glyphosate treatment of weed and crop species can result in increased colonisation of the roots by *Fusarium* species and increased numbers of *Fusarium* propagules in soil (Levesque *et al.* 1987; Levesque *et al.* 1993).

PGR and lodging

Few reports have detailed any effect of PGR on FEB parameters. One study found an increase in FIG when a PGR (ethophon) was used (Martin *et al.* 1991). A second study found the use of PGR with foliar fungicides resulted in increased FEB and DON concentration in harvested wheat (Oerke *et al*. 2002 as reported in (Oldenburg 2004). This maybe due to a direct effect of the altered crop physiology due to the application of the PGR or due the reduction in height resulting in greater numbers of *Fusarium* spores splash dispersed from the soil surface (Jenkinson and Parry 1994). Such an effect has to be balanced against the risk of lodging, as PGR are primarily used in cereal production to reduce lodging risk. An early observational study of wheat fields (n=28) in Illinois identified that a higher incidence of FEB occurred in lodged areas of fields (Holbert *et al.* 1919). Similar results of high levels of DON in lodged plots were reported during fungicide efficacy experiments (Nicholson *et al.* 2003).

Host resistance

Many studies have been conducted on host resistance to FEB and resultant reduction in fusarium mycotoxin in harvested grain (Miedaner 1997). There are a number of wheat varieties worldwide which have good resistance to FEB. However, these varieties are not amenable to UK agriculture and the polygenic nature of the resistance means that the resistance available can not be readily incorporated into UK breeding lines. In Germany, several varieties are known to have moderate-good resistance to FEB, and consistently have lower DON concentration than more susceptible varieties (Koch *et al.* 2006)

Adjacent crops

No evidence that the adjacent crop has an effect on DON concentration of wheat (Schaafsma *et al.* 2005). If any effect does occur it would be expected to be limited to the field margin and therefore unlikely to be detectable in observational studies of samples from whole fields.

Drilling date and seed rate

In a large survey conducted in Saskatchewan, Canada from 1999 to 2002 (n=659), drilling date or seed rate had no impact on FEB infection (Fernandez *et al.* 2005). Field trials have also failed to identify an effect of seed rate on FEB incidence or DON concentration (Schaafsma and Tamburic-Ilincic 2005). In Croatia, field trials of cultivar and drilling date identified that over a three year period, latest drilling date resulted in significantly higher FIG (5th Nov compared to 25th Sept and 15th Oct) (Jurkovic *et al.* 2006). Differences between countries may be due to differences in prevailing weather conditions when early and late drilled crops are in flower.

Organic production

Limited studies have been done to compare the mycotoxin concentration in organic production. A survey of French wheat identified no significant difference between organic (n=11) and conventional (n=11) wheat (Malmauret *et al.* 2002). A survey of wheat flour in Germany in 2001 found that organic samples (n=24, DON median = 120 ppb) had a significantly lower median than conventional ones (n=36, DON median = 295 ppb) (Schollenberger *et al.* 2002). In a study of organic and conventional production at three sites over three years, there was lower DON from the organic wheat fields compared to the conventional ones (Birzele *et al.* 2002). However, this study was confounded by several different agronomic practices.

Interactions

As DON concentration largely depends on suitable weather conditions for FEB infection, would expect a significant temporal (year) and spatial (location) interaction. If suitable weather conditions do not occur at a specific location in a particular season then DON contamination of grain will not occur irrespective of agronomic practices employed.

All evidence available indicates that particular crop residues are an important source of *Fusarium* inoculum. As a result of this, previous studies have shown an interaction between previous crop and crop residue management. If the previous crop is a host of *Fusarium* then it is more important to reduce the amount of crop debris on the soil surface. However, *Fusarium* can also be detected on non-host crop debris.

1.2.5 Visual analysis

Fusarium ear blight can result in *Fusarium* damaged grains (FDG) at harvest. Various researchers have analysed the relationship between FDG and DON in harvest wheat. The relationship between FDG and DON appears to be better at higher concentrations, as shown during an assessment of this relationship in Minnesota grain after the ear blight epidemics of 1993 and 1994, which had a mean DON content of 8300 ppb (Jones and Mirocha 1999). The relationship tends to vary between years and is not considered strong enough or robust enough to allow a FDG assessment to be used as an estimate of DON concentration (Anon. 2002). However, a visual assessment is an ideal diagnostic test in other respects (i.e. cheap, quick, non-hazardous) and is currently used in Canada as a quality criterion for wheat. Maximum limits of FDG is assessed as a percentage by weight and maximum limits range from 0.25 to 5% depending on the class and grade of wheat (Anon. 2003a). In the UK there are no set standards although a number of feed mills use an intake threshold of 5 FDG per kg which is approximately 0.025% by weight.

1.2.6 PCR analysis

Numerous PCR assays have been developed for the *Fusarium* genus. They have been targeted at the whole genus, individual species or are gene specific. Gene-specific assays have been primarily designed towards mycotoxin genes to detect and quantify chemotaxonomic groups. The *Tri*5 gene codes for trichodiene synthase, which is the enzyme which catalyses the first step in the trichothecene pathway. As a consequence the *Tri*5 gene is present in all trichothecene-producing *Fusarium* species. A quantitative PCR assay was developed for the *Tri*5 gene which allows all trichothecene-producing *Fusarium* species to be quantified in a single assay (Edwards *et al.* 2001).

1.2.7 Statistical analysis

Statistical analysis can be used to identify the importance of agronomic factors on the mycotoxin content of grain. By modelling the concentration of mycotoxins in field samples against the agronomy used within those fields, the statistical significance and extent to which an agronomic factor affected the mycotoxin content at harvest can be determined.

1.3 Aims and objectives

Determine how agronomic factors affect the concentration of trichothecenes and zearalenone in harvested wheat grain in the UK. These factors included organic production, rotation, cultivation, T3 fungicide, variety.

Determine the range of trichothecene and zearalenone contamination within harvested UK wheat grain over a five year period (2001 – 2005).

Determine the relationships between the amount of fusarium mycotoxins, the amount of trichothecene-producing *Fusarium* and the amount of fusarium damaged grain within grain samples.

2. Methods

2.1 Sampling

Each year three hundred grain samples and related agronomic data were collected by crop consultants and conventional and organic growers.

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

Requested a similar number from each region:

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

(Northern Ireland was included from 2002 harvest).

Regions were based on UK corn return regions (Figure 2.1). Scottish regions were combined as a single region. North east and north west were combined, as were south east and south west.

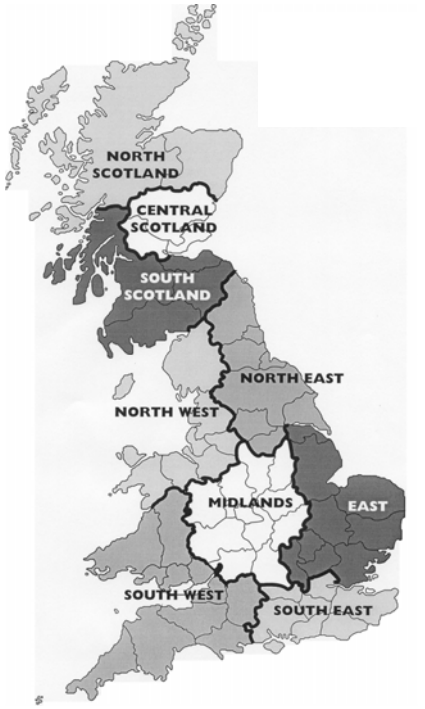


Figure 2.1 HGCA corn return regions

Requested a similar number from each of the following categories:

1. Organic production
2. Conventional production with no T3 application
3. Conventional production with straight strobilurin T3 application
4. Conventional production with strobilurin /triazole mixture T3 application
5. Conventional production with straight triazole T3 application

Agronomy details requested were:

Field name or reference number
Acreage of wheat grown
County
Variety
Intended end use
Cultivation technique
Previous crop
Maize in the rotation?
Maize next to this crop?
What fungicides were applied at T3,, at what growth stage, on what date?
What fungicides were applied at T2, at what growth stage, on what date?

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 visual assessment. The remaining sample was milled with a 1 mm screen, mixed in a tumbler mixer before three 200 g sub-samples were collected. One sample was sent to RHM Technology for GC-MS analysis of trichothecenes while a second sample was sent to CSL for HPLC analysis of zearalenone. A third sample was held at Harper Adams as an archive sample at –20°C.

2.2 Mycotoxin analysis

The GC-MS analysis of trichothecenes was performed by RHM Technology (High Wycombe). The trichothecenes analysed for were deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-acetylDON, fusarenone X, T2 toxin, HT2 toxin, diacetoxyscirpenol (DAS), neosolaniol and T2 triol. Spiked samples were included in each batch to determine extraction recovery. The UKAS accredited method had acceptable recovery range for each trichothecene of 70-110%. Results were adjusted according to recovery. For this study the calculation of the measurement uncertainty was carried out using in-house data, performance in international collaborative trials and Food Analysis Performance Assessment Scheme (www.fapas.co.uk/fapas.cfm) thus incorporating repeatability and reproducibility data. 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 ±25% for all trichothecenes across the quantification range of the assay. The limit of quantification (LoQ) was determined as six times the baseline noise and calculated to be 10 ppb. Samples

below the LoQ were entered as (LoQ)/6, ie 1.667 ppb in the calculation of mean values.

The HPLC analysis of zearalenone was performed by CSL (York). Spiked samples were included in each batch to determine extraction recovery. The UKAS accredited method had acceptable recovery range for zearalenone of 70-110%. Results were adjusted according to recovery. For this study the calculation of the measurement uncertainty was carried out using performance in an international collaborative trial (MacDonald *et al.* 2005). 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 for wheat flour was calculated to be ±34% at the concentration of 227 ppb zearalenone. Similar measurements of uncertainty were achieved for other cereal matrices with zearalenone concentration ranging from 67 to 143 ppb. The limit of quantification (LoQ) was calculated to be 5 ppb. Samples below the LoQ were entered as (LoQ)/6, ie 0.833 ppb in the calculation of mean values.

2.3 Visual and PCR-based analysis

Each year, sixty samples were selected which covered the whole range of mycotoxin contamination. Each selected sample was assessed for *Fusarium* infection based on visual symptoms and using a trichothecene gene (*Tri*5) PCR assay. One thousand grains were visually scored for *Fusarium* damaged grains (scored as red or white FDG) by the same individual. For the PCR assay, DNA was extracted from 10 g of flour and amplified using a quantitative PCR assay for the *Tri5* gene as detailed by Edwards *et al*. (2001). This gene is the first in the trichothecene pathway and present in all trichothecene-producing *Fusarium* species. Other quality parameters; thousand grain weight and specific weight were also measured.

2.4 Visual assessment workshop

A visual assessment workshop was run at Harper Adams on the 9th June 2005. Twenty delegates from the cereal industry attended. Delegates had between zero and twenty years experience of visual assessments. Nine of the delegates had responsibility for accepting grain at intake. Most delegates (18) had a brief training session on identification of FDG before starting the assessment.

Sixty samples were selected from 2002-2004 harvests to cover the whole range of DON concentrations. Samples were either accepted or rejected based on a visual assessment. Each assessor was scored for average mycotoxin content of accepted samples and number of false negative (rejected when below legal limit) and false positive (accepted when above legal limit) samples.

2.5 Statistical analysis

For summary statistics, samples with a mycotoxin content below the limit of quantification (LoQ) were assigned a value of (LoQ)/6 for calculation of mean values according to the methodology of the fusarium mycotoxin SCOOP project (Anon 2003a). Summary statistics (percentage greater than 10 ppb, mean, median, 90^{th} percentile, 95^{th} percentile and maximum) were calculated using Excel (Microsoft v.2002). All other statistical analysis was completed using Genstat (Lawes Agricultural Trust, v8) unless stated otherwise. Relationships between mycotoxin concentrations, visual assessments and quantified *Tri*5 DNA were determined using regression analysis. Statistical analysis to determine agronomic factors on the fusarium mycotoxin concentration of wheat was performed using a stepwise selection ANOVA. For modelling the mycotoxin concentration of samples, samples with a mycotoxin concentration below the LoQ were assigned a value of (LoQ)/2 and $\log_{10}$ transformed and analysed using a normal distribution. For mycotoxins with a low incidence, the incidence and concentration of positive samples was modelled. For modelling the incidence of samples, samples below the LoQ were assigned a value of 0 and those above the LoQ a value of 1 and analysed using a Bernoulli distribution. Models of mycotoxin concentrations were validated using residual plots and models of incidence were validated by Receiver Operating Characteristic (ROC) curve analysis (SPSS, v14). The predictive ability of the DON model was assessed by observing the stability of the parameter estimates for each year and by calculating the Prediction Error Sum of Squares (PRESS) (Montgomery & Peck, 1992).

3. Results

3.1 Summary of samples received

In the first year of the project the target number of samples was not achieved. This was primarily due to the outbreak of foot and mouth disease which resulted in restricted access to farms with livestock in 2001. In the following years the target was exceeded by about 10% each year (Table 3.1.1).

Table 3.1.1 Number of samples received compared to target.

Year	Target	Received
2001	300	283
2002	300	343
2003	300	328
2004	300	344
2005	300	326
Total	1500	1624

Samples were not collected from Northern Ireland in 2001. From 2002 onwards the Department of Agriculture and Rural Development (DARD) collected samples in Northern Ireland. Numbers of samples collected from Scotland and Northern Ireland were lower than for other regions, this was due to the smaller areas of wheat in these regions and possibly due to the lower concern for fusarium ear blight and mycotoxins in those countries (Table 3.1.2). Northern Ireland may have a lower concern as nearly all wheat is grown for animal feed. Scotland may have a lower concern as the colder climate results in less ear blight occurring in Scotland

Table 3.1.2 Sample distribution by year and region.

Year	Region						Total
	South	East	Midlands	North	Scotland	N.Ireland	
2001	50	79	63	58	33	0	283
2002	64	62	93	67	38	19	343
2003	75	62	69	77	29	16	328
2004	60	73	93	72	33	13	344
2005	61	67	81	73	31	13	326
Total	310	343	399	347	164	61	1624

Samples receiving a strobilurin without an azole at T3 became rare after 2002 (Table 3.1.3). In 2002, due to fungicide resistance problems, strobilurin applications to wheat were restricted to two applications. Many growers preferred to use a strobilurin at T1 and T2 (traditional first and second spray timings of growth stage (GS) 31 in spring and GS 39 (flag leaf fully emerged). Strobilurins must also be used in combination with other fungicide chemistry, at T3 this would usually be an azole partner.

Table 3.1.3 Sample distribution by year and T3 fungicide category.

Year	T3 fungicide					Total
	Organic	No T3	Strobilurin	Azole/Strob	Azole	
2001	17	107	51	64	44	283
2002	57	104	49	91	42	343
2003	61	91	17	94	65	328
2004	54	129	10	88	63	344
2005	58	85	12	114	57	326
Total	247	516	139	451	271	1624

3.2 Summary statistics

Of the eleven mycotoxins analysed only seven were detected, of these only four, DON, nivalenol, HT2 and zearalenone were detected above 100 ppb. Tables 3.2.1 to 3.2.5 below shows 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 European legal limits will be based on a combined concentration.

Table 3.2.1 Mycotoxin concentrations for all mycotoxins detected in UK wheat in 2001 (283 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	80	80	32	133	223	5175
NIV	80	34	23	71	97	428
15AcDON	1	<10	<10	<10	<10	22
HT2	29	<10	<10	22	32	193
T2	2	<10	<10	<10	<10	21
T2 triol	1	<10	<10	<10	<10	15
HT2+T2	30	<20	<20	22	32	214
ZEAR	5	<5	<5	6	9	188

Means are based on an imputation of 1.667 (0.833 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 ppb for zearalenone).

Table 3.2.2 Mycotoxin concentrations for all mycotoxins detected in UK wheat in 2002 (343 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	78	116	30	211	470	3065
NIV	55	21	11	46	68	430
15AcDON	2	<10	<10	<10	<10	45
3AcDON	1	<10	<10	<10	<10	21
HT2	14	<10	<10	14	17	54
T2	5	<10	<10	<10	<10	32
HT2+T2	16	<20	<20	<20	22	75
ZEAR	17	11	<5	19	38	707

Means are based on an imputation of 1.667 (0.833 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 ppb for zearalenone).

Table 3.2.3 Mycotoxin concentrations for all mycotoxins detected in UK wheat in 2003 (328 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	89	218	38	346	594	10626
NIV	82	34	22	77	106	237
3AcDON	1	<10	<10	<10	<10	42
15AcDON	5	<10	<10	<10	<10	217
HT2	62	13	12	26	35	150
T2	51	<10	10	19	23	52
T2 triol	0.3	<10	<10	<10	<10	45
HT2+T2	69	22	<20	44	55	199
ZEAR	13	7	<5	14	28	209

Means are based on an imputation of 1.667 (0.833 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 ppb for zearalenone).

Table 3.2.4 Mycotoxin concentrations for all mycotoxins detected in UK wheat in 2004 (344 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	92	469	65	694	1467	20333
NIV	58	24	12	63	103	276
15AcDON	5	<10	<10	<10	<10	164
3AcDON	3	<10	<10	<10	<10	44
HT2	13	<10	<10	12	15	121
T2	7	<10	<10	<10	11	28
HT2+T2	14	<20	<20	<20	26	149
ZEAR	49	54	10	108	261	1292

Means are based on an imputation of 1.667 (0.833 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 ppb for zearalenone).

Table 3.2.5 Mycotoxin concentrations for all mycotoxins detected in UK wheat in 2005 (326 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	92	242	65	500	734	11306
NIV	65	21	15	45	74	276
15AcDON	1.2	<10	<10	<10	<10	46
3AcDON	1.2	<10	<10	<10	<10	19
HT2	44	10	<10	24	34	102
T2	21	<10	<10	13	15	27
HT2+T2	44	<20	<20	35	44	127
ZEAR	13	8	<5	13	26	559

Means are based on an imputation of 1.667 (0.833 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 ppb for zearalenone).

Table 3.2.6 Mycotoxin concentrations for all mycotoxins detected in UK wheat in 2001-2005 (1624 samples).

		Mycotoxin concentration (ppb)				
	%>10ppb	Mean	Median	90th%	95th%	Max
DON	86	230	42	368	722	20333
15AcDON	2.7	<10	<10	<10	<10	217
3AcDON	1.2	<10	<10	<10	<10	44
NIV	67	27	16	64	95	430
T2 triol	0.4	<10	<10	<10	<10	45
T2	16	2.7	<10	13	17	52
HT2	32	6.7	<10	20	28	193
HT2+T2	34	9.4	<20	31	43	214
ZEAR	19	17	<5	27	61	1292

Means are based on an imputation of 1.667 (0.833 for zearalenone) for all samples below the limit of quantification (10 ppb; 5 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 3.2.1). nivalenol was the next most common fusarium mycotoxin detected although it was never detected at a high concentration (maximum = 430 ppb). This would support the decision not to set a maximum limit for this mycotoxin, although nivalenol should not be considered a co-contaminant with DON, as regression analysis shows the relationship between the two is complex (See Section 3.3 below). *F. graminearum* and *F. culmorum* isolates either produce DON or nivalenol. Low levels detected in UK wheat would indicate that the production of nivalenol in UK wheat is limited. It should be noted that the relationship between pathogens and hosts is a dynamic one, and the current situation may change. HT2 and T2 was detected in 31 and 19% of samples respectively, the concentration was usually low but some samples did exceed the proposed combined limit of 100 ppb HT2 and T2 (Table 3.2.9). Zearalenone was detected in 39% of samples (LoQ=5%), 19% of samples exceeded 10 ppb. However, due to the lower legal limits for this mycotoxin, more samples exceeded the legal limit for zearalenone than for DON (Table 3.2.7 and 3.2.8). As for DON, the zearalenone distribution was also skewed (Fig 3.2.2). Comparison of the 95th percentile and maximum values indicates that all mycotoxin detected had a skewed distribution similar to DON and zearalenone.

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

Table 3.2.7 Percentage of samples exceeding 200, 500, 750 and 1250 ppb DON.

Year	Percentage of samples			
	> 200	> 500	> 750	> 1250
2001	6.3	1.4	1.4	0.4
2002	12.0	4.9	3.5	1.7
2003	16.5	6.4	4.0	2.4
2004	29.0	12.5	10.0	5.5
2005	23.9	10.1	4.9	1.5
Overall	18.5	7.3	4.8	2.4

Table 3.2.8 Percentage of samples exceeding 20, 50, 75 and 100 ppb zearalenone.

Year	Percentage of samples			
	> 20	> 50	> 75	> 100
2001	3.2	3.2	3.2	0.7
2002	9.6	3.8	2.6	0.6
2003	7.3	3.4	1.5	1.2
2004	34.0	18.3	14.5	11.3
2005	6.7	2.5	1.5	1.2
Overall	12.6	6.0	4.4	3.1

Table 3.2.9 Percentage of samples exceeding 20, 50, 75 and 100 ppb HT2 + T2.

	Percentage of samples			
Year	>20	>50	>75	>100
2001	29.7	2.8	1.1	0.7
2002	16.0	1.2	0.0	0.0
2003	68.9	7.0	1.2	0.6
2004	13.7	1.2	0.9	0.9
2005	44.5	3.7	0.9	0.3
Overall	34.3	3.1	0.8	0.5

It should be noted that the legal limits for DON and zearalenone include a measurement of uncertainty. Therefore for a consignment of unprocessed wheat intended for human consumption to exceed the legal limit for DON and zearalenone the concentration as determined by the analytical procedures employed in this project would have to exceed 1563 ppb DON or 134 ppb zearalenone.

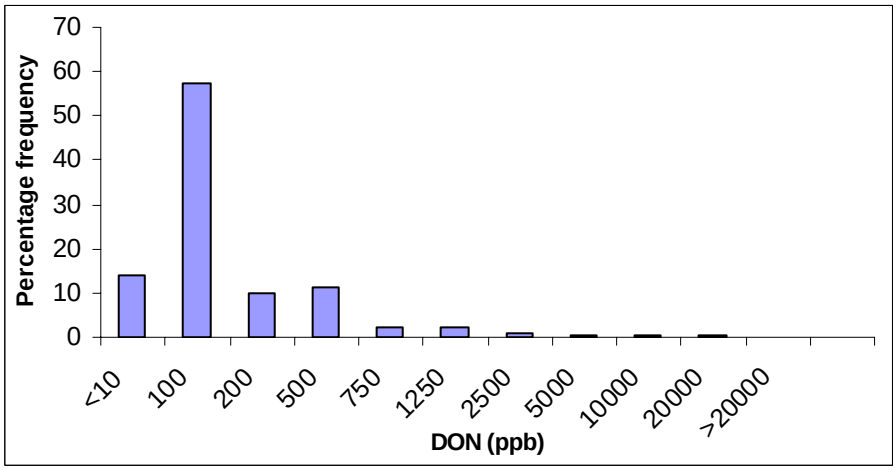


Fig 3.2.1 Percentage frequency of DON contamination in UK wheat in 2001-2005 (n = 1624).

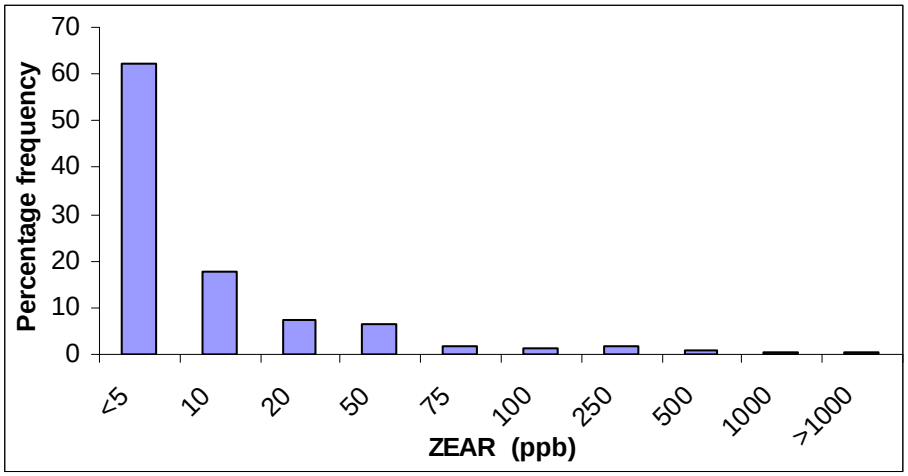


Fig 3.2.2 Percentage frequency of zearalenone contamination in UK wheat in 2001-2005 (n = 1624).

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 will bias the summary data. For example, growers were requested to provide wheat samples following maize and minimum cultivation if available. Fifteen samples were obtained from wheat following maize and minimum cultivation; their mean and median DON content of these samples was 2621 and 522 ppb respectively. The proportion of UK wheat crops which follow maize and minimum tillage is very low as maize only accounts for about 3% of all arable land and minimum tillage only accounts for about 25% of land cultivation.

3.3 Regression analysis

There was a positive relationship (r^2=0.39) between DON and zearalenone (Fig 3.3.1). This is to be expected as DON and zearalenone are produced by the same species, namely *F. culmorum* and *F. graminearum*, but isolates of these species may or may not produce DON and zearalenone. An important point to note in Figure 3.3.1 is that samples which exceed 1250 ppb DON may, or may not exceed 100 ppb zearalenone, and vice versa. It is therefore important that both DON and zearalenone are analysed in samples which are thought may exceed legal limits for fusarium mycotoxins. The percentage of samples which exceeded 1250 ppb DON and 100 ppb zearalenone fluctuated each year and the relationship between the two was not stable between years (Figure 3.3.2, Table 3.2.7 and 3.2.8). The relationship between DON and zearalenone concentration was analysed with regression analysis of logarithmic transformed values, grouped by year. The regression and year were both highly significant (P<0.01). The regression was best fitted by separate, non-parallel lines (r^2 = 0.56). The major difference was the high ratio of zearalenone to DON in 2004. Zearalenone is known to be produced as the crop senesces at the end of the growing season. It is likely that the high ratio of zearalenone to DON in 2004 is a result of the delayed, wet harvest that year.

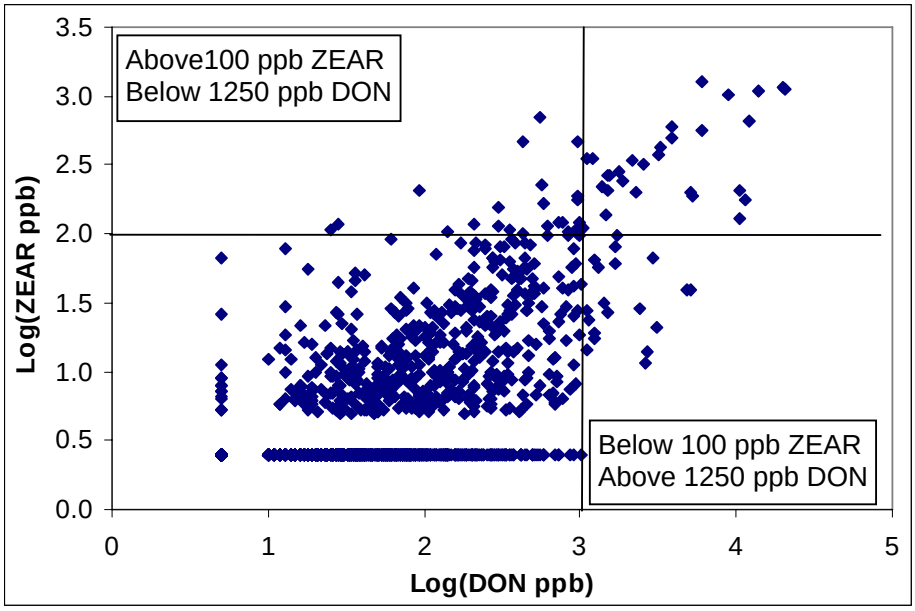


Figure 3.3.1 Zearalenone against DON (log log plot) for wheat 2001-2005 (n=1624). Lines represent 100 ppb zearalenone and 1250 ppb DON.

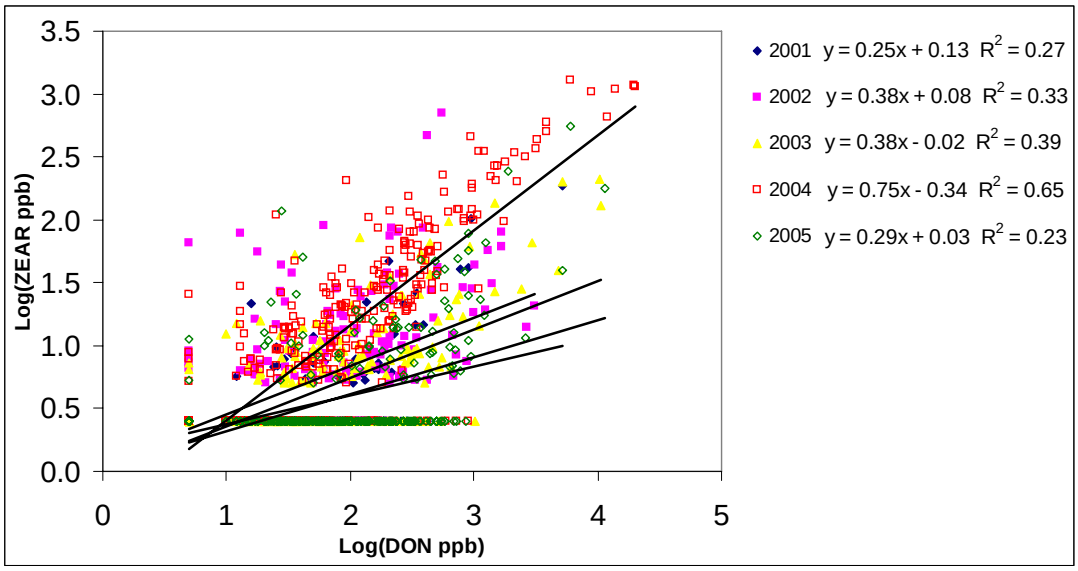


Figure 3.3.2 Zearalenone against DON (log log plot) for wheat for each year from 2001 to 2005 (n = 1624).

There are no other positive relationships between the concentrations of other commonly detected fusarium mycotoxins detected in UK wheat. In fact both nivalenol and HT2+T2 show signs of mutual exclusion with DON, ie when one mycotoxin is present at high concentration then the other is low. For HT2 and T2 this appears to be simple mutual exclusion (Figure 3.3.3); this is probably because HT2 and T2 are produced by *F. langsethiae* which appears to have different environmental requirements to *F. culmorum* and *F. graminearum*. As a consequence of this relationship, DON concentration cannot be used to predict HT2 and T2 concentration.

For nivalenol the relationship is more complex (Figure 3.3.4) as:
a) nivalenol and DON are produced by different chemotypes of the same species (*F. culmorum* and *F. graminearum*),
b) nivalenol is produced as a low level co-contaminant by DON chemotypes (and hence always some nivalenol present in samples with high DON concentration),
c) nivalenol is also produced by *F. poae*, which has different environmental requirements to *F. culmorum* and *F. graminearum*.

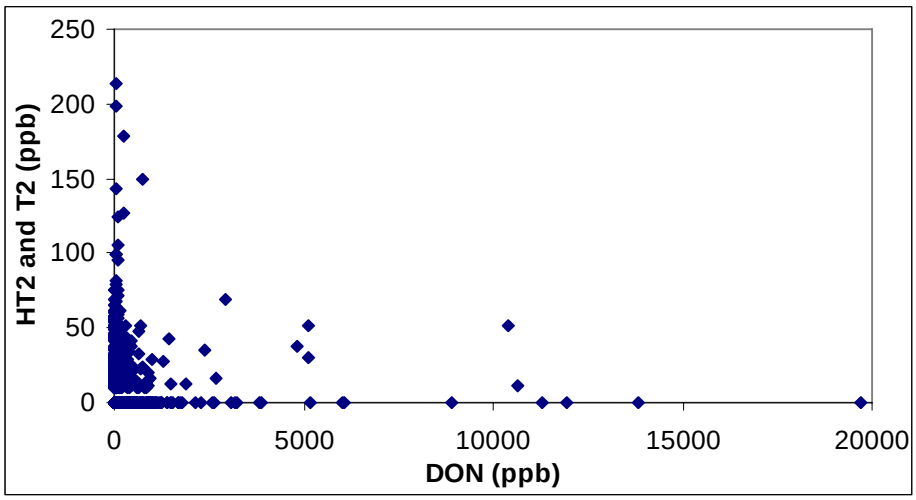


Figure 3.3.3 HT2+T2 against DON concentration for wheat 2001-2005 (n=1624).

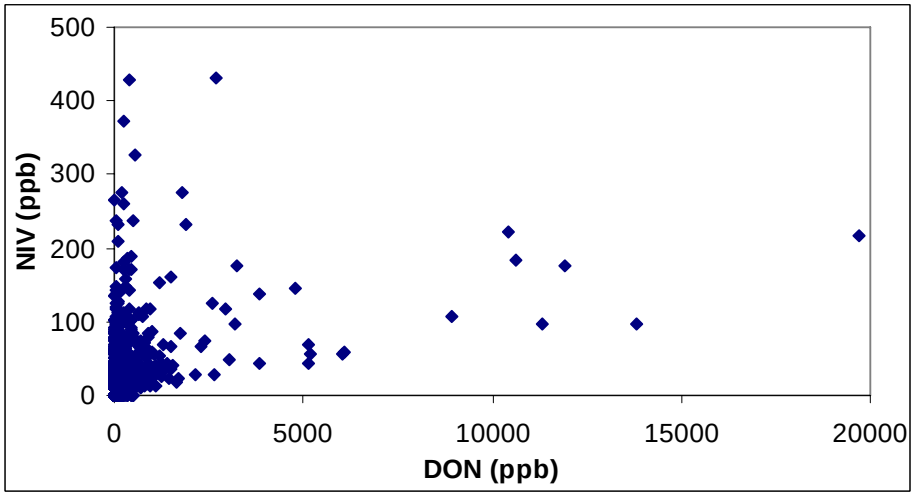


Figure 3.3.4 NIV against DON concentration for wheat 2001-2005 (n=1624).

The acetylated versions of DON (3-acetyl and 15-acetyl DON) are co-contaminants of DON which occur at a low percentage of the DON concentration, and as such are only normally detected when DON is present at a high concentration. As such legislation that reduces consumer exposure to DON also reduces consumer exposure to acetylated DON in cereals and cereal products intended for human consumption. *F. graminearum* and *F. culmorum* DON chemotypes are either 3-acetyl or 15-acetyl DON producers. The regression of acetylated DON against DON (Figure 3.3.5) shows that this relationship fits for the majority of samples with no acetylated DON detected when DON concentration was low and a low concentration of acetylated DON when the DON concentration was high. For these samples, the percentage of acetylated DON to DON was between 0.25 and 2.5%.

However, Figure 3.3.5 shows two anomalies:
a) Samples with high DON exist with no detectable acetylated DON suggesting a chemotype of *F. culmorum* and/or *F. graminearum* may exist which does not produce an acetylated DON.

b) In five samples (circled in Figure 3.3.5), acetylated DON occurred as a high proportion of the total DON concentration with a percentage acetylated DON to DON concentration of 25 to 150%. All five samples were from the 2005 harvest, four samples were from the midlands and one from Suffolk. This anomaly is of concern as legislation is set for DON alone on the assumption that acetylated forms of DON occur as a co-contaminant at a low percentage of DON concentration. Therefore there is a need to monitor acetylated DON as well as DON in cereals and cereal products to ensure that high concentrations of acetylated DON do not occur, or only occur infrequently.

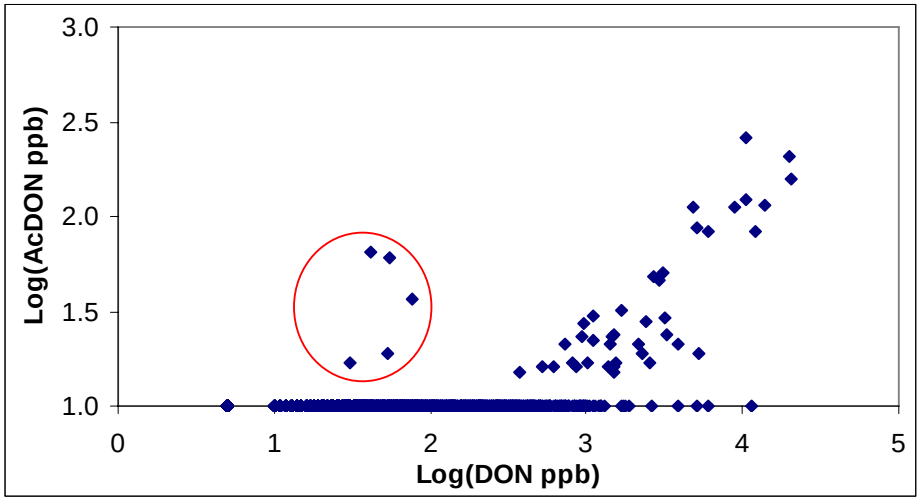


Figure 3.3.5 Acetylated DON (3- and 15-acetyl DON) against DON concentration for wheat 2001-2005 (n=1624). Samples with high ratio of acetylated DON to DON are circled in red.

There was a weak relationship between HT2 and T2 concentration (Fig 3.3.6). A relationship between these two mycotoxins could have been expected as the two mycotoxins are produced by the same species on the same metabolic pathway. A good relationship over a much wider concentration range was found for HT2 and T2 in UK oats over the same period (See related project FSA CO4034/HGCA 2706).

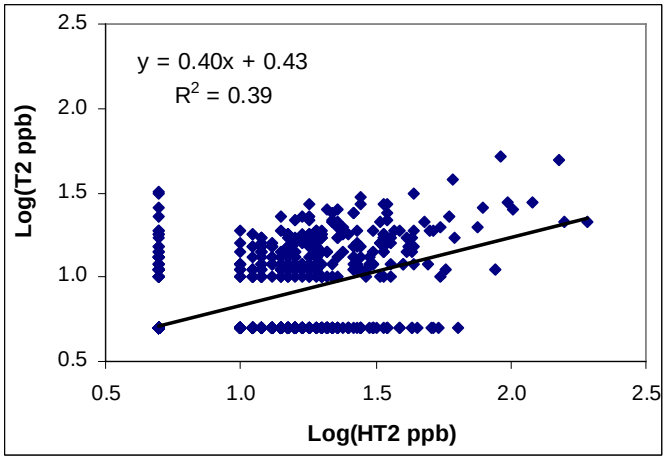


Figure 3.3.6 T2 against HT2 concentration for wheat 2001-2005 (n=1624).

3.4 Statistical analysis of DON

The aim of the statistical analysis was to determine the effect of agronomic factors on the fusarium mycotoxin contamination of wheat. Results will determine "Good Agricultural Practice" for growers to minimise fusarium mycotoxins in wheat.

Samples with less than the LoQ were given a value of ½(LoQ) i.e. 5 ppb and all samples log_{10} transformed (logd = Log_{10} of deoxynivalenol) to stabilise the variance.

Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v8, 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. See Appendix 1 for a description of agronomic factors. 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 and the method of cultivation (± ploughing). Agronomic factors entered for selection were:

Year*region
Practice
Previous crop*plough
Type(Variety resistance score)
T3

(* indicates an interaction)

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, plough, variety FEB resistance scores and T3 fungicides were all significant. There were significant interactions between year and region and between previous crop and cultivation. The model accounted for 41% of the observed variance. 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, this is usually indicated by the large confidence limits.

The majority of conventional samples were collected by agronomists, and these agronomists were either employed by an agrochemical distribution company or were independent advisors. The source of samples was added to the model after year and region to determine if DON concentration of samples was significantly different depending on the source of the sample. Which type of agronomist collected the sample (and therefore provided agronomic advice to the grower) had no significant ($P>0.05$) effect on DON concentration.

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

monitoring DON in wheat as it would suggest that the intended use was not a factor necessary to consider. However, this may change with increased pressure on growers to minimise fusarium mycotoxin content of wheat intended for human consumption to conform to new legislation.

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

Frequency of samples within agronomic factors, after removal of blanks, is displayed in Appendix 2. Statistical analysis of deoxynivalenol is detailed in Appendix 3.

Although there was a significant interaction between year and region, there was a consistent trend of DON contamination decreasing northwards (Fig 3.4.1). This difference was probably due to differences in weather (some *Fusarium* pathogens prefer warmer conditions). The relative difference in DON contamination in the South and East was probably a result of regional differences in weather conditions between the years.

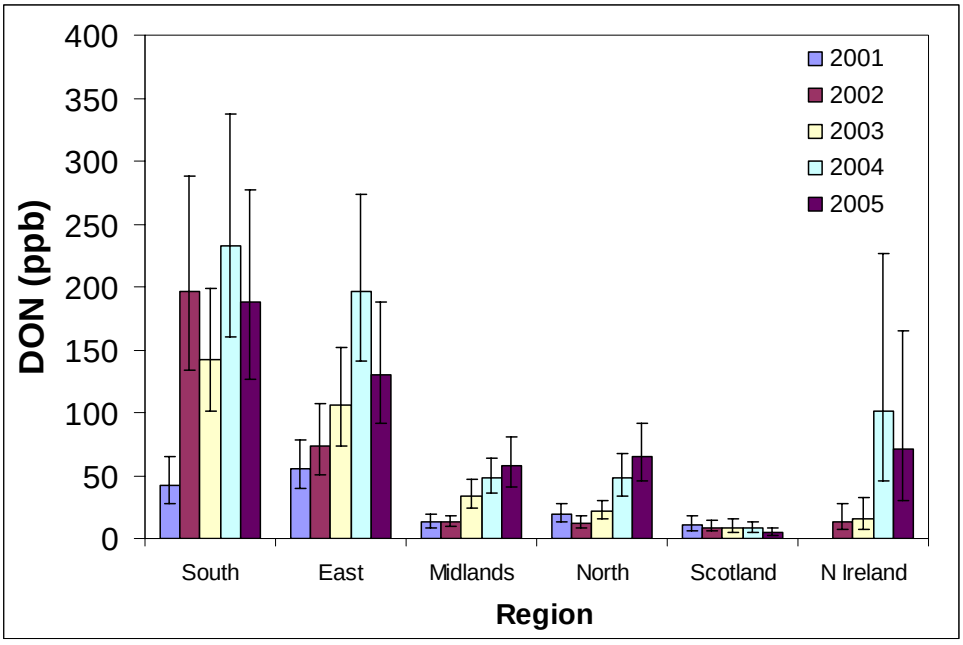
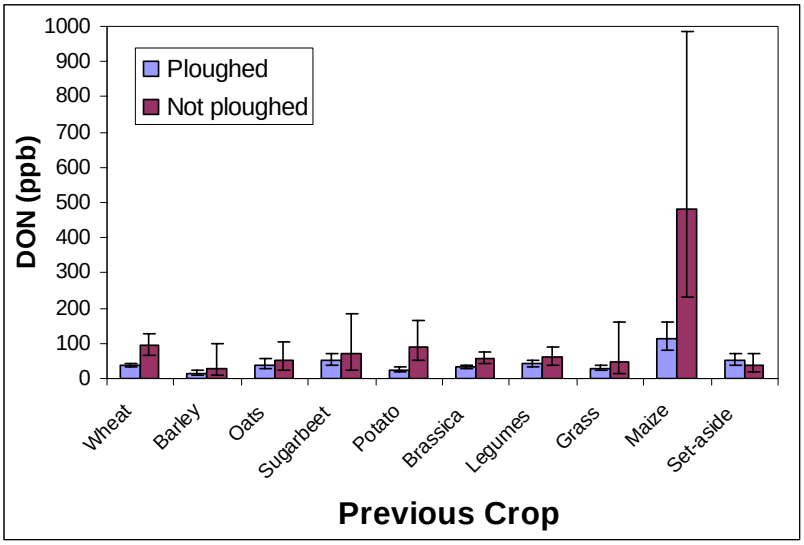
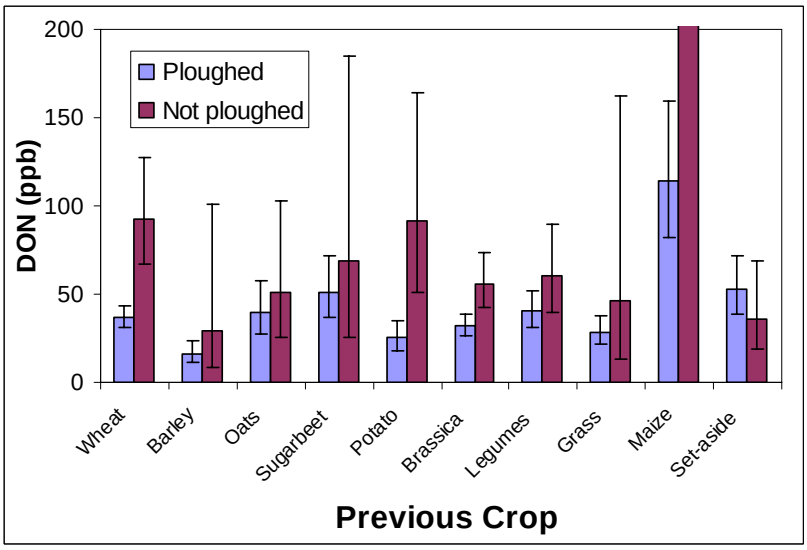


Figure 3.4.1 DON contamination of wheat by region for each year. Bars represent 95% confidence limits for predictions.

Ploughing after maize, wheat, potatoes and brassicas reduced DON contamination of wheat significantly (Fig 3.4.2). The difference was greatest for maize and least for brassicas. There was a consistent trend of ploughing reducing DON content after all crops except set-aside. The low number of samples following minimum cultivation for some crops resulted in large confidence intervals and the inability of statistical analysis to identify significant differences.



A



B

Figure 3.4.2 A. Effect of cultivation and previous crop on DON contamination of wheat. B. As A, but scale modified to show differences for crops other than maize. Bars represent 95% confidence limits for predictions.

Results show an inverse relationship between the Fusarium ear blight (FEB) resistance rating and the DON content of grain samples for winter wheat cultivars (Fig 3.4.3). UK spring wheats are not assessed for FEB resistance but appeared to have a similar DON content to winter wheats with a resistance rating of 6 and 7; this

maybe due to differences in agronomy, i.e. sowing date, rather than differences in disease resistance. Petrus is a German variety with no UK rating but is known to have moderate-good resistance to FEB (Koch *et al.* 2006).

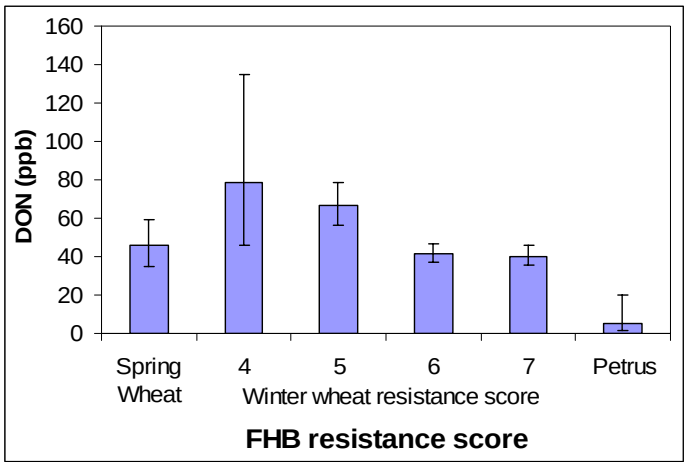


Figure 3.4.3 DON content of samples grouped by Fusarium ear blight resistance rating. Bars represent 95% confidence limits for predictions.

Wheat receiving a T3 azole had a significantly lower DON content compared to wheat which received no T3 (Fig 3.4.4). The reduction achieved is not as good as would be expected for some azoles, this is probably due to the low number of samples which received azoles recommended against FEB at optimum rates and timings. Field trials with natural infection of FEB typically show a 50% reduction in DON when using a fungicide recommended for the control of FEB. There was no significant difference between wheat samples from conventional and organic farms as practice was removed from the model as not significant ($p>0.05$) during stepwise selection.

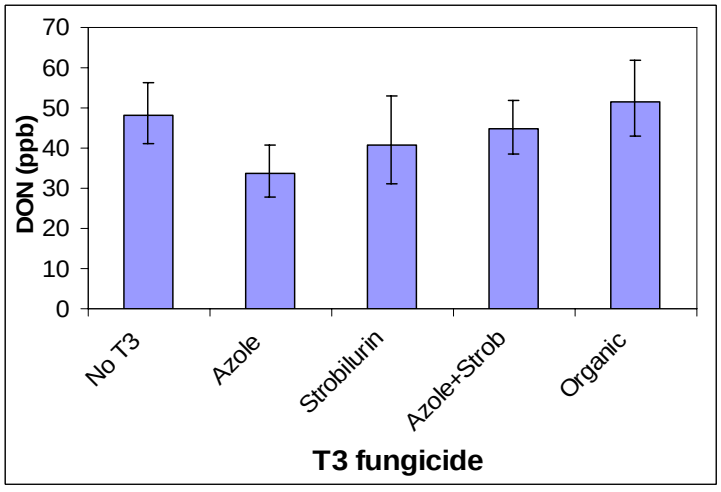


Figure 3.4.4 DON content of wheat samples grouped by T3 fungicide regime. Bars represent 95% confidence limits for predictions.

3.4.1 Predictive quality of DON model

For a model to be used to predict the concentration of DON based on its known agronomy the predictive ability of the model developed must be assessed. The DON model was tested in two ways. Firstly, the stability of the effect of the agronomic factors on DON concentration was observed over the five year period (Appendix 3.5). The scatterplot of parameter estimate versus year showed that the estimates were relatively stable over the five year period for each agronomic factor. Factor levels which did show greatest variation over time were those with low numbers of samples and therefore expected to be less accurate, e.g. Northern Ireland as a region and Petrus as a variety.

Secondly the predictive ability of the model was tested using the Prediction Error Sum of Squares (PRESS) statistic (Appendix 3.6; Montgomery & Peck, 1992). This method calculates $R^2_{prediction}$, which if close to the R^2 of the model indicates it may be a good predictive model.

The $R^2_{prediction}$ was calculated to be 36% compared to the overall R^2 of the model of 41% indicating that the model may be a good predictor of new observations.

3.5 Statistical analysis of Zearalenone

As for DON, the aim of the statistical analysis was to determine the effect of agronomic factors on the fusarium mycotoxin contamination of wheat. Results will determine "Good Agricultural Practice" for growers to minimise fusarium mycotoxins in wheat. Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v8, Lawes Agricultural Trust). Temporal (year) and spatial (region) factors were forced into the model. All other agronomic factors were ordered based on the order in which they occur within a growing season. 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.

Due to the low frequency of samples with detectable levels of zearalenone (39% with quantifiable zearalenone) the dataset was analysed by incidence and the sub-set of positive samples was analysed by concentration.
Frequency of samples within agronomic factors, after removal of blanks, is displayed in Appendix 4. Statistical analysis of zearalenone is detailed in Appendix 5.

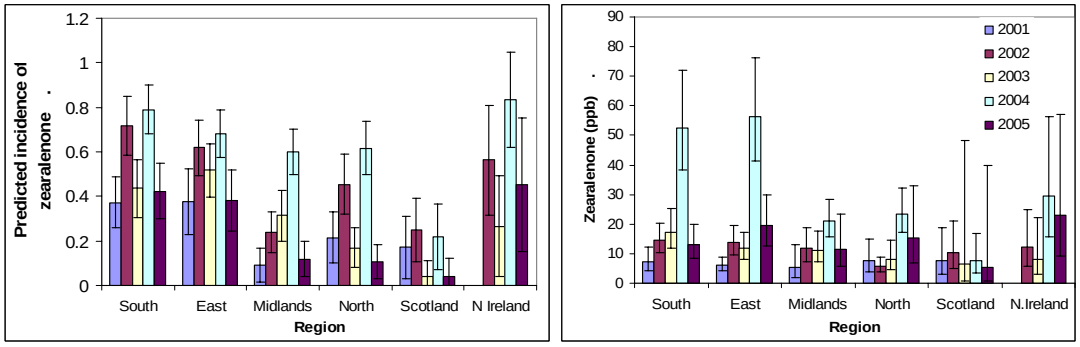
Samples with less than the LoQ were given a value of 0 (absence) and those above the LoQ a value of 1 (presence). A logistic model with a Bernoulli distribution was used to model the incidence of zearalenone for each individual sample.

For positive samples the combined concentration was $\log_{10}$ transformed (logz = Log_{10} [zearalenone]) to stabilise the variance.

Of the factors tested for incidence, only year, region and year*region interaction were significant. The figures below show the estimated mean proportions of samples with quantifiable levels of HT2+T2 for year*region.

Of the factors tested; year*region, previous crop*cultivation and type were all significant for concentration of positive samples. The model accounted for 38% of the observed variance. 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, this is usually indicated by the large confidence limits.

Results show that zearalenone incidence varies between year and region (Fig 3.5.1). There is a general trend for incidence and concentration to be lower the further north that samples were collected from. Incidence and concentration were highest for most regions in 2004.



A. B.

Fig. 3.5.1 A. Predicted proportion of UK wheat samples with quantifiable zearalenone by region. B. Predicted concentration of zearalenone in positive samples (>LoQ). Bars represent 95% confidence limits for predictions.

As for DON in wheat, highest levels were found in samples following maize after minimum tillage (Fig 3.5.2). The general trend was for higher levels of zearalenone to occur after minimum tillage, however, as for DON, the opposite was observed for set-aside.

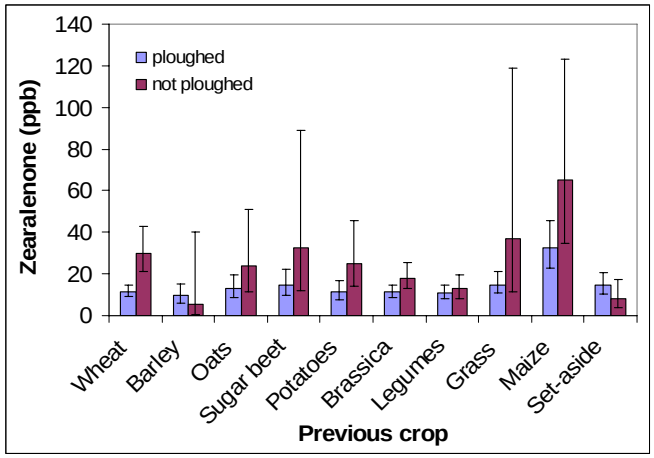


Fig. 3.5.2 Predicted concentration of zearalenone in UK wheat samples with quantifiable zearalenone for each previous crop. Bars represent 95% confidence limits for predictions.

The significant (p=0.047) difference between zearalenone concentration of positive samples of winter and spring varieties (Fig 3.5.3) is different to what was observed for DON. For DON spring varieties had a DON level similar to winter wheat varieties with a FEB resistance score of 6 or 7. For zearalenone, positive spring wheat samples have a high concentration than winter wheat samples. The difference between DON and zearalenone may be due to the different plant growth stages when production occurs. Zearalenone is produced most as the crop senesces near to harvest, as spring wheats senesce later than winter wheats this may affect zearalenone production.

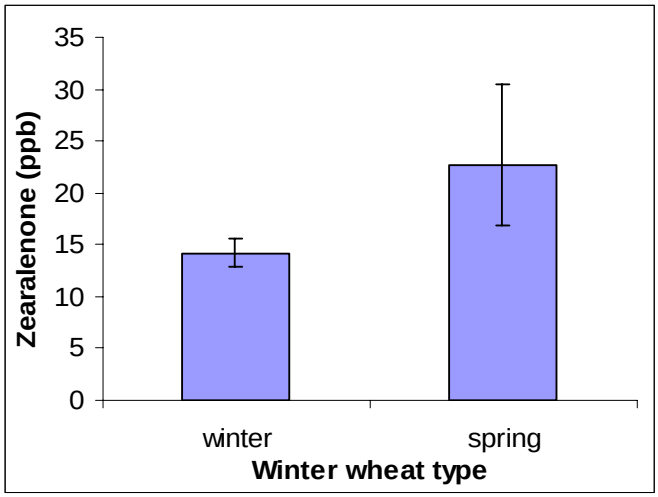


Fig. 3.5.3 Predicted concentration of zearalenone in UK wheat samples with quantifiable zearalenone for wheat type (winter and spring). Bars represent 95% confidence limits for predictions.

3.6 Statistical analysis of HT2+T2 toxins

As for DON and zearalenone, the aim of the statistical analysis was to determine the effect of agronomic factors on the fusarium mycotoxin contamination of wheat. Results will determine “Good Agricultural Practice” for growers to minimise fusarium mycotoxins in wheat. Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v8, Lawes Agricultural Trust). Temporal (year) and spatial (region) factors were forced into the model. All other agronomic factors were ordered based on the order in which they occur within a growing season. 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.

As for zearalenone, due to the low frequency of samples with detectable levels of HT2 and T2 (33% with quantifiable HT2 and/or T2) the dataset was analysed by incidence and the sub-set of positive samples was analysed by concentration. Frequency of samples within agronomic factors, after removal of blanks, is displayed in Appendix 6. Statistical analysis of HT2 and T2 is detailed in Appendix 7.

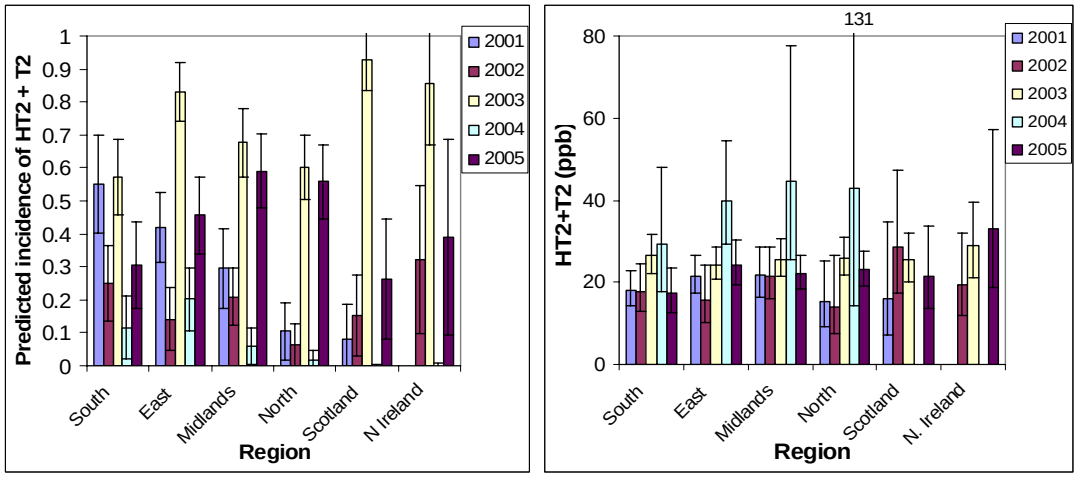
Samples with less than the LoQ were given a value of 0 (absence) and those above the LoQ a value of 1 (presence). A logistic model with a Bernoulli distribution was used to model the incidence of HT2+T2 for each individual sample.

For positive samples the combined concentration was $\log_{10}$ transformed (loga = Log_{10} [HT2+T2]) to stabilise the variance.

Of the factors tested for incidence, year*region, practice, variety FEB resistance scores, and T3 fungicide were all significant. The figures below show the estimated mean proportions of samples with quantifiable levels of HT2+T2 for each significant factor.

Of the factors tested; year, practice, previous crop and T3 fungicides were all significant for concentration of positive samples. There was no significant interaction between year and region. The model accounted for 18% of the observed variance. 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, this is usually indicated by the large confidence limits.

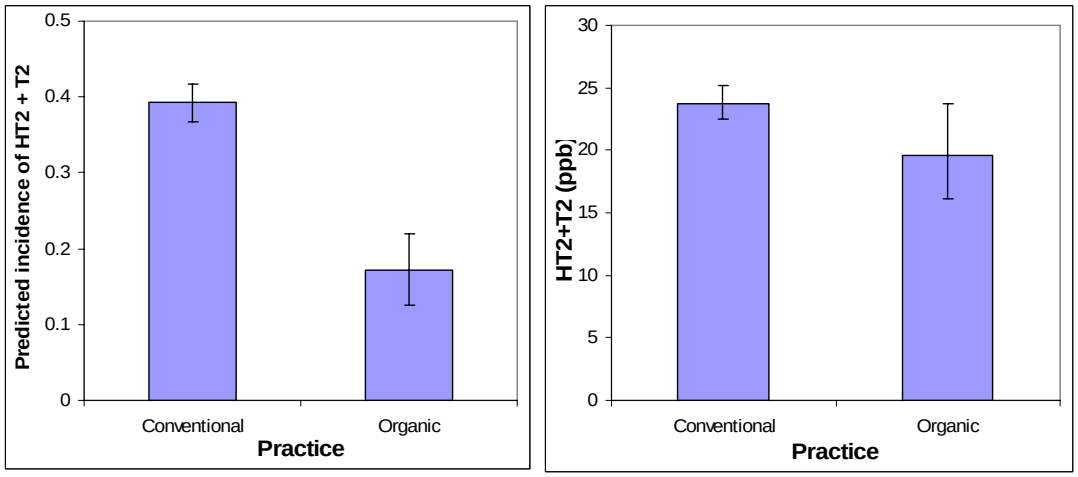
Results indicate that HT2 and T2 can occur anywhere in the UK at equivalent frequency and the distribution across regions varies between years (Fig 3.6.1). Incidence was high in 2003 and low in 2004. In positive samples the concentration is consistent across all regions. Although the incidence of HT2+T2 was lowest in 2004, when present the concentration was significantly higher than in other years.



A. B.

Fig. 3.6.1 A. Predicted proportion of UK wheat samples with quantifiable HT2+T2 by region. B. Predicted concentration of HT2+T2 in positive samples (>LoQ). Bars represent 95% confidence limits for predictions.

HT2+T2 occurred less frequently in organic samples and when it did occur it was present at lower concentrations (Fig 3.6.2)



A. B.

Fig. 3.6.2 A. Predicted proportion of UK wheat samples with quantifiable HT2+T2 in organic and conventional samples. B. Predicted concentration of HT2+T2 in positive samples (>LoQ). Bars represent 95% confidence limits for predictions.

Previous crop did not affect the incidence of HT2+T2. Significantly higher HT2+T2 concentration occurred in positive samples in wheat after oats than wheat following any other crops (Fig 3.6.3).

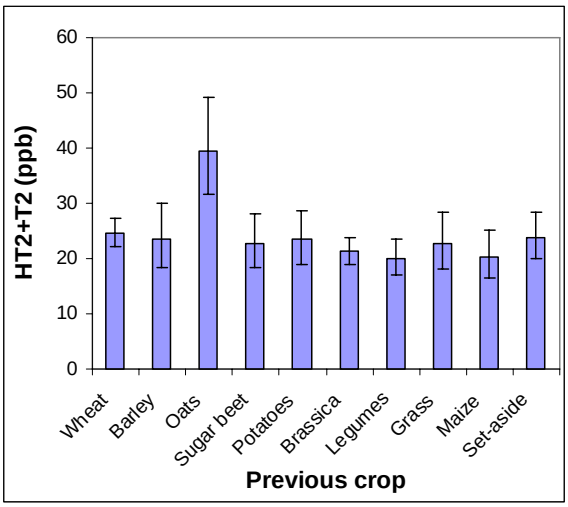


Fig 3.6.3 Concentration of HT2+T2 in positive samples (>LoQ) for each previous crop. Bars represent 95% confidence limits for predictions.

The incidence of HT2+T2 on varieties of wheat appeared to follow a similar stepwise decline as observed for DON concentration; with the highest incidence occurring on wheat varieties with the lowest FEB resistance scores (Fig 3.6.4). There was no significant difference in HT2+T2 concentration between wheat varieties with different FEB resistance scores.

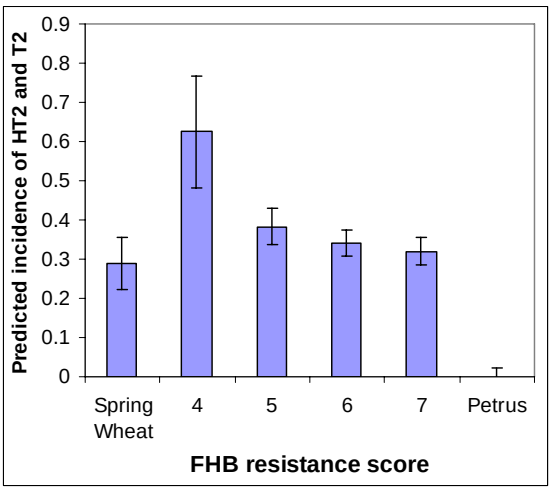
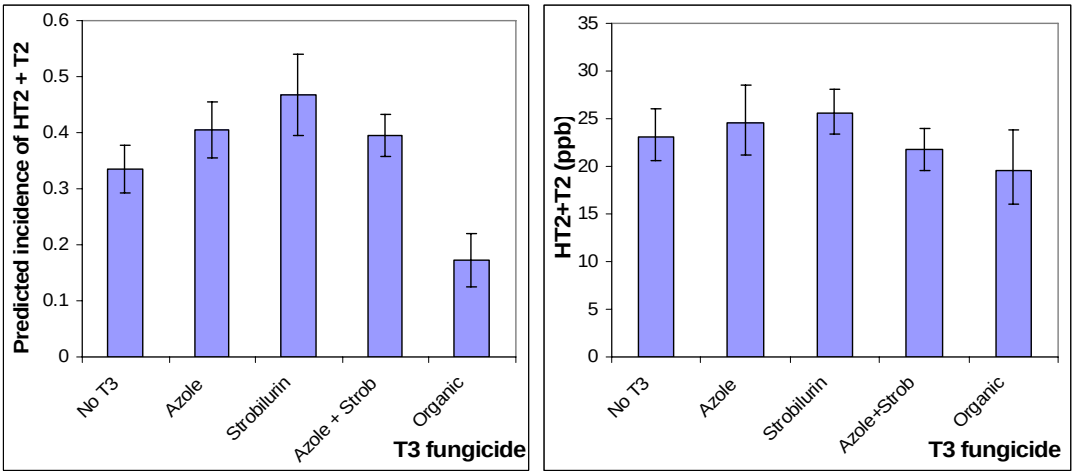


Fig. 3.6.4 Predicted proportion of UK wheat samples with detectable HT2+T2 grouped by Fusarium ear blight resistance rating. Bars represent 95% confidence limits for predictions.

As detailed in Fig 3.6.2, organic samples had significantly lower incidence and concentration of HT2+T2 than conventional samples. Within conventional samples the incidence of HT2+T2 was significantly higher for crops receiving a strobilurin without an azole partner at T3 compared to crops which received no T3. There was no significant difference between the concentration of HT2+T2 in positive conventional samples (LSD at 1%) (Fig 3.6.5).



A. B.

Fig. 3.6.5 A. Predicted proportion of UK wheat samples with detectable HT2+T2 grouped by fungicide regime. B. Predicted concentration of HT2+T2 in positive samples (>LoQ). Bars represent 95% confidence limits for predictions.

3.7 PCR-based analysis

Quantitative PCR of the *Tri5* gene has shown a significant ($p<0.01$) relationship exists between the concentration of genes coding for the production of the trichothecenes and the resulting trichothecene contamination of grain (Fig 3.7.1). The relationship differed significantly ($p<0.01$) between years. As DON was the predominant trichothecenes present in UK wheat, regression analysis of Tri5 DNA against DON produces similar results although the variance accounted for is slightly lower (Figure 3.7.1). For use as a predictive tool, then the influence of year would be unknown, regression analysis with all years combined resulted in an r^2 of 61% for total trichothecenes and 54% for DON. However the variance accounted for varied between years ranging from 28% in 2001 to 76% in 2005 for DON.

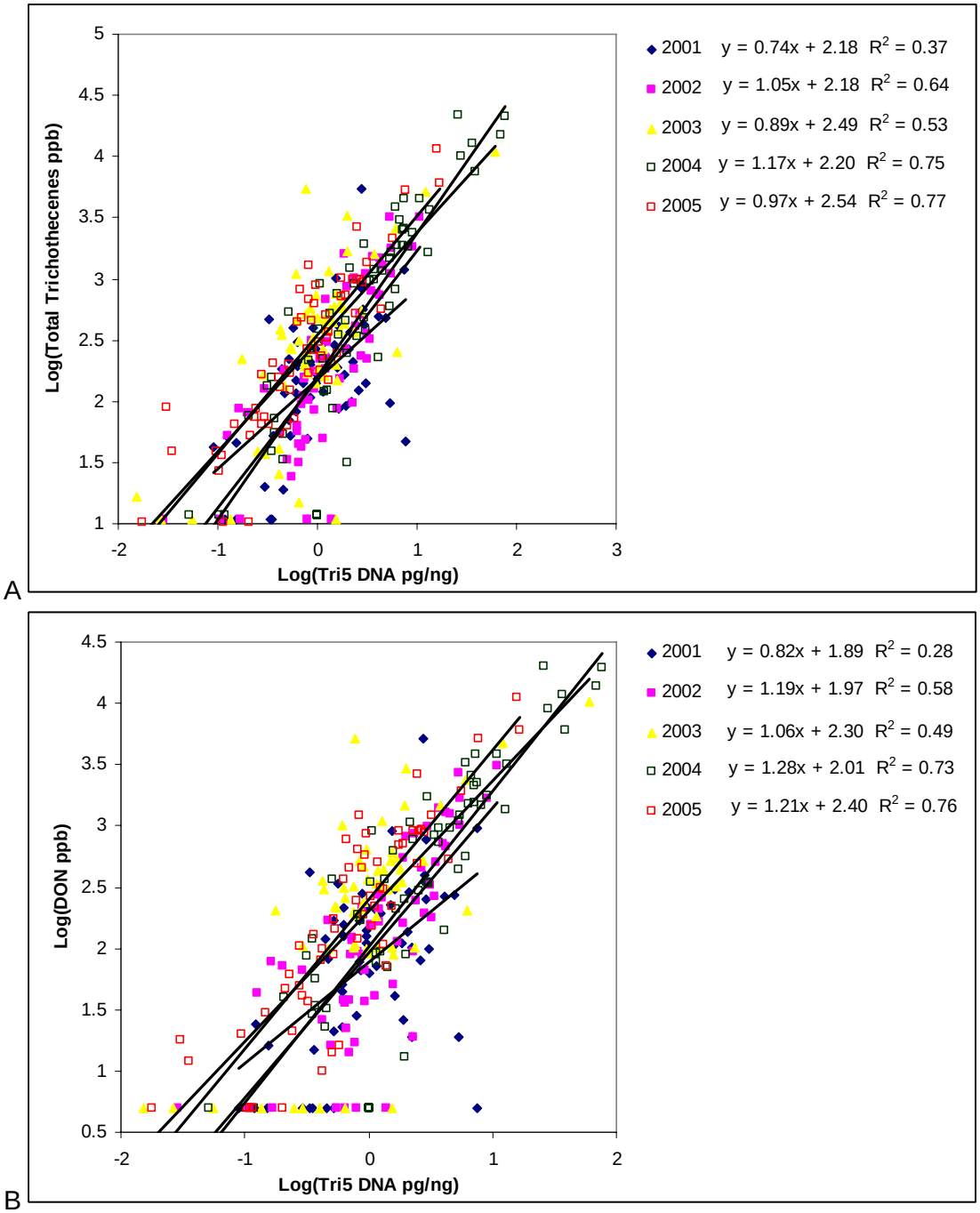


Figure 3.7.1 Regression analysis of A. total trichothecenes and B. DON against *Tri*5 DNA concentration in UK wheat samples from 2001 to 2005.

3.8 Visual analysis

One thousand grains were assessed for *Fusarium* damaged grains (FDG) for sixty selected samples from each year. Samples were selected to represent the range of DON contamination present each year. FDG were recorded as red or white grains; samples either contained no FDG or red, white or a mixture of both colours of FDG.

More red grains were present than white (mean FDG in selected samples was 5.3 and 2.5 for red and white FDG respectively). Figure 3.8.1A shows the relationship between FDG and DON concentration. All samples with more than 40 (0.4%) FDG had a DON concentration of more than 1250 ppb. Below this level of contamination the relationship was highly variable (Figure 3.8.1B). No samples with four (0.04%) or less FDG exceeded 1250 ppb DON. Of the samples between four and forty FDG, 26% exceeded 1250 ppb.

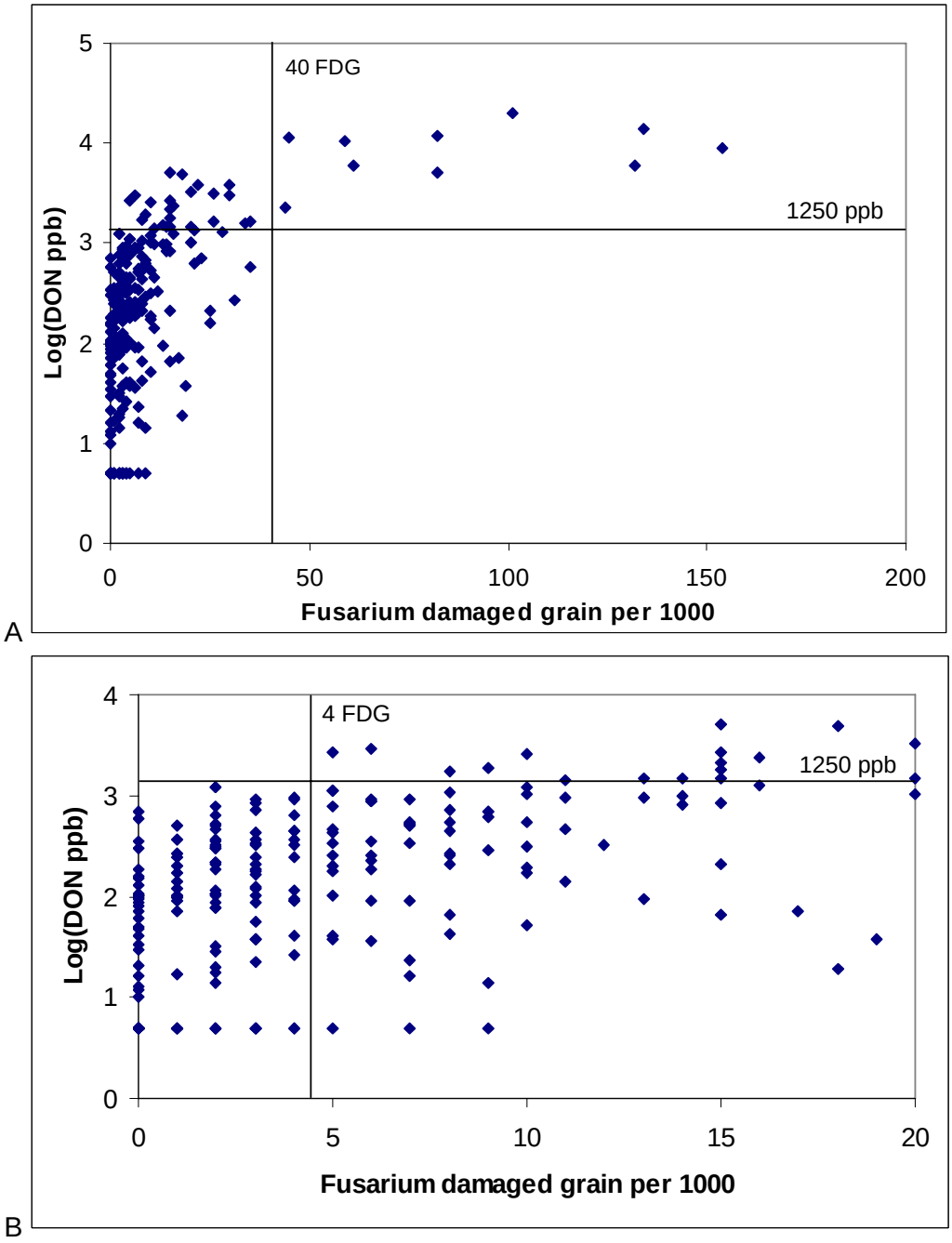


Figure 3.8.1 A. Regression of DON against Fusarium damaged grain count for wheat (2001-2005). B. As A but x-axis scale limited to 20 FDG.

Regression analysis showed a significant regression ($p<0.01$) occurred between logarithmic transformed values for DON (logd) and FDG (log[FDG+1]). Analysis of all years combined showed FDG accounted for 36% of the variance. Regression analysis with grouping by year showed there were significant differences between years ($p<0.01$), and 43% of variance was accounted for. Regression analysis for each year showed the variation accounted for varied from 8% in 2001 to 75% in 2004. Regressions tended to be better in years with more samples with high DON concentration (>1000 ppb).

Modelling DON using counts of red (R) and white (W) FDG allowed weighting of red and white FDG but only slightly improved the regression with 38% of variance accounted for when all years combined. The equation below shows that red FDG had a greater weighting than white. This agrees with a previous finding that red FDG contained about 60% more DON on average than white FDG (Bechtel *et al.* 1985).

Equation: $Logd = 1.44 + 0.99(log[R+1]) + 0.55(log[W+1])$

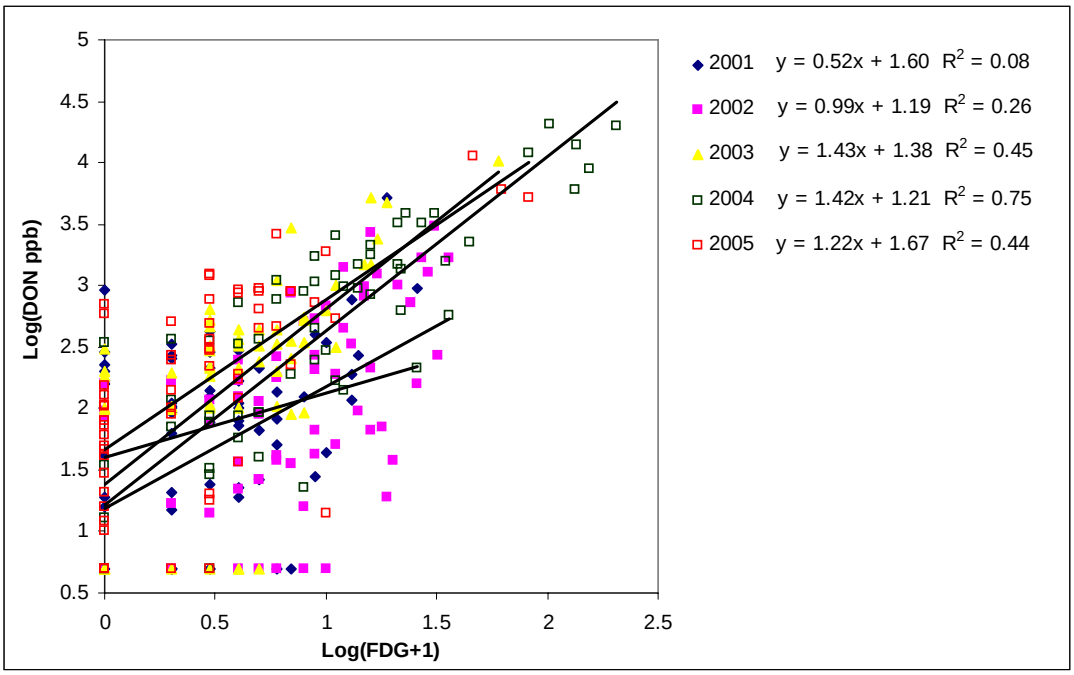


Figure 3.8.2 DON concentration against Fusarium damaged grain (FDG) count for UK wheat from 2001 to 2005.

3.9 Visual assessment workshop

A workshop on visual assessments was run at Harper Adams on the 9th June 2005 with twenty delegates from the cereal industry. Delegates had between zero and twenty years experience of visual assessments. Nine of the delegates had responsibility for accepting grain at intake. Most delegates (18) had a brief training session on identification of FDG before starting the assessment.

Sixty samples were selected from 2002-2004 harvests to cover the whole range of DON concentration. Samples were either accepted or rejected based on a visual assessment. Each assessor was scored for average mycotoxin content of accepted samples and number of false negative and false positive samples.

Nearly all delegates could identify all samples with more than 5000 ppb DON. The average DON and zearalenone content for all samples was 2010 and 141 ppb. The average DON and zearalenone for all samples below both thresholds (DON less than 1250 ppb and zearalenone less than 100 ppb) was 340 and 19 ppb. The average DON and zearalenone content of all samples accepted by delegates was 719 and 32 ppb. The average concentration of HT2+T2 was low for all samples and remained low for all selected samples.

The average number of false positive and false negative selections by delegates was 11 and 13% respectively.

Table 3.9.1 Average mycotoxin concentrations from the Fusarium visual assessment workshop.

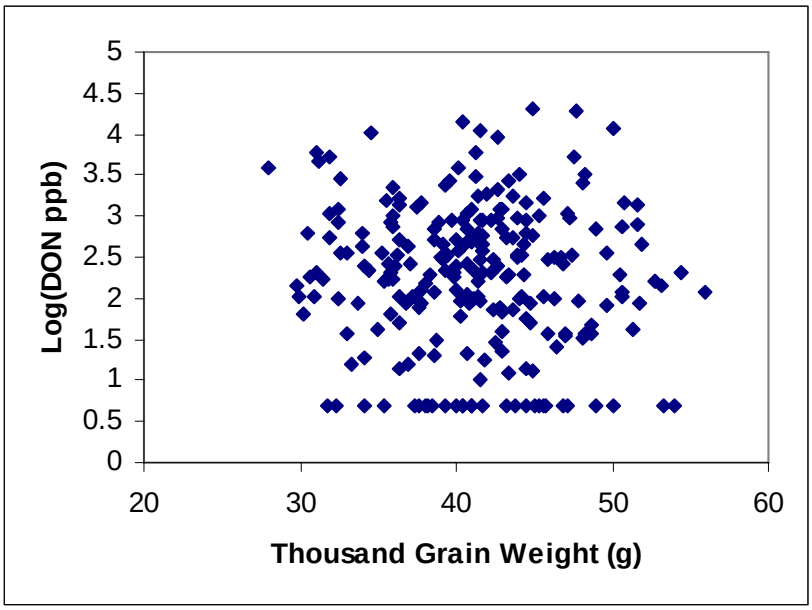
	Mycotoxin concentration (ppb)		
	DON	ZEAR	HT2+T2
All samples (n=60)	2010	141	18
Samples below thresholds (n=34)	340	19	17
Average concentration of samples 'passed' by delegates	719	32	17
Lowest average concentration of samples 'passed' by a single delegate	494	25	18
Highest average concentration of samples 'passed' by a single delegate	1260	47	18

It must be remembered that these samples were selected to cover a wide range of mycotoxin concentrations with a high proportion of samples close to, and well above, the legal limits for DON and zearalenone. As such the concentration distribution of the selected samples was very different to the true distribution of these mycotoxins in wheat. Therefore the mean values and percentage false positives and negatives do not reflect those that would be expected during commercial cereal intake inspections.

3.10 Grain quality

As well as visual assessments, grain quality was measured using two standard measurements of grain quality, thousand grain weight and specific weight. As can be seen in Figure 3.10.1, there was no relationship between thousand grain weight and DON concentration. For specific weight it appeared that samples with a low weight (<68 kg/hl) had high DON concentrations (>300 ppb). This was not part of the workshop referred to in Section 3.9.

A.



B.

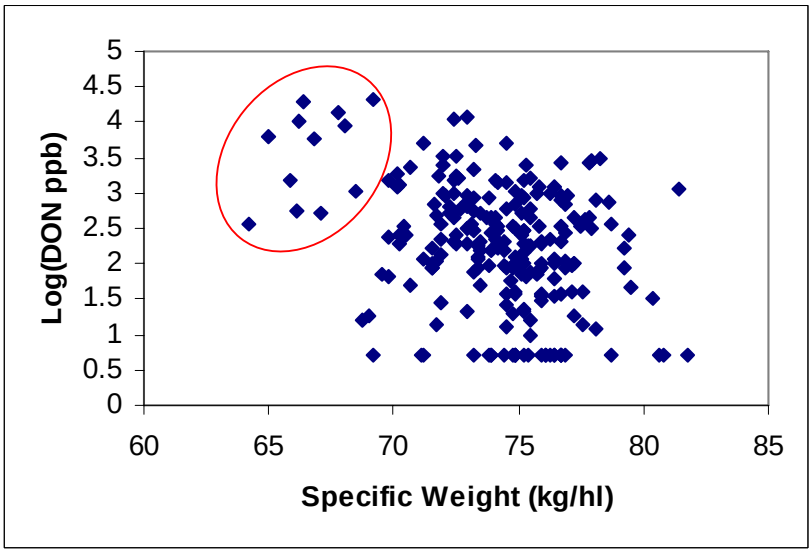


Figure 3.10.1 Regression analysis of DON against A. thousand grain weight and B. specific weight.

Discussion

Of the eleven fusarium mycotoxins analysed only seven were detected, of these only four, DON, nivalenol, HT2 and zearalenone were detected above 100 ppb. DON was the most frequently detected fusarium mycotoxin, present in 86% of samples, and was usually present at the highest concentration. DON and zearalenone were occasionally detected at concentrations above newly introduced legal limits for wheat grain intended for human consumption. HT2+T2 was occasionally detect above the proposed combined limit of 100 ppb. The incidence and concentration of DON, zearalenone and HT2+T2 were modelled against agronomic practices applied to each field.

DON and zearalenone contamination was shown to vary between seasons and between regions, with a consistent trend of lower levels in the north. This is in agreement with the CSL Crop Monitor project which has rarely detected *F. culmorum* and has not detected *F. graminearum* in wheat ear samples at GS 73-75 collected above North Yorkshire (Anon. 2004c; Jennings *et al.* 2004; Jennings *et al.* 2004). HT2+T2 incidence and concentration also varied with year, but only incidence varied with region. There was no decline in concentration towards the north indicating that the HT2+T2-producing *Fusarium* species distribution is less temperature dependent than DON-producing *Fusarium* species. The annual differences in DON contamination between the South and East are probably a result of regional differences in weather conditions between each year. The higher level of DON in the East compared to the Midlands was unexpected as the Midlands had more maize in rotation and higher rainfall. The higher level in the East was probably a result of the greater intensity of wheat and min-till in rotations in the East compared to in the Midlands. This study's result agrees with previous studies which have identified that climate is a major factor in DON and zearalenone content of cereals.

The highest average DON and zearalenone content in harvested wheat occurred after maize. Ploughing reduced DON and zearalenone contamination of subsequent wheat crops. Ploughing appeared to reduce DON and zearalenone contamination for all other previous crops, to a varying degree, except set-aside. The greatest difference in DON concentration was between ploughing and not ploughing after maize (four-fold). This agrees with data from other countries. The risk is greater in other countries where large amounts of grain maize are grown compared to in the UK, where the majority of maize is grown for forage. Studies in France and Germany have shown greater DON in wheat following grain maize compared to forage maize, this has been attributed to the greater amount of crop debris after grain maize. Large replicated field trials in Germany identified that there was a significant interaction between previous crop and cultivation technique (Koch *et al.* 2006). Following sugar beet there was no significant difference in DON concentration between wheat plots receiving different methods of cultivation, however, following a wheat crop without straw removal, direct drilled wheat had a significantly higher DON compared to wheat from plots which were either ploughed or min-tilled (Koch *et al.* 2006) Studies in France have determined that crop debris management can have a large impact on DON concentration at harvest, particularly after maize. Highest DON concentration was found after no-till, followed by min-till and then lowest levels after ploughing. The reduction in DON has been linked to the reduction in crop residue on the soil surface. However, the reduction in DON with min-till, compared to no-till is usually greater than the reduction of crop residue on the soil surface (Labreuche *et al.* 2005; Maumene 2005). This is probably due to the fact that min-till increases the

colonisation of crop debris with soil saprophytic microorganisms, which compete with *Fusarium* species. Chopping of maize debris before minimum tillage also caused a marked decrease in DON concentration in the following wheat crop (Maumene 2005), again this is likely to increase the mixing of crop debris with soil. In this study, samples were split by ploughed and not ploughed as too few samples were collected from no-till fields (eg one in 2005) to allow analysis of min-till versus no-till.

Recent results therefore indicate the following factors are important in the host crop and cultivation interaction:

a) Previous crop,
b) Amount of crop debris,
c) Removal/burial of crop debris
d) Mixing of soil and crop debris on the soil surface.

The one anomaly in the trend showing reduced DON and zearalenone in wheat after ploughing was for set-aside. Although not significantly different it is the only previous crop resulting in both higher DON and zearalenone predicted means in wheat after ploughing compared to after minimum cultivation. One common feature of set-aside fields is that they are usually treated with a broad spectrum herbicide such as glyphosate prior to cultivation. In a Canadian study it was shown that glyphosate use resulted in increased DON concentration in the following spring wheat crops (Fernandez *et al.* 2005). As this was an observational study there is no evidence of a "cause and effect" relationship. It has also been shown that glyphosate treatment results in the colonisation of plant roots by *Fusarium* species (Levesque *et al.* 1987; Levesque *et al.* 1993). Therefore the use of glyphosate on regeneration set-aside may result in an increase in *Fusarium* inoculum in plant roots, and by ploughing, this would bring this inoculum to the soil surface.

For HT2+T2 there was no statistical difference with cultivation but concentration of positive samples was significantly higher after oats than any other previous crop. It is known from the barley and oat project (see Project Report FSA CO4030/HGCA 2706) that oats have high HT2+T2 concentrations and are therefore likely to be an inoculum source for HT2+T2-producing *Fusarium*.

Winter wheat varieties in the UK are assessed for FEB resistance as part of the HGCA recommended list trials. Resistance is scored from one to nine with nine equalling high resistance. From 2001 to 2005 the range of resistance available on the recommended list was from four to seven, most varieties been five, six or seven. Results for DON and HT2+T2 showed an inverse relationship between the FEB resistance rating and the DON content of grain samples for winter wheat cultivars. Petrus is a German variety with a score of two on the German national assessment scheme (one to nine scale with one equalling high resistance) (Koch *et al.* 2006), and would probably therefore equal to an eight on the UK scale. Spring wheats are not assessed for FEB resistance. They have a similar DON and HT2+T2 content to winter wheats with a resistance rating of six or seven, this may be due to differences in agronomy (eg drilling date) as well as/rather than inherent differences in resistance. The fact that the results for DON and HT2+T2 are very similar would suggest that the polygenic resistance within UK wheat varieties is equally active against DON- and HT2+T2-producing species of *Fusarium*. Although Petrus is not a commercially viable variety under UK growing conditions it does illustrate the potential of varietal resistance in the reduction of fusarium mycotoxins.

Results for zearalenone are different with no significant difference between varietal resistance scores but a significantly higher concentration of zearalenone in positive samples of spring wheat compared to winter wheat samples. This difference may well be due to differences in the agronomy of the two wheat types. Spring wheat is later developing and therefore ripens later in the season. It is known that zearalenone is produced in late summer (unlike DON which is produced predominantly in early summer) (Matthaus *et al.* 2004) and therefore conditions maybe more conducive for zearalenone production when spring wheat ripens compared to when winter wheat ripens.

This is the first observational study with a large number of organic samples over several seasons to allow a robust comparison of organic and conventional samples of wheat. Results show there was no significant difference in DON and zearalenone concentration between organic and conventional samples. There was a lower incidence and concentration of HT2+T2 in organic samples. This matches data from the barley and oat project (FSA CO4030/HGCA 2706) which found lower levels of HT2+T2 in organic compared to conventional oats. Reasons for such a difference to occur are not readily identifiable as many confounding factors exist when comparing organic and conventional agronomy.

There was a significant difference in DON concentration between fungicide regimes by comparing T3 fungicide regimes. Wheat which had received an azole fungicide at T3 had a significantly lower DON content than samples which had received no T3 fungicide (organic or conventional). Previous experiments have shown that a good reduction in DON can be achieved in artificially inoculated field trials when inoculation of *Fusarium* and treatments are closely synchronised (ca. 90% reduction) (Nicholson *et al.* 2003). Reduction achieved is generally less in field trials with natural infection, which occurs over a longer period of time (ca. 50% reduction) (Simpson *et al.* 2001; Loos *et al.* 2005). The reduction seen in this observational study was not as good (ca. 30% reduction).

This is likely to be due to:

a) Not all azoles applied have good activity against FEB pathogens.
b) Not all azoles were applied at recommended rate (42% applied at below half recommended rate, 44% at half rate and 14% above half rate).
c) Not all azoles were applied at the recommended crop growth stage.

Some studies have indicated that certain strobilurin fungicides when applied in the absence of an azole partner can result in an increase in DON (Simpson *et al.* 2001; Ioos *et al.* 2005; Ellner 2006). When the strobilurin was applied in mixture with an azole partner, a reduction in DON occurred, but the reduction was not as good as the azole partner applied alone (Edwards *et al.* 2001; Nicholson *et al.* 2003). These results have been linked to the activity of these strobilurins towards other fungi which usually compete with *Fusarium* on the wheat ear (Edwards *et al.* 2001; Simpson *et al.* 2001). In this observational study such an effect was not detected for DON, however such an increase was seen for incidence of HT2+T2.

The predictive ability of the DON model was determined using two methods. Firstly, the parameter estimates for each agronomic factor where determined for each year. The results showed that the estimates were stable for the majority of factors over the

course of the project. The levels within a factor which showed relatively large variation were those levels with low sample numbers and therefore known to be less accurate. Using the Prediction Sum of Squares analysis (Montgomery and Peck 1992) the calculated $R^2_{prediction}$ was not much lower than the model's overall R^2 indicating that the model may be a good predictor of new observations. Consequently the model could be used to predict mycotoxin content of wheat crops based on the agronomy of the crop.

There are two advancements which would improve the predictive ability of the DON model. The first would be to include weather parameters. The variance accounted for by year and year*region interaction, is probably largely attributable to differences in weather between years and regions and would be unknown in a predictive model. The inclusion of weather parameters from flowering to harvest would account for some of this variance. The more accurate the weather data was for a particular field, then the more accurate the prediction would be. The second improvement would be to investigate other possible key agronomic factors. Based on this and other recent studies these would appear to be:

a) Intensity of host crops in the rotation
b) Intensity of min-till cultivation in the rotation
c) Crop debris management (removal/chopping/burial)

Due to the large number of samples with unquantifiable zearalenone (62% of samples below LoQ), the role of agronomic factors cannot be identified as accurately for zearalenone as for DON. As zearalenone and DON are produced by the same *Fusarium* species, *F. graminearum* and *F. culmorum*, and largely by the same isolates of these species; then it can be assumed that the effect of agronomy on zearalenone is the same as that for DON. This is supported by the correlation of DON and zearalenone concentrations determined in this study. The only difference is that zearalenone is produced at the end of the growing season whereas DON is produced primarily earlier in the season (GS65 -79); therefore any agronomy at the end of the season is likely to affect zearalenone concentration more than DON. There is little agronomic input that occurs after GS69. No agronomic factors analysed within the model occur after GS 69. The main factor would be weather conditions before and during harvest. One factor identified as significant is type (winter versus spring wheat varieties). As spring wheat ripens later than winter wheat then type is one agronomic factor that can have an influence on agronomy (later harvest) at the end of the season.

The models of HT2+T2 incidence and concentration indicate that the production of these mycotoxins is affected by some agronomic factors differently to that of DON and zearalenone. This is probably due to the differences in the epidemiology of the *Fusarium* species that produce HT2 and T2 compared to *F. culmorum* and *F. graminearum* which produce DON and zearalenone.

This project has clearly identified the extent to which UK wheats are contaminated with fusarium mycotoxins. Overall, levels of Fusarium mycotoxins in UK wheat are lower than those frequently found in continental Europe and in North America (Jones and Mirocha 1999; Schaafsma 2002; Anon. 2003b). The majority of samples contained fusarium mycotoxins, the predominant one being DON, however the vast majority of samples had a concentration close to the limit of quantification with only a low percentage of samples exceeding the new legal limits for DON and zearalenone.

Numbers of samples exceeding 1250 ppb DON were between about 0.5 and 5% over the five year period. More samples, between 1 and 10% exceeded 100 ppb zearalenone over the five year period. It is important to note that some processors of wholewheat products cannot reduce the mycotoxin content during processing, consequently they use an intake limit of 500 ppb DON. Between 1 and 10% of samples exceeded 500 ppb DON.

HT2+T2 concentrations were generally lower. The number of samples exceeding 100 ppb HT2+T2 varied from 0 to 1% between years. The poor relationship between HT2 and T2 is important as legislation will be introduced as a combined limit for HT2+T2. Currently there are no ELISA-based assays for HT2+T2. ELISA assays exist for T2 but these kits cross-react to a low percentage (ca. 10%) with HT2. Consequently, with a poor relationship between HT2 and T2 in wheat, ELISA assays for T2 are of very limited use. Consequently there is no quick or cheap method to allow the industry to monitor these mycotoxins at intake or other critical control points during processing.

Nivalenol was not detected at high concentrations. This maybe because nivalenol-producing isolates and species of *Fusarium* are less virulent on wheat than DON-producing isolates (Wong *et al.* 1995; Desjardins *et al.* 2004). Consequently, without very high levels of initial inoculum, infection can not cause high nivalenol concentrations in harvested grains. However, nivalenol is not a co-contaminant of DON, either based on the known chemotaxonomy or based on observational data such as this study, and as such nivalenol should not be treated as a co-contaminant. High nivalenol may occur in different cereals and/or countries and should be monitored accordingly.

Acetylated versions of DON did occur as low level co-contaminants of DON in a few samples, however five samples were analysed in 2005 with equivalent levels of DON and acetyl DON. These were not of concern as the combined concentration of these mycotoxins was not high. However, it is important to be aware that some isolates of *Fusarium* may produce equivalent concentrations of acetyl DON compared to DON and as such the acetylated versions of DON should be monitored as well as DON itself.

Highest concentration of DON and zearalenone occurred in 2004. In 2004, most of the country was drier than average during June, when most UK wheat is in flower. This resulted in lower than average fusarium ear blight at GS73-75 as recorded during the CSL Crop Monitor project (Anon. 2004c). However, a wetter than average summer followed for the whole of the UK resulting in high levels of ear blight reported at harvest, particularly where harvest was delayed due to continued wet weather. The worst affected area appeared to be around the Wash, an area which had higher than average rainfall in June that year. 2004 was a good example of how the relationship between FEB severity early in the summer and the final mycotoxin content of grain at harvest depends on weather conditions in the intervening period.

Highest levels of HT2+T2 occurred in 2003, which was not the highest year for DON and zearalenone indicating that the *Fusarium* species that produce HT2+T2 differ in their environmental requirements compared to those that produce DON and zearalenone.

It should be noted that:

a) This was not a stratified survey and as such the summary data may not be an exact representation of the UK situation.
b) Samples collected were for both human consumption and animal feed.
c) Samples intended for human consumption may have been rejected as unsuitable for that end use.
d) The legal limit includes an expanded measurement of uncertainty (MU), this approximates to a 95% confidence interval.

The *Tri*5 gene, which is present in all trichothecene-producing *Fusarium* species, was quantified in 60 samples selected from each of the five years. Regression analysis identified that there was a reasonable correlation with total trichothecene and the correlation was slightly less for DON alone. The correlations achieved in years with higher DON content (2004 and 2005) are similar to those achieved by Waalwijk *et al*. using individual assays for *F. culmorum* and *F. graminearum* (Waalwijk *et al.* 2004). It is believed the correlation from this assay is not good enough to use the PCR assay to estimate DON content in harvested wheat. A more accurate correlation may be achieved if primers were available for the DON chemotype of *F. graminearum* and *F. culmorum*. Unfortunately the only assays available for DON chemotypes are species specific and not quantitative. Diagnostic PCR of the 20 samples with highest DON in 2004 identified that all samples contained high amounts of *F. graminearum* and one sample contained some *F. culmorum* as well. A complete DNA screen of high DON samples (>1000 ppb) from this study would be useful to identify the key species responsible for high DON in UK wheat samples. It may be determined that *F. graminearum* must be present for high DON to occur in commercial crops.

Visual assessments, using fusarium damaged grain (FDG) counts, have previously proved to be inconsistent predictors of DON contamination of wheat (Schaafsma *et al.* 2004). Results from this project, where 1000 grains from each sample were carefully examined for FDG identified the following: Samples with less than 5 FDG had less than 1250 ppb DON and samples with more than 40 FDG had more than 1250 ppb. However, samples with between 5 and 40 FDG could contain anywhere between less than 10 and up to 5000 ppb DON. Part of the poor regression of FDG to DON is due to the error of counting low numbers (eg a threshold of 5 FDG per 1000); this error could be reduced by assessing a greater number of grains. However, this would be more labour intensive (eg a threshold of 50 FDG per 10,000 grains). For processors with an intake limit lower than the legal limit then it would be increasingly inaccurate to use visual assessments as part of a screening system.

The visual assessment workshop at Harper Adams identified that:

a) Visual assessments can reduce the amount of fusarium mycotoxins entering the cereal processing industry.
b) Individuals can, to varying degrees of ability, identify grain with high concentrations of fusarium mycotoxins based on visual assessment.
c) Visual assessment alone will not stop all grain consignments exceeding legal limits entering the cereal processing industry (false negatives).
d) Visual assessment alone will also result in grain consignments below the thresholds for fusarium mycotoxin also being rejected (false positives).

Results from this and other relevant studies have been used to inform the UK Code of Good Agricultural Practice to reduce fusarium mycotoxin in cereals produced by the FSA (Anon, 2007). The agronomic advice is summarised below:

a) Avoid maize as previous crop
b) Minimise previous crop residue on soil surface
c) Select resistant varieties
d) Consider an ear spray to control ear blight
e) Timely harvest

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Appendix 1 - Description of agronomic factors.

All agronomic factors are detailed below.

Year	2001 to 2005
Region	South, East, Midlands, North, Scotland, Northern Ireland HGCA defined regions except combined two Northern and two Southern Regions into two (North and South) as two regions have low sample number (North West and South East).
Practice	organic or conventional.
Previous crop	wheat, barley, oats, sugar beet, potatoes, brassicas (mainly oil seed rape), legumes (mainly peas and beans), grass, maize, set-aside. These encompass nearly all previous crops, few "others" were removed from dataset.
Plough	Method of cultivation; ploughed or not ploughed. Collected data on min-tilled and direct drilled, but very few direct drilled so combined as "not ploughed".
Type	winter or spring wheat variety.
Varress	Fusarium ear blight resistance score for UK winter wheat varieties plus spring wheats plus Petrus (German FEB resistant variety).
T3	T3 fungicide regime (T3 applied at flowering, growth stage 59-69) categories include azole, strobilurin, azole plus strobilurin mixture, no T3, organic. As includes all organic and conventional samples this is a sub-set of practice above.
Varnabim	Nabim end-use categories for bread, biscuit and feed wheats.
Use	Intended end use – Seed, feed, human consumption, other (usually distillation).
Maize in rotation	Yes/No.
Maize adjacent	Yes/No.
Source	Who supplied sample – agrochemical distributor, independent agronomist, farmer.

Appendix 2 – Number of samples for each level within each agronomy factor from dataset with blanks removed.

Table A2.1 Number of observations for year x region

	Year				
Region	2001	2002	2003	2004	2005
South	41	52	69	55	51
East	70	54	60	71	59
Midlands	55	84	66	92	68
North	50	55	68	68	65
Scotland	29	36	26	32	24
Northern Ireland	0	15	15	12	11

Table A2.2 Number of observations for cultivation x previous crop

Previous crop	Cultivation	
	ploughed	not ploughed
Wheat	273	78
Barley	54	5
Oats	59	16
Sugar beet	68	8
Potato	73	23
Brassicas	224	100
Legumes	129	48
Grass	99	5
Maize	72	15
Set-aside	85	19

Table A2.3 Number of observations for each variety resistance category

Varietal resistance	Number
Spring Wheat	120
FEB resistance score 4	27
FEB resistance score 5	283
FEB resistance score 6	566
FEB resistance score 7	453
Petrus	4

Table A2.4 Number of observations for fungicide use at flowering (T3)

T3 fungicide	Number
No T3	465
Azole	244
Strob	120
Azole/Strob	422
Organic	202

Appendix 3 - DON statistical analysis

DON concentration was not normally distributed. Log_{10} transformation resulted in a distribution which approached normality.

Logd = log_{10}(DON)

A3.1 Stepwise model selection

All years data sets were combined and significant agronomic factors were selected for the model using a stepwise model selection ANOVA on Genstat 8. Temporal (year) and spatial (region) factors were forced into the model. All other agronomic factors were ordered based on the order in which they occur within a growing season. 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.

Table of accumulated ANOVA of log_{10}(DON) using selected factors is shown below.

Table A3.1 Accumulated analysis of variance table for Log_{10}DON concentration

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ year	4	54.8145	13.7036	37.28	<.001
+ region	5	214.2586	42.8517	116.57	<.001
+ year.region	19	33.3683	1.7562	4.78	<.001
+ previous crop	9	13.7884	1.532	4.17	<.001
+ plough	1	4.9513	4.9513	13.47	<.001
+ pcrop.plough	9	8.2462	0.9162	2.49	0.008
+ varress	5	20.7477	4.1495	11.29	<.001
+ T3	4	3.7296	0.9324	2.54	0.039
Residual	1396	513.1896	0.3676		
Total	1452	867.0943	0.5972		

The model accounted for 41% of the observed variance. The majority of variation accounted for, 35% of the observed variance, was attributable to the temporal and spatial factors (year, region and year*region).

A3.2 Assessment of goodness of fit for logd by residual plots

Normal plot of a good model should have residual values in a straight line 45° diagonally through the axis. Fitted values plot of a good model should show a random scatter. Samples below the Limit of Quantification (LoQ) result in the straight line of residuals in the bottom left of the plot.

These plots show that the model is not a bad fit (Fig A3.2.1).

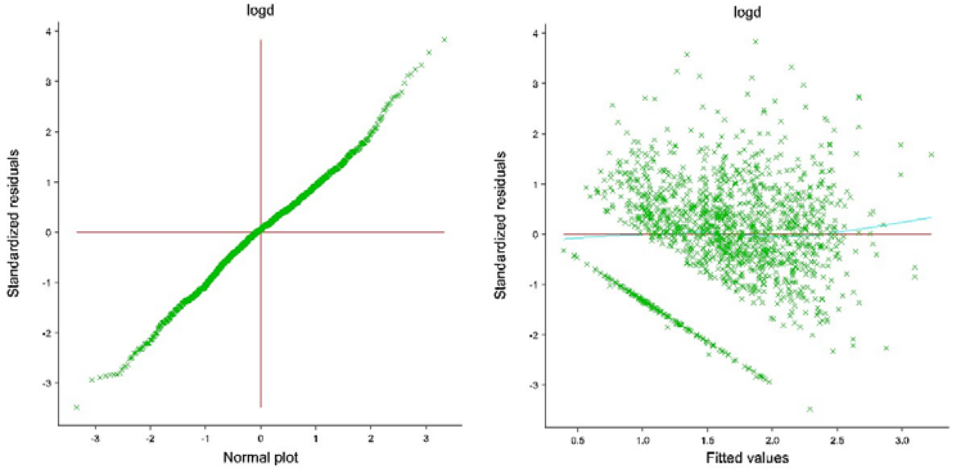

Figure A3.2.1 Residual plots of logd (log_{10} transformed DON concentration).

A3.3 Tables of predicted means and standard error of the predicted mean for DON concentration on the $\log_{10}$ scale (logd)

Graphs presented in the main text of report are the back-transformed predicted values below (10^{logd}) and the bars are the 95% confidence limits ($10^{[logd \pm 2*s.e.]}$).

Table A3.3.1 Predicted mean and standard error for each region/year combination

Region	2001		2002		2003	
	Prediction	s.e.	Prediction	s.e.	Prediction	s.e.
South	1.5913	0.0951	2.2549	0.0844	2.0962	0.0734
East	1.734	0.0727	1.8613	0.0829	2.0066	0.0785
Midlands	1.0911	0.082	1.1141	0.0665	1.5002	0.0749
North	1.259	0.0859	1.0914	0.0821	1.3011	0.0741
Scotland	1.0458	0.1134	0.9671	0.1016	0.9428	0.1192
N Ireland			1.0276	0.1569	1.1889	0.1567

Region	2004		2005	
	Prediction	s.e.	Prediction	s.e.
South	2.3725	0.082	2.3043	0.0856
East	2.2961	0.0724	2.0559	0.0791
Midlands	1.6533	0.0642	1.7378	0.0737
North	1.6416	0.0738	1.7818	0.0755
Scotland	0.8857	0.108	0.6608	0.1241
N Ireland	2.0052	0.175	1.8819	0.1832

Table A3.3.2 Predicted mean and standard error for each previous crop/cultivation combination

Previous crop	Ploughed Prediction	s.e.	Not ploughed Prediction	s.e.
Wheat	1.5612	0.0373	1.9656	0.0690
Barley	1.2053	0.0832	1.4628	0.2712
Oats	1.5984	0.0793	1.7057	0.1527
Sugar beet	1.7082	0.0737	1.8391	0.2144
Potato	1.4041	0.0712	1.9628	0.1266
Brassica	1.5105	0.0410	1.7466	0.0613
Legumes	1.6070	0.0539	1.7776	0.0877
Grass	1.4564	0.0616	1.6688	0.2712
Maize	2.0580	0.0718	2.6807	0.1569
Set-aside	1.7239	0.0663	1.5598	0.1391

Table A3.3.3 Predicted mean and standard error for each varietal resistance category of wheat. Categories include winter wheat with disease resistance 4, 5, 6 and 7; spring wheats and Petrus)

Resistance score	Prediction	s.e.
FEB resistance 5	1.8078	0.0363
FEB resistance 4	1.8957	0.1168
FEB resistance 6	1.5780	0.0260
FEB resistance 7	1.5757	0.0288
Spring Wheat	1.7099	0.0563
Petrus	0.7039	0.3037

Table A3.3.4 Predicted mean and standard error for wheat crops receiving a T3 fungicide application

T3 fungicide	Prediction	s.e.
No T3	1.6822	0.0287
Azole	1.5274	0.0391
Strobilurin	1.6087	0.0556
Azole+Strobilurin	1.6509	0.0299
Organic	1.7115	0.0434

A3.4 DON parameter estimates

Parameter for each factor are differences compared with a reference level. Table 3.4.1 shows reference levels set for each agronomic factor.

Table 3.4.1 Reference levels for DON concentration model

Factor	Reference Level	Reason
year	2001	first year of study
region	East	region with most wheat grown
previous crop	wheat	most common previous crop
cultivation	ploughed	most common cultivation
variety resistance	score 6	most common resistance score
T3 fungicide	no T3 spray	most common T3 option

Table 3.4.2 Parameter estimates for DON with standard error, t value and t probability

			estimate	s.e.	t(1396)	t pr.
Constant			1.654	0.084	19.720	<.001
Year	2002		0.063	0.111	0.570	0.567
	2003		0.249	0.108	2.300	0.022
	2004		0.458	0.104	4.380	<.001
	2005		0.264	0.109	2.420	0.016
Region	Midlands		-0.602	0.110	-5.460	<.001
	North		-0.410	0.114	-3.610	<.001
	N Ireland		-0.002	0.205	-0.010	0.993
	South		-0.146	0.121	-1.200	0.231
	Scotland		-0.695	0.138	-5.050	<.001
Year*Region	2002	Midlands	-0.078	0.153	-0.510	0.609
	2002	North	-0.273	0.162	-1.680	0.092
	2002	N Ireland	-0.686	0.269	-2.550	0.011
	2002	South	0.627	0.169	3.710	<.001
	2002	Scotland	-0.164	0.189	-0.870	0.387
	2003	Midlands	0.109	0.156	0.700	0.484
	2003	North	-0.283	0.157	-1.800	0.072
	2003	N Ireland	-0.684	0.268	-2.560	0.011
	2003	South	0.163	0.163	1.000	0.318
	2003	Scotland	-0.440	0.199	-2.210	0.027
	2004	Midlands	-0.025	0.147	-0.170	0.864
	2004	North	-0.104	0.155	-0.680	0.499
	2004	N Ireland	-0.110	0.279	-0.400	0.693
	2004	South	0.298	0.163	1.830	0.068
	2004	Scotland	-0.625	0.192	-3.260	0.001
	2005	Midlands	0.325	0.155	2.100	0.036
	2005	North	0.182	0.158	1.150	0.248
	2005	N Ireland	0.000	*	*	*
	2005	South	0.402	0.169	2.380	0.017
	2005	Scotland	-0.613	0.202	-3.030	0.002

Table 3.4.2 cont. Parameter estimates for DON with standard error, t value and t probability

			estimate	s.e.	t(1396)	t pr.
Previous crop	Barley		-0.090	0.096	-0.940	0.349
	Oats		0.079	0.090	0.870	0.383
	Sugar beet		0.085	0.084	1.010	0.315
	Potato		-0.013	0.085	-0.150	0.879
	Brassicas		0.060	0.057	1.050	0.292
	Legumes		-0.016	0.068	-0.240	0.810
	Grass		-0.034	0.081	-0.420	0.672
	Maize		0.336	0.082	4.080	<.001
	Set-aside		0.150	0.079	1.890	0.059
Cultivation	Not ploughed		0.337	0.080	4.240	<.001
	Barley	NP	-0.301	0.298	-1.010	0.313
	Oats	NP	-0.001	0.194	-0.010	0.994
	Sugar beet	NP	-0.332	0.241	-1.380	0.167
	Potato	NP	-0.079	0.167	-0.470	0.636
	Brassicas	NP	-0.311	0.109	-2.850	0.004
	Legumes	NP	-0.207	0.132	-1.570	0.116
	Grass	NP	-0.458	0.292	-1.570	0.117
	Maize	NP	0.288	0.194	1.480	0.139
	Set-aside	NP	-0.419	0.176	-2.380	0.017
Variety resistance	Spring Wheat		0.084	0.082	1.030	0.305
	Score 4		0.177	0.122	1.450	0.148
	Score 5		0.245	0.045	5.400	<.001
	Score 7		-0.079	0.040	-1.970	0.049
	Petrus		-0.657	0.313	-2.100	0.036
T3 fungicide	Azole		-0.135	0.050	-2.730	0.006
	Strobilurin		0.027	0.066	0.400	0.686
	Azole/Strob		-0.026	0.044	-0.590	0.555
	Organic		0.047	0.073	0.640	0.520

NP = Not ploughed

Constant estimate is estimated $\log_{10}$ transformed DON concentration (logd) for wheat samples from 2001, from the East, after wheat, after ploughing, winter wheat variety with a ear blight resistance score of 6 and not sprayed with a fungicide at T3. Other estimates are the ratio of that factor level and the constant. T probability indicates significance of difference between DON concentration of factor level and the reference level.

Back transformed parameter estimates and 95% confidence intervals are shown in Table 3.4.3. Constant estimate is estimated DON concentration for wheat samples from 2001, from the East, after wheat, after ploughing, winter wheat variety with a ear blight resistance score of 6 and not sprayed with a fungicide at T3. Other estimates are the ratio of that factor level and the constant. The lower and upper values are the 95% confidence limits for the estimated ratio.

Table 3.4.3 Back-transformed (10^X) parameter estimates for DON with 95% confidence intervals

			Ratio	Lower	Upper
Constant			45.05	30.85	65.79
Year	2002		1.157	0.702	1.907
	2003		1.772	1.088	2.888
	2004		2.868	1.790	4.595
	2005		1.835	1.122	3.000
Region	Midlands		0.250	0.152	0.411
	North		0.389	0.233	0.650
	N Ireland		0.996	0.394	2.514
	South		0.715	0.413	1.237
	Scotland		0.202	0.108	0.376
Year*Region	2002	Midlands	0.835	0.418	1.669
	2002	North	0.533	0.256	1.109
	2002	N Ireland	0.206	0.061	0.695
	2002	South	4.240	1.975	9.100
	2002	Scotland	0.686	0.292	1.612
	2003	Midlands	1.286	0.636	2.598
	2003	North	0.521	0.256	1.060
	2003	N Ireland	0.207	0.062	0.692
	2003	South	1.454	0.697	3.034
	2003	Scotland	0.363	0.148	0.893
	2004	Midlands	0.943	0.485	1.836
	2004	North	0.786	0.391	1.581
	2004	N Ireland	0.776	0.220	2.733
	2004	South	1.988	0.950	4.161
	2004	Scotland	0.237	0.100	0.564
	2005	Midlands	2.112	1.051	4.247
	2005	North	1.521	0.746	3.104
	2005	N Ireland	1.000	*	*
	2005	South	2.522	1.178	5.401
	2005	Scotland	0.244	0.098	0.607

Table 3.4.3 cont. Back transformed (10^x) parameter estimates for DON with 95% confidence intervals

			Ratio	Lower	Upper
Previous crop	Barley		0.814	0.529	1.253
	Oats		1.199	0.797	1.804
	Sugar beet		1.215	0.831	1.776
	Potato		0.971	0.661	1.426
	Brassicas		1.149	0.887	1.487
	Legumes		0.963	0.707	1.311
	Grass		0.924	0.642	1.331
	Maize		2.168	1.494	3.147
	Set-aside		1.412	0.987	2.021
Cultivation	Not ploughed		2.172	1.517	3.111
	Barley	NP	0.500	0.130	1.922
	Oats	NP	0.997	0.416	2.389
	Sugar beet	NP	0.465	0.157	1.379
	Potato	NP	0.833	0.391	1.775
	Brassicas	NP	0.489	0.299	0.800
	Legumes	NP	0.621	0.343	1.125
	Grass	NP	0.348	0.093	1.302
	Maize	NP	1.939	0.807	4.661
	Set-aside	NP	0.381	0.172	0.844
Variety resistance	Spring Wheat		1.214	0.838	1.758
	Score 4		1.502	0.866	2.606
	Score 5		1.759	1.433	2.159
	Score 7		0.834	0.696	0.999
	Petrus		0.22	0.054	0.906
T3 funigicide	Azole		0.733	0.586	0.916
	Strobilurin		1.063	0.789	1.433
	Azole/Strob		0.942	0.774	1.148
	Organic		1.113	0.802	1.545

NP = Not ploughed

Constant estimate is estimated DON concentration for wheat samples from 2001, from the East, after wheat, after ploughing, winter wheat variety with a ear blight resistance score of 6 and not sprayed with a fungicide at T3. Other estimates are the ratio of that factor level and the constant. The lower and upper values are the 95% confidence limits for the estimated ratio.

A3.5 Stability of each agronomic factor's effect on DON concentration over time

When data are collected across time, the stability of the coefficients over a shorter time span can be examined i.e. fit the same model for each year separately and compare the magnitude of estimates over time. Consistent estimates give support that the chosen model is applicable to broader circumstance than those related to the original data i.e. the model is stable over time.

The coefficients shown in Fig A3.5.1 are arranged alphabetically. In general they appear quite stable over time. Coefficients that show appreciable changes over time include various previous crop/cultivation interactions and the main effects region = Northern Ireland, variety resistance = FEB score 4 and Petrus. The common feature of these predictors is that they have low sample numbers.

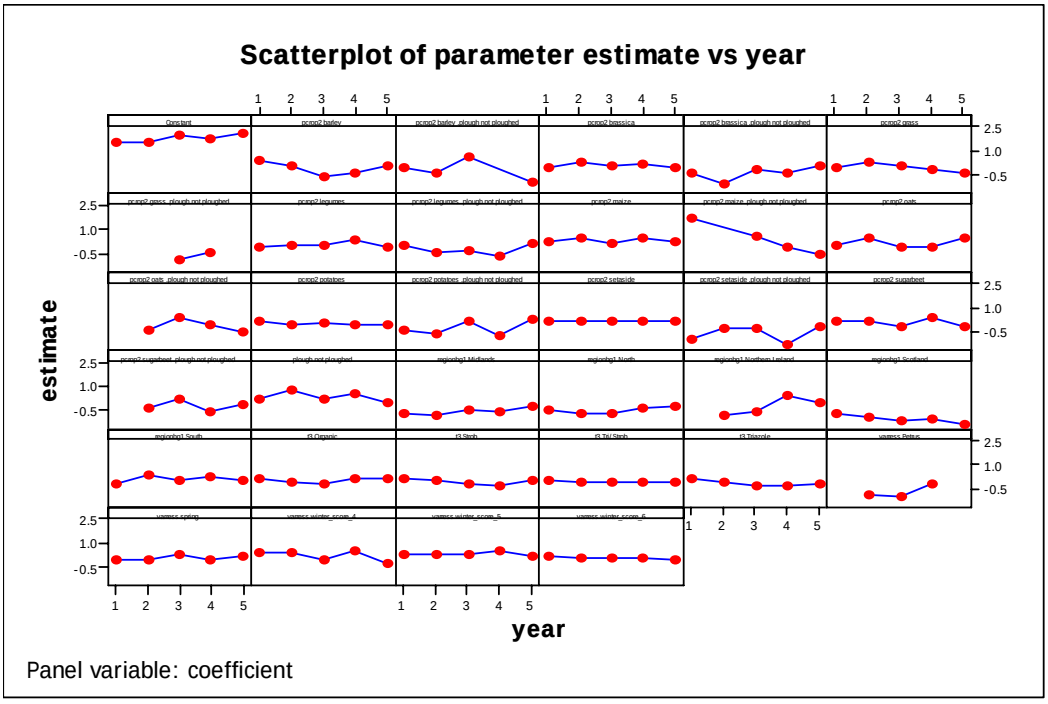

Figure A3.5.1 Scatterplot of parameter estimate for each year

A3.6 Cross-validation by splitting the dataset

The dataset was split into a training set and a validation set. The regression coefficients are derived from the training set and used to form predictions for the observed responses in the validation set.
Then the discrepancy between observed and predicted responses in the validation set was used to compute $R^2_{prediction}$ as a summary measure that indicates roughly how much of the variability in new observations the selected model might be expected to explain.

Due to the observational nature of the study and the number of factors and levels in each factor it was difficult to split the dataset into a balanced validation and training set. Therefore the PRESS statistic was used to calculate $R^2_{prediction}$ (Montgomery & Peck, 1992).

PRESS stands for Prediction Error Sum of Squares and is the analogous of the Residual Sum of Squares from a model fitted omitting one observation at a time. Thus PRESS uses each possible subset of *(n-1)* observations as the training set, and every omitted observation in turn is predicted.

So: $R^2_{prediction} = 1 - \frac{PRESS}{Syy} = 0.355 = 35.5\%$

where S_{yy} is the Total (corrected) Sum of Squares for the entire dataset.

$R^2_{prediction}$ is not much lower than R^2 of 40.7% from the full dataset, indicating the model may be a good predictor of new observations.

Appendix 4 – Number of samples for each level within each agronomy factor from dataset of positive zearalenone samples

Table A4.1 Number of samples with quantifiable zearalenone for year x region

	Year					
Region	2001	2002	2003	2004	2005	Total
South	15	37	30	44	22	148
East	27	33	31	48	22	161
Midlands	5	20	21	55	8	109
North	9	25	12	42	7	95
Scotland	5	8	1	7	1	22
N.Ireland	0	8	4	10	5	27
Total	61	131	99	206	65	562

Table A4.2 Number of samples with quantifiable zearalenone for previous crop x cultivation

Previous	Cultivation	
crop	Ploughed	Not ploughed
Wheat	100	35
Barley	19	1
Oats	26	8
Sugar beet	25	4
Potatoes	28	12
Brassica	71	38
Legumes	45	23
Grass	36	3
Maize	37	11
Set-aside	33	7
Total	420	142

Table A4.3 Number of samples with quantifiable zearalenone for each type of wheat

Type	Number
winter	515
spring	47

Appendix 5 - Zearalenone statistical analysis

Due to the low number of quantifiable samples (above LoQ) for zearalenone then the incidence of zearalenone was modelled using the Bernoulli distribution and then the concentration (log_{10} transformed) of the quantifiable samples was modelled using a normal distribution.

A5.1 Stepwise model selection for zearalenone incidence

As for DON, significant agronomic factors were selected for the model using a stepwise model selection method on Genstat 6. Temporal (year) or spatial (region) factors were forced into the model. All other agronomic factors were ordered based on the order in which they occur within a growing season.

To analyse incidence, samples above LoQ were set a value of one, samples below the LoQ for zearalenone were given a value of zero.

Table of accumulated analysis of deviance of zearalenone incidence using selected factors is shown below.

Table A5.1 Accumulated analysis of deviance table for zearalenone incidence

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
year	4	135.896	33.974	33.97	<.001
region	5	134.077	26.815	26.82	<.001
year.region	19	36.717	1.932	1.93	0.009
Residual	1442	1659.247	1.151		
Total	1470	1965.937	1.337		

A5.2 Assessment of goodness of fit by ROC curve analysis

Receiver Operating Characteristic curve (or ROC curve) is a plot of the true positives [sensitivity] against the false positives [1 - specificity]. The closer the curve follows the left-hand and top border of the ROC space (area under curve approaches 1), the more accurate the classification based on the model used. The null hypothesis is that the model is not a good fit (ie area under curve = 0.5)

Table 5.2.1 ROC curve case processing summary

Zearalenone	Valid N (listwise)
Positive[a]	562
Negative	891

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.
[a]The positive actual state is 1.

Of 1453 samples, 891 (61%) were below the LoQ.

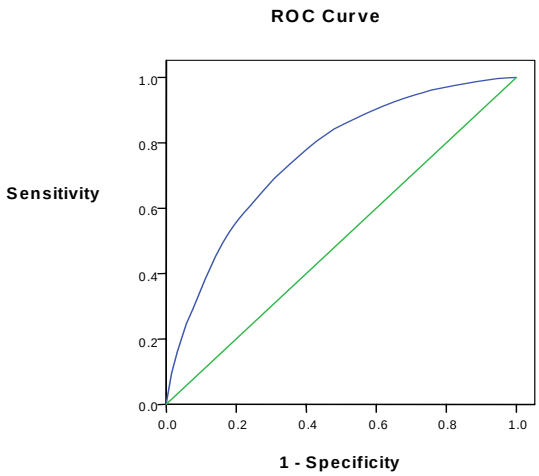

Fig 5.2.1 ROC curve for zearalenone incidence

Table 5.2.2 Area under the ROC Curve

Area	Std. Error	Asymptotic Sig.[a]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.758	.013	.000	.733	.783

[a]Null hypothesis: true area = 0.5

Area under curve = 0.758; therefore the model is not a bad fit

A5.3 Tables of predicted proportions and standard error of the predicted proportions for zearalenone incidence (>LoQ).

Table 5.3.1 Predicted proportion of samples with zearalenone greater than the LoQ and standard error for each region/year combination

Region	2001 Prediction	s.e.	2002 Prediction	s.e.	2003 Prediction	s.e.
South	0.3721	0.057	0.717	0.0653	0.4348	0.0645
East	0.375	0.0736	0.6182	0.0617	0.5167	0.0597
Midlands	0.0893	0.0381	0.2381	0.0463	0.3134	0.0565
North	0.2157	0.0575	0.4545	0.0672	0.169	0.0444
Scotland	0.1724	0.0701	0.25	0.0719	0.0385	0.0372
N Ireland			0.5625	0.1239	0.2667	0.1138

Region	2004 Prediction	s.e.	2005 Prediction	s.e.
South	0.7895	0.0548	0.4231	0.0627
East	0.6805	0.054	0.3833	0.0685
Midlands	0.5978	0.051	0.1176	0.0391
North	0.6176	0.0588	0.1077	0.0384
Scotland	0.2188	0.0729	0.0417	0.0404
N Ireland	0.8333	0.1074	0.4545	0.1502

A5.4 Stepwise model selection for positive zearalenone dataset

As for DON, significant agronomic factors were selected for the model using a stepwise model selection ANOVA on Genstat 8 for a dataset containing samples above the LoQ for zearalenone. Zearalenone concentration was $\log_{10}$ transformed to normalise the data (logz). The table of accumulated ANOVA of logz using selected factors is shown below.

Table 5.4.1 Accumulated analysis of variance table

Change	d.f.	s.s.	m.s.	v.r.	F pr.
year	4	31.0235	7.7559	40.29	<.001
region	5	9.3089	1.8618	9.67	<.001
year.region	19	6.1989	0.3263	1.69	0.033
previous crop	9	6.4628	0.7181	3.73	<.001
plough	1	3.4926	3.4926	18.14	<.001
pcrop.plough	9	3.394	0.3771	1.96	0.042
type	1	0.7636	0.7636	3.97	0.047
Residual	513	98.7472	0.1925		
Total	561	159.3915	0.2841		

The model accounted for 38% of the variance.

A5.5 Assessment of goodness of fit for logz by residual plots

Plots show the model is not a bad fit (Fig A5.5.1)

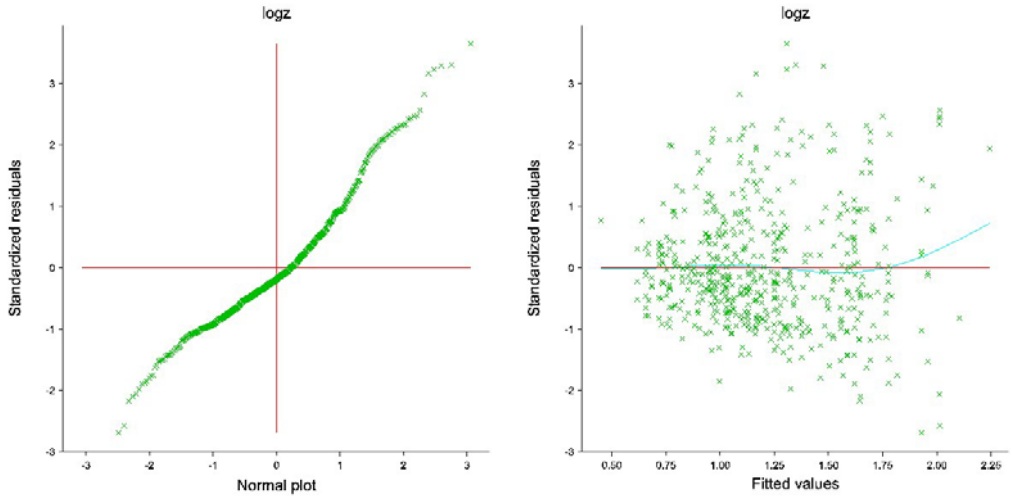

Figure A5.5.1 Residual plots of logz ($\log_{10}$ transformed zearalenone concentration)

A5.6 Tables of predicted means and standard error of the predicted mean for zearalenone concentration on the log_{10} scale (logz)

Graphs presented in the main text of the report are the back-transformed predicted values from the tables below (10[logz]) and the bars are the 95% confidence limits (10[logz±2*s.e.])

Table 5.6.1 Predicted mean and standard error for each region/year combination

	2001		2002		2003	
Region	Prediction	s.e.	Prediction	s.e.	Prediction	s.e.
South	0.8574	0.1143	1.1607	0.0751	1.2365	0.0841
East	0.7781	0.0862	1.1364	0.0777	1.0701	0.0817
Midlands	0.7147	0.1968	1.0677	0.1012	1.0481	0.0967
North	0.8776	0.1468	0.7629	0.0893	0.9055	0.1279
N.Ireland			1.0846	0.1564	0.9076	0.2197
Scotland	0.8774	0.1970	1.0103	0.1551	0.8062	0.4387

	2004		2005	
Region	Prediction	s.e.	Prediction	s.e.
South	1.7188	0.0689	1.1115	0.0948
East	1.7491	0.0670	1.2888	0.0944
Midlands	1.3273	0.0640	1.0557	0.1554
North	1.3705	0.0693	1.1833	0.1678
N.Ireland	1.4719	0.1395	1.3641	0.1967
Scotland	0.878	0.1725	0.7243	0.4387

Table 5.6.2 Predicted mean and standard error for each previous crop/cultivation combination

	Cultivation			
Previous	Ploughed		Not ploughed	
crop	Prediction	s.e.	Prediction	s.e.
Wheat	1.0654	0.0467	1.4774	0.0762
Barley	0.9784	0.1017	0.7236	0.4387
Oats	1.1178	0.0872	1.3818	0.1635
Sugar beet	1.1648	0.0907	1.5116	0.2194
Potatoes	1.0548	0.0838	1.4002	0.1290
Brassicas	1.0530	0.0556	1.2556	0.0740
Legumes	1.0385	0.0671	1.1056	0.0938
Grass	1.1733	0.0741	1.5688	0.2533
Maize	1.5101	0.0739	1.8143	0.1380
Set-aside	1.1593	0.0782	0.9060	0.1665

Table 5.6.3 Predicted mean and standard error for each wheat type

Type	Prediction	s.e.
winter	1.1517	0.0216
spring	1.3545	0.0647

Appendix 6 - Number of samples for each level within each agronomy factor from dataset of positive HT2+T2 samples

Table A6.1 Number of observations with quantifiable HT2+T2 by year and region

	Year				
Region	2001	2002	2003	2004	2005
South	22	12	39	5	13
East	28	7	49	13	25
Midlands	16	15	42	4	39
North	5	3	43	1	37
N. Ireland	0	5	13	0	4
Scotland	2	5	24	0	6

Table A6.2 Number of observations with quantifiable HT2+T2 for previous crop

Previous crop	Number
Wheat	137
Barley	21
Oats	26
Sugar beet	27
Potatoes	30
Brassicas	95
Legumes	48
Grass	24
Maize	28
Set-aside	41

Table A6.3 Number of observations with quantifiable HT2+T2 for fungicide use at flowering (T3)

T3 fungicide	Number
No T3	94
Azole	55
Strobilurin	163
Azole+Strob	132
Organic	33

Appendix 7 - HT2+T2 statistical analysis

Due to the low number of quantifiable samples (above LoQ) for HT2+T2 then the incidence of HT2+T2 was modelled using the Bernoulli distribution and then the concentration (log_{10} transformed) of the quantifiable samples was modelled using a normal distribution.

A7.1 Stepwise model selection for HT2+T2 incidence

As for DON, significant agronomic factors were selected for the model using a stepwise model selection method on Genstat 6. Temporal (year) or spatial (region) factors were forced into the model. All other agronomic factors were ordered based on the order in which they occur within a growing season.

To analyse incidence, samples above LoQ were set a value of one, samples below the LoQ for HT2+T2 were given a value of zero.

Table of accumulated analysis of deviance of HT2+T2 incidence using selected factors is shown below.

Table A7.1 Accumulated analysis of deviance table for HT2+T2 incidence

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ year	4	356.76	89.19	89.19	<.001
+ region	5	16.6	3.32	3.32	0.005
+ year.region	19	94.367	4.9667	4.97	<.001
+ practice	1	42.4589	42.4589	42.46	<.001
+ varress	5	17.3802	3.476	3.48	0.004
+ T3	3	15.1667	5.0556	5.06	0.002
Residual	1415	1296.644	0.9164		
Total	1452	1839.377	1.2668		

A7.2 Assessment of goodness of fit by ROC curve analysis

Receiver Operating Characteristic curve (or ROC curve) is a plot of the true positives [sensitivity] against the false positives [1 - specificity]. The closer the curve follows the left-hand and top border of the ROC space (area under curve approaches 1), the more accurate the classification based on the model used. The null hypothesis is that the model is not a good fit (ie area under curve = 0.5)

Table 7.2.1 ROC curve case processing summary

HT2+T2	Valid N (listwise)
Positive[a]	477
Negative	976

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.
[a]The positive actual state is 1.

Of 1453 samples, 976 (67%) were below the LoQ.

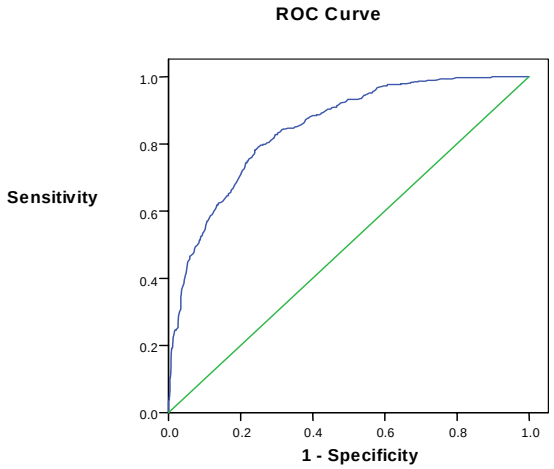

Fig 7.2.1 ROC curve for HT2+T2 incidence

Table 7.2.2 Area under the ROC Curve

Area	Std. Error	Asymptotic Sig.[a]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.847	.010	.000	.827	.867

[a]Null hypothesis: true area = 0.5

Area under curve = 0.847; therefore the model is not a bad fit

A7.3 Tables of predicted proportions and standard error of the predicted proportions for HT2+T2 incidence (>LoQ).

Table 7.3.1 Predicted proportion of samples with HT2+T2 greater than the LoQ and standard error for each region/year combination

	2001		2002		2003	
Region	Prediction	s.e.	Prediction	s.e.	Prediction	s.e.
South	0.5754	0.07594	0.2358	0.05714	0.5722	0.05705
East	0.4232	0.05417	0.1427	0.04808	0.8336	0.04426
Midlands	0.3017	0.06094	0.2102	0.04292	0.6689	0.05261
North	0.1081	0.04451	0.0619	0.03368	0.6040	0.04948
N.Ireland			0.3368	0.11544	0.8551	0.09157
Scotland	0.0801	0.05385	0.1560	0.06237	0.9302	0.04743

	2004		2005	
Region	Prediction	s.e.	Prediction	s.e.
South	0.1214	0.04920	0.2922	0.06574
East	0.2045	0.04778	0.4659	0.05910
Midlands	0.0577	0.02774	0.5906	0.05511
North	0.0153	0.01511	0.5586	0.05630
N.Ireland	0.0006	0.00427	0.3885	0.14831
Scotland	0.0006	0.00265	0.2656	0.09165

Table 7.3.2 Predicted proportion of samples with HT2+T2 greater than the LoQ and standard error for each variety resistance category

Variety resistance category	Prediction	s.e.
Spring Wheat	0.2931	0.03435
FEB Score 4	0.6244	0.07207
FEB Score 5	0.3884	0.02394
FEB Score 6	0.3381	0.01698
FEB Score 7	0.3218	0.01793
Petrus	0.0010	0.01046

Table 7.3.3 Predicted proportion of samples with HT2+T2 greater than the LoQ and standard error for each T3 fungicide category

T3 fungicide	Prediction	s.e.
No T3	0.3353	0.02123
Triazole	0.4078	0.02538
Strob	0.4695	0.03635
Tri/Strob	0.3980	0.01855
Organic	0.1699	0.02344

A7.4 Stepwise model selection for positive HT2+T2 dataset

As for DON, significant agronomic factors were selected for the model using a stepwise model selection ANOVA on Genstat 8 for a dataset containing samples above the LoQ for HT2+T2. HT2+T2 concentration was $\log_{10}$ transformed to normalise the data (loga). The table of accumulated ANOVA of loga using selected factors is shown below.

Table 5.4.1 Accumulated analysis of variance table

Change	d.f.	s.s.	m.s.	v.r.	F pr.
year	4	1.99171	0.49793	8.53	<.001
region	5	0.25479	0.05096	0.87	0.499
year.region	17	0.72622	0.04272	0.73	0.771
practice	1	0.22888	0.22888	3.92	0.048
previous crop	9	1.78253	0.19806	3.39	<.001
T3	3	0.69684	0.23228	3.98	0.008
Residual	437	25.51754	0.05839		
Total	476	31.19852	0.06554		

The model accounted for 18% of the variance.

A7.5 Assessment of goodness of fit for loga by residual plots

Plots show the model is not a bad fit (Fig A7.5.1).

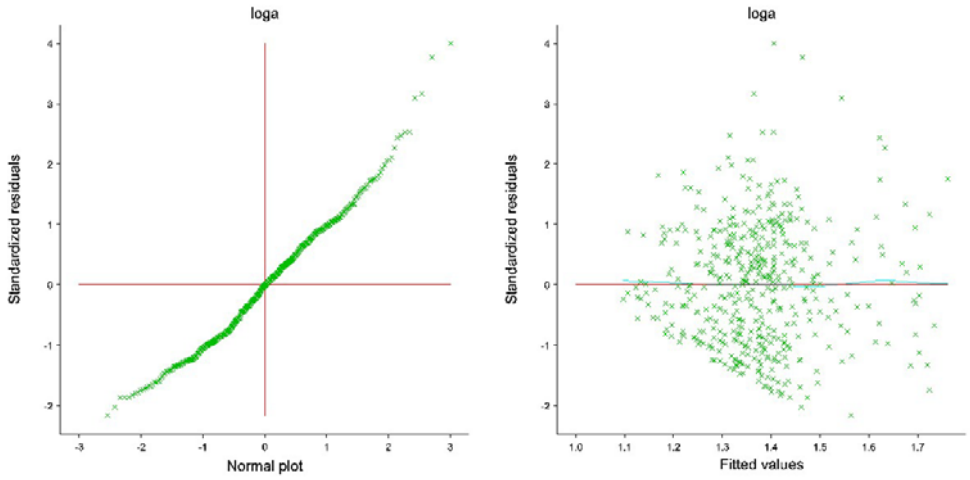

Figure A7.5.1 Residual plots of loga ($\log_{10}$ transformed HT2 + T2 concentration)

A7.6 Tables of predicted means and standard error of the predicted mean for HT2+T2 concentration on the $\log_{10}$ scale (loga)

Graphs presented in the main text of the report are the back-transformed predicted values from the tables below (10[loga]) and the bars are the 95% confidence limits (10[loga±2*s.e.])

Table 7.6.1 Predicted mean and standard error for each year combination

Year	Prediction	s.e.
2001	1.2869	0.0292
2002	1.2817	0.0356
2003	1.4129	0.0174
2004	1.5722	0.054
2005	1.3511	0.0225

Table 7.6.2 Predicted mean and standard error for each practice

Practice	Prediction	s.e.
Organic	1.2909	0.0425
Conventional	1.3763	0.0122

Table 7.6.3 Predicted mean and standard error for each previous crop

Previous crop	Prediction	s.e.
Wheat	1.3923	0.0221
Barley	1.3727	0.0529
Oats	1.5964	0.0477
Sugar beet	1.3566	0.047
Potatoes	1.3694	0.0448
Brassicas	1.3273	0.0252
Legumes	1.2991	0.0353
Grass	1.3548	0.0497
Maize	1.3079	0.046
Set-aside	1.3776	0.0381

Table 7.6.4 Predicted mean and standard error for each T3 fungicide type

T3 fungicide	Prediction	s.e.
No T3	1.3636	0.0257
Azole	1.3903	0.0329
Strobilurin	1.4083	0.0197
Azole+Strob	1.3365	0.0221
Organic	1.2909	0.0425