

June 2016



Project Report No. 566

Improved Modelling of Fusarium to Aid Mycotoxin Prediction in UK Wheat

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This is the final report of a 5 year project (RD-2008-3573) which started in April 2009. The work was funded by a contract of £283,527 from AHDB Cereals & Oilseeds. The project obtained grain samples and agronomic information from the Defra funded disease survey of winter wheat conducted by the Food and Environment Research Agency.

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1. Abstract

European legal limits for fusarium mycotoxins deoxynivalenol (DON) and zearalenone (ZON) for unprocessed cereals intended for human consumption and cereal products were introduced in 2006. The aim of this study was to monitor the fusarium mycotoxins in UK wheat from 2009 to 2013. Collated data was then used to develop models to predict fusarium pathogen incidence and mycotoxin risk at different timings (early and late season) and at different levels (national, regional and field). Results showed large seasonal differences in fusarium pathogen incidence and mycotoxin risk with high levels in 2012 (10% and 15% exceeding legal limit for DON and ZON, respectively) and low levels in 2011 (no samples exceeding legal limits for DON and ZON). Prediction of mycotoxin content early season was not successful with poor prediction in the validation years of 2012 and 2013. This is likely due to a number of factors. This includes the large number of zero values in the model, the poor ability to predict the key growth stages for fusarium infection in winter wheat and the paucity of local meteorological data for some fields. This study has highlighted the need for greater recording and/or prediction of growth stages to improve mycotoxin prediction alongside greater availability of in-field weather data. Due to the multi-step infection process of fusarium head blight (spore production, spore release, infection and mycotoxin production) then mixed or mechanistic models maybe more appropriate.

Analysis of agronomic factors identified the same factors as previously determined, namely previous crop, cultivation and variety. A new factor determined was harvest date with a one month delay in harvest resulting in a large increase in risk for both DON and ZON, as was experienced with the delayed harvest of 2008.

Prediction of mycotoxin late season, based on a combination of fusarium head blight pathogen incidence (as recorded at growth stage 73) and agronomy was a better predictor of risk and proved reasonable at the national and field scale. At field scale, the DON model was a better predictor of risk than the ZON model and could be used to predict ZON as well as DON risk. If a consignment of wheat was deemed negative by the prediction model, it would not require a mycotoxin test and would enter the food chain. If it was subsequently found to be above the legal limit, then the consignment was a false negative and considerable costs may be incurred as well as a potential risk to human health. As false negatives have a greater consequence for the industry (consignments of wheat exceeding legal limits entering the food chain), then the probability of exceeding the legal limit can be calculated and this can be used to determine a lower risk threshold for predicting false negatives. If a threshold of 5% is set, then a sample is deemed to be below the legal limit if the probability that the actual concentration will exceed 1250 ppb DON is below 5%. With a 5% probability that a sample exceeded the DON limit, no samples above the legal limit were predicted not to exceed the DON limit (zero false negatives) and only 0.4% of ZON samples exceeded the ZON limit of 100 ppb but were predicted not to (0.4% false negatives). This study shows the benefit of the continued determination of fusarium head blight incidence data as generated by Fera from the Defra funded winter wheat disease survey. Late season national risk

prediction could be used by the cereal chain to identify the availability of home-grown food grade wheat in the UK and the extent of due diligence testing required at harvest each season. Late season field scale risk prediction could be used by the cereal chain to determine which consignments of wheat require testing for fusarium mycotoxins prior to delivery into the human food chain.

2. Introduction

2.1. Fusarium head blight

Fusarium head blight (FHB) of UK cereals may be caused by several fungal pathogens. The predominant species in the UK are *Fusarium graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum*, *Microdochium nivale* and *M. majus*. The disease is also referred to as fusarium ear blight or scab. Some of the fungi that cause FHB produce fusarium mycotoxins while the *Microdochium* spp. do not. Fusarium head blight can be detected in crops around the milky ripe stage (Growth Stage 73-75) as premature ripening (bleaching) of individual spikelets. Orange/pink spores of *Fusarium* may be seen on infected spikelets. Infection can result in bleaching of the head above the point of infection. As the whole crop ripens the symptoms are less visible. At harvest, fusarium head blight can result in fusarium-damaged grains that may be shrivelled with a chalky white or pink appearance. The presence of fusarium-damaged grains is an indication that fusarium mycotoxins may be present.

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

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

There is strong evidence that rain is important in the dispersal of *F. culmorum* and *F. graminearum*. For *F. culmorum*, macroconidia which are produced at ground level are splashed onto the wheat heads during rainfall (Horberg, 2002, Jenkinson and Parry, 1994). This may occur in a stepwise manner, from leaf to leaf, and finally onto the head. It was noted that during epidemic years in Idaho in 1982 and 1984, when *F. culmorum* was the dominant FHB pathogen, sprinkler-irrigated fields had severe FHB whereas surface-irrigated fields had little or no FHB (Mihuta-Grimm 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 FHB was much more prevalent in fields with irrigation compared to fields with no irrigation (Strausbaugh and Maloy, 1986).

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

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

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

2.2. Fusarium mycotoxins

The trichothecene mycotoxins are produced by some of the fusarium head blight pathogens and their levels within grain depend on weather conditions. High humidity during and after flowering is conducive to head blight epidemics and mycotoxin production. DON and nivalenol (NIV) are Type B trichothecenes produced predominantly by *F. culmorum* and *F. graminearum*. Isolates of both these species are either DON or NIV producers. DON producers are referred to as Type 1 chemotype, this chemotype is further divided into 1A and 1B depending on the acetylated DON that is produced as a co-contaminant, 3- or 15-acetyl DON, respectively. *F. poae* has also been linked to high levels of NIV. HT2 and T2 are Type A trichothecenes, which are thought to be produced predominantly by *F. langsethiae* in the UK (Edwards et al., 2012).

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 ones currently being considered for legislation are HT2 and T2 toxins.

Zearalenone is another mycotoxin produced predominantly by *F. culmorum* and *F. graminearum*. Zearalenone function in the fungus is not known and is predominantly produced late in the crop growing season, near to harvest (Matthaus et al., 2004). Zearalenone has low cellular toxicity but is problematic as it has high estrogenic activity causing hyperestrogenism in animals and humans. In animals, the mycotoxin causes a range of fertility problems, with young female pigs being particularly susceptible (Anon, 2004b). There are no proven cases of human exposure but the mycotoxin has been implicated in cases of premature puberty in girls (Anon, 2000).

2.3. Fusarium mycotoxin legislation

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

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

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

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

The maximum levels set for unprocessed cereals apply to cereals placed on the market for processing. Cereal grains may have been cleaned, dried and/or sorted prior to being placed on the market; these grains are still classified as unprocessed cereals. The European Commission states that maximum levels are set on unprocessed cereals to avoid highly contaminated cereals entering the food chain and to encourage all measures to minimise fusarium mycotoxin contamination to be taken in the field and storage stages of the production chain.

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

The European Commission also set guideline limits in 2006 for fusarium mycotoxins in animal feed (Anon, 2006a). The lowest guidance limits have been set for pigs owing to their higher sensitivity to fusarium mycotoxins. The DON guidance value for complementary and complete feedingstuffs for pigs is 900 ppb. The zearalenone guidance value for complementary and complete feedingstuffs for sows and fattening pigs is 250 ppb and for piglets and gilts is 100 ppb.

In 2013 the European Commission published a Recommendation on the mycotoxins HT2 and T2 (Anon, 2013). The Recommendation included indicative levels for the combined concentration of HT2 and T2 (HT2+T2) in unprocessed cereals and cereal products. The indicative level for unprocessed wheat is 100 ppb. The Recommendation states that Member States in collaboration with industry should continue to monitor the occurrence of the mycotoxins HT2 and T2 and where they exceed the indicative level then an investigation should be conducted as to why exceedances occurred and what mitigation can be implemented to avoid future exceedances.

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. These factors were analysed in a previous FSA and AHDB Cereals & Oilseeds-funded studies during 2001-2005 and 2006-2008 (Edwards, 2007, Edwards, 2011); results from these studies are summarised below.

Year, region, previous crop, cultivation, variety and fungicide application all had statistically significant effects on DON concentration. Statistical tests of the predictive quality of the model indicated it may be a good predictor of new observations. There was a significant interaction between year and region, which is probably due to fluctuation in weather between years and regions. Extreme years occurred in 2006 (dry summer, low mycotoxins) and 2008 (wet summer with delayed harvest, high mycotoxins). 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 head blight. Highest predicted DON concentration occurred in wheat following maize, which the primary host for *Fusarium graminearum*, the most potent DON-producing species. Ploughing generally reduced DON concentration; this reduction was greatest following maize. 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 is predicted to increase with climate change (Kenny and Harrison, 1992).

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

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

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

ripens, and therefore conditions may be more conducive to zearalenone production later in the summer.

The concentration of HT2 and T2 was low across all years. 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 limiting factor in HT2 and T2 production in the UK.

AHDB Cereals & Oilseeds published “G34: Guidelines to minimise risk of fusarium mycotoxins in cereals” (Anon, 2010b) which included a “Fusarium mycotoxin risk assessment” (Anon, 2010a) based on the results of the previous study (Edwards, 2007). Updates are included in the Information Sheet “Risk assessment for fusarium mycotoxins in wheat (Anon, 2015). The risk assessment was integrated into cereal crop assurance schemes. Results from the previous project identified the importance of weather for DON and ZON contamination of wheat, as indicated by the seasonal and regional variation. The regional variation, in combination with what is known of the lifecycle of *Fusarium* species indicated that other factors, such as cereal intensity within rotations and crop debris management, may also be important agronomic factors for DON and ZON contamination of cereals.

2.5. Aims and objectives

The overall aim of the project was to improve the prediction of fusarium mycotoxin risk at key stages of the growing season and at different scales (national, regional and farm level).

The project was originally for three years (2009-2012). A two year extension was awarded in 2012 to continue monitoring fusarium mycotoxins in the 2013 and 2014 harvests and to test the predictive ability of the fusarium mycotoxin risk model developed within the project.

3. Materials and methods

3.1. Annual build-up of fusarium head blight (FHB) pathogen inoculum through early season monitoring

During 2009, 2010 and 2011 winter wheat trials were established at five locations (York, Caythorpe, Morley, Andover (or Sutton Scotney 2009 only) and Cirencester. Trials other than that

at York were managed and sampled by NIABTAG. These locations provided a network which reflected differences in regional risk from the different species responsible for FHB within England. At each site, in each year, three replicate plots of the winter wheat (Robigus) were drilled and the FHB pathogen levels at the stem base and on the different leaf layers determined fortnightly between GS31 and 65. At each sampling, 10 tillers were collected from each replicate plot and the stem bases plated onto potato dextrose agar (PDA). To determine the presence of FHB pathogens on leaves, the top four to six leaf layers (depending on growth stage) from three tillers per replicate plot were plated onto PDA. All agar plates were incubated at 20°C for four days and any fusarium-like colonies transferred onto sucrose nutrient agar and fresh PDA to determine the species present. All isolation data were averaged across the replicated plots and reported on the Fusarium monitoring pages of CropMonitor™ (www.cropmonitor.co.uk) to provide in season information on FHB pathogen inoculum levels.

3.2. Seasonal and regional distribution of the *Fusarium* species responsible for fusarium head blight symptoms

With the exception of 1983 and 1984, the Defra funded disease survey of winter wheat has been carried out annually at Fera since 1970. Fusarium head blight symptoms have been assessed as part of the survey since 1986 and the species responsible for symptoms determined since 1998. The survey was carried out in July of each year when crops were at the early- to medium-milk growth stage (GS73-75). For the period covered by this project 300 samples, from eight geographical regions (North East, North West, Yorkshire and Humberside, East Midlands, West Midlands, East, South East and South West) were assessed, with the number of samples from each region stratified by farm size and proportional to the area of winter wheat grown in that region. For each sample 50 tillers were collected and assessed for FHB symptoms at Fera. Where symptoms were assessed the pathogen responsible was determined through isolation. To visualise the isolation, maps showing the distribution of *F. culmorum* and *F. graminearum* were produced. The maps also provided information from which national and regional risk predictions for the likely mycotoxin contamination of grain could be based.

3.3. Grain sample collection

Each year 300 grain samples of winter wheat and related agronomic data were requested by the Fera. These samples were collected by growers involved in the Defra funded winter wheat disease survey as part of CropMonitor™ (www.cropmonitor.co.uk). The winter wheat disease survey is a stratified survey of wheat within England. As such, mycotoxin results generated provide an

accurate assessment of fusarium mycotoxins in England. The target number of samples for each year was 150 (50% return rate).

Samples were collected at harvest from specific fields either from the combine or from trailers leaving the field. Approximately 300 g sub-samples were taken from ten arbitrary points around the field and combined to provide a 3 kg sample.

On receipt of samples their moisture content was determined. Any samples with a moisture content greater than 18% were dried overnight on a heated-air dryer, the moisture content was re-assessed the next day and then processed. A 500 g sub-sample of grain was removed using a ripple divider, dried to 12% moisture content and stored at room temperature as a grain archive. The remaining sample was milled (ZM200, Retsch) with a 1 mm screen and mixed in a tumbler mixer before two 300 g sub-samples were collected. One sample was used for mycotoxin analysis, the remaining sample was held at Harper Adams University as an archive sample at -20°C .

3.4. Mycotoxin analysis of grain samples

All samples were analysed by Campden BRI using UKAS accredited procedures. The trichothecenes (deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-acetylDON, fusarenone X, T2 toxin, HT2 toxin, diacetoxyscirpenol and neosolaniol) and the non-trichothecene, zearalenone (ZON) were analysed by liquid chromatography with tandem mass spectrometry (LC/MS/MS). Spiked samples were included in each batch to determine extraction recovery. The method had acceptable recovery range for each trichothecene of 60-120%. Results were corrected for recovery. The expanded measurement of uncertainty was calculated using a standard coverage factor of two, equivalent to a confidence of approximately 95% that the actual level of the mycotoxin being measured lies within the quoted range. The expanded measurement of uncertainty was calculated to be 22.7% for DON and 24.0% for ZON. The Limit of Quantification (LoQ) for the trichothecenes was 10 ppb and for ZON was 2 ppb.

3.5. Summary statistics

For summary statistics, samples with a mycotoxin content below the limit of quantification (LoQ) were assigned a value of (LoQ)/2 for calculation of mean values. Summary statistics (percentage greater than 10 ppb, mean, median, 90th percentile, 95th percentile and maximum) were calculated using Excel (Microsoft v.2013).

3.6. Modelling FHB severity.

3.6.1. Modelling crop growth stage

As flowering (GS61-GS69) is the most critical period for FHB infection, it was important to have a good estimation of when this occurred for each crop surveyed. Data on the date of the T3 application to commercially managed crops was sourced from the Defra winter wheat survey. This application timing varied from GS55 (half of ear emerged above flag leaf ligule) to GS65 (mid flowering). However, as the accuracy of the dates provided for these timing could not be verified, modelling was used as a means to more accurately determine the start of flowering for each crop surveyed. Two approaches for the growth stage modelling were used, the first based on data from the CropMonitor™ live monitoring sites and the second on data collected from AHDB Recommended List trials.

CropMonitor™ data

Data for the years 2003-2010 were obtained from CropMonitor™ on weekly growth stage (GS) development of five winter wheat varieties assessed weekly for 12 weeks starting when a crop reached GS31 (first node detectable). The number of sites used each year during this period ranged from 5 to 16. The five varieties used were not consistent over the period the data were collected which resulted in data collected for seven different varieties (Ambrosia, Claire, Consort, Einstein, Malacca, Robigus and Solstice). For each site in each year meteorological data were obtained from the National Climatic Data Centre (NCDC) database.

These data were used in a linear discriminant analysis (LDA), with growing degree-days (GDD) and the number of Julian days from sowing used to discriminate between the different growth stages. GDD was calculated using the following equation:

$$GDD = \left[\frac{(T_{\max} + T_{\min})}{2} \right] - T_{base} \quad \text{where } T_{base} = 0$$

Based on the fitted linear function, a probability for any of the six growth stages observed (31, 37, 39, 59, 61 and 69) was derived for any given combination of growing degree-days and number of days from sowing.

AHDB Recommended Lists trials data

A second approach to the growth stage modelling was to apply GDD estimations to assessment data made at GS61 in the AHDB Recommended Lists (RL) trials between 2006 and 2012. These trials were carried out at two locations (one in West Yorkshire and the other in Essex) with data from early, mid and late sowings available for each year. For each site in each year meteorological data were obtained from the National Climatic Data Centre (NCDC) database.

The GDD data to GS61 were calculated based on three start dates: 1) from sowing date, 2) from 15th January and 3) from 31st January. For each of these timings, GDD were calculated with a base

temperature of either 0 or 5°C, that is days where the average daily temperature was below either 0 or 5°C were not included in the GDD calculation.

A total of 70 varieties were used in the trials, these were ranked in terms of maturity compared to a control variety (Solstice). Based on these rankings GDD data for similar varieties were combined. The maturity value applied was based on the AHDB RL ripening score with a negative value indicating a variety which ripened earlier than Solstice and a positive value one which ripened later.

3.6.2. Modelling FHB

Fusarium data collected during the Defra-funded winter wheat survey described in Section 3.2 were used for the modelling of FHB severity. The main mycotoxins found in UK wheat are deoxynivalenol (DON) and zearalenone (ZON). The main producers of these toxins in the UK wheat crop are *F. graminearum* and *F. culmorum*, with *F. graminearum* predominating over *F. culmorum* since 2002. Chemotyping results from the surveys showed that over 90% of the *F. graminearum* population produced DON and ZON (the remainder producing nivalenol (NIV) and ZON) whereas for the *F. culmorum* population it was nearer a 50/50 split between DON and NIV producers. The predominance of *F. graminearum* and the fact that half of *F. culmorum* isolates do not produce DON would suggest that the majority of the DON found in UK grain resulted from *F. graminearum* infections. As a result the FHB modelling was based on data on the *F. graminearum* isolations from the winter wheat survey.

Two approaches were undertaken to the modelling of *F. graminearum*: the first estimated incidence at the regional level, with the second providing information at the site level.

Regional level

For accurate risk predictions of disease at a 'regional' level, or to model disease by risk parameter, it is important to aggregate 'like' samples together. Cluster analysis can be used to objectively determine spatially contiguous zones of disease (Luo, 2008). Four cluster analysis methods were used to determine the most appropriate for use with *F. graminearum*: 1) Complete linkage, 2) Average linkage, 3) Wards algorithm and 4) K-means. The analyses were undertaken on *F. graminearum* data obtained between 2005 and 2010.

Weather data were obtained from the NCDC database between 2005 and 2011, an example of the spread of the weather stations available for 2010 is shown in Figure 1.

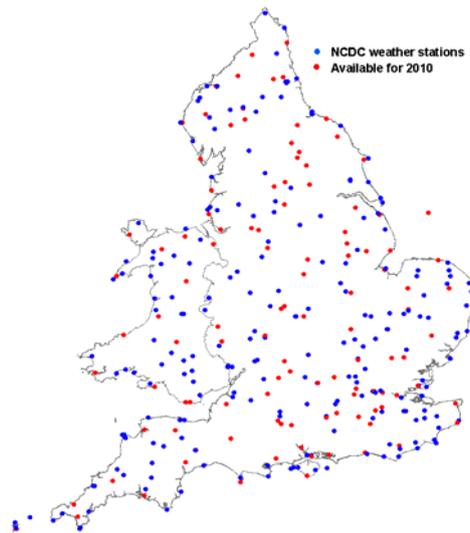


Figure 1. Weather stations available on the National Climatic Data Centre (NCDC) database for 2010.

The weather variables selected for testing were mean, min and max temperature, rainfall, the number of frost days and relative humidity (calculated using $RH=100-5(T-T_d)$, where T was the temperature and T_d was the dew point). The quality of data from each station was assessed based on the following criteria, with stations falling into the following categories removed:

- 1) Missing rainfall or temperature (mean, min or max) data between 6th and 20th June inclusive
- 2) Six or more consecutive days missing between 1st April and 5th June inclusive
- 3) Four or more consecutive days missing between 20th June and 30th September inclusive
- 4) More than 10% of data missing between 1st April and 30th September

As the data quality was checked each year it was possible for a weather station to be removed in one year but not in another.

For each year, 2005 to 2010, mean daily weather data for the stations occurring in a cluster and mean *F. graminearum* incidence data within the same cluster were analysed through Window Pane analysis to determine the weather variables most likely to be necessary for infection. The analysis used data starting at the end of July and ran backwards with a time lag between 210 and 10 days in steps of 5 days and an analysis window between 5 and 180 days again in steps of 5. The weather functions tested in the analysis are shown in

Table 2. Correlation analyses were carried out on the weather parameters selected and a high dimensional regression model constructed.

Table 2. Weather functions tested in the Window Pane analysis.

Weather function	Description	Thresholds
$V_{nod;>i}$ or $V_{nod;<i}$	Number of days the weather variable (V) above (>) or below (<) a threshold (i) in a window	Tmin: $i = -2, \dots, 7$; Tmax: $i = 20, \dots, 25$;
$V_{cnod;>i}$ or $V_{cnod;<i}$	Number of consecutive days above or below a threshold	Tmean: $i = 7, \dots, 14$; Rain: $i = 0, \dots, 9$;
$V_{acc;>i}$ or $V_{acc;<i}$	Accumulation above or below a threshold	Hmin: $i = 50, 60, 70$; Hmax: $i = 92, 95, 99$;
V_{avg}	Average value of the weather variable	Hmean: $i = 70, 80, 90$;

Model validation was carried out using data collected in 2011 and then with data collected during 2012 and 2013. Weather data for 2012 and 2013 were obtained from NCDC database, however the data for 2013 was not of sufficient quality so alternative data were obtained from the Met Office Integrated Data Archive System (MIDAS).

Site level

In this approach, the data were analysed at the site level with the *F. graminearum* incidence data for each site linked to its nearest weather station provided it was within 23 km; sites where the nearest weather station was further than 23 km were removed from the analysis.

The model was derived using data collected between 2005 and 2011, with the model validated using data collected in 2012 and 2013. The underlying approach was similar to that described for the regional modelling with the weather variables combined through a stepwise linear regression. The analysis started with data from the end of June and ran backwards with a time lag from 170 down to 40 in steps of 1 and an analysis window from 40 down to 5 again in steps of 1.

As the models were derived using individual sites it was also possible to examine the effects of agronomic factors such as previous cropping (legume, oilseed rape, wheat or other), soil cultivation (ploughing or minimum tillage) and harvest period (early, or late harvest).

3.7. Modelling fusarium mycotoxin risk – agronomy data

Significant agronomic factors were selected for the model using a stepwise selection ANOVA on Genstat (v16, Lawes Agricultural Trust). Temporal (year) and spatial (region) factors were forced into the model. Other agronomic factors were ordered based on the order in which they occur within a growing season. Interactions between factors were entered into the model where there was a biological reason to expect one to occur. As weather is an important parameter of fusarium head blight epidemiology one could expect a temporal (year) and spatial (region) interaction. As

crop debris is an important parameter of fusarium head blight epidemiology, as in the type and amount of crop debris, then an interaction between previous crop, crop debris management and the method of cultivation (\pm ploughing) could be expected (i.e. removal of straw and/or ploughing would be beneficial for some crops but not others). After selection of factors to be used in the model the data file was filtered of all samples containing blanks within these factors and the data was re-analysed.

3.8. Modelling fusarium mycotoxin risk – meteorological data

As with the FHB modelling, the modelling of the fusarium mycotoxin deoxynivalenol (DON) and zearalenone (ZON) were carried out at both the regional and individual site level.

Regional level

Data used were collected as described in section 3.3, with the weather data obtained as described in section 3.4.2. Cluster analyses were carried out on both the DON and ZON data to determine whether: 1) Complete linkage, 2) Average linkage, 3) Wards algorithm or 4) K-means, were the most appropriate to use. The analyses were undertaken on data obtained between 2006 and 2010 and due to the skewedness of the toxin data, values were \ln_e transformed prior to analysis. The weather variables selected for testing were mean, min and max temperature, rainfall, the number of frost days and relative humidity (calculated using $RH=100-5(T-T_d)$).

For each year (2006 to 2010) weather and toxin data within a cluster region were analysed through Window Pane analysis to determine possible weather variable involved in infection. The analysis started at the end of September and ran backwards with a time lag between 270 and 10 days in steps of 5 days and an analysis window between 5 and 180 days again in steps of 5 days. The weather functions tested in the analysis were the same as those used in the *F. graminearum* incidence modelling (

Table 2). Correlation analyses were carried out on the weather parameters selected and a high dimensional regression model constructed. Model validation was carried out as described for *F. graminearum*.

Site level

As for the *F. graminearum* incidence modelling, site level toxin data (both DON and ZON) were linked to its nearest weather station provided it was within 23 km, with sites further than 23 km from a weather station removed from the analysis.

The model was derived using data collected between 2006 and 2011, with the model validated using data collected in 2012 and 2013. Due to the skewed nature of the toxin data an \ln_e transformation was used before any analysis. The underlying approach was similar to that described for the regional modelling with the weather variables combined through a stepwise linear regression. The analysis started at the end of June and ran backwards with a time lag from 170 down to 40 in steps of 1 and an analysis window from 40 down to 5 again in steps of 1.

As the models were derived using individual sites it was also possible to address the effects of agronomic factors such as previous cropping (legume, oilseed rape, wheat or other), soil cultivation (ploughing or minimum tillage) and harvesting period (each site with “-3 to -1”, “0” and “1 to 6”, defining early, on-time or late harvest respectively).

3.9. Modelling fusarium mycotoxin risk – pathogen incidence data

National level

For the national risk prediction the percentage of samples exceeding legal limits for DON and ZON were analysed using general linearized models using two national datasets. The first was the national Fusarium Head Blight pathogen incidence data generated from the Defra funded winter wheat disease survey and the second was the English average monthly rainfall data.

Regional level

As for national risk, a similar methodology was performed for regional risk using the FHB incidence data for each Fera region and the nearest appropriate regional rainfall data. As the regional dataset had a low number of samples within each region for each year, there were a large number of zero values for the percentage of samples that exceeded legal limits. Such data is unsuitable for linear models. For this reason, the log₁₀ transformed mean values for DON and ZON were modelled against the percentage incidence of FHB pathogen and the regional rainfall data. The

model was conducted on the 2001 to 2011 dataset and validated against the 2012 and 2013 dataset.

Site level

For the site risk, national, regional and individual site data for FHB species incidence generated from the Defra winter wheat disease survey were tested for significant effects with site agronomy data; year was not included as seasonal variation for the current harvest season is not known when the prediction is made. As the FHB species incidence data is based on samples collected at early-medium milk ripe (GS73-75), this data is only available near harvest time and as such is suitable for a harvest prediction of risk. A model was selected using a forwards stepwise process with agronomy added in a chronological order and interactions only included where expected based on the known biology of the fusarium wheat pathosystem. Models were constructed based on the 2006-2011 (6 year) dataset and then validated using the 2012 and 2013 dataset. Statistical analysis was completed using Genstat (Lawes Agricultural Trust, v16). Statistical analysis to determine agronomic factors on the fusarium mycotoxin concentration of wheat was performed using a stepwise selection ANOVA. An additional agronomic factor included in this study, compared to the previous study (Edwards, 2011), was harvest week. This was calculated for each site based on each county six-year average harvest date with samples within +/- 3 days of this date harvested in week 0 and sites harvested a week earlier recorded as -1 and a week late as +1 etc. As levels within harvest week are on a linear scale it was entered into the model as a polynomial sub-model. Samples with a mycotoxin concentration below the LoQ were assigned a value of (LoQ)/2. All concentration were log₁₀ transformed and analysed using a normal distribution.

4. Results

4.1. Annual build-up of FHB pathogen inoculum through early season monitoring

4.1.1. Stem base and leaf layer isolation data

2009

Five sites (York, Caythorpe, Morley, Cirencester and Sutton Scotney) were monitored to measure changes in FHB pathogens at the stem-base and on the different leaf layers between GS31 and GS65.

At all five sites the predominant FHB pathogen isolated from the stem-base at the start of the season was either *M. nivale* or *M. majus* (Figure 2). However by mid to late June, the level of *Fusarium* species isolation from the stem bases at 3 of the 5 sites (York, Caythorpe and Morley) was at an equivalent level or higher than the *Microdochium* species. Throughout the isolation period, *Microdochium* levels recorded at the stem base in 2009 were much lower than those

recorded in either of the two previous years (AHDB Cereals & Oilseeds Project Report 459), perhaps reflecting the colder winter conditions. However, the levels of *Fusarium* species isolated were generally higher, with *F. graminearum* only isolated from stem bases at Caythorpe and Cirencester.

Leaf layer isolations (Figure 3) indicated that *F. culmorum* and *F. graminearum* were present on leaves at all but the York site throughout the isolation period. At York, *F. culmorum* was not detected until the flag leaf sheath was visibly swollen (GS43) and *F. graminearum* not until complete ear emergence (GS59). This was in contrast to isolation data from 2008 (AHDB Cereals & Oilseeds Project Report 459) when *F. graminearum* was not isolated from leaves until crop flowering. Either *F. culmorum* or *F. graminearum* were detected on the flag leaf at Caythorpe, Morley and Sutton Scotney during the flowering period, suggesting that given the correct weather conditions infection by the deoxynivalenol producing species would occur at these sites. In contrast, the *Microdochium* species were only isolated from the flag leaf during flowering at Cirencester. No isolation of the *Microdochium* species occurred after the first node detectable growth stage (GS31) at Morley or Sutton Scotney. The level of isolation for all species from leaves was lower than in 2008, in particular around flowering, which suggested FHB symptoms in the crop during 2009 would be lower than those seen in 2008.

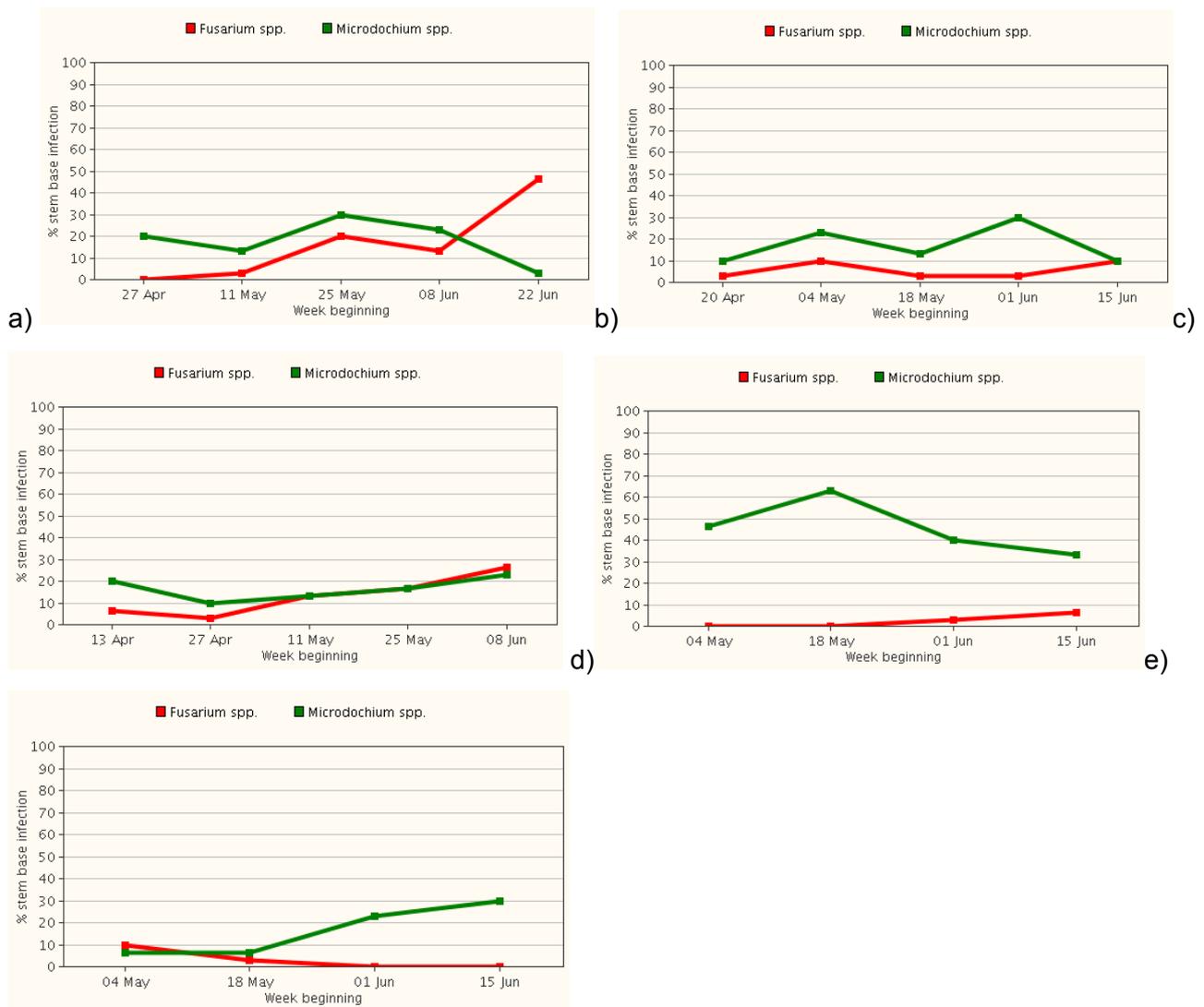


Figure 2. Isolation of fusarium head blight pathogens from winter wheat stem bases at the five monitoring sites during 2009 (a) York, (b) Caythorpe, (c) Morley, (d) Sutton Scotney and (e) Cirencester.

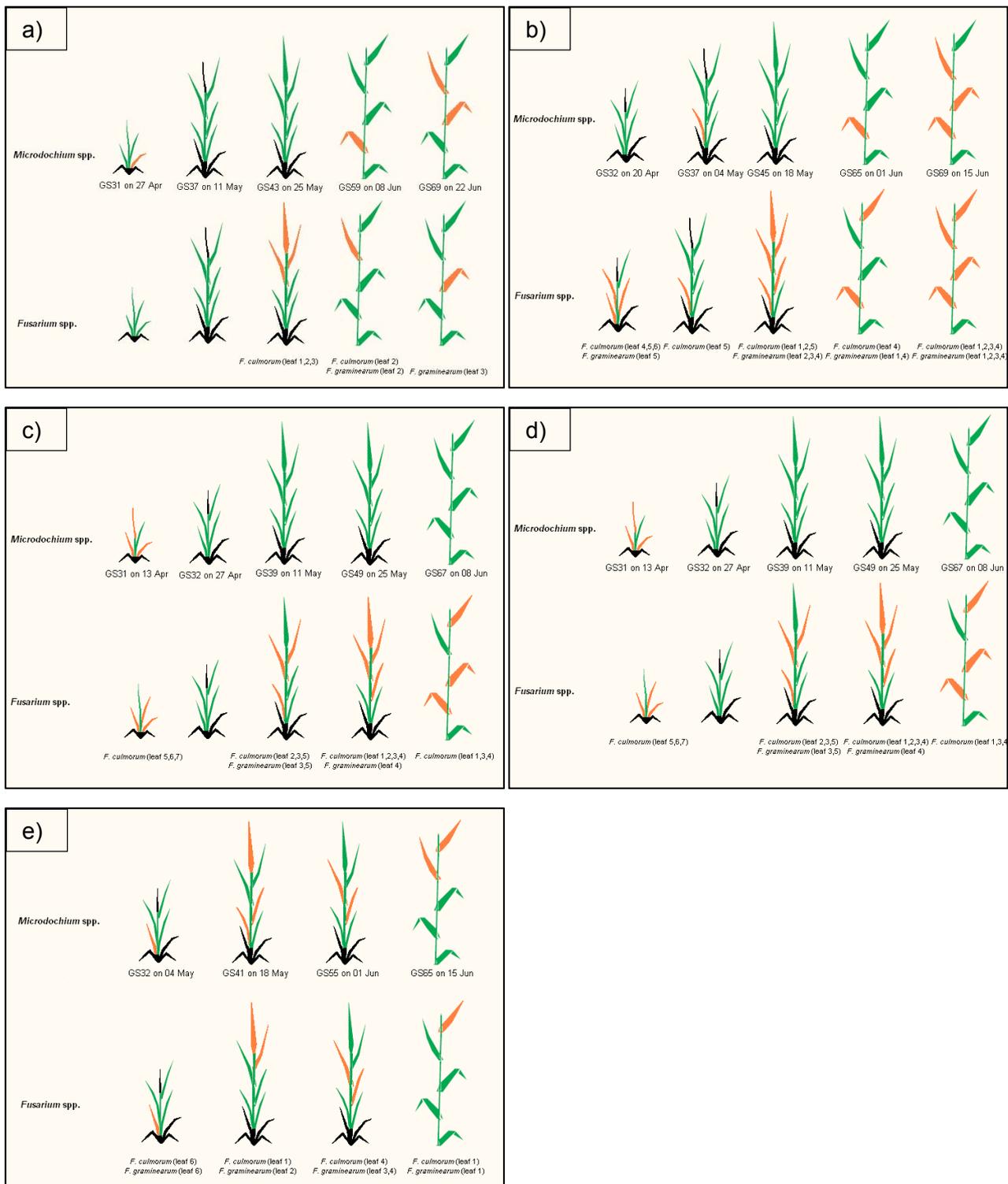


Figure 3. CropMonitor™ outputs for the leaf layer isolation of the fusarium head blight (FHB) pathogens from winter wheat leaf layers at the five monitoring sites in 2009. Orange coloured leaves indicate the presence of a FHB pathogen and green the leaf layers assessed. (a) York, (b) Caythorpe, (c) Morley, (d) Sutton Scotney and (e) Cirencester.

2010

At all five sites the predominant FHB pathogen isolated from the stem-base at the start of the season was either *M. nivale* or *M. majus* (Figure 4). However, by mid to late June, the frequency of *Fusarium* species isolation from the stem bases was at an equivalent level or higher than those of the *Microdochium*. Throughout the isolation period levels of stem base infection caused by *Microdochium* species were lower than those recorded in 2009 which in turn were much lower than those in 2007 and 2008.

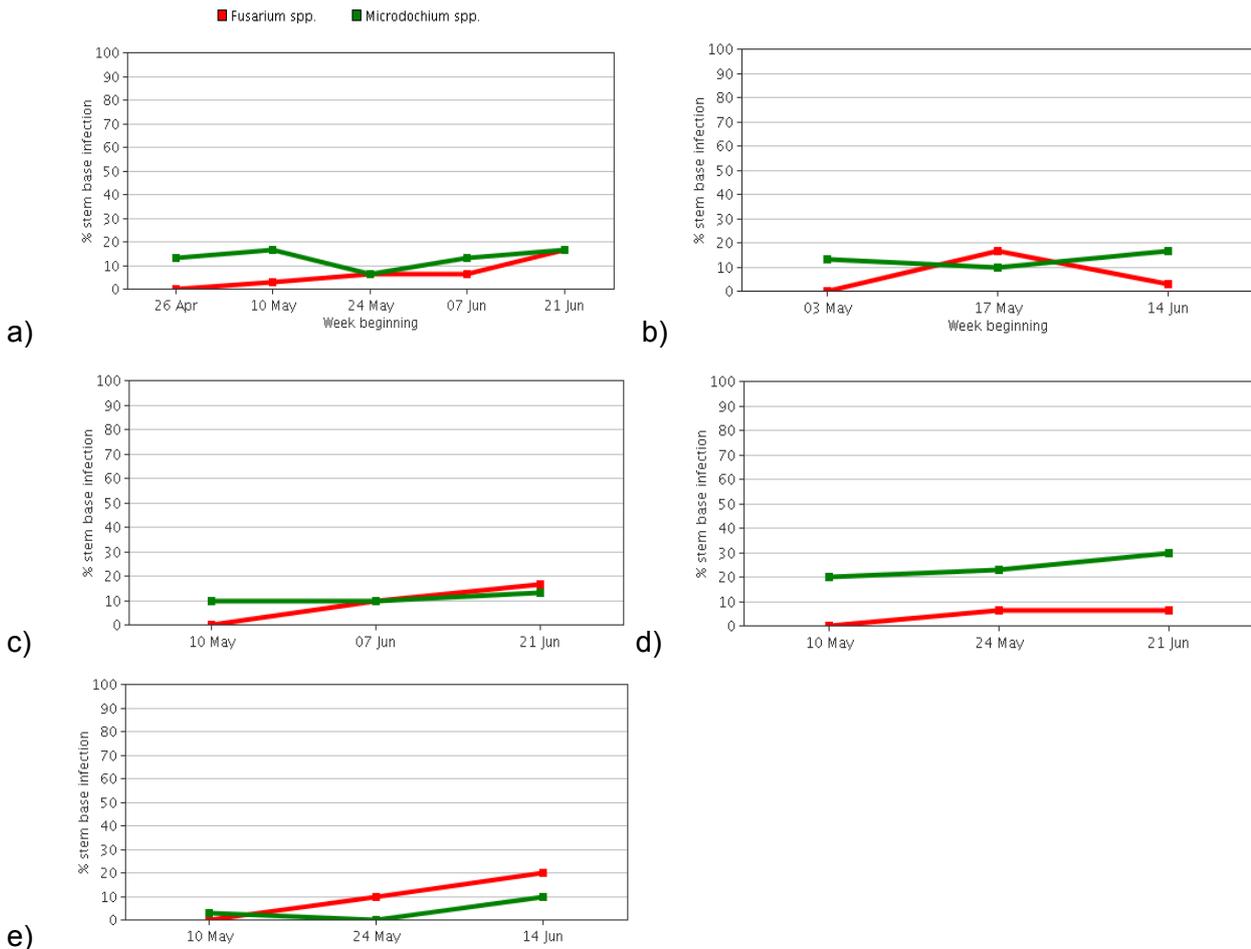
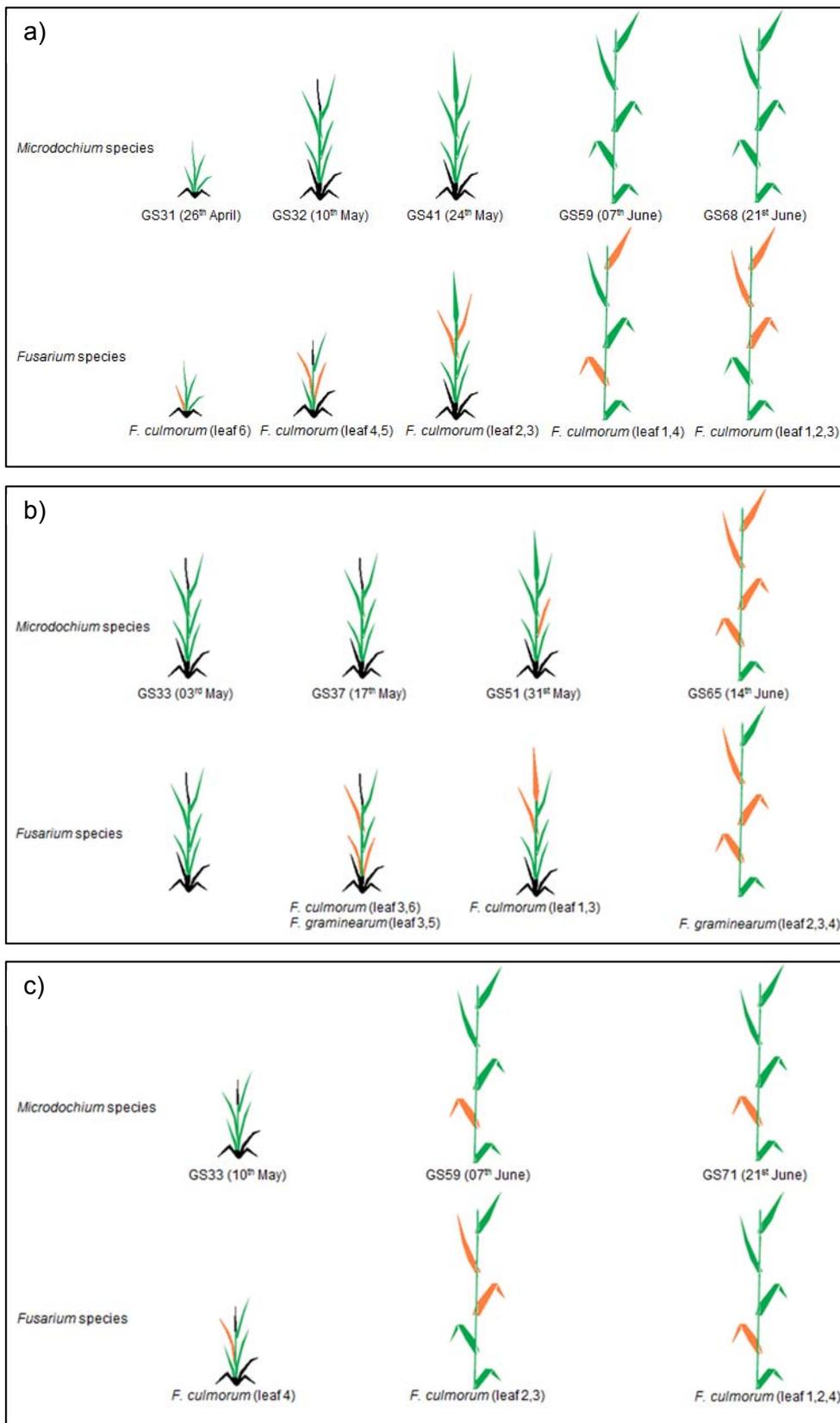


Figure 4. Isolation of fusarium head blight pathogens from winter wheat stem bases at the five monitoring sites during 2010 (a) York, (b) Caythorpe, (c) Morley, (d) Andover and (e) Cirencester.

Leaf layer isolations in 2010 (Figure 5) showed that *F. culmorum* was present on leaves at all five sites throughout monitoring period. *F. graminearum* was isolated from leaves at Caythorpe, Andover and Cirencester. There was no isolation of *F. graminearum*, before flowering at either the Caythorpe or Cirencester sites. *Microdochium* species were isolated from leaf layers at Caythorpe, Morley and Andover, again the first detection did not occur until later in the season (GS51-65), and was only isolated on the flag leaf during flowering at Caythorpe leaf layers. The level of isolation of

F. graminearum and *Microdochium* species from leaves was much lower than in 2008 and similar to 2009; levels of *F. culmorum* were higher than seen in both years.



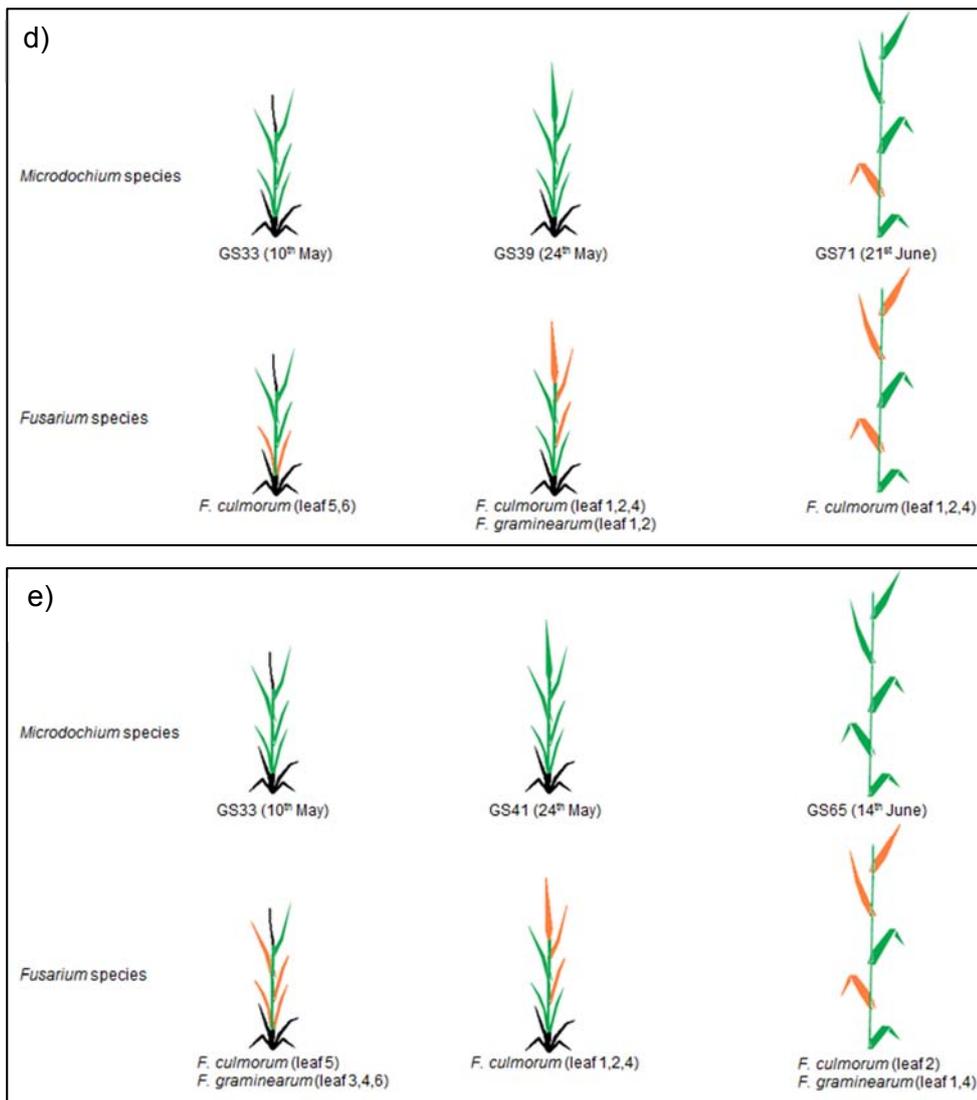


Figure 5. CropMonitor™ outputs for the leaf layer isolation of the fusarium head blight (FHB) pathogens from winter wheat leaf layers at the five monitoring sites in 2010. Orange coloured leaves indicate the presence of a FHB pathogen and green the leaf layers assessed. (a) York, (b) Caythorpe, (c) Morley, (d) Andover and (e) Cirencester.

2011

At three sites (Morley, Cirencester and Andover), *M. nivale*/*M. majus* predominated stem-base isolations at the start of the season (Figure 6). Isolation levels at this stage were generally less than 20% of stems infected, with the exception of Andover where *M. nivale*/*majus* were isolated from 30% of stem bases. At Andover, the levels of the *Microdochium* species increased up to the week beginning 23rd May, reaching a maximum of 70% stem bases affected. After this period of increase, levels dropped off dramatically. Levels of both *Fusarium* and *Microdochium* species were similar at York and Caythorpe throughout the season. At Morley and Cirencester fusarium levels (*F. culmorum*) at the stem base were higher than those of the *Microdochium* species by the week beginning 20th June.

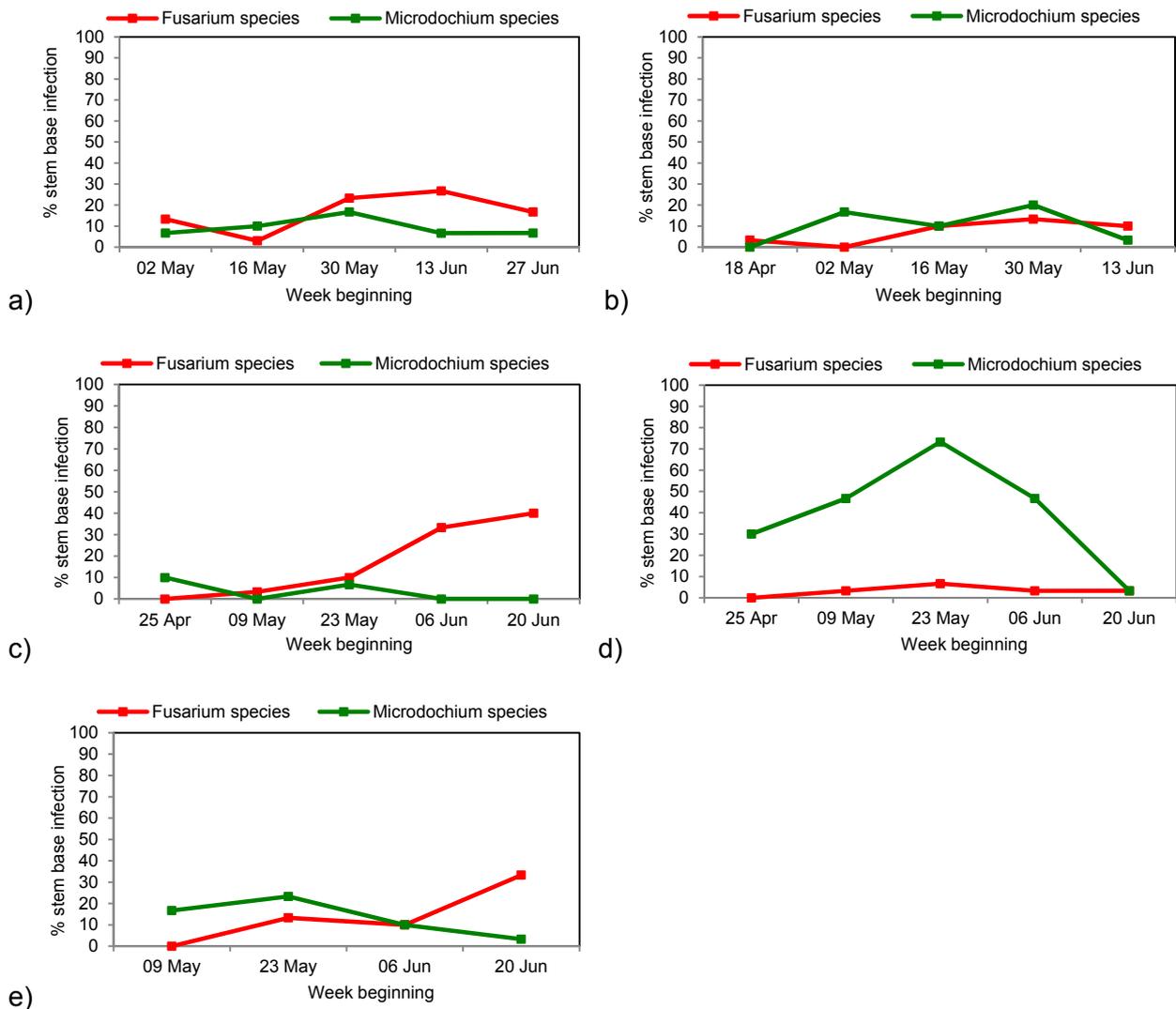
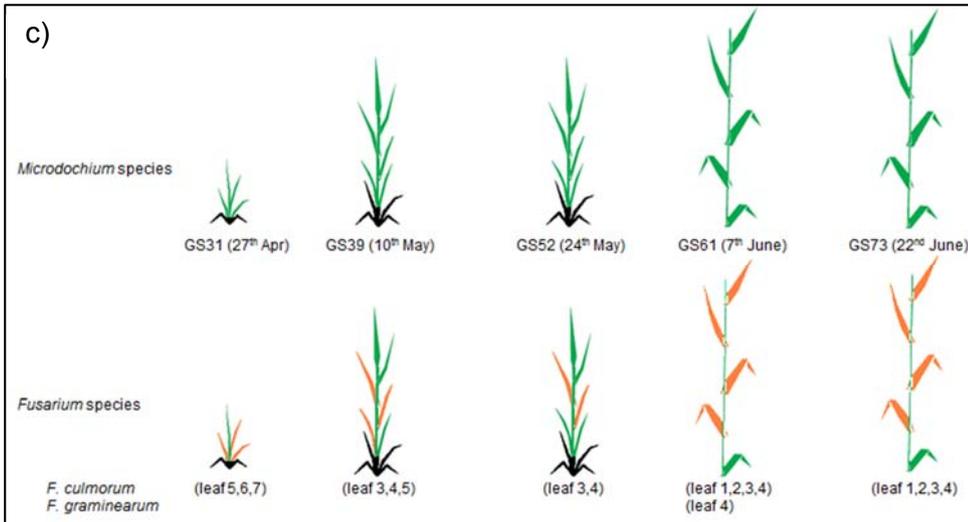
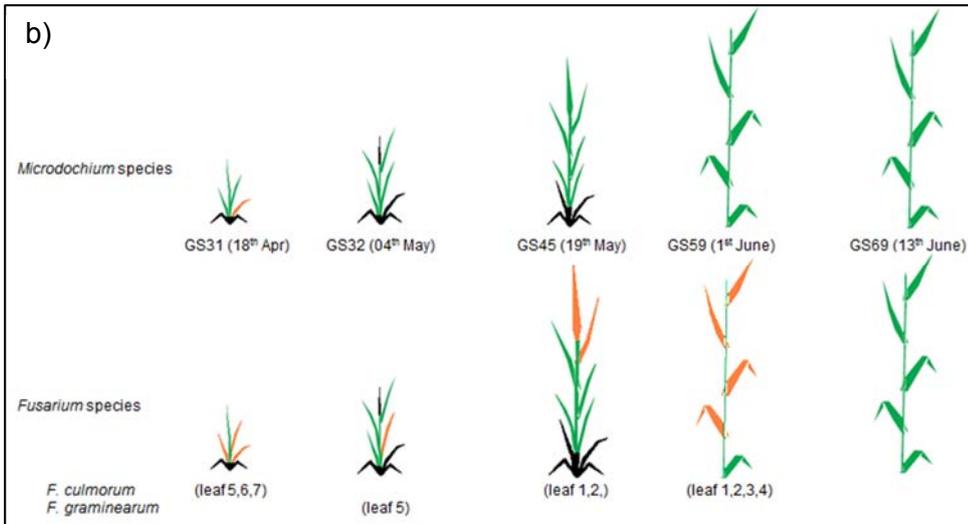
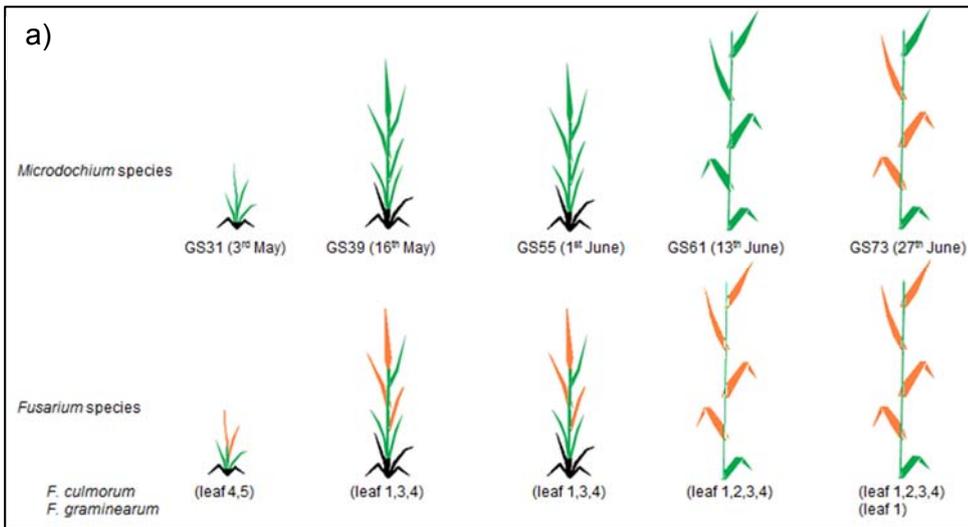


Figure 6. Isolation of fusarium head blight pathogens from winter wheat stem bases at the five monitoring sites during 2011 (a) York, (b) Caythorpe, (c) Morley, (d) Andover and (e) Cirencester.

Leaf layer isolations for 2011 (Figure 7) showed that *F. culmorum* was present on leaves at all five sites throughout the monitoring period. *F. graminearum* was only isolated from leaves at three sites (York, Caythorpe and Morley) and was only detected during the critical flowering period (GS61) at Morley. *Microdochium* species were isolated from leaf layers at York, Andover and Cirencester. As with *F. graminearum*, the first detection was later in the season (GS69-73) and generally only on the lower leaf layers. The level of isolation of *F. graminearum*, *F. culmorum* and the *Microdochium* species from leaves was similar to 2010.



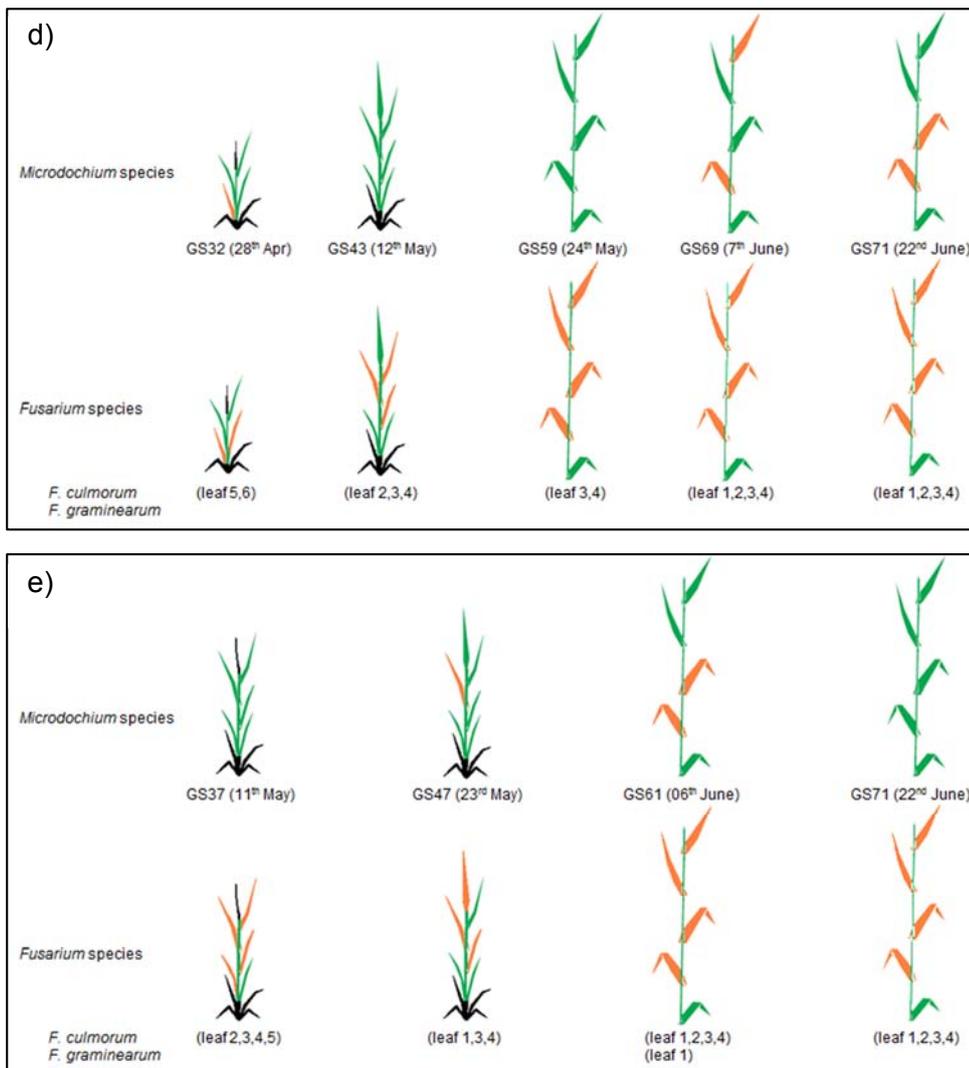


Figure 7. CropMonitor™ outputs for the leaf layer isolation of the fusarium head blight (FHB) pathogens from winter wheat leaf layers at the five monitoring sites in 2011. Orange coloured leaves indicate the presence of a FHB pathogen and green the leaf layers assessed. (a) York, (b) Caythorpe, (c) Morley, (d) Andover and (e) Cirencester.

4.2. Seasonal and regional distribution of the *Fusarium* species responsible for fusarium head blight symptoms

FHB symptoms assessed during the Defra funded winter wheat disease survey between 2000 and 2011 are shown in Figure 8. Data indicate that the levels of FHB symptoms varied between growing seasons with levels over this period highest in 2007 (86% crops affected). The levels of FHB symptoms post 2007 were generally higher than those pre-2007 with an average of 62% crops showing symptoms compared to 30%.

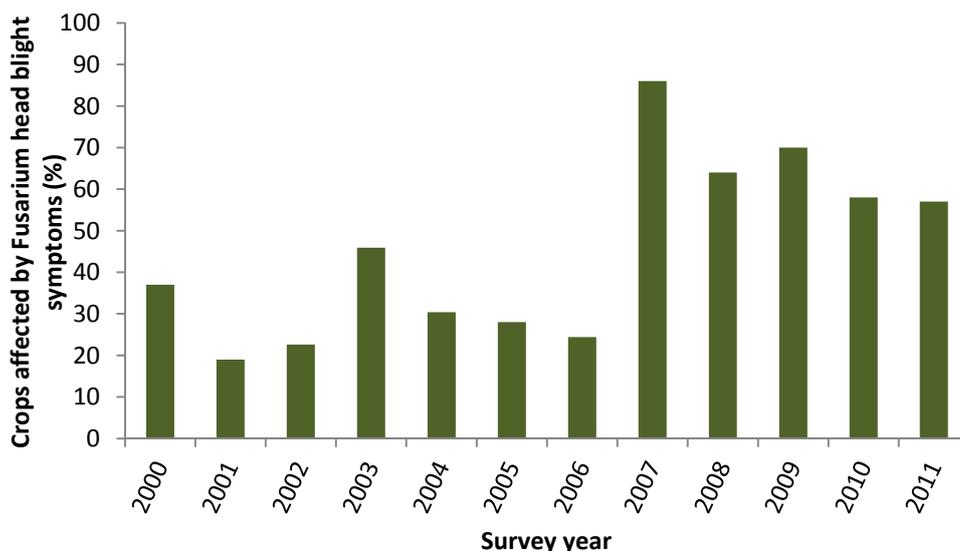


Figure 8. Levels of fusarium head blight symptoms measured during the Defra funded winter wheat survey between 2000 and 2011.

Isolation results for *F. culmorum* and *F. graminearum*, the main deoxynivalenol producing species responsible for FHB symptoms in winter wheat, between 2000 and 2011 (Figure 9) indicated that *F. graminearum* generally predominated over *F. culmorum*. Infections caused by *F. culmorum* were at their highest in 2007 (13% of crops affected) with levels in the other years relatively consistent ranging from 1 to 6% of crops affected (average 3.5%). Generally, levels of *F. graminearum* were much more variable than those of *F. culmorum* ranging from a high of 26% in 2007 and 2008 to 2% in 2001. Between 2008 and 2011 the level of symptoms (Figure 8) were high with over 50% of crops affected, in contrast isolation of *F. graminearum* reduced from 26% of crops affected in 2008 to 2% in 2011. The symptoms seen in 2010 and 2011 were primarily caused by *F. poae* which generally predominated isolations during years where the flowering period was dry, conditions which are known to reduce infection by *F. graminearum*.

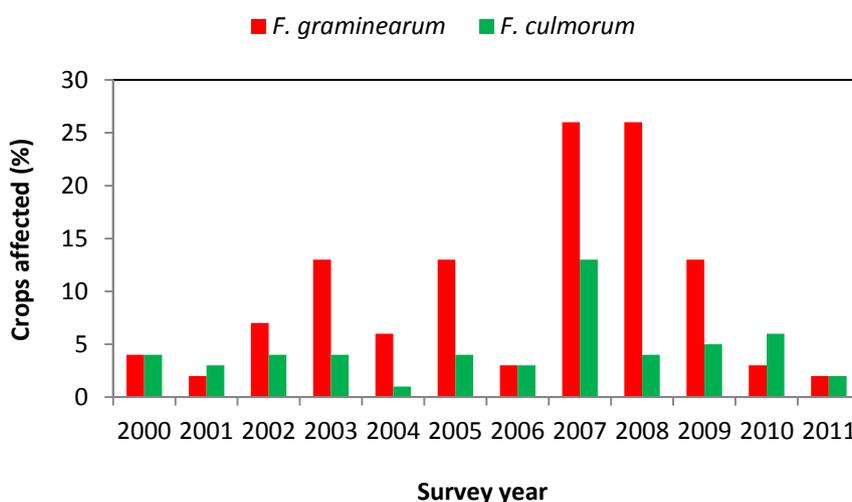


Figure 9. Crops affected by fusarium head blight symptoms caused by *Fusarium graminearum* and *F. culmorum* during the Defra funded winter wheat survey 2000-2011.

The distribution of *F. culmorum* in eight English regions (North West (NW), Yorkshire and Humberside (Y+H), East Midlands (EM), East (E), South East (SE), South West (SW), West Midlands (WM) and North West (NW)) was mapped between 2000 and 2011 (Figure 10). Averaging the data across the whole time period (Figure 10a) showed that the proportion of fields containing FHB symptoms caused by *F. culmorum* was similarly low (between 3 and 5%) in six of the eight regions. For the NW and SE region the proportion of crops infected by *F. culmorum* was slightly higher (7 and 8% respectively), but still low. To determine whether differences existed over time the data were split to produce maps for 2000 to 2005 (Figure 10b) and 2006 to 2011 (Figure 10c). These maps showed that between 2000 and 2005 (Figure 10b) all but one region (NW) had infections caused by *F. culmorum*, with the proportion of crops infected low for the remaining seven regions at between 2 and 5% of crops infected. Between 2006 and 2011 (Figure 10c) the level of infection in four regions (Y+H, EM, E and MW) remained similar to those seen between 2000 and 2005, however levels in the other four regions (NE, SE, SW and NW) were higher. The increase was particularly noticeable in the NW and SE where the proportion of crops infected by *F. culmorum* increased from 0 to 14% and from 4 to 12% respectively. The increase in these two regions primarily resulted from increases in infection levels in 2007 (Figure 9) when 53 and 23% of crops in the NW and SE respectively had symptoms caused by *F. culmorum* (data not presented).

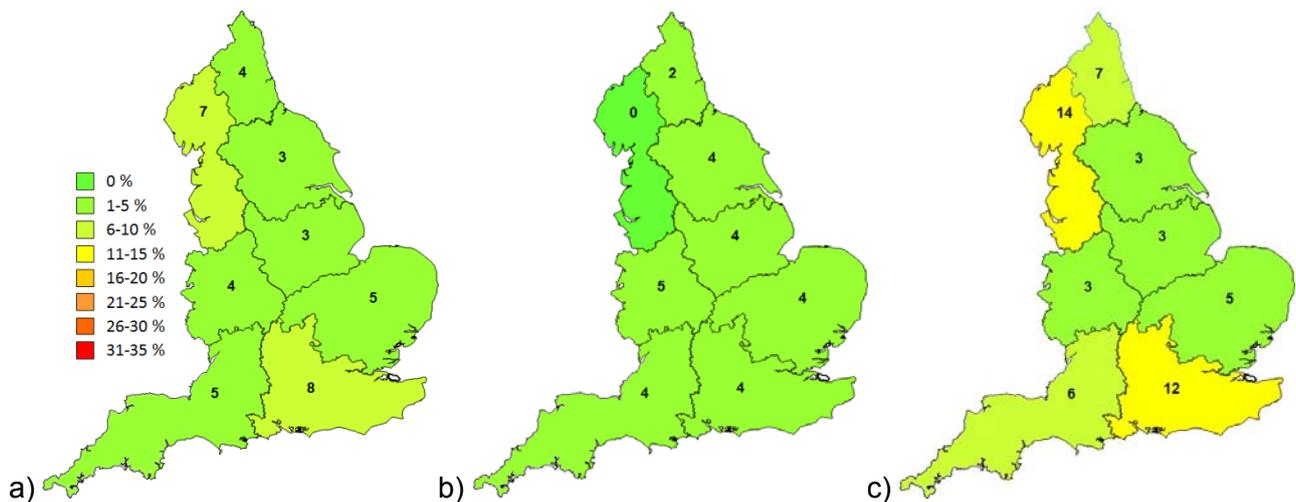


Figure 10. Regional distribution (% crops affected) of *Fusarium culmorum*, a) 2000-2011, b) 2000-2005 and c) 2006-2011.

The distribution of *F. graminearum* was also mapped for eight regions between 2000 and 2011 (Figure 11). Averaging the data for the whole time period (Figure 11a) showed the proportion of fields containing FHB symptoms caused by *F. graminearum* varied between regions with levels low (between 1 and 5%) in four of the eight regions (NE, Y+H, WM and NW). In the remaining four regions (EM, E, SE and SW) the proportion of crops infected by *F. graminearum* was higher

between 9 and 16%. The data were split to produce maps from 2000 to 2005 (Figure 11b) and 2006 to 2011 (Figure 11c). These maps showed that between 2000 and 2005 (Figure 11b) infections caused by *F. graminearum* were highest in the EM and SW regions (15 and 12% respectively). For the remaining six regions the levels of *F. graminearum* infection were low ranging between 0 and 6%. Between 2006 and 2011 (Figure 11c) the level of infection increased, compared to the earlier time period, in all but two regions (NW and SW). Infection levels were still highest in the EM, however the greatest difference between the two time periods was seen in the SE. The increase in these two regions primarily results from increases in infections in 2007 and 2008 (Figure 9) when 44 and 35% of crops in the EM and 33 and 49% of crops in the SE had symptoms caused by *F. graminearum* (data not presented).

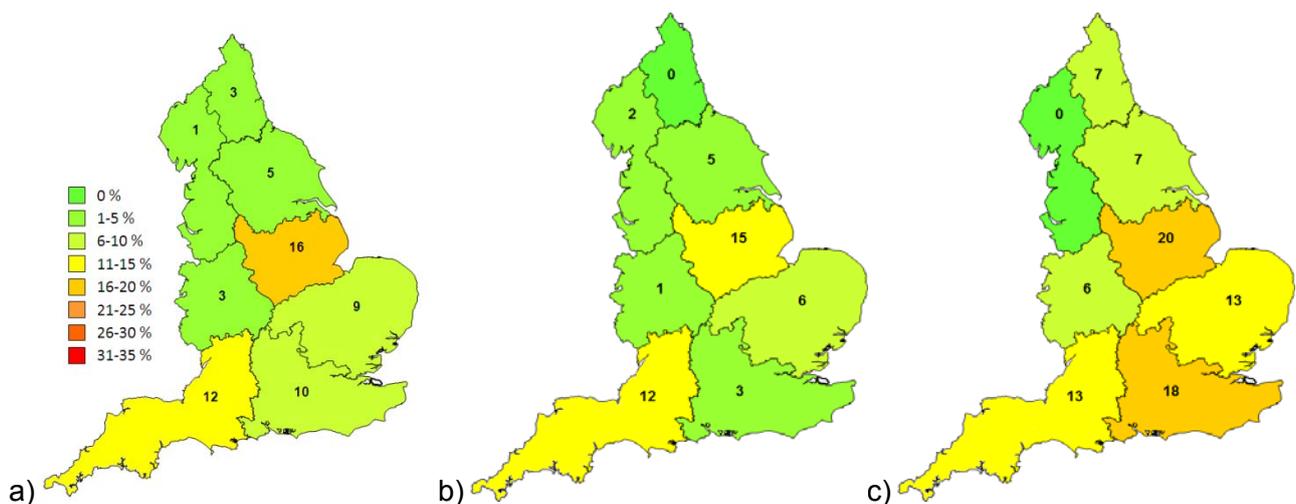


Figure 11. Regional distribution (% crops affected) of *Fusarium graminearum*, a) 2000-2011, b) 2000-2005 and c) 2006-2011

Overall, the data presented in Figure 11b and c suggest that *F. graminearum* inoculum has progressed east and north over time. To provide detail on when this movement may have occurred, the data for *F. graminearum* infections were further broken down to cover 2000-2003, 2003-2005, 2005-2007, 2007-2009 and 2009-2011 (Figure 12a-e). Between 2000 and 2003 (Figure 12a) the incidence of *F. graminearum* was low (0 to 3%) in the majority of regions. Regions with the highest incidence were the E, SE and EM with 12, 11 and 9%, respectively. The low incidence in most regions between 2000 and 2003 reflected the national incidence of *F. graminearum* which was less than 5% in two of the four years (Figure 9). For the time period 2003 to 2005 (Figure 12b) the pattern of incidence was similar to that during 2000 and 2003, with low incidence (0 to 3%) in the NE, NW, WM and SE. The most significant difference was the increased incidence in the EM where the incidence had risen from 9 to 28% of samples infected by *F. graminearum*. This primarily reflected disease incidence in 2005 when *F. graminearum* was isolated from 45% of the samples in the EM. The major difference between the mapping for 2005-2007 and the two previous periods mapped was the increased proportion of crops infected by *F.*

graminearum in the SE, although levels were also raised in the E and EM regions (Figure 12c). These increases were principally a result of infections in 2007. The period 2007 to 2009 (Figure 12d) suggested northerly movement of *F. graminearum* inoculum with increased incidence in the WM, Y+H and NE regions. The high incidence between 2007 and 2009 was undoubtedly influenced by the June weather conditions with two (2007 and 2008) out of the three years having conditions which favoured *F. graminearum* infection. During the last mapping period (2009 to 2011) weather during June tended not to favour *F. graminearum* infection and so infection levels in all regions but the NE and E reduced compared to the 2007 to 2009 mapping period (Figure 12e). However, higher levels of infection in the NE confirmed the northerly movement of *F. graminearum* inoculum.

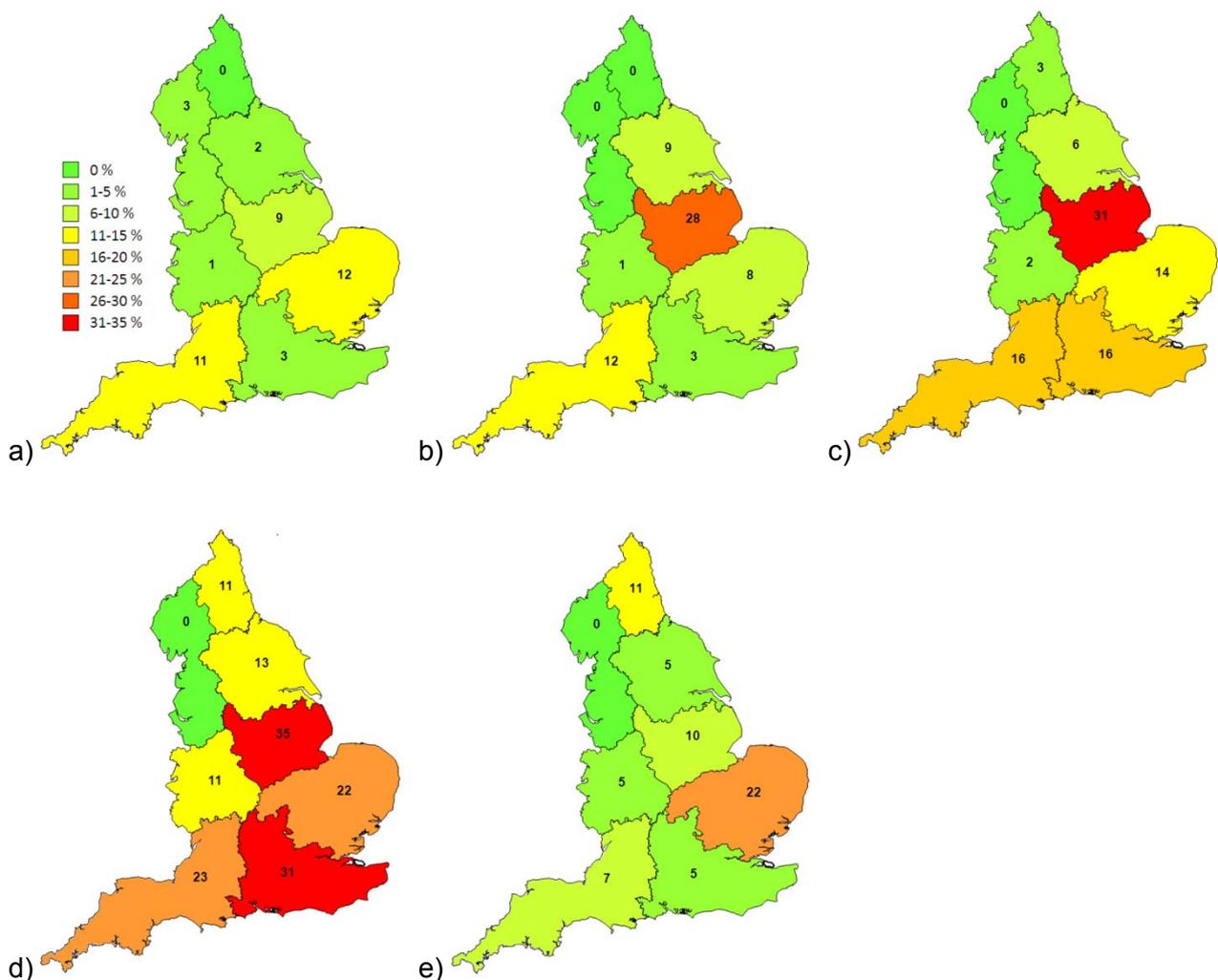


Figure 12. Regional distribution (% crops affected) of *Fusarium graminearum*, a) 2000-2003, b) 2003-2005, c) 2005-2007, d) 2007-2009 and e) 2009-2011.

Although the main driver for FHB infection is weather during flowering, agronomic factors such as reduced cultivation and previous cropping are known to influence disease. Between 2000 and 2011, data from the survey show that the proportion of crops using reduced cultivation methods

increased from below 20% to about 45% (Figure 13). This may account for some of the increase at the national scale, however breaking the data down to a regional level did not provide any evidence of a regional effect.

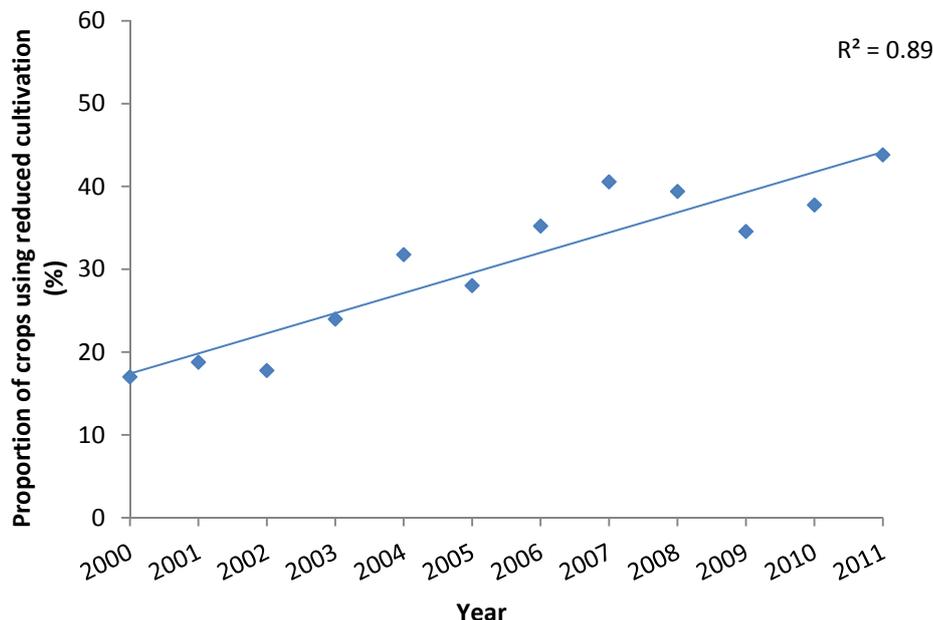


Figure 13. Proportion of crops surveyed between 2000 and 2011 using reduced soil cultivation practices.

4.3. Quantification of fusarium mycotoxins in winter wheat across England

4.3.1. Summary of grain samples received

Total number of samples was 2% greater than the target number of 750 although the target of 150 samples was not achieved in 2013 (Table 3). As the collection of samples was organised from growers who participated in the Defra funded winter wheat disease survey the samples were stratified based on the amount of wheat grown in an area, as such a greater density of samples came from the East of England with a similar, lower number from the North, South and Midlands.

Table 3. Number of samples received for each region in each year

Year	Region				Total
	East	Midlands	North	South	
2009	54	29	36	33	152
2010	71	33	38	35	177
2011	56	35	31	28	150
2012	61	31	36	30	158
2013	45	27	20	38	130
Total	287	155	161	164	767

4.3.2. Mycotoxin summary statistics

Of the ten mycotoxins analysed only six were detected, of these only three, DON, NIV and ZON, were detected above 100 ppb. Table 4 and Table 5 below show the percentage above 10 ppb (the limit of quantification for trichothecenes), the mean, median, the 90th percentile, the 95th percentile and the maximum concentration for DON, NIV, HT2+T2 and ZON from previous studies of UK wheat from 2001-2005 and in English wheat from 2006-2008 for comparison. Table 6-10 show the same data for each individual year from 2009 to 2013. For calculation of the mean values, samples below the limit of quantification (LoQ) were allocated a value of half the LoQ (5 ppb for DON and NIV, 10 ppb for HT2+T2 and 1 ppb for ZON).

Table 4. Fusarium mycotoxin summary statistics for UK wheat in 2001-2005 (n=1624)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	86	230	42	368	722	20333	2.4
NIV	67	27	16	64	95	430	
HT2+T2	34	<20	<20	31	43	214	0.5
ZON	19	17	<5	27	61	1292	3.1

Table 5. Fusarium mycotoxin summary statistics for UK wheat in 2006-2008 (n=958)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	77	342	82	777	1304	11805	5.2
NIV	29	17	<10	36	57	332	
HT2+T2	0.1	<20	<20	<20	<20	36	0.0
ZON	37	51	<2	118	225	1862	11.2

Note in 2006 samples were not analysed for NIV, HT2 or T2.

Table 6. Fusarium mycotoxin summary statistics for English wheat in 2009 (n=152)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	95	202	77	407	590	4059	1.9
NIV	9	7	5	5	16	71	
HT2+T2	1	<20	<20	<20	<20	35	0.0
ZON	41	22	7	51	102	280	8.0

Table 7. Fusarium mycotoxin summary statistics for English wheat in 2010 (n=177)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	41	14	<10	31	42	190	0.0
NIV	0	<10	<10	<10	<10	<10	
HT2+T2	2	<20	<20	<20	<20	48	0.0
ZON	3	4	<2	<2	<2	452	0.6

Table 8. Fusarium mycotoxin summary statistics for English wheat in 2011 (n=150)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	27	18	<10	26	57	558	0.0
NIV	0	<10	<10	<10	<10	<10	
HT2+T2	0	<20	<20	<20	<20	<20	0.0
ZON	0	<2	<2	<2	<2	9	0.0

Table 9. Fusarium mycotoxin summary statistics for English wheat in 2012 (n=158)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	100	615	333	1263	1768	6767	10.1
NIV	51	18	11	43	62	146	
HT2+T2	1	<20	<20	<20	<20	73	0.0
ZON	59	56	16	126	281	859	15.2

Table 10. Fusarium mycotoxin summary statistics for English wheat in 2013 (n=130)

	%>10ppb	Mycotoxin concentration (ppb)					%>LL
		Mean	Median	90th%	95th%	Max	
DON	99	309	95	576	1202	8106	5.0
NIV	45	18	<10	43	62	134	
HT2+T2	5	<20	<20	<20	<20	48	0.0
ZON	15	10	<2	27	43	214	2.3

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

Acetylated derivatives, 3-acetylDON and 15-acetylDON were detected in a few samples in 2012 and 2013 and always as a low concentration secondary contaminant in the presence of a high concentration of a primary contaminant, DON. The highest concentrations for 3-acetyl DON and 15-acetyl DON were 72 and 142 ppb, respectively. Fusarenone X is an acetylated version of nivalenol and was detected in 2012 and 2013 in a total of six samples with a maximum concentration of 32 ppb. The type A trichothecenes, T2 and HT2 were not detected in 2011 and

only in a very low number of samples and at low concentrations (combined HT2+T2 < 100 ppb). Diacetoxyscirpenol and neosolaniol were not detected in any samples (LoQ = 10 ppb).

It should be noted that the legal limits for DON and ZON include a measurement of uncertainty. Therefore, for a consignment of unprocessed wheat intended for human consumption to exceed the legal limit for DON and ZON the concentration as determined by the analytical procedures employed would have to exceed 1534 ppb DON or 124 ppb ZON.

4.4. Modelling FHB incidence and fusarium mycotoxins – meteorological data

4.4.1. Modelling crop growth stage

CropMonitor™ data

Growth stage data, collected at the CropMonitor™ live monitoring sites, were analysed against meteorological data collected from the nearest met station on the NCDC database to provide the number days (Figure 14) and the GDD (Figure 15) from sowing to crop growth stages between 31 (stem extension) and 69 (late flowering). The box plots for number of days from sowing (Figure 14) suggested that the different growth stages for each of the varieties could be separated on this basis. The median values indicated that each variety took a similar time from sowing to reach a particular growth stage, e.g. the median range for the different varieties to reach GS61 was between 240 and 250 days. At GS61, two variety groupings were apparent: Ambrosia/Einstein/Solstice which took approximately 244 days to reach GS61 and Claire/Consort/Malacca/Robigus which took 250 days. The tight interquartile range (the box in the plot) for the different varieties at GS61 (ranged between 7 days (Malacca and Robigus) and 16 days (Ambrosia)) also suggested that estimating a date for the start of flowering was possible.

The use of GDD (Figure 15) again indicated that the different growth stages could be estimated. However, the interquartile range was large for Robigus at all growth stages and for Claire and Malacca at GS61. The plots of GDD again suggested that varieties could be grouped together with growth stage median values similar for Ambrosia/Einstein/Solstice and Claire/Consort/Malacca. The median values for Robigus generally fell between these two variety groupings.

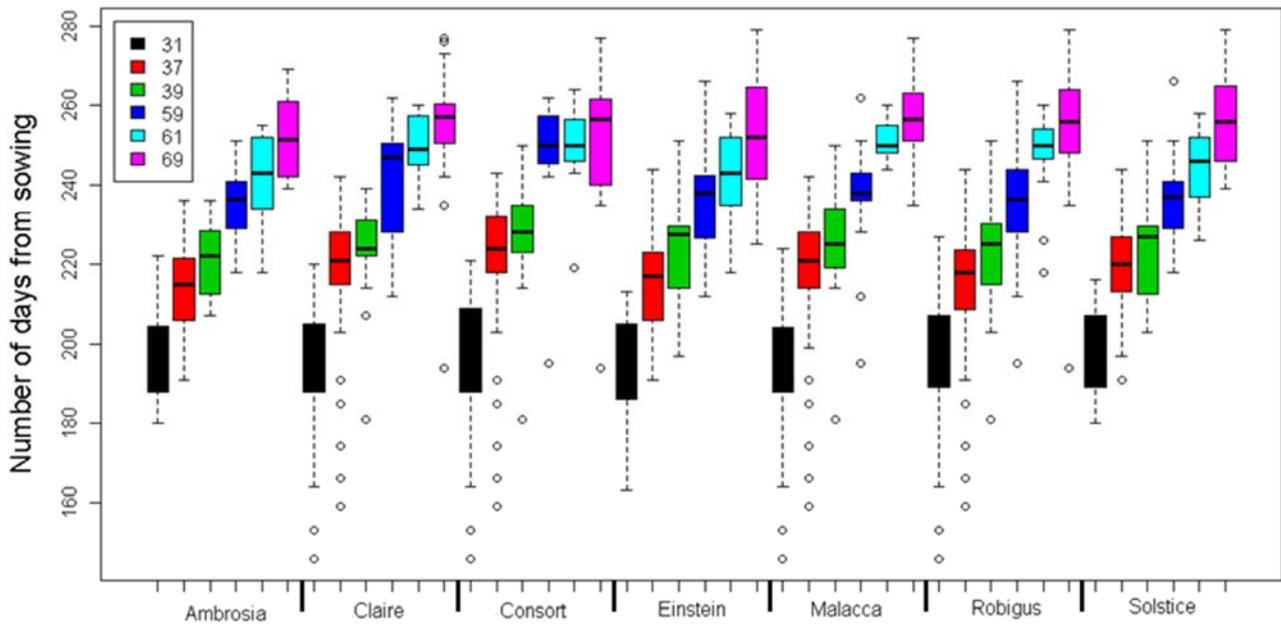


Figure 14. The number of days from sowing to growth stages between 31 (stem extension) and 69 (late flowering) for seven winter wheat varieties grown at CropMonitor trial sites between 2003 and 2010.

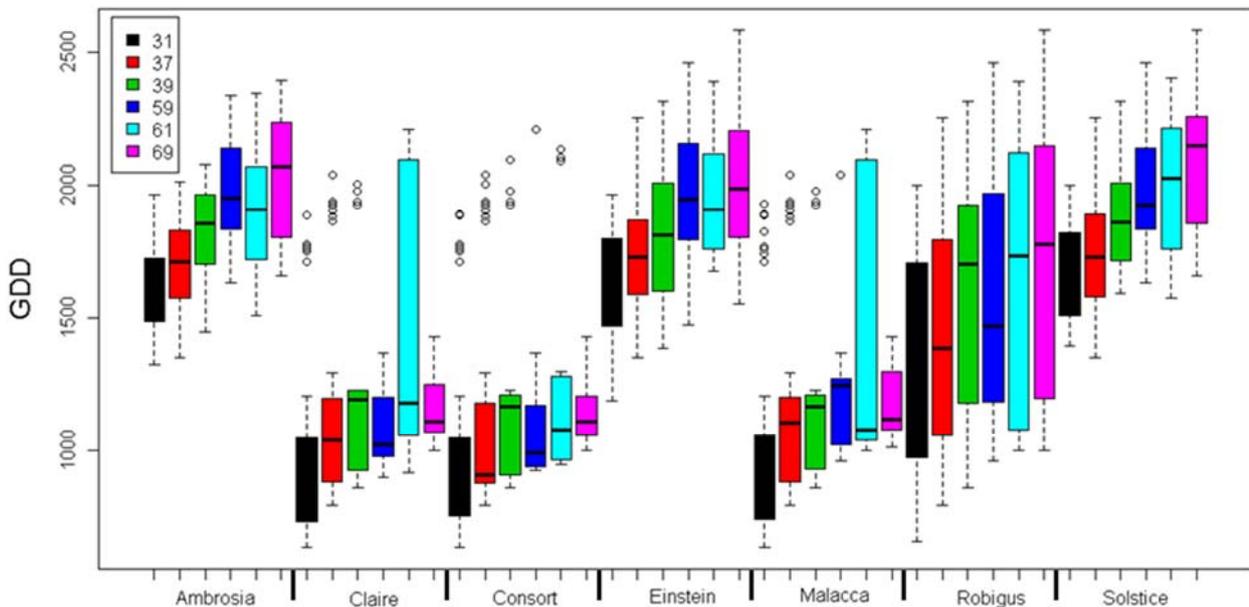
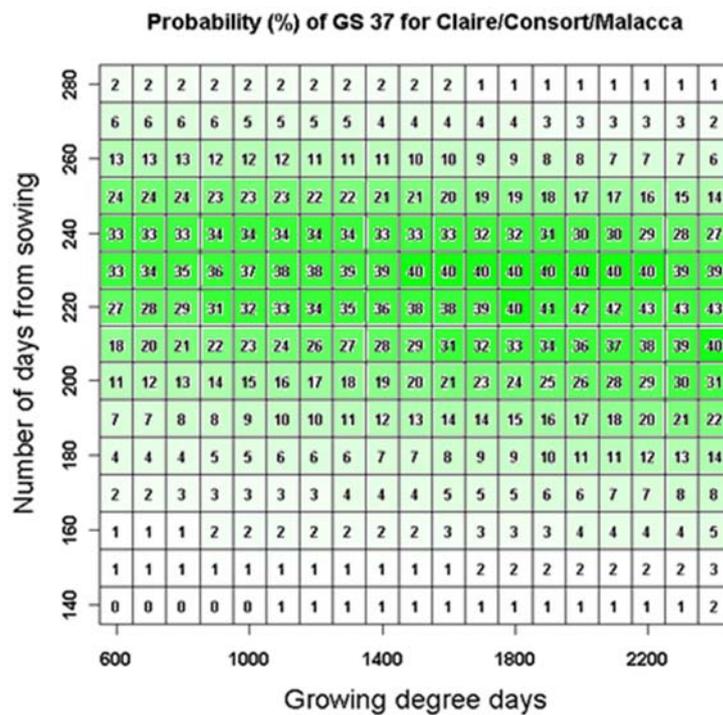


Figure 15. The number of growth degree days (GDD) from sowing to growth stages between 31 (stem extension) and 69 (late flowering) for seven winter wheat varieties grown at CropMonitor trial sites between 2003 and 2010.

Regression analysis to test the relationship between GDD and number of days from sowing showed significant differences between individual growth stages ($p=0.01$). From this it was also apparent that varieties could be grouped together, in particular Ambrosia with Einstein and Solstice

and Claire with Consort and Malacca. Robigus could not be grouped with any other variety. The similarity in reaching the different growth stages for the different variety grouping can be seen when comparing growth degree-days (Figure 15). These analyses suggested that a model combining both days from sowing and growth degree days may provide a good estimation for the date when flowering started. A linear discriminant analysis (LDA) was carried out to test this. Results from this analysis provided an estimated probability for a particular growth stage for any combination of GDD and days from sowing. Example outputs from the LDA for the Claire/Consort/Malacca variety grouping are shown in Figure 16.

a)



b)

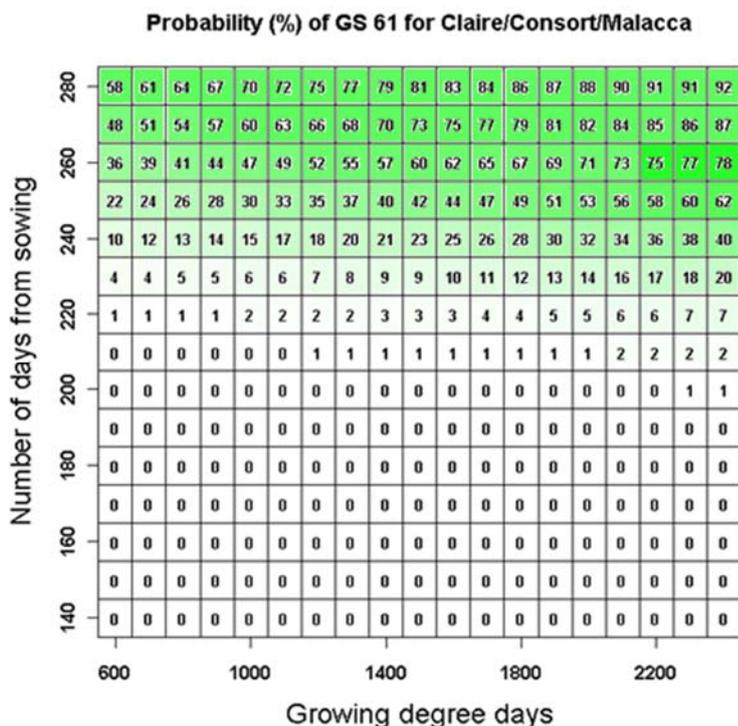


Figure 16. Linear discrimination analysis for number of days from sowing against growing degree days showing the probability of growth stage, a) 37, b) 39, c) 59 and d) 61 for the Claire/Consort/ Malacca variety grouping.

The model produced was validated by comparing the model output using data from the 2008 winter wheat survey with the growth stage recorded by farmers following fungicide spray applications (Table 11). These data suggested that the model was relatively good at predicting GS31 for all the variety groups tested. However, prediction of the flowering period, critical for FHB infection, was less accurate. From the data for the Robigus crops surveyed in 2008, the model gave eight predictions for GS61. However, the actual growth stages recorded by the growers for these were 3 at GS37, 3 at GS39 and two at GS59. A similar over-prediction for GS61 compared to the recorded growth stage was observed for both the Claire/Consort/Malacca and the Ambrosia/Einstein/Solstice variety groupings. For these variety groups, 13 and 43 spray timings were predicted as GS61 by the model, respectively. However, for the Claire grouping, 5 and for the Ambrosia group, 25 of these were recorded as GS39 or below by the farmer.

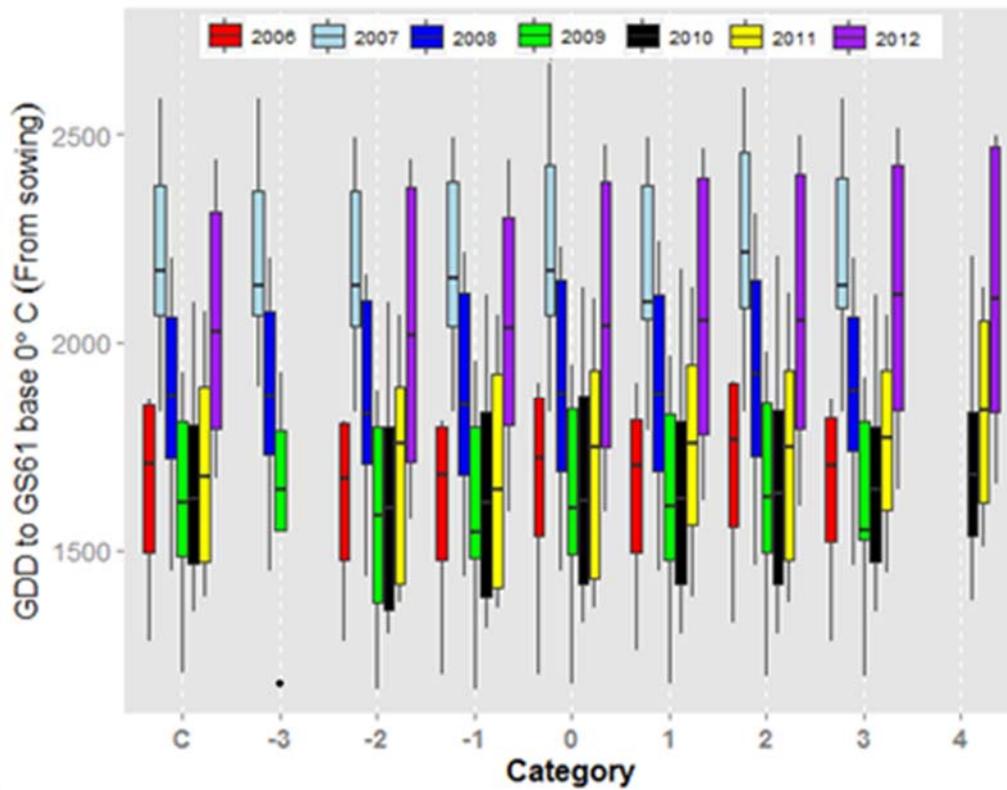
Table 11. Contingency table showing the accuracy of growth stage prediction by the growth stage model compared to the growth stage recorded by farmers during 2008 winter wheat survey

		Growth stage prediction from growth stage model														
		Ambrosia/Einstein/Solstice					Claire/Consort/Malacca					Robigus				
		31	37	39	59	61	31	37	39	59	61	31	37	39	59	61
Growth stage recorded by farmer	31	100	24	0	0	1	19	8	0	0	1	29	2	0	0	0
	37	5	3	0	0	4	0	4	0	0	1	2	2	0	0	3
	39	10	19	0	0	20	2	3	0	0	3	3	6	0	2	3
	59	1	4	0	1	14	0	1	0	0	7	0	1	0	0	2
	61	0	3	0	0	3	0	0	0	0	1	0	0	0	0	0

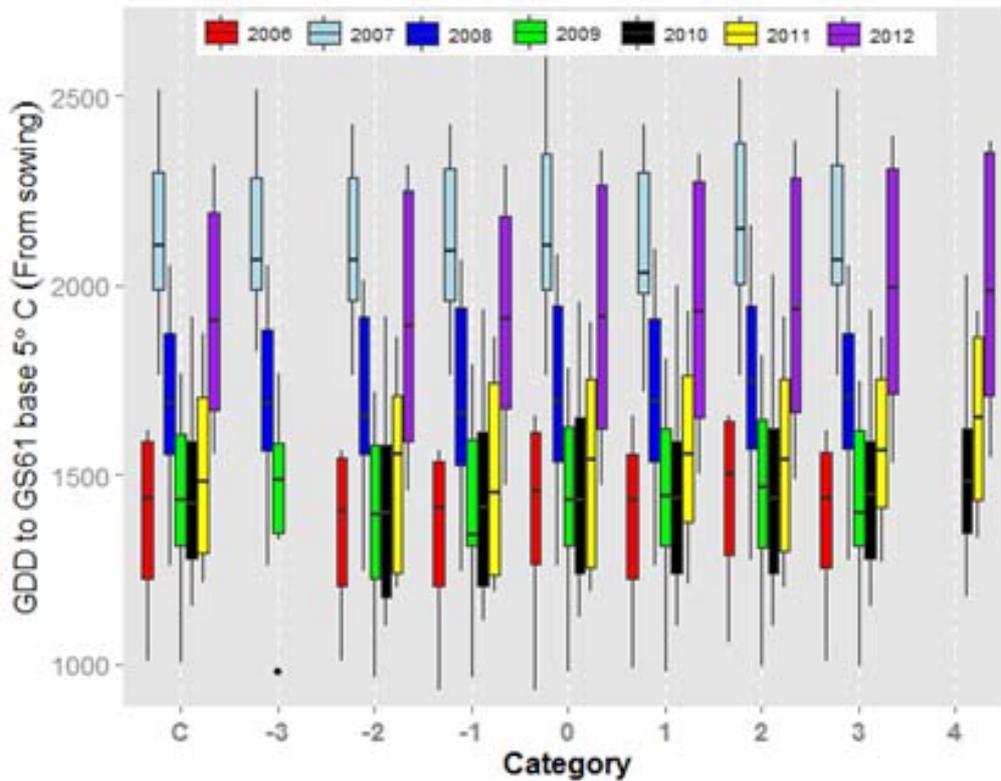
As the growth stage model produced using the CropMonitor™ data was not able to accurately predict the start of flowering for the surveyed crop, the model was not used in the modelling of FHB severity or mycotoxin production.

AHDB Recommended Lists trials data

The calculation of GGD from sowing (Figure 17) produced a similar set of results irrespective of whether the base temperature was set at 0°C (Figure 17a) or 5°C (Figure 17b). The influence of year can clearly be seen with the median number of GDD taken from sowing to the start of flowering in 2007 being highest across all the ripening categories. The GDD calculated for four years (2006, 2009, 2010 and 2011) was similar, with median GDD between 1500 and 1700 using a base temperature of 0°C (Figure 17a) and 1300 to 1600 with a base temperature of 5°C (Figure 17b). However, the median GDD seen for the remaining years was much higher which suggested that the calculations for the start date of flowering for the surveyed crops would need to be different for each year. The large box in the plots (the interquartile range) suggested that there was a large variation within the data; in other words, the data were not consistent. This was particularly true for 2012, where the average interquartile range was 580 GDD. Assuming an average daily temperature of 9°C from sowing to flowering, this gives a difference of 64 days for time to flowering. Even for data from 2006, where the interquartile range was shortest at around 320 GDD, this gave a difference of 36 days. As flowering generally only lasts 10 days, the difference was too great and meant that the GDD from sowing was not a suitable for calculating the start of flowering.



a)

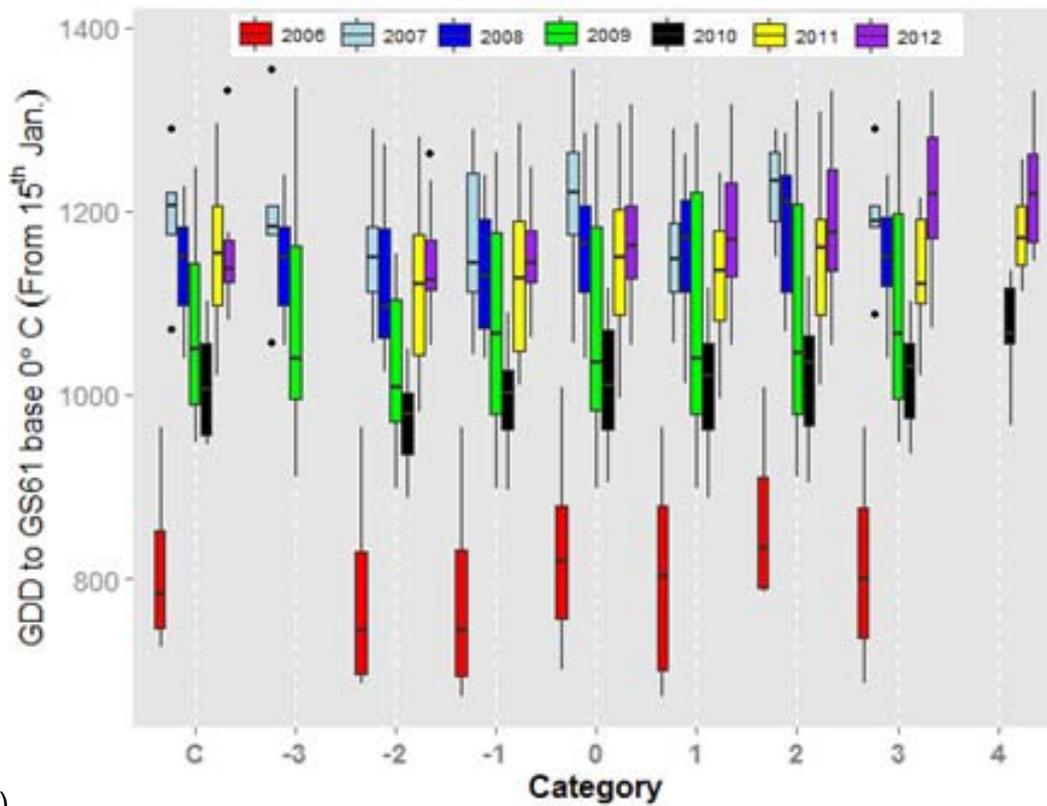


b)

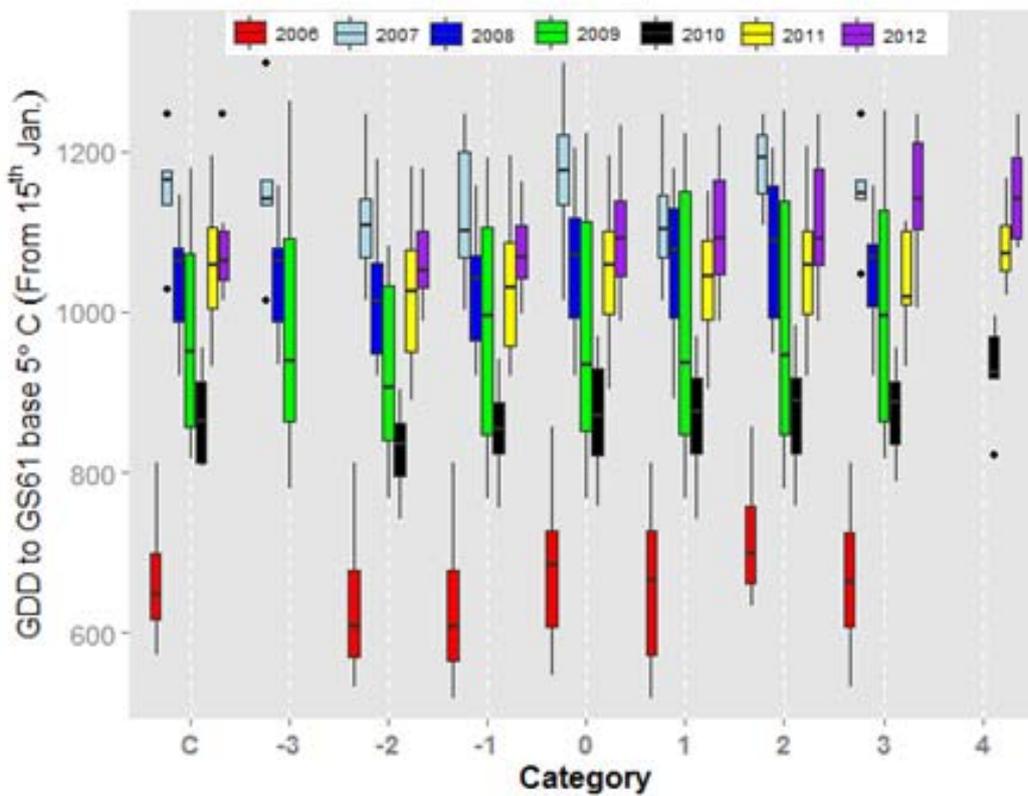
Figure 17. Number of growth degree days (GDD) from sowing to GS61 (early flowering) calculated using a base temperature of, a) 0°C and b) 5°C for winter wheat varieties grouped by ripening and compared to the control variety Solstice (C). Minus ripening categories ripen earlier than Solstice whereas positive categories ripen later.

Calculating the GDD from the middle of January to the start of flowering (Figure 18) produced a different outcome from starting at sowing date. Again, differences were seen between years with crops in 2006 needing a shorter GDD to reach flowering. The median values obtained using a base temperature of 0°C for the other years were relatively consistent ranging between about 1000 GDD and 1200 GDD (Figure 18a). The median range based on a base temperature of 5°C was wider (800 to 1200 GDD (Figure 18b)) which suggested using a base temperature of 0°C would provide an intermediate estimated date for the start of flowering. Using the 15th Jan start date for the GDD calculation generally produced narrower interquartile ranges than those produced when the calculation started from the sowing date. The year with the widest range was 2009 which equated to 21 days, whereas 2007 had the narrowest range and equated to 7 days. Although the range produced using the 15th Jan start date was narrower than that produced using the sowing date, the possible variation was still considered too great.

Moving the start date of the calculation of GDD to 31st January (Figure 19) produced outputs very similar to those produced using a 15th of January start date and so the variation was once again considered to be too large for the accurate prediction required.

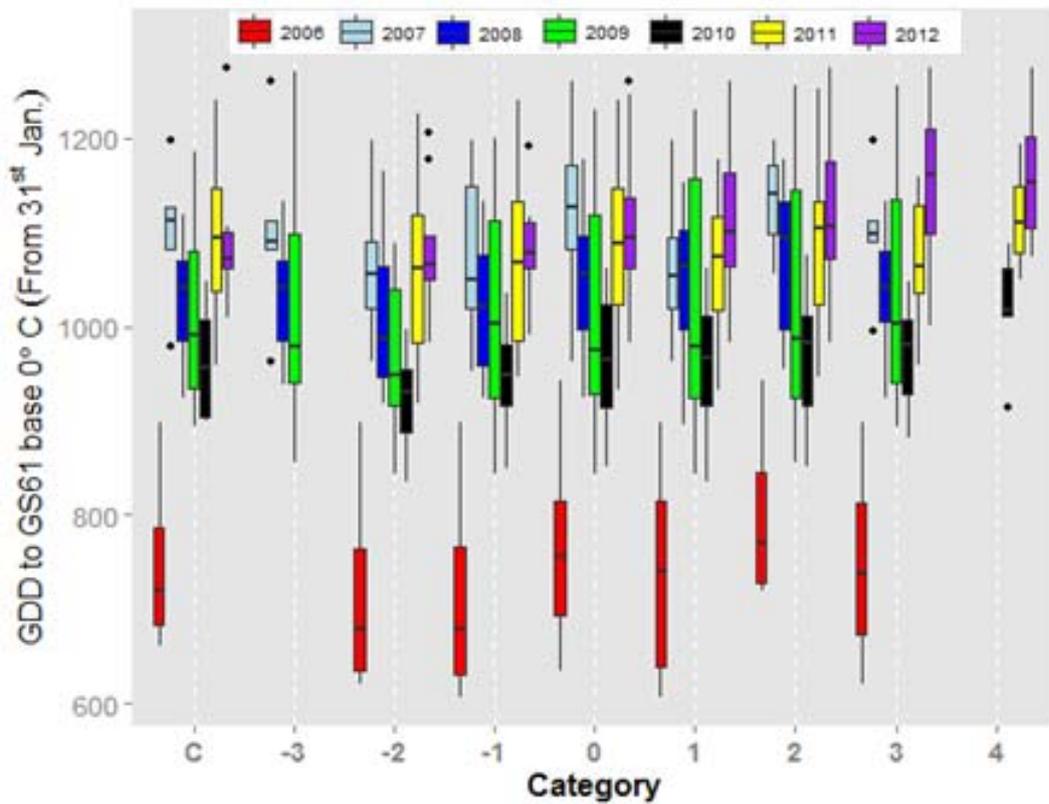


a)

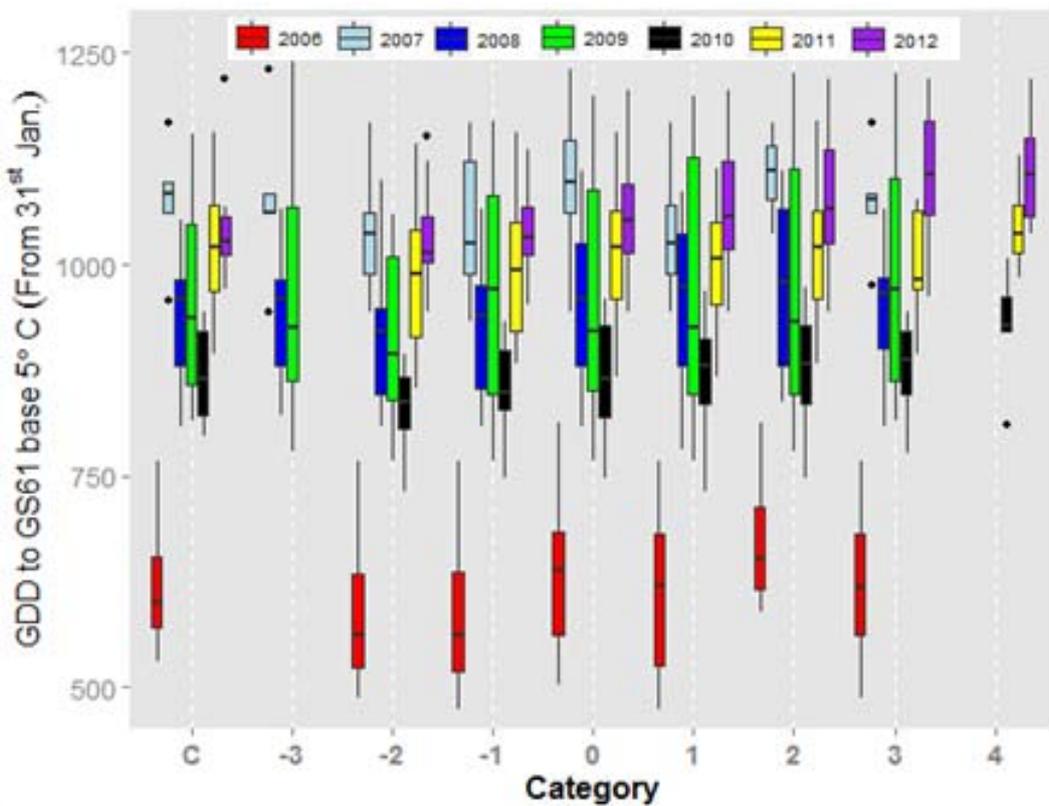


b)

Figure 18. Number of growth degree days (GDD) from 15th January to GS61 (early flowering) calculated using a base temperature of, a) 0°C and b) 5°C for winter wheat varieties grouped by ripening and compared to the control variety Solstice (C). Minus ripening categories ripen earlier than Solstice whereas positive categories ripen later.



a)



b)

Figure 19. Number of growth degree days (GDD) from 31st January to GS61 (early flowering) calculated using a base temperature of, a) 0°C and b) 5°C for winter wheat varieties grouped by ripening and compared to the control variety Solstice (C). Minus ripening categories ripen earlier than Solstice whereas positive categories ripen later.

4.4.2. Modelling FHB

Regional level

Four cluster analysis methods (complete linkage, average linkage, Ward's algorithm and K-means) were used to analyse the incidence data for *F. graminearum* between 2005 and 2010. Initial analyses evaluated each cluster method for matching disease pattern and predictive ability, with greatest emphasis placed on the latter. From these analyses Ward's algorithm and K-means were a better fit than the complete linkage and average linkage methods. The use of the 'elbow criterion' showed that the most appropriate cluster method for use with *F. graminearum* incidence data collected between 2005 and 2010 was Ward's algorithm. Using this method, a cluster map was produced with twelve disease risk regions (Figure 20).

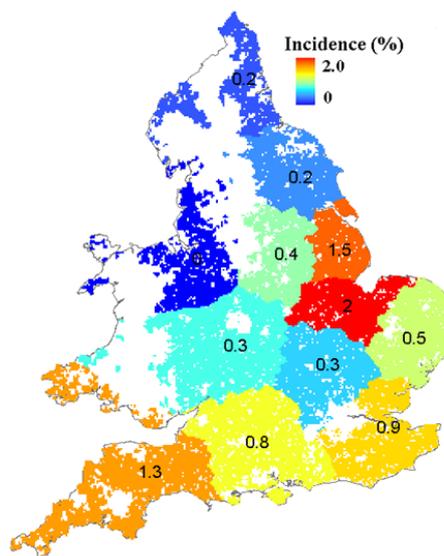


Figure 20. Cluster analyses, as produced by Ward's algorithm showing risk regions for *Fusarium graminearum* disease incidence using data collected at GS73-75 between 2005 and 2010.

The window pane analyses produced seven weather variables of interest (

Table 12.) with the average maximum temperature (Tmax_ave) and the average rainfall (rain_ave) selected for correlation analyses (Figure 21). Stepwise-partial least square (PLSR) analysis, showed that for these weather parameters the only significant windows for the development *F. graminearum* infections were the Tmax_ave during February and March and the rain_ave from late May to early June; this is represented diagrammatically in Figure 22. The model produced appeared to be a relatively good fit to current knowledge on the conditions required for *F. graminearum* development, with warm temperatures in the spring encouraging inoculum development on crop debris etc., rainfall in late May encouraging the development of perithecia and the rainfall in early June leading to infection during crop flowering.

Table 12. Correlation range and number of significant correlations for weather parameters highlighted of potential interest in fusarium head blight infections caused by *Fusarium graminearum*.

Weather Variable	Correlation range	Number ($\rho < 0.001$)
Tmean_avg	-0.32 -0.48	164
Tmin_avg	-0.31 - 0.42	6
Frost days (number)	-0.42 - 0.29	0
Tmax_avg	-0.27 - 0.52	241
Tmax_nod20	-0.27 - 0.45	101
RHMean_avg	-0.27 - 0.47	0
Rain_avg	-0.3 - 0.51	8

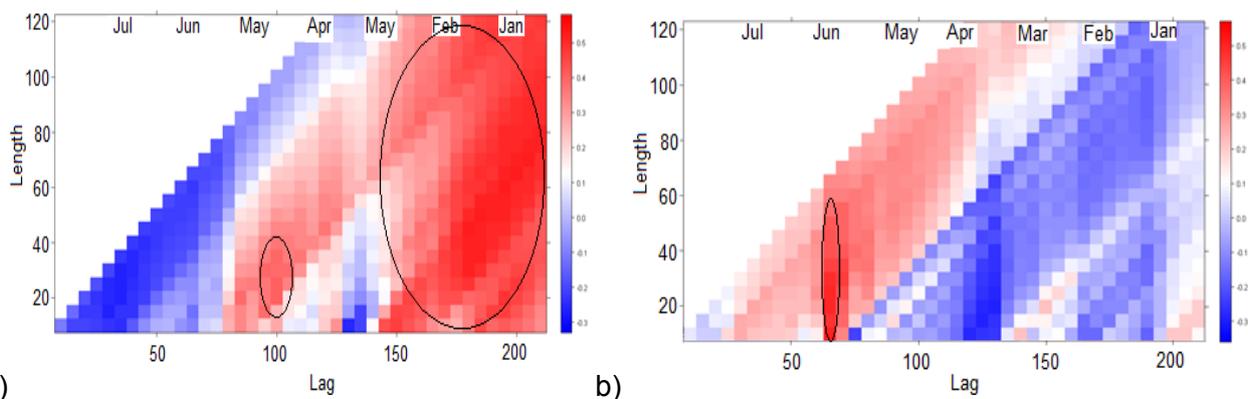


Figure 21. Correlation patterns with significant windows highlighted (circled) for *Fusarium graminearum* infection, a) average maximum temperature and b) average rainfall.

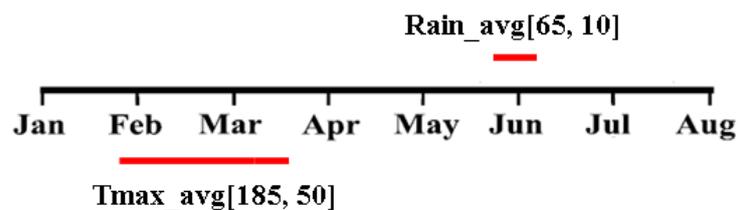


Figure 22. Diagrammatic representation of the weather parameters determined as important for fusarium head blight development caused by *Fusarium graminearum* at the regional level.

Regression analysis for observed *F. graminearum* incidence and that predicted by the high dimensional regression model (Figure 23) produced a relatively good fit with an R^2 of 0.43. The regression indicated the model was better at predicting low level *F. graminearum* incidence than high. At high incidence levels, the model overestimated *F. graminearum* infection compared to the observed data.

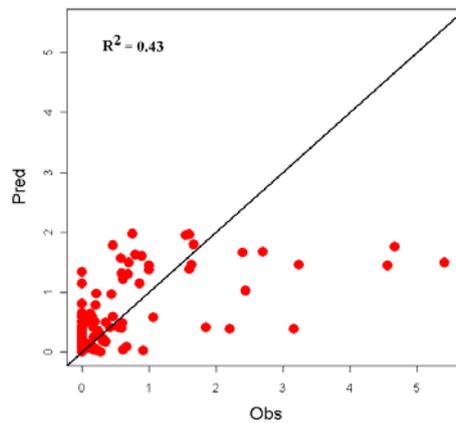


Figure 23. Goodness of fit for observed versus predicted regional incidence of *Fusarium graminearum* as determined by High Dimensional Regression Modelling (black line indicates a perfect fit).

Although the goodness of fit of models can be a reliable indication of the usefulness of a model, the ultimate test of a predictive model is to test it on independent data. For this purpose disease monitoring data and weather data were sourced for 2012 and 2013 from CropMonitor™, however the poor quality of rain data in 2013 (both from the NCDC and MIDAS databases) meant that only data from 6 stations were suitable for use. These stations did not cover the range of clusters and so could not be used to validate the model. As the model was initially produced using data from 2005 to 2010, the data for 2011 were not incorporated into the model but instead used for validation. The predictive power of the *F. graminearum* model for the 2011 and 2012 data is shown in **Figure 24**. In both years the model was unable to successfully predict the incidence of *F. graminearum*, over-predicting incidence in 2011 and largely underpredicting in 2012. The observed incidence in the two validation years differed in that they were at the extremes of incidence, with levels amongst the lowest recorded in 2011 (cluster incidence range 0 to 0.4%) and the highest recorded in 2012 (cluster incidence range 0.2 to 10%).

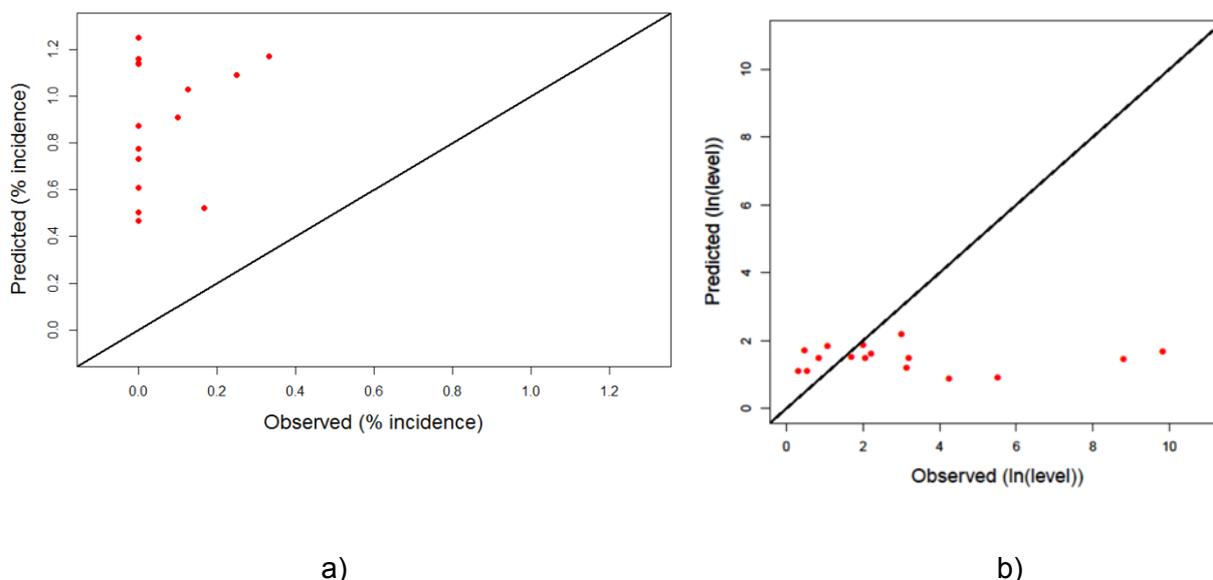


Figure 24. Goodness of fit validation for observed versus predicted regional incidence of *Fusarium graminearum* using data collected in a) 2011 and b) 2012. For both plots the black line indicates a perfect fit.

Site level analysis

The window pane analysis for *F. graminearum* incidence at the site level produced two significant weather variables (average maximum temperature (Tmax_ave) and average rainfall (rain_ave)). The lag time for Tmax_ave was 169 days with a 40 day window, whereas the lag for rain_ave was 94 days with a window of 14 days (Figure 25).

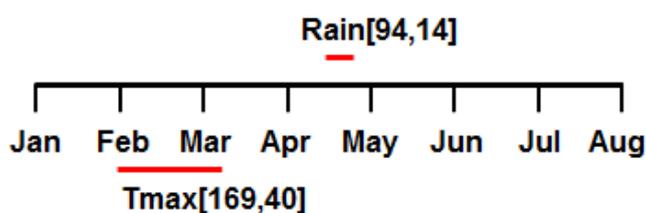


Figure 25. Diagrammatic representation of the weather parameters determined as important for fusarium head blight development caused by *Fusarium graminearum* at the individual site level.

Three agronomic factors (previous crop, cultivation and harvest period) were tested for inclusion in the stepwise regression. Even though previous crop and cultivation are known to influence *F. graminearum* incidence neither were determined significant in these analyses. Unlike the parameters produced during the regional modelling for *F. graminearum*, the weather variables identified for the individual site modelling did not seem to entirely fit with current knowledge. In particular, there was no rain variable indicated during crop flowering (June); the condition known to be required for infection by *F. graminearum*.

The fitted regression for the observed versus predicted incidence (Figure 26) showed that the model did not perform well, particularly at high incidence when the model vastly underestimated disease.

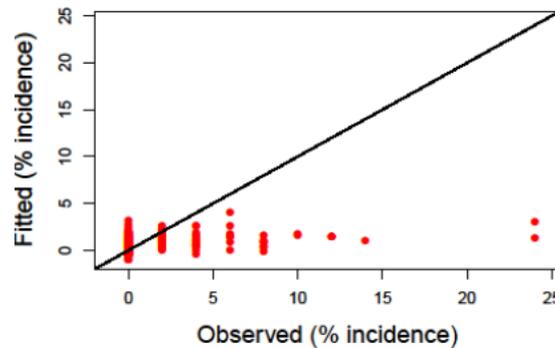


Figure 26. Goodness of fit for observed versus predicted site level incidence of *Fusarium graminearum* as determined by Stepwise Regression Modelling (black line indicates a perfect fit).

The predictive power of the *F. graminearum* site level model was tested using incidence data collected during 2012 and 2013 (Figure 27). The issues over the quality of the rainfall data in 2013 described previously meant that the validation of the model in 2013 could only be tested on six sites. The pattern for the fit seen in 2012 was very similar to that for the original model fit where the incidence of *F. graminearum* was underestimated particularly when the observed incidence was high e.g. at two sites where the observed incidence was 24% the model predicted the incidence to be less than 5%. Of the six sites used to validate the model in 2013, only one had FHB symptoms caused by *F. graminearum*. The model predicted an incidence of 0.5% which was again an underestimation as the observed incidence was 2%.

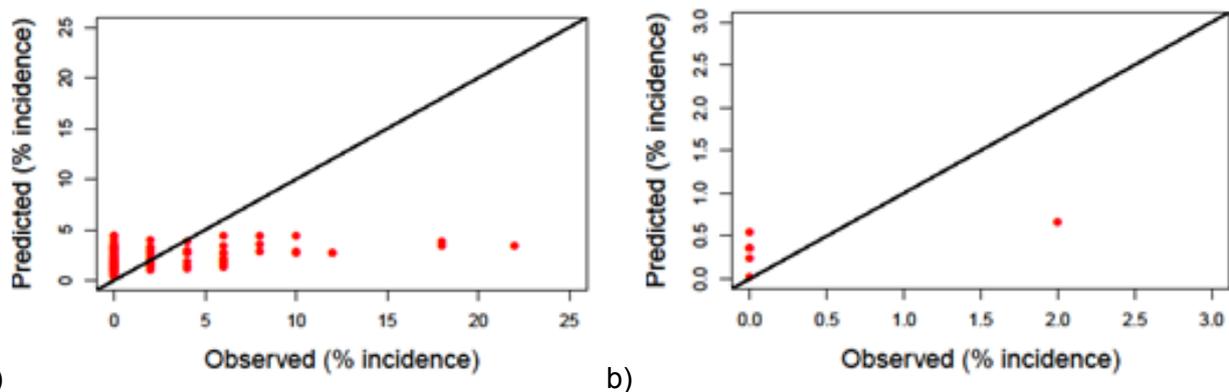


Figure 27. Goodness of fit validation for observed versus predicted site level incidence of *Fusarium graminearum* using data collected in a) 2012 and b) 2013. For both plots the black line indicates a perfect fit.

Overall, FHB symptoms increased between 2005 and 2011 (the years used to produce the model). However, the majority of these symptoms were not produced by *F. graminearum* which resulted in the majority of the data consisting of zeroes (Figure 28). Even in the years where *F. graminearum* incidence was at its highest, 2007 and 2008, the data still consisted of over 200 zeroes. A large proportion of zeroes in a data set has a large influence on the regression modelling and may go some way to explain the lack of fit to the data. To try and overcome this, the analyses were repeated on a modified dataset where the zeroes were not included.

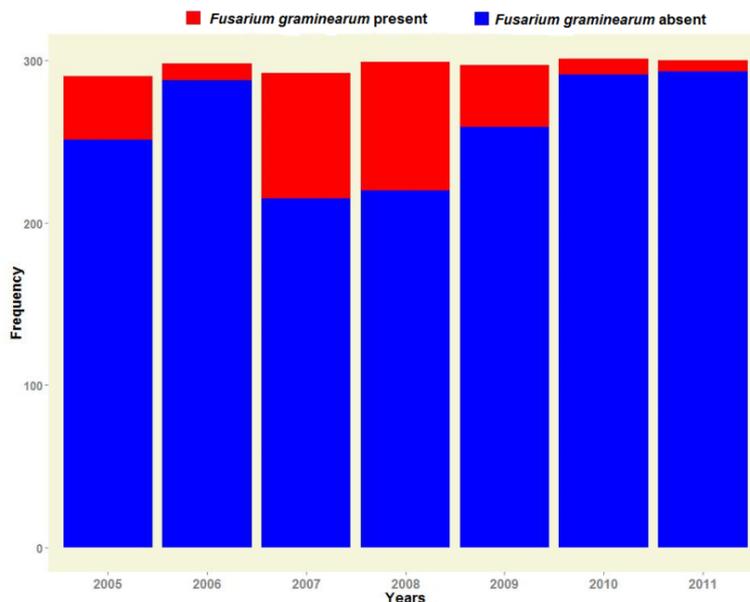


Figure 28. Incidence of *Fusarium graminearum* in crops sampled in the winter wheat survey between 2005 and 2011.

Repeating the analysis on the dataset which only included sites where *F. graminearum* had been present again produced two significant weather variables, the average minimum temperature (Tmin_ave) and average rainfall (rain_ave). The lag time for Tmin_ave was 60 days with a 7 day window, whereas the lag for rain_ave was 87 days with a window of 7 days (Figure 29). As was found using the full data set (with zeroes), no significant correlation was detected for any of the agronomic variables tested using the data set minus zero values.

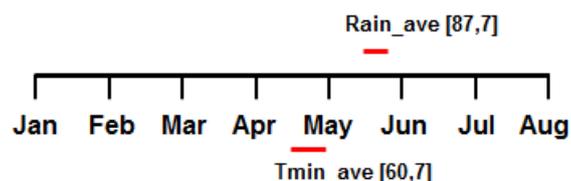


Figure 29. Diagrammatic representation of the weather parameters determined as important for fusarium head blight development caused by *Fusarium graminearum* at the individual site level based on a data set with zeroes removed.

The fitted regression for the observed versus predicted incidence (Figure 30) showed that removal of the zeroes from the analysis did improve the model, although the model fitted values were larger than those for the model where zeroes were included.

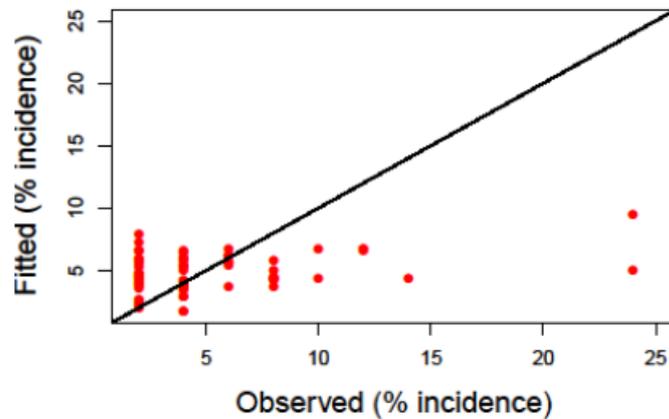


Figure 30. Goodness of fit for observed versus predicted site level incidence of *Fusarium graminearum* as determined by Stepwise Regression Modelling based on data with the zeroes removed (black line indicates a perfect fit).

Validation of the model using data collected in 2012 and 2013 once again showed a lack of predictive power (Figure 31). The regression for 2012 (Figure 31a) showed that the range of observed values for the incidence (for all but the three largest) matched the range of predicted values i.e. up to 10% observed and up to 10% predicted, however within this range, prediction at the individual site level was not accurate. The validation using the limited data for 2013 suggested that the model produced with the zeroes removed had poorer predictive power than the individual site model produced with the zeroes included. The predicted incidence values produced for the zeroes removed model ranged between 4 and 8% (Figure 31b) compared to 0 and 0.5% for the zeroes included model (Figure 27b), when the observed incidence range was between 0 and 2%.

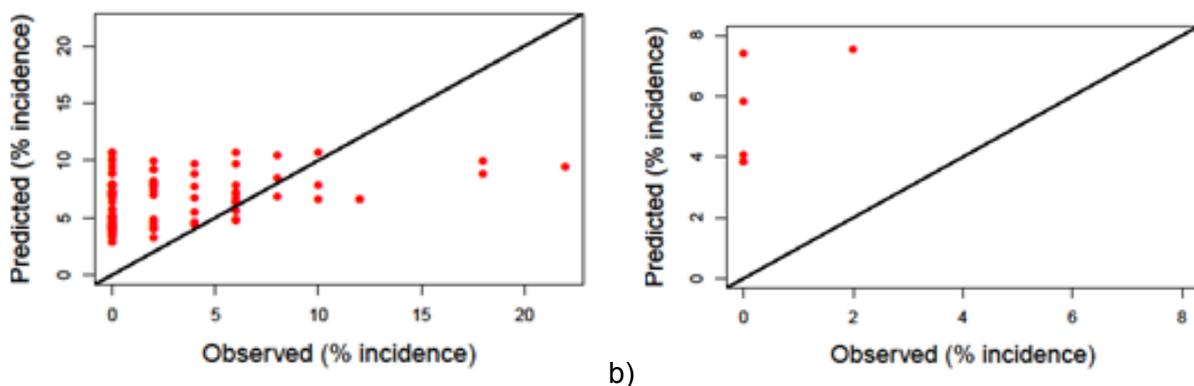


Figure 31. Goodness of fit validation for observed versus predicted site level incidence of *Fusarium graminearum* using data collected in a) 2012 and b) 2013. For both plots the black line indicates a perfect fit.

4.4.3. Modelling fusarium mycotoxins

Regional level

Deoxynivalenol (DON)

Four cluster analysis methods (complete linkage, average linkage, Ward's algorithm and K-means) were used to analyse the DON data between 2006 and 2010. Initial analyses evaluated each cluster method for matching contamination pattern and predictive ability, with greatest emphasis placed on the latter. From these analyses and the use of the 'elbow criterion' K-means linkage was superior to the other three methods. Using this method, a cluster map was produced with fourteen DON contamination risk regions (Figure 32).

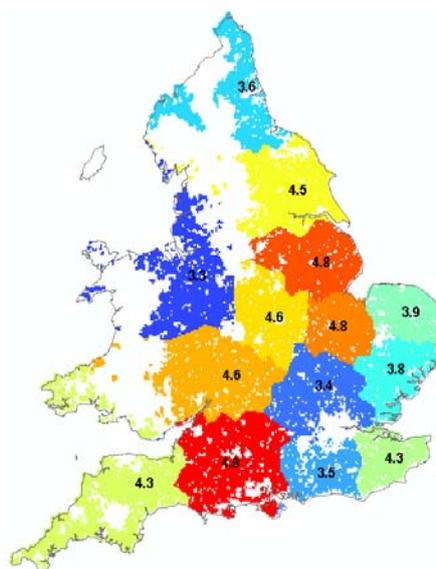


Figure 32. Cluster analyses, as produced by k-means showing risk regions for deoxynivalenol contamination of grain using data collected 2006 and 2010.

The window pane analyses produced 11 weather variables of interest for DON contamination of grain (Table 13), with the average mean temperature (Tmean_ave), the average mean relative humidity (RHmean_ave) and the average rainfall (rain_ave) selected for further correlation analyses (Figure 33). Stepwise-partial least square analysis, showed that for these weather parameters there were two main windows of significance for Tmean_ave and one each for RHmean_ave and rain_ave which influenced DON contamination of grain. For Tmean_ave, these windows were from late February to mid-March and late April to mid-May, for rain_ave from early April to early June and for RHmean_ave mid-May; this is represented diagrammatically in Figure 34. The model produced (parameters and windows) was similar to that produced for *F. graminearum* and as a result was a good fit in terms of current knowledge on conditions required for inoculum development, perithecial maturation and infection. In terms of parameters responsible for increased grain contamination by DON, rainfall during July has been implicated as having a significant effect. Although the correlation analysis picked up significant positive correlations for

rainfall and relative humidity during early July these were only weakly significant compared to the other parameters and so were not included in the model.

Table 13. Correlation range and number of significant correlations for weather parameters highlighted of potential interest in deoxynivalenol contamination of grain.

Weather Variable	Correlation range	Number (p<0.001)
Tmean_avg	-0.73 ~ 0.81	291
Tmin_avg	-0.73 ~ 0.80	238
Frost days (number)	-0.77 ~ 0.56	135
Tmax_avg	-0.66 ~ 0.83	266
Tmax_nod20	-0.59 ~ 0.59	112
RHMean_avg	-0.73 ~ 0.66	249
Rain_avg	-0.55 ~ 0.73	278
Rain_nod0	-0.66 ~ 0.54	6
Rain_nod1	-0.66 ~ 0.62	74
Rain_nod5	-0.45 ~ 0.70	178
Rain_nod9	-0.59 ~ 0.68	129

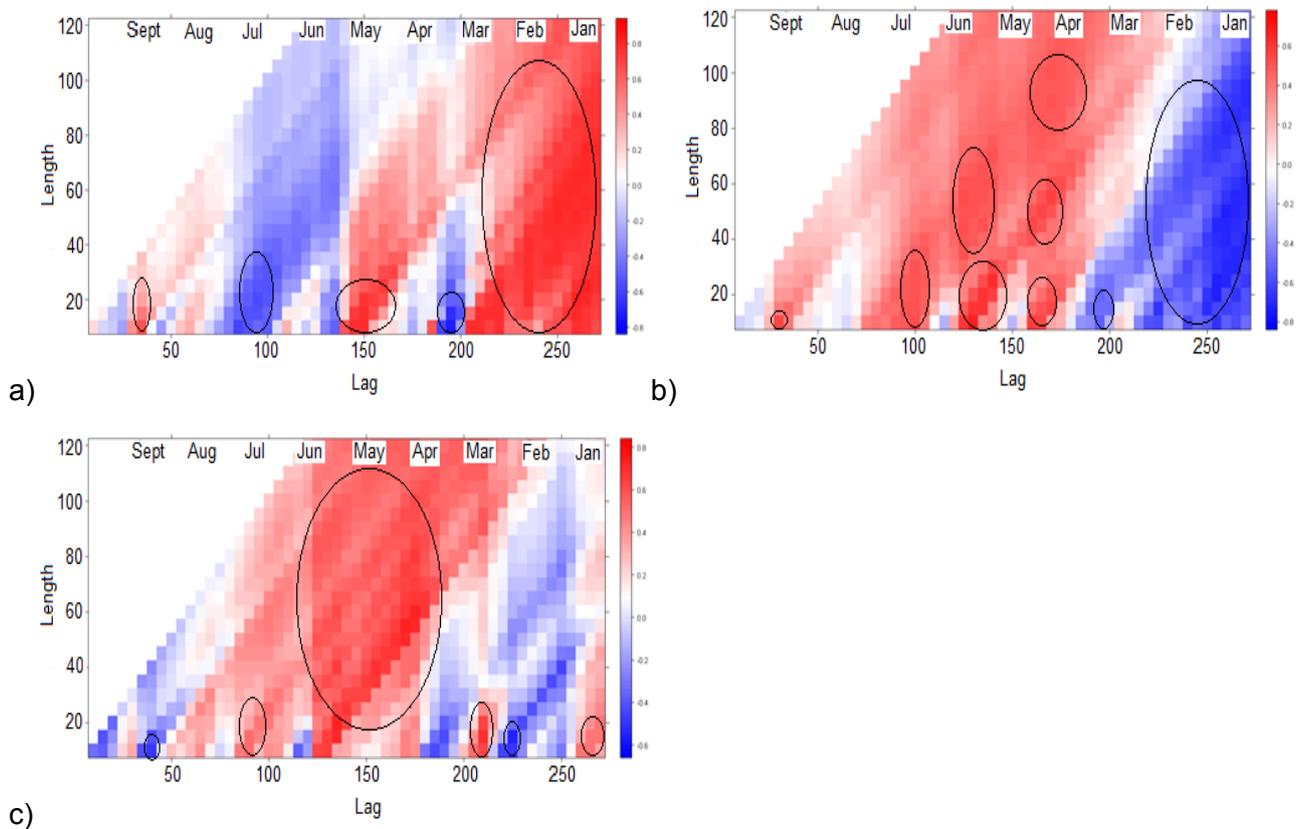


Figure 33. Correlation patterns with significant windows highlighted (circled) for deoxynivalenol contamination of grain, a) average mean temperature, b) average mean relative humidity and c) average rainfall.

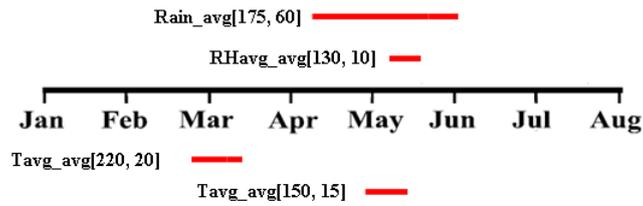


Figure 34. Diagrammatic representation of the weather parameters determined as important for deoxynivalenol contamination of grain.

Goodness of fit analysis for observed DON contamination of grain compared to predicted estimates produced by the high dimensional regression model (Figure 35) indicated a good fit across the whole range of observed data ($R^2 = 0.78$), although at the lower levels of contamination the regression indicated the model was likely to underestimate DON contamination. Even so, it appeared that a model had been developed which should allow the prediction of DON contamination of grain at a regional level.

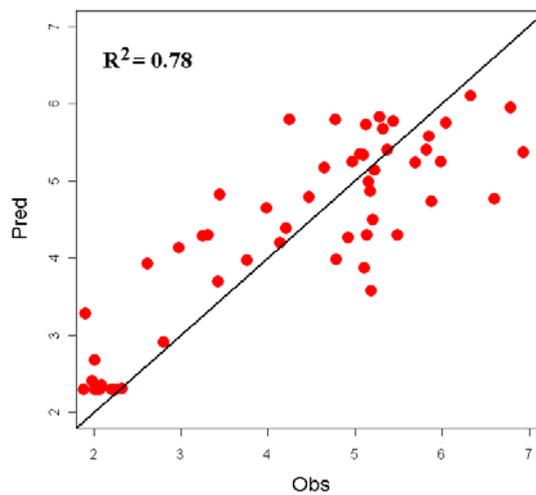


Figure 35. Regression analysis showing observed versus predicted regional incidence deoxynivalenol contamination of grain as determined by High Dimensional Regression Modelling.

Model validation for prediction of regional DON contamination was carried out as described for modelling of *F. graminearum* incidence. The regression outputs showing the predictive power of the model using data collected in 2011 and 2012 are presented in Figure 36. In both years, the model predicted higher levels of DON contamination at the regional level than was observed.

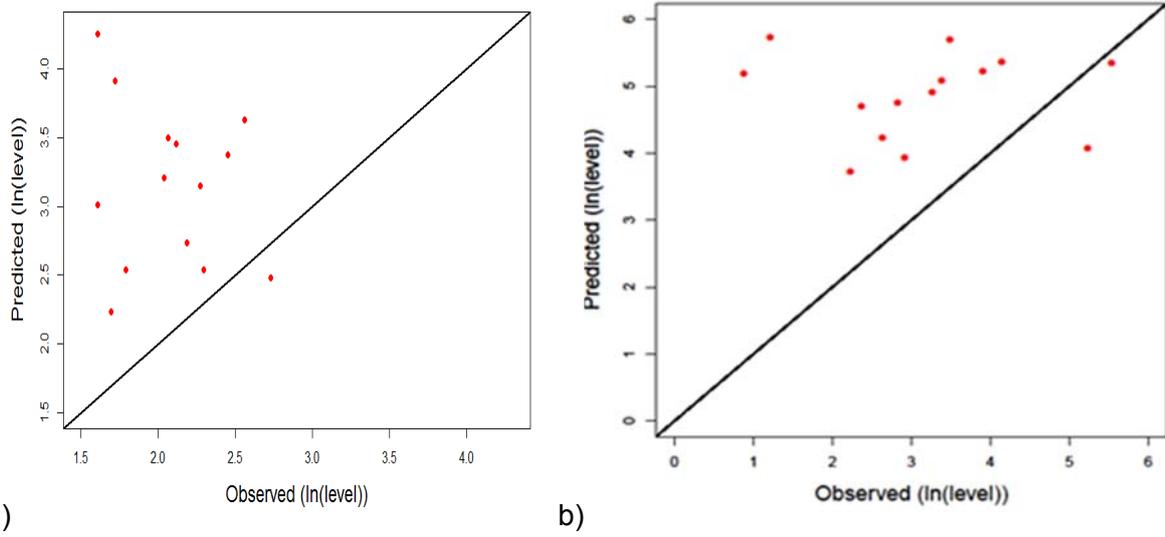


Figure 36. Goodness of fit validation for observed versus predicted regional deoxynivalenol contamination of grain using data collected in a) 2011 and b) 2012. For both plots the black line indicates a perfect fit.

Zearalenone

Four cluster analysis methods (complete linkage, average linkage, Ward’s algorithm and K-means) were used to analyse the ZON data between 2007 and 2010. Initial analyses evaluated each cluster method for matching contamination pattern and predictive ability, with greatest emphasis placed on the latter. From these analyses and the use of the ‘elbow criterion’ average linkage was superior to the other three methods. Using this method, a cluster map was produced with fourteen ZON contamination risk regions (Figure 37).

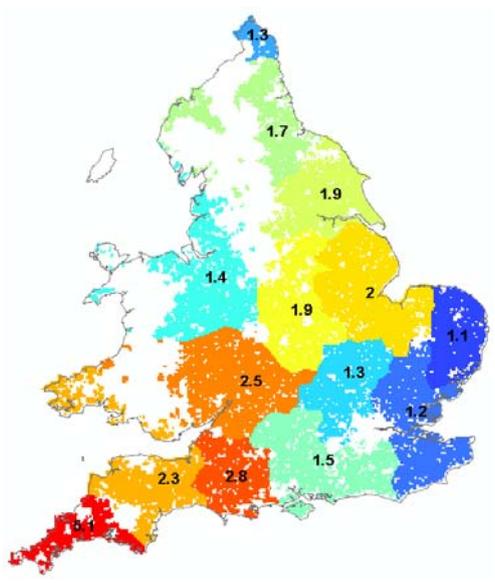


Figure 37. Cluster analyses, as produced average showing risk regions for zearalenone contamination of grain using data collected 2007 and 2010.

The window pane analyses for ZON contamination of grain produced 11 weather variables of interest (Table 14) with average maximum temperature (Tmax_ave), average mean relative humidity (RHmean_ave) and average rainfall (rain_ave) selected for correlation analyses (Figure 38). Stepwise-partial least square analysis, showed that for these weather parameters there were two main windows of significance for RHmean_ave (February and late August to early September) and one long window for rain_ave (March to early June) which influenced ZON contamination of grain. A diagrammatic representation of this is shown in Figure 39.

Table 14. Correlation range and number of significant correlations for weather parameters highlighted of potential interest in zearalenone contamination of grain.

Weather Variable	Correlation range	Number ($p < 0.001$)
Tmean_avg	-0.62 ~ 0.84	75
Tmin_avg	-0.59 ~ 0.74	16
Frost days (number)	-0.69 ~ 0.67	85
Tmax_avg	-0.70 ~ 0.83	126
Tmax_nod20	-0.68 ~ 0.64	76
RHMean_avg	-0.72 ~ 0.76	198
Rain_avg	-0.53 ~ 0.78	403
Rain_nod0	-0.67 ~ 0.55	57
Rain_nod1	-0.64 ~ 0.76	306
Rain_nod5	-0.43 ~ 0.72	197
Rain_nod9	-0.34 ~ 0.64	141

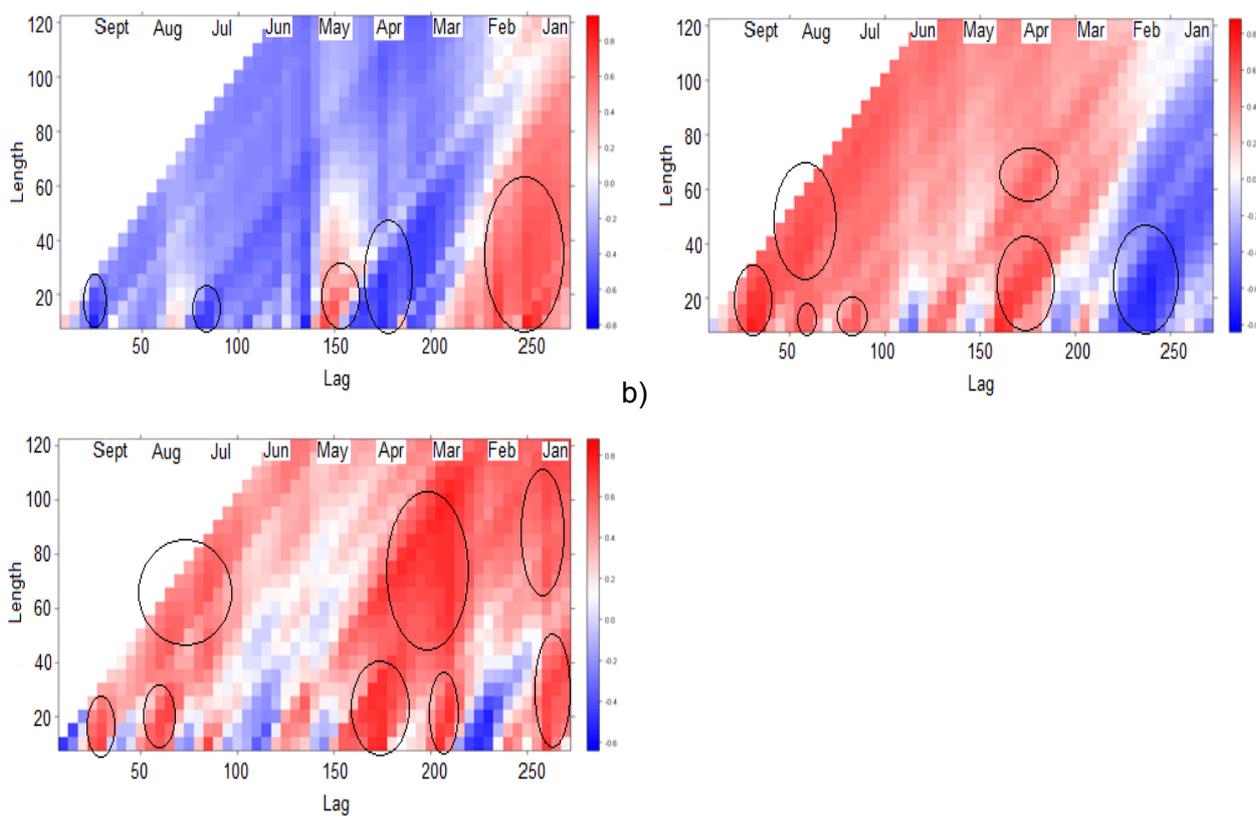


Figure 38. Correlation patterns with significant windows highlighted (circled) for zearalenone contamination of grain, a) average maximum temperature, b) average mean relative humidity and c) average rainfall.

The model produced for ZON differed from those produced for *F. graminearum* infection and DON contamination of grain as the criteria for inoculum development were not so well defined. ZON production is generally associated with delayed harvest usually resulting from wet weather just prior to harvest. The influence of the delayed harvest seems to be inferred in the model by the inclusion of increased relative humidity during late August and early September. There were weakly significant correlations for increased rainfall towards the end of August/beginning of September (Figure 38c) which may also indicate the influence of a delayed harvest however the stronger significance of relative humidity meant they were not included in the model.

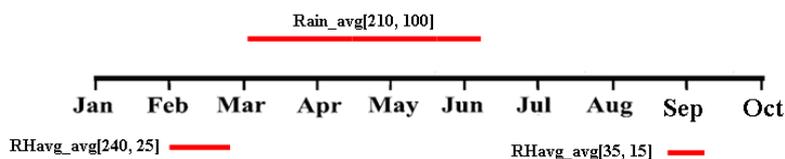


Figure 39. Diagrammatic representation of the weather parameters determined as important for zearalenone I contamination of grain.

A goodness of fit analysis for observed ZON contamination of grain compared to predicted estimates produced by the high dimensional regression model (Figure 40) indicated a good fit

across the whole range of observed data with an R^2 of 0.75 and suggested that prediction of ZON contamination at the regional level might be possible.

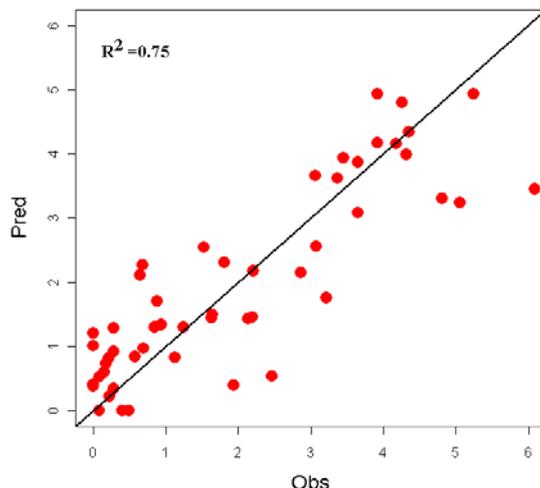


Figure 40. Regression analysis showing observed versus predicted regional incidence zearalenone contamination of grain as determined through High Dimensional Regression Modelling.

Output for the goodness of fit analysis to show the predictive power of the ZON model for 2011 and 2012 is presented in Figure 41. In 2011, levels of ZON were low and the model performed reasonably well with the predicted level of ZON ranging from 0 to 3 ppb (back transformed data) compared to between 0 and 1.5 ppb for the observed data. ZON values in 2012 contrasted with those in 2011, as levels of ZON contamination ranged from 3 to 250 ppb across the cluster regions (back transformed data). Generally, the model underestimated the level of ZON compared to the observed, in particular, at the higher ZON levels. In two regions the observed ZON concentrations was above the legislative limit of 100 ppb, however the level predicted was below the limit.

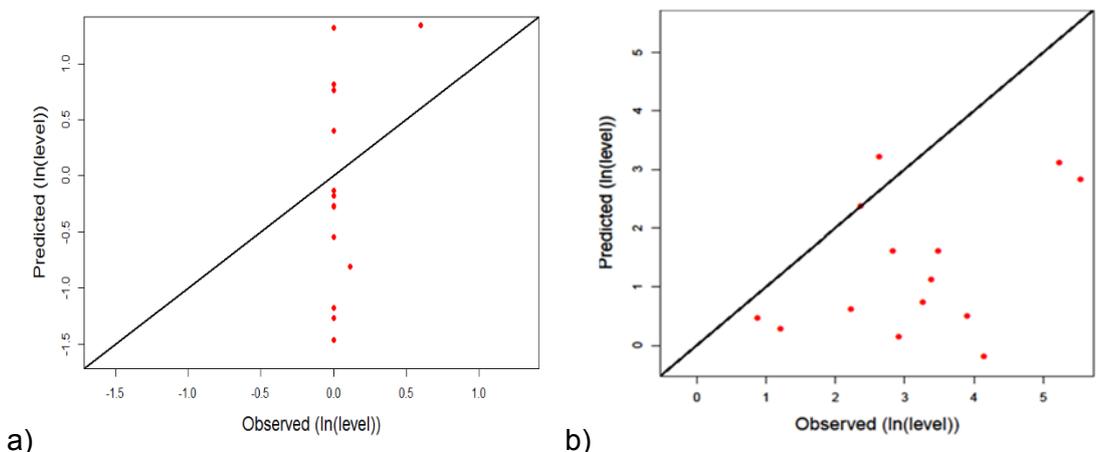


Figure 41. Goodness of fit validation for observed versus predicted regional zearalenone contamination of grain using data collected in a) 2011 and b) 2012. For both plots the black line indicates a perfect fit.

Site level

Deoxynivalenol

The window pane analysis for DON contamination at the site level produced four significant weather variables; average maximum temperature (Tmax_ave), average minimum temperature (Tmin_ave), average rainfall (rain_ave) and number of days with rainfall greater than 0 mm (Rain_nod0). The lag time and window for each of these variables is shown in Figure 42, the variables and timing present appeared to reflect conditions for *F. graminearum* inoculum development and infection but did not represent the conditions required for increased levels of DON contamination. Of the agronomic factors tested, only harvesting period had a significant effect on the level of the DON, with levels increasing as delays to harvest increased.

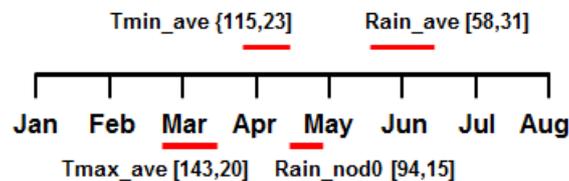


Figure 42. Diagrammatic representation of the weather parameters determined as important for deoxynivalenol contamination of grain.

The goodness of fit regression for observed DON contamination at the site level compared to predicted estimates (Figure 43) indicated the model did not produce a good fit for the data, with the model generally underestimating DON levels.

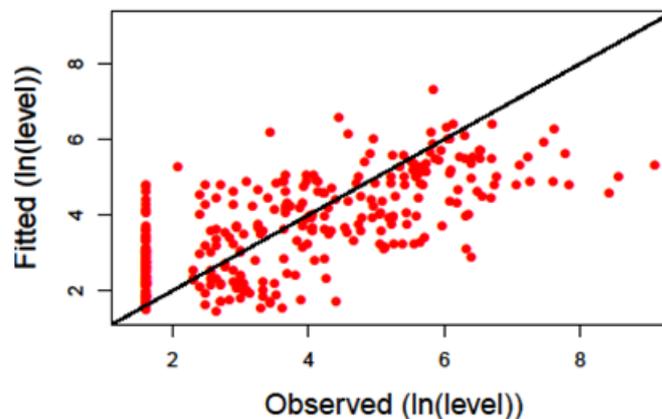


Figure 43. Goodness of fit for observed versus predicted site level deoxynivalenol contamination as determined by Stepwise Regression Modelling (black line indicates a perfect fit).

Validation of the model using DON contamination data collected in 2012 and 2013 (Figure 44) indicated a poor model fit. In 2012, the model overestimated at low DON contamination levels but underestimated at high levels. Six sites had DON contamination at or above the legislative limit of 1250 ppb (\ln_e value ~ 7.1). However, the model predicted the DON concentration for these to be

between 244 and 812 ppb. In 2013, the model underestimated DON contamination at all six sites. The highest level of contamination observed at the sites able to be used in the validation was 365 ppb (\ln_e value 5.9) however the predicted DON contamination for this site was 20 ppb (\ln_e value 3).

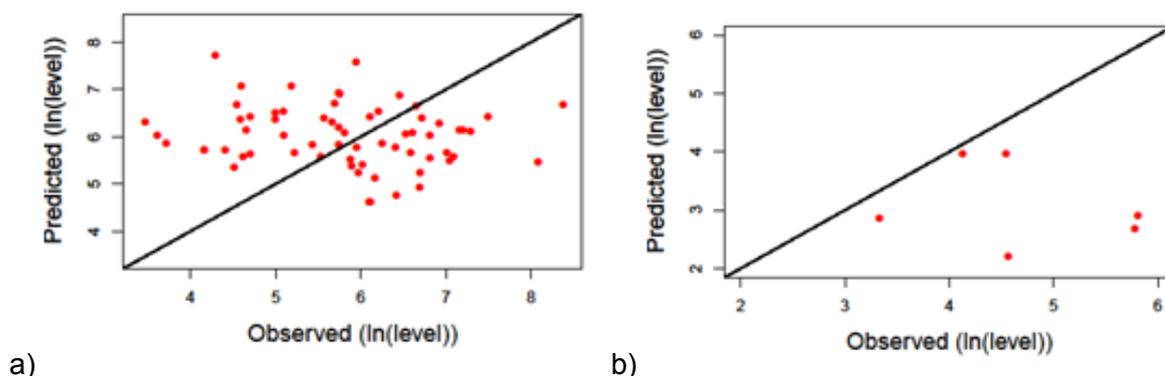


Figure 44. Goodness of fit validation for observed versus predicted site deoxynivalenol contamination of grain using data collected in a) 2012 and b) 2013. For both plots the black line indicates a perfect fit.

It was highlighted for the *F. graminearum* site level modelling that a large number of zeroes in a dataset can adversely affect the regression analysis. As the DON dataset also contained a large proportion of data below the level of detection (and so classed as zero) the analysis was repeated on a dataset with these values removed. Using these data the window pane analysis produced three significant weather variables; number of days with a maximum temperature above 20°C (Tmax_nod20), average minimum temperature (Tmin_ave) and number of days with rainfall (Rain_nod0); the lag time and window for each of these variables is shown in Figure 45. The variables and timing appeared to reflect conditions for *F. graminearum* inoculum development, infection and increase in DON contamination as the window for Rain_nod0 covered a 40 day period from early June to mid-July. As seen with the site level model for DON where zeroes were included, the harvesting period had a significant effect on the level of the DON, with levels increasing with later harvesting periods. This was more than likely due to rainfall just prior to harvest.

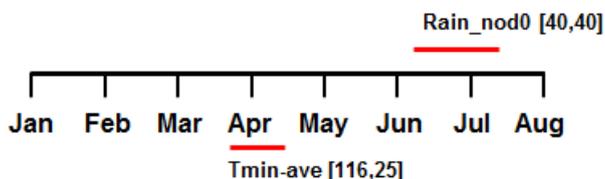


Figure 45. Diagrammatic representation of the weather parameters determined as important for deoxynivalenol contamination of grain where zeroes had been removed from the data set.

The goodness of fit regression (Figure 46) indicated the model produced on the data set with zeroes removed was no better than the fit for the model produced on the full data set. The validation using data collected in 2012 and 2013 showed a flat prediction pattern in both years (Figure 47) which confirmed the models lack of predictive power. In 2012 there were six sites where the observed DON contamination was at or above the legislative limit of 1250 ppb (\ln_e value ~ 7.1) however the model predicted the DON concentration for these to be between 244 and 665 ppb.

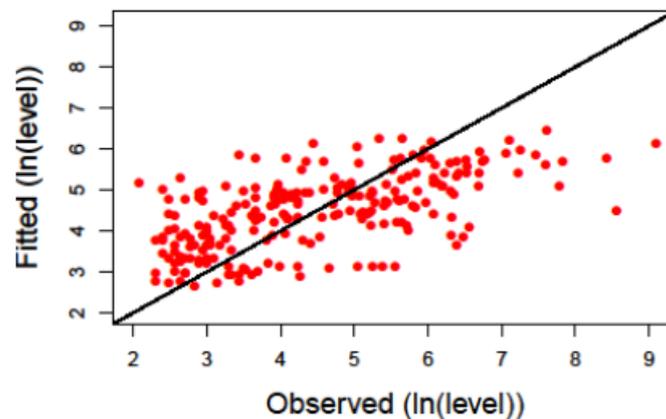


Figure 46. Goodness of fit for observed versus predicted site level deoxynivalenol contamination of grain as determined by Stepwise Regression Modelling based on data with the zeroes removed (black line indicates a perfect fit).

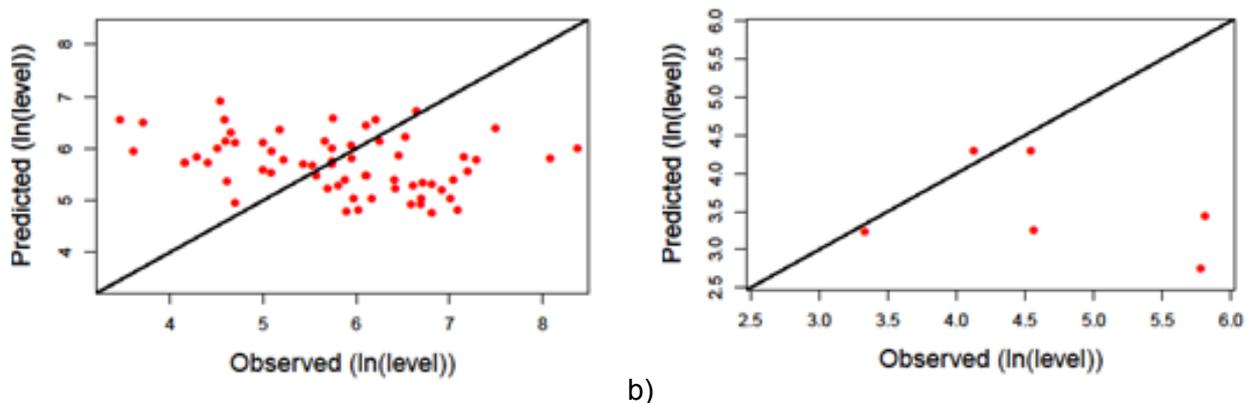


Figure 47. Goodness of fit validation for observed versus predicted site level deoxynivalenol contamination model (produced with zero values removed) using data collected in a) 2012 and b) 2013. For both plots the black line indicates a perfect fit.

Zearalenone

The window pane analysis for ZON contamination at the site level produced three significant weather variables; average maximum temperature (Tmax_ave), average minimum temperature (Tmin_ave) and number of days with rainfall greater than 0 mm (Rain_nod0). The lag time and window for each of these variables is represented in Figure 48. Of the agronomy variables tested,

delayed harvest was the only one which produced a significant effect, giving increased ZON levels when a delay occurred.

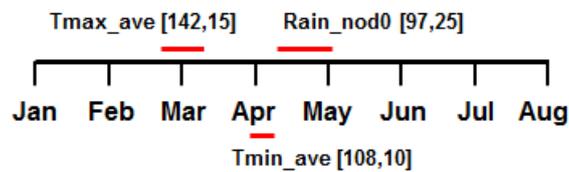


Figure 48. Diagrammatic representation of the weather parameters determined as important for zearalenone contamination of grain.

The goodness of fit regression for observed ZON contamination at the site level compared to predicted estimates (Figure 49) indicated the model did not produce a good fit for the data, with the model generally underestimating ZON levels.

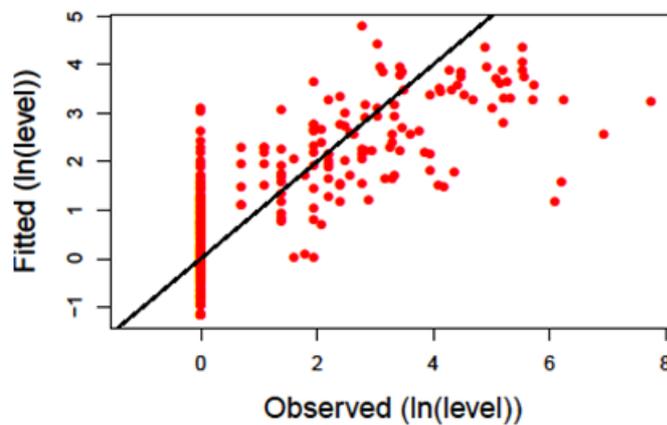
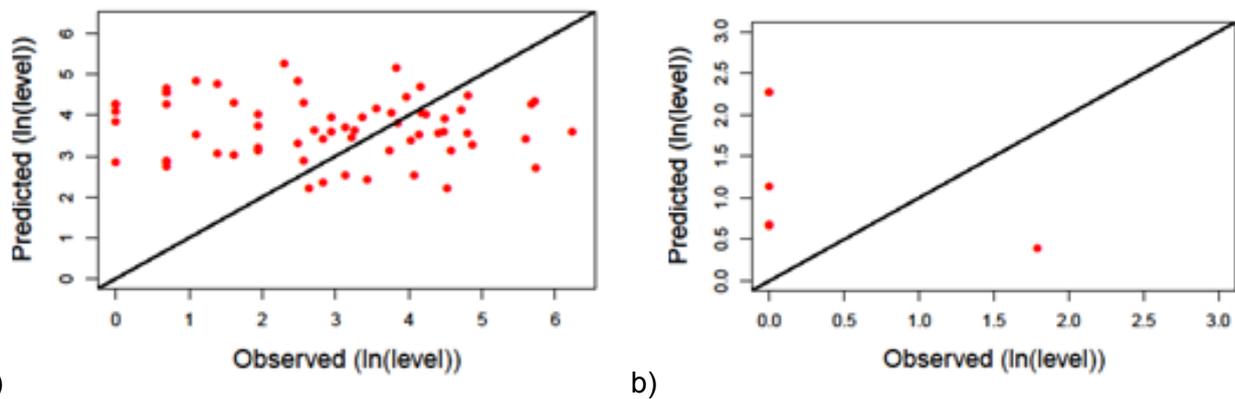


Figure 49. Goodness of fit for observed versus predicted site level zearalenone contamination of grain as determined by Stepwise Regression Modelling (black line indicates a perfect fit).

Validation of the model using ZON contamination data collected in 2012 and 2013 (Figure 50) indicated a poor model fit. In 2012, the model overestimated at the low levels of ZON contamination but underestimated at high levels. In some cases, the model predicted that the ZON content would be over the legislative limit of 100 ppb when the observed value was well below e.g. an observed value of 3 ppb ZON (\ln_e value 1) was predicted to be 148 ppb (\ln_e value 5). The reverse was also seen where the observed value was over the 100 ppb limit but the model predicted a value well below the limit e.g. an observed value of 299 ppb ZON (\ln_e value 5.7) was predicted to be 12 ppb (\ln_e value 2.5) by the model. In 2013, the model underestimated the level of ZON contamination at the only site where ZON was detected.



a) b) **Figure 50. Goodness of fit validation for observed versus predicted site zearalenone contamination of grain using data collected in a) 2012 and b) 2013. For both plots the black line indicates a perfect fit.**

4.5 Modelling fusarium mycotoxin risk – agronomy

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

For DON, the model accounted for 74% of the observed variance; 69% of variance was accounted for by year ($P < 0.01$) and region ($P < 0.01$) and their interaction ($P < 0.01$). For ZON, the model accounted for 69% of the observed variance; 61% of variance was accounted for by year ($P < 0.01$) and region ($P < 0.01$) and their interaction ($P < 0.01$). Consequently, the agronomic factors only accounted for an additional 5 and 8% of the variance for the DON and ZON model, respectively. Of this additional variance, the main difference was the harvest timing which accounted for an additional variance of 0.6% for DON and 5.3% for ZON

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, the dataset is highly unbalanced with low numbers of samples for some levels, these can be identified by the large confidence limits.

As can be seen in Figure 51, there was a large seasonal variation in predicted DON and ZON with high means experienced in 2008 and 2012 for DON and only high for ZON in 2008. There were

also regional differences and these fluctuated between seasons but there has been a consistent trend of higher risk DON in the East Midlands in recent years.

Cultivation was not a significant factor for DON ($p=0.071$) or ZON ($p=0.333$) but previous crop ($p<0.001$ and 0.004 , respectively) and the interaction of previous crop and cultivation ($p<0.001$ and 0.002 , respectively) were both significant for DON and ZON. The clear difference in the predicted means for previous crop and cultivation (Figure 52) shows that growing wheat after maize and min-till is a major risk factor. The predicted mean for this agronomy was nearly two-times the legal limit for DON and close to the legal limit for ZON. For all other previous crops, the risk is relatively low, although following sugar beet after ploughing or min-till or following grass after min-till had slightly greater predicted means compared to other previous crops.

Varieties present in more than ten samples were analysed individually and this did result in significant differences for DON and ZON ($p<0.001$ and $p=0.001$, respectively). The predicted means for each variety appear to bear little relation to the resistance rating for FHB recorded for the varieties based on AHDB RL trials (Figure 53). However, as this is an observational study the dataset is unbalanced with varieties only present within some years or regions of the survey. For example, KWS Santiago was only present in 2012 and 2013, which had a higher average DON compared to the full 8 year dataset. Also, other varieties such as Xi19 were preferred for late drilling which in itself may increase mycotoxin risk.

Figure 54 shows the impact of harvest week on the mycotoxin content of harvested wheat. The average harvest day was calculated based on the six year average for each county. The harvest day of each sample was then calculated relative to the long term average, this was then categorised into weeks with a minus score for early harvests and positive score for late harvests. The figure shows that there is a slight increase in risk as harvest moves from early to average timing but then the risk increases exponentially as harvests are delayed. The increase in risk is greater for ZON with a 25-fold increase in the predicted mean at week 4 compared to week 0. This compares to a 10-fold increase in the predicted mean for DON over the same time period.

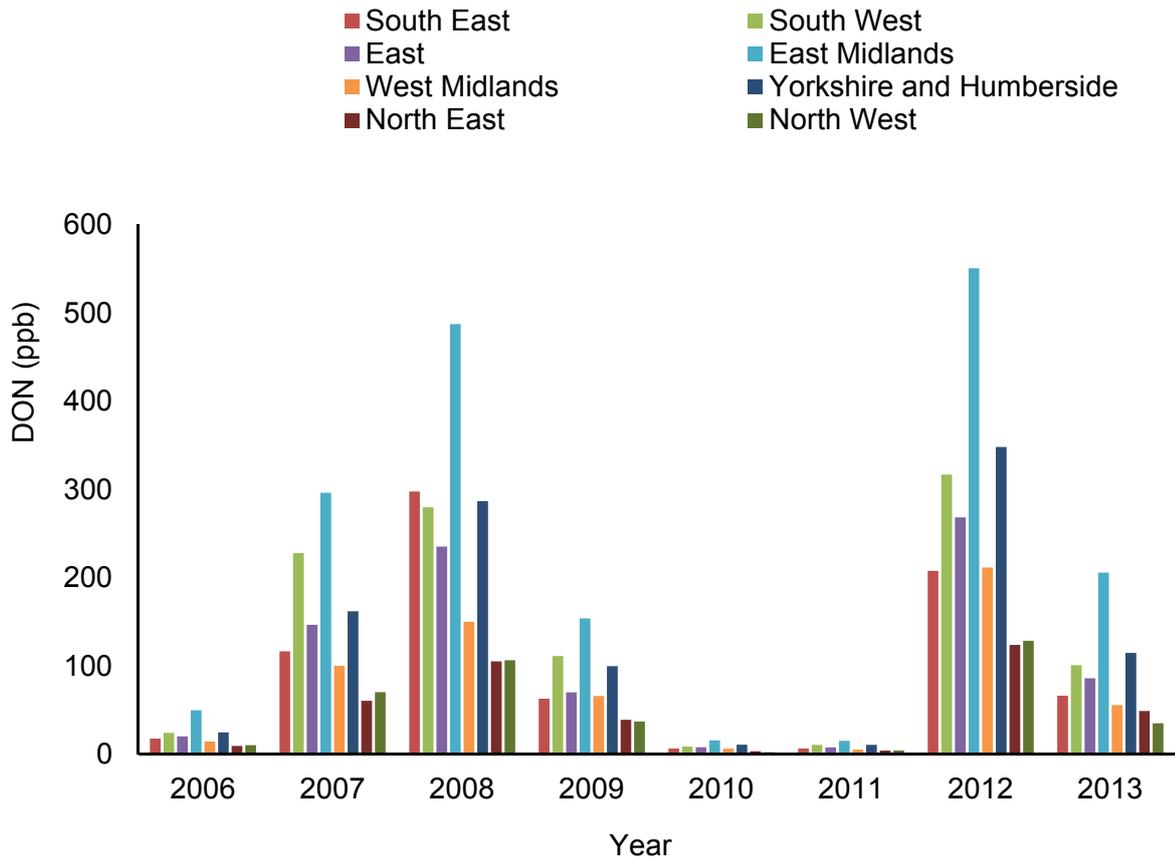
Crop debris management, i.e. the baling and removal of straw, compared to incorporation had no significant effect on DON in the subsequent wheat crop. This occurred even when analysed as an interaction with previous crop and cultivation. Based on the known importance of crop debris within the *Fusarium* lifecycle one could expect that straw removal for some previous crops could result in a reduction in inoculum, and this would interact with method of cultivation. However, this was not identified as significant within the DON or ZON models.

There was no significant difference in the DON or ZON content of wheat crops which received different fungicide regimes. Seed treatment was analysed based on the product used. There was no difference identified, although this may be because very few wheat samples came from crops with no seed treatment applied and most single purpose dressings have good activity towards *Fusarium*.

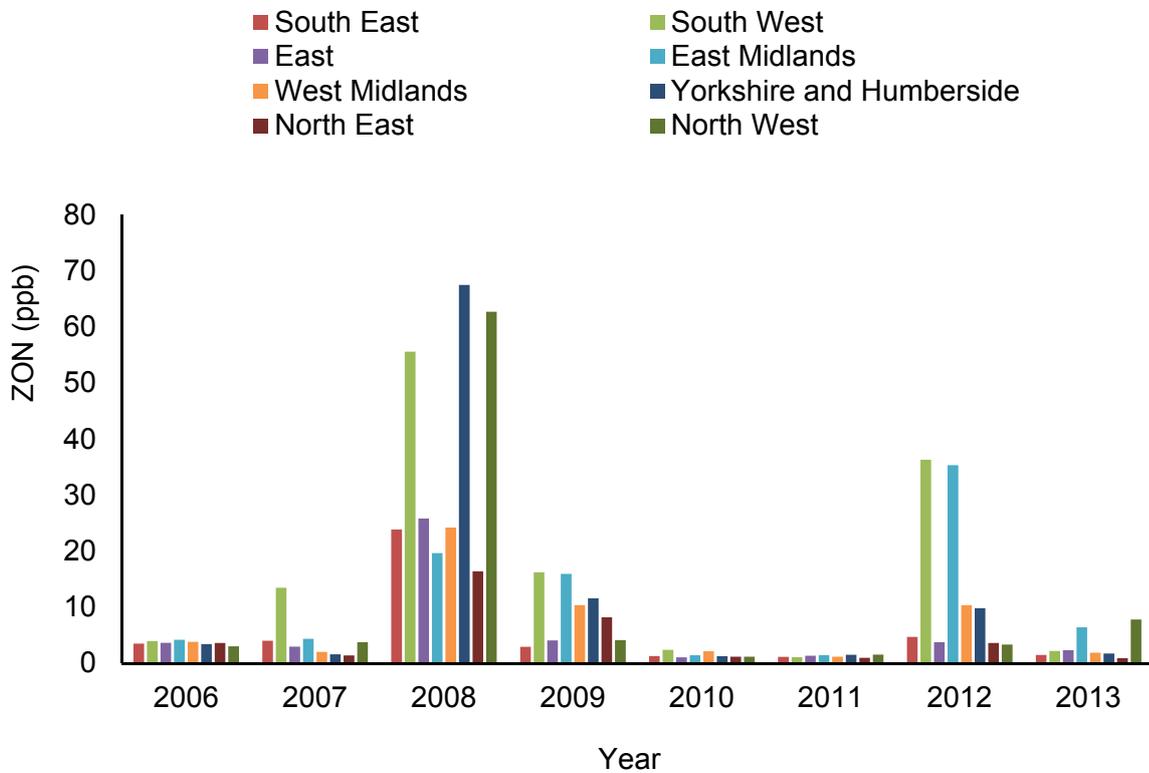
T3 treatment (fungicide application at flowering, GS59 – 61) was analysed based on:

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

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

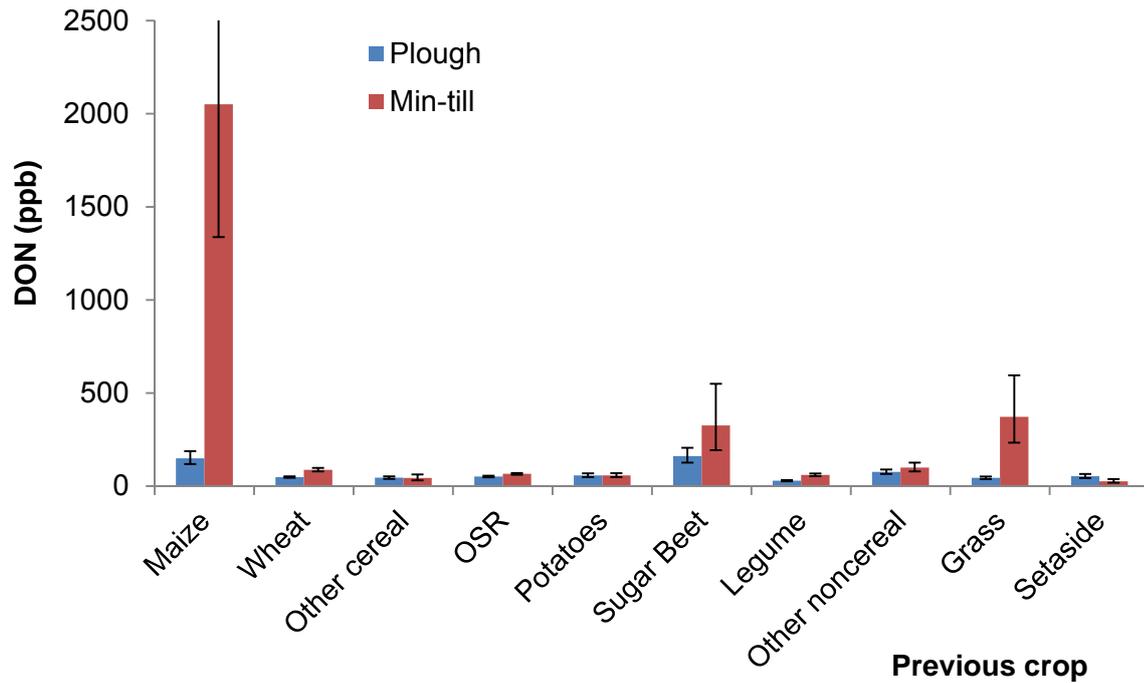


a)

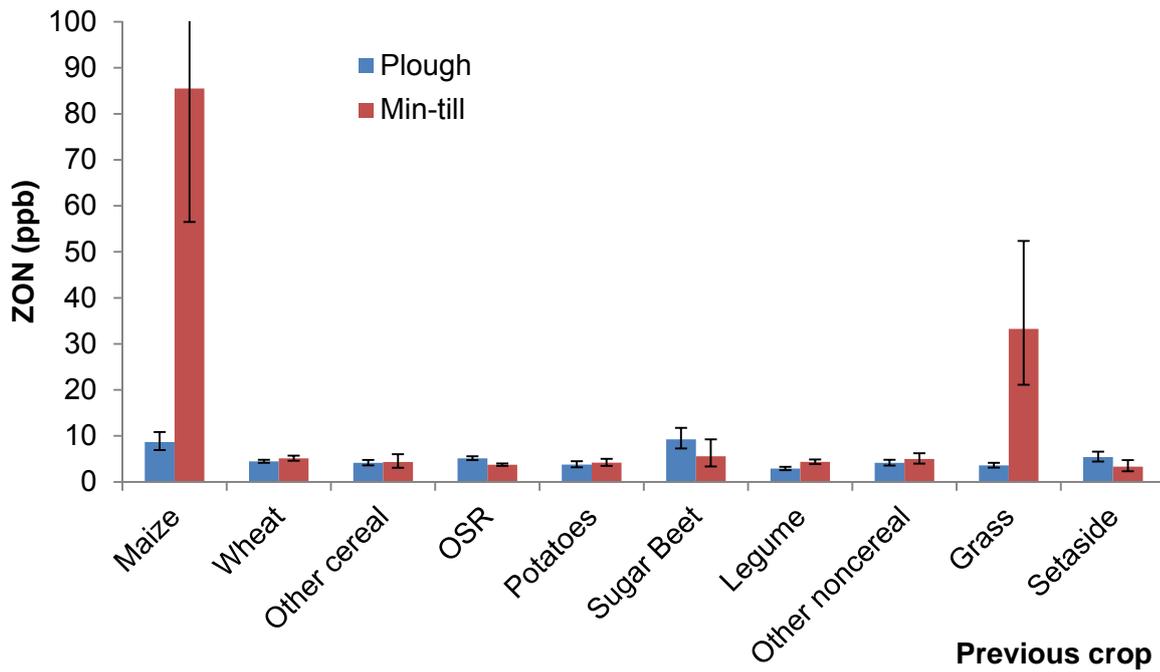


b)

Figure 51. a) DON and b) ZON predicted mean concentration wheat by region for each year.

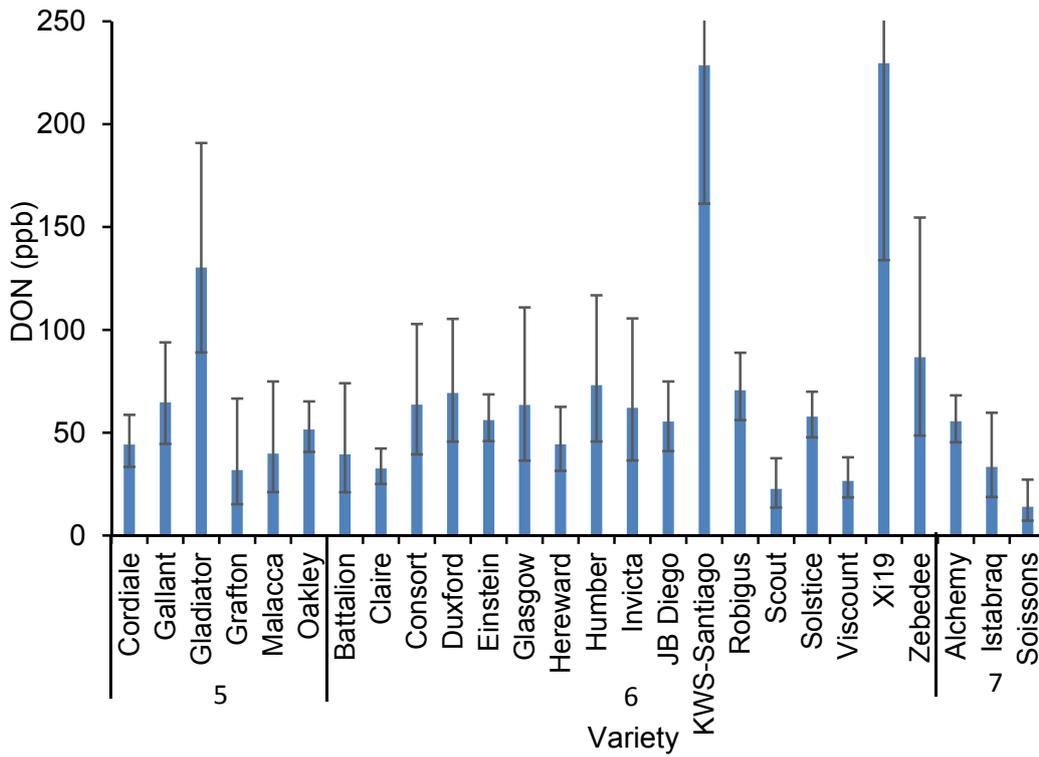


a)

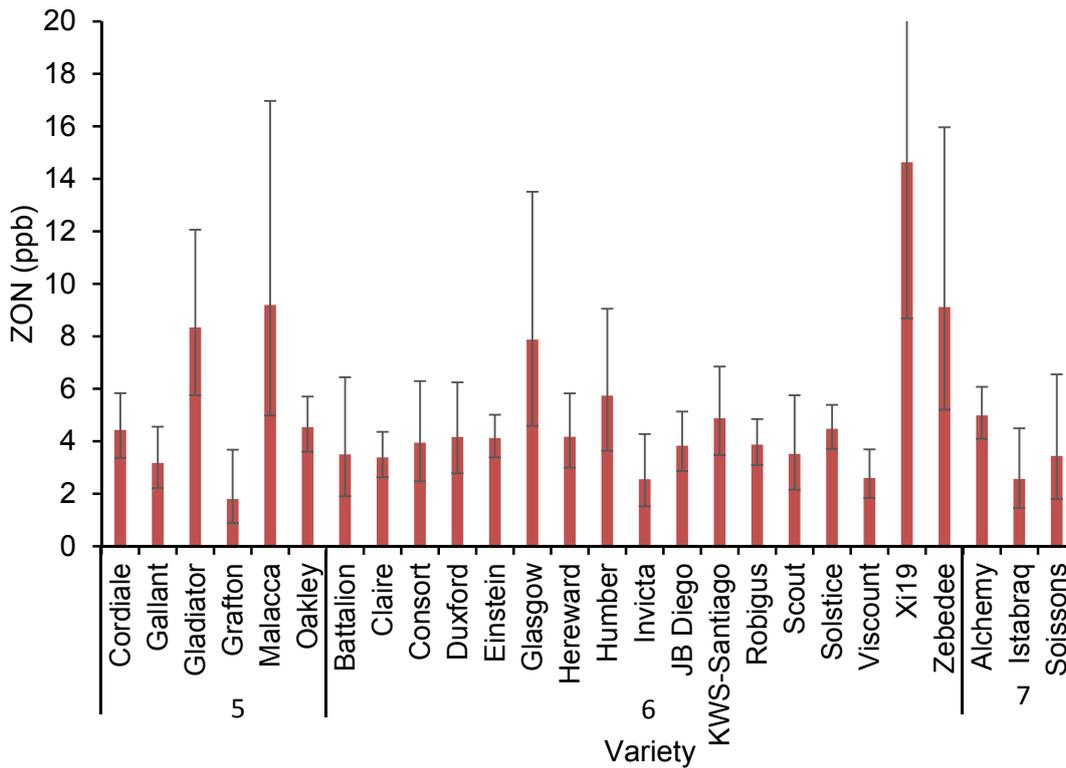


b)

Figure 52. a) DON and b) ZON predicted mean concentration of wheat after different previous crop and cultivations. Bars represent 95% confidence limits for predictions.

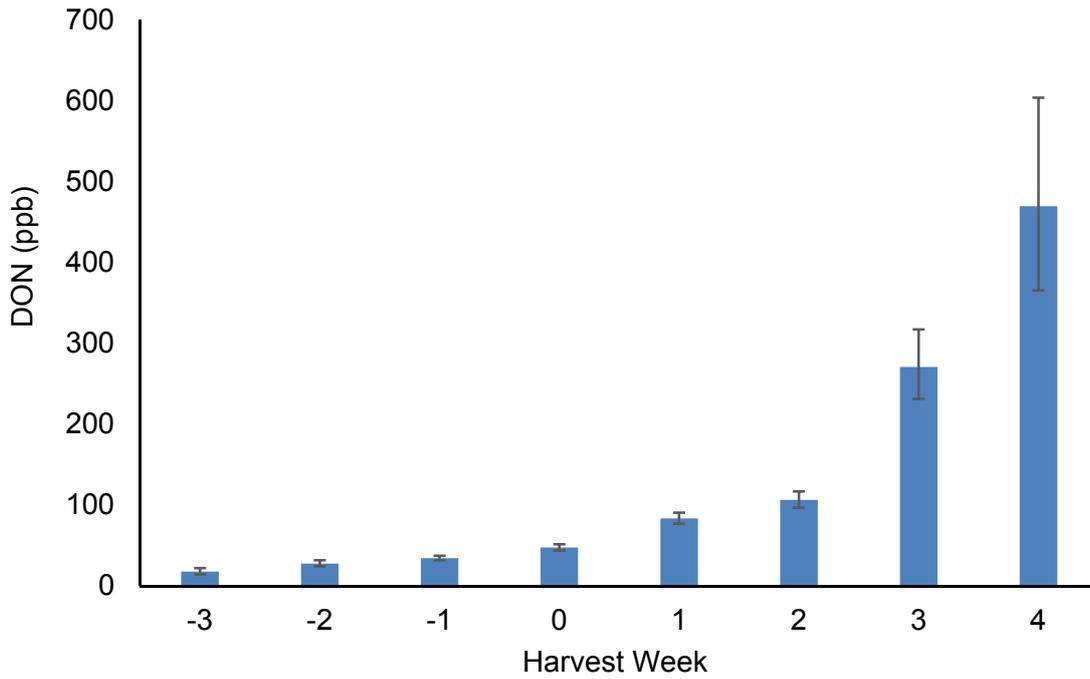


a)

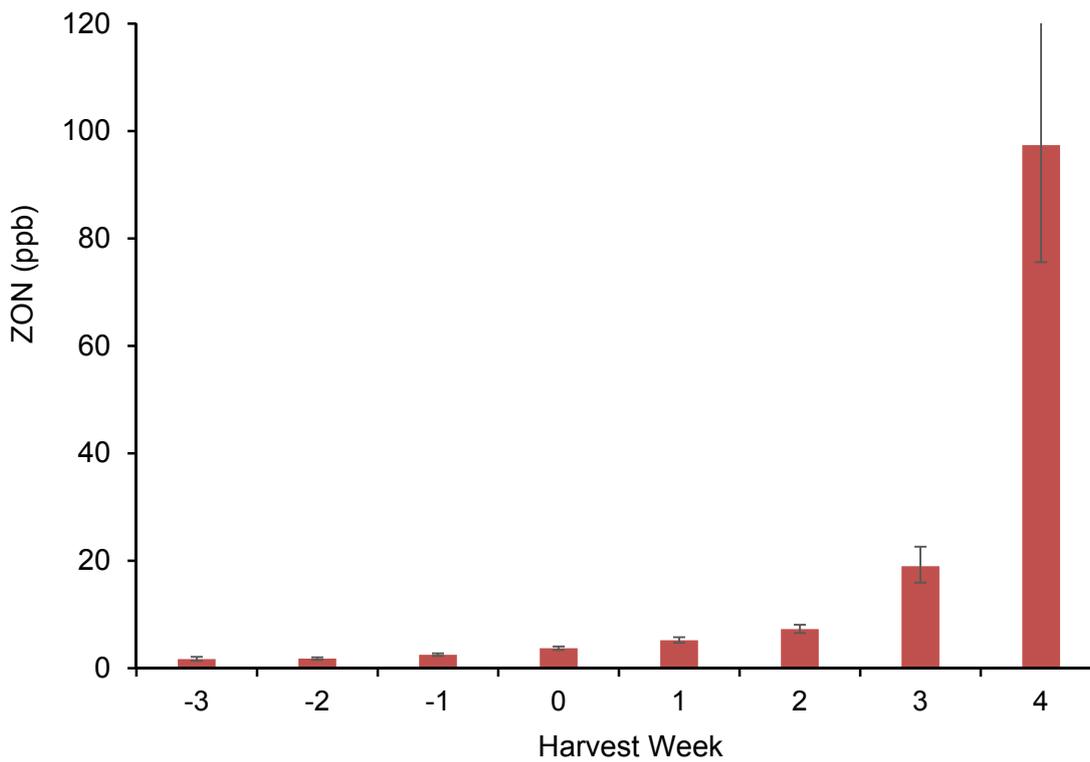


b)

Figure 53. a) DON and b) ZON predicted mean concentration of wheat samples grouped by variety. Bars represent 95% confidence limits for predictions. Numbers in boxes indicate FHB resistance scores.



a)



b)

Figure 54. a) DON and b) ZON predicted mean concentration of wheat samples grouped by harvest week. Week zero represent the long term average harvest date. Minus weeks are early harvests, plus weeks are late harvests. Bars represent 95% confidence limits for predictions.

4.6 Modelling fusarium mycotoxin risk – pathogen incidence data

As the FHB species incidence data is based on samples collected at early milky ripe (GS73-75), this data is only available near harvest time and as such is suitable for a harvest prediction of risk.

4.6.1 National Risk

For the national risk, the percentage of samples exceeding legal limits for DON and ZON were analysed using general linearized models using the national Fusarium Head Blight pathogen incidence data generated from the Defra funded winter wheat disease survey and the English average monthly rainfall data were used. The best model for both DON and ZON were generated from the variables *F. graminearum* incidence, *F. culmorum* incidence and harvest rainfall. Harvest rainfall is a weighted calculation based on the August and September rainfall (August rainfall + ((August rainfall – 50)*September rainfall*2)). This formula weights the impact of September rainfall according to the level of August rainfall. The greater August rainfall is above 50 mm then the greater the impact of September rainfall will have on national harvest delays. This is a crude assessment of harvest rainfall as it does not take into account when during the harvest period the rainfall occurs or the period of time over which the rainfall occurs.

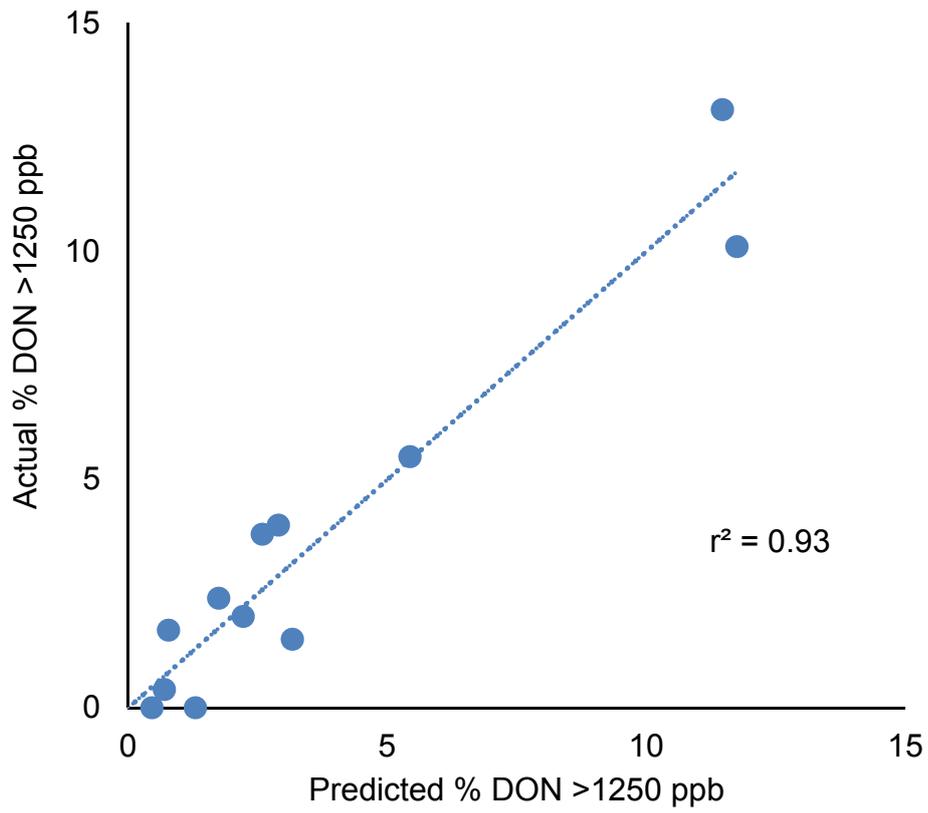
Linear equations for percentage of samples exceeding legal limits for DON and ZON generated from the national *F. graminearum* incidence, *F. culmorum* incidence and harvest rainfall are presented below:

$$\% \text{DON} > 1250 \text{ ppb} = -0.95 + 0.385F_g - 0.573F_c + 0.0177 \text{Harvest rainfall}$$

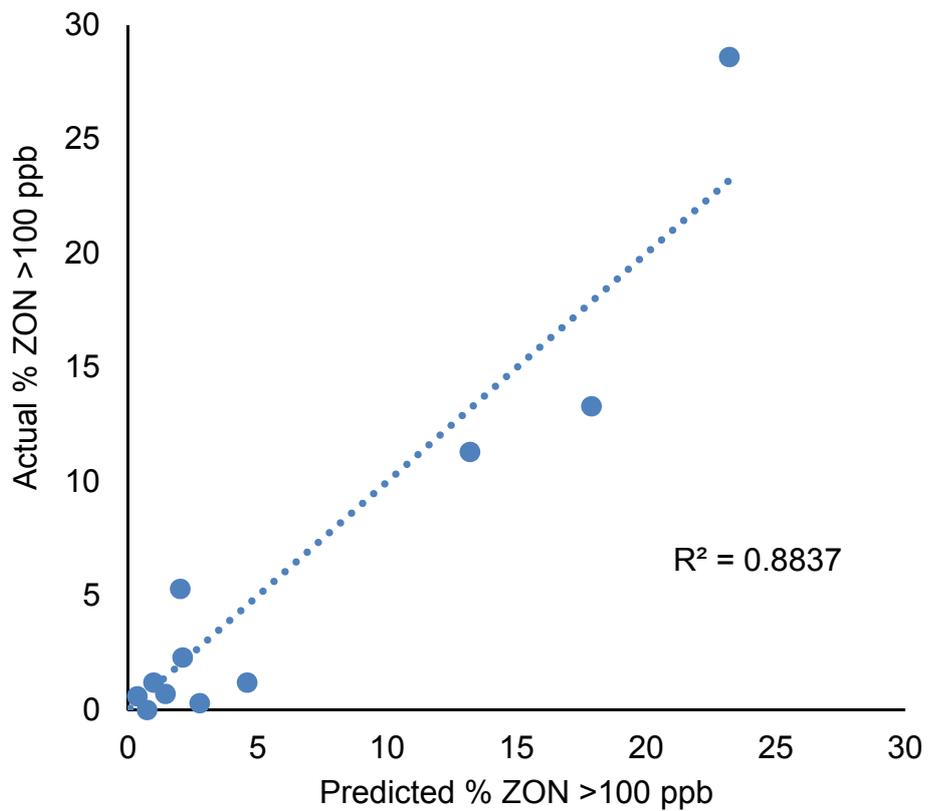
$$\% \text{ZON} > 100 \text{ ppb} = -2.6 + 0.728F_g - 1.55 F_c + 0.0494 \text{Harvest rainfall}$$

The DON model was highly significant ($p < 0.001$) and accounted for 93% of the variance indicating the model was a good fit for the percentage of samples exceeding the legal limit of 1250 ppb (Fig 55a). Due to the small number of values (13) it was decided to use all years within the model development and use the Prediction Error Sum of Squares (PRESS) statistic to validate the model. This calculation assesses the predictive ability of the model to new observations by calculating the predicted missing value using each possible n-1 dataset. The predicted r^2 for the DON model was 0.89 which was close to the actual r^2 of 0.93 indicating the model may be a good predictor of new observations.

The same calculation were performed for ZON with a similar linear regression fitted (Fig 55b). However the predicted r^2 was only 0.58 compared to the r^2 from the model of 0.88 indicating that this relationship is not as stable over different seasons and as such the model may not be a good indicator of new observations.



(a)



(b)

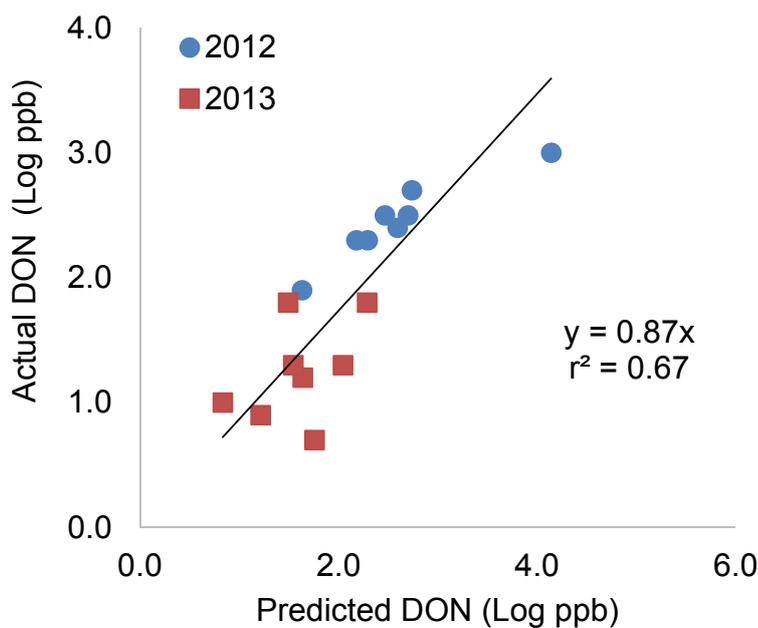
Figure 55. Plotted linear regression of the predicted versus the actual percentage of samples exceeding the legal limit of 1250 ppb for DON (a) and the legal limit for of 100 ppb for ZON (b) each year at the national scale.

4.6.2 Regional Risk

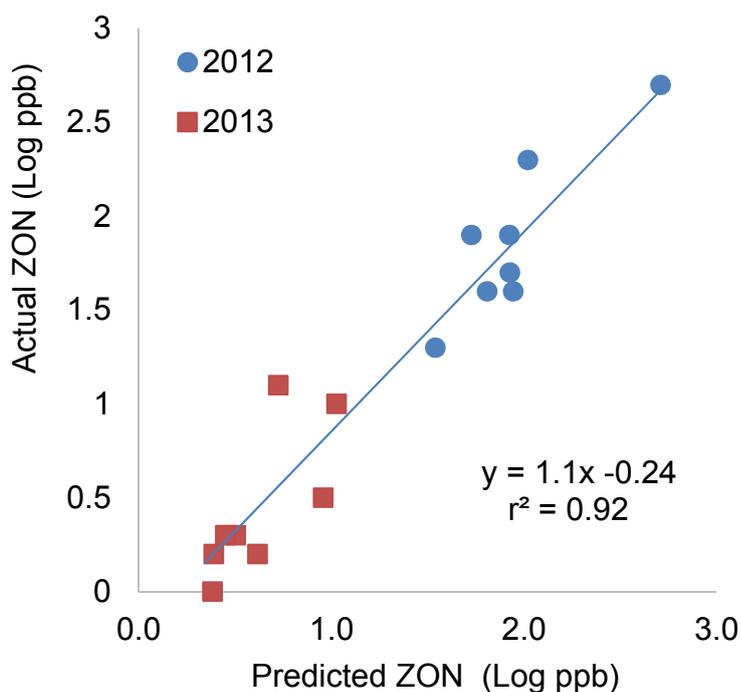
As for national risk, a similar methodology was performed for regional risk using the FHB incidence data for each Fera region and the nearest appropriate regional rainfall data. As the regional dataset had a low number of samples within each region for each year there were a large number of zero values for the percentage of samples that exceeded legal limits. Such data is unsuitable for linear models. For this reason the log₁₀ transformed mean values for DON and ZON were modelled against the percentage incidence of FHB pathogens and the regional rainfall data. The model was conducted on the 2001 to 2011 dataset and validated against the 2012 and 2013 dataset. It was identified that for DON the significant variables to include in the model was just *F. graminearum*; no other variable was significant. For ZON the significant variables to include in the model were *F. graminearum*, *M. nivale* and harvest rainfall. Both models were very highly significant ($p < 0.001$). The formulae and r^2 for DON and ZON regional models were:

DON:	$\text{LogDON} = 1.35 + (0.029Fg)$	$r^2 = 0.35$
ZON:	$\text{LogZON} = 0.127 + (0.016Fg) + (0.011Mic) + (0.0013HarvestRainfall)$	$r^2 = 0.41$

The low r^2 (< 0.5) indicated that although the models were statistically significant there was not a close relationship between the variables within the models and the mean mycotoxin concentrations. Fig 56 shows the predicted versus actual plot for the log₁₀ transformed mean DON and ZON concentration for each region in 2012 and 2013. As the figure shows the fit for DON (Fig 56a) was statistically significant ($p = 0.002$) but not particularly good with an r^2 of 0.67. The data is actually better fitted by two non-parallel lines for each year indicating that some seasonal variation is still not accounted for within the model. The fit was better for the ZON data with an $r^2 = 0.92$. For this data set, there was a slightly better model by fitting parallel lines for each year although this only accounted for an additional 2% of the variance ($r^2 = 0.94$) and hence there was negligible difference between the model fit between the two validation years and data points from both years fit a single regression line reasonably well (Fig 56b).



(a)



(b)

Figure 56. Plotted linear regression of the predicted versus the actual log₁₀ transformed mean DON (a) and ZON (b) concentration for each region in 2012 and 2013.

4.6.3 Site Risk

For the site risk, national, regional and individual site data for FHB species incidence generated from the Defra winter wheat disease survey were tested for significant effects with site agronomy data. Models were constructed based on the 2006-2011 (6 year) dataset and then validated using

the 2012 and 2013 dataset the best model included the national average % incidence for *Fusarium graminearum*, *F. culmorum* and *Microdochium* spp.; it also included the national average in-field % incidence of *F. graminearum*. The significant agronomy factors included in the model were previous crop and cultivation interaction and harvest timing. The regional and site pathogen data had no significant impact on the model once the national data and the geographic regions were already included in the model.

The DON model accounted for 67% of the variance with *F. graminearum* % national incidence accounting for 56% of the variance. This indicates that the *F. graminearum* % national incidence is a good indicator of the DON seasonal risk for any given year and this can be identified pre-harvest.

The ZON model had the same parameters as the DON model and accounted for 54% of the variance, however for ZON, the *F. graminearum* % national incidence only accounted for 28% of the variance. This indicates that the *F. graminearum* % national incidence alone is a not such a good indicator of the ZON seasonal risk for any given year.

The predictive ability of the DON and ZON models were validated by predicting the DON and ZON content of samples harvest in 2012 and 2013. Figure 57 and 58 show plots of the predicted DON and ZON values against the actual DON and ZON values (log log plot). The legislative limit is highlighted to indicate the samples that were correctly predicted to be above or below the legislative limit (shaded green) and the samples predicted to be below the limit but were actually above the limit (false negatives, shaded red) and samples that were predicted to be above the limit but were actually below the legal limit (false positives, shaded orange). As can be seen from the plot there is a good correlation between the predicted and actual DON content with a regression line with a gradient close to one ($y = 0.96x$; $r^2 = 0.48$).

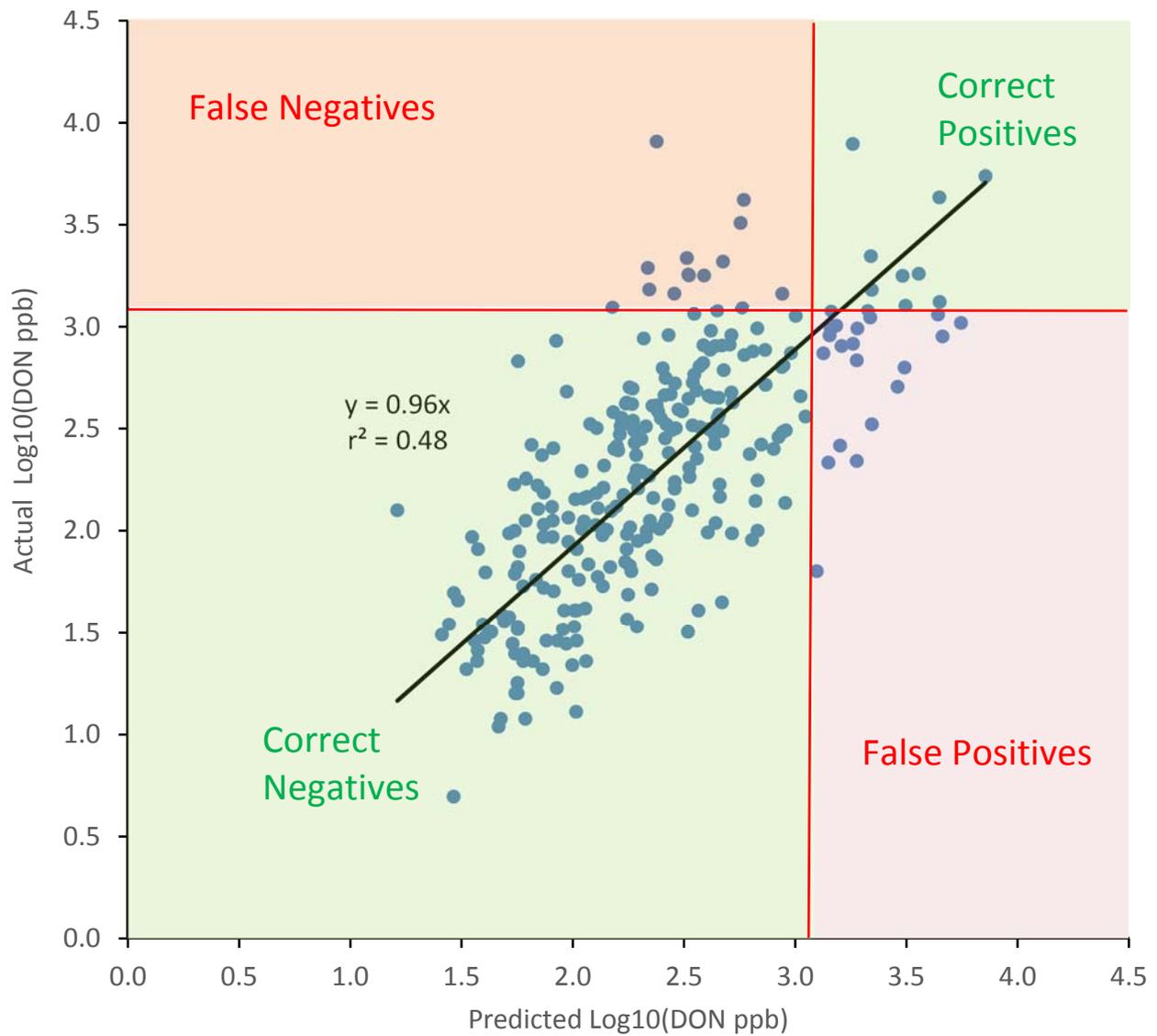


Figure 57. Log plot of actual DON concentration of samples from 2012 and 2013 against predicted DON content based on the model generated from the 2006-2011 dataset.

Figure 58 shows the ZON model is a poorer fit of the 2012 and 2013 validation samples compared to the DON model. There are a number of samples predicted to have a wide range of ZON which actually had undetectable concentrations (samples on the x-axis). The regression line shows a poor fit with an r^2 of 0.32 and a gradient greater than one. This prediction resulted in a high number of false negative samples compared to a single false positive sample.

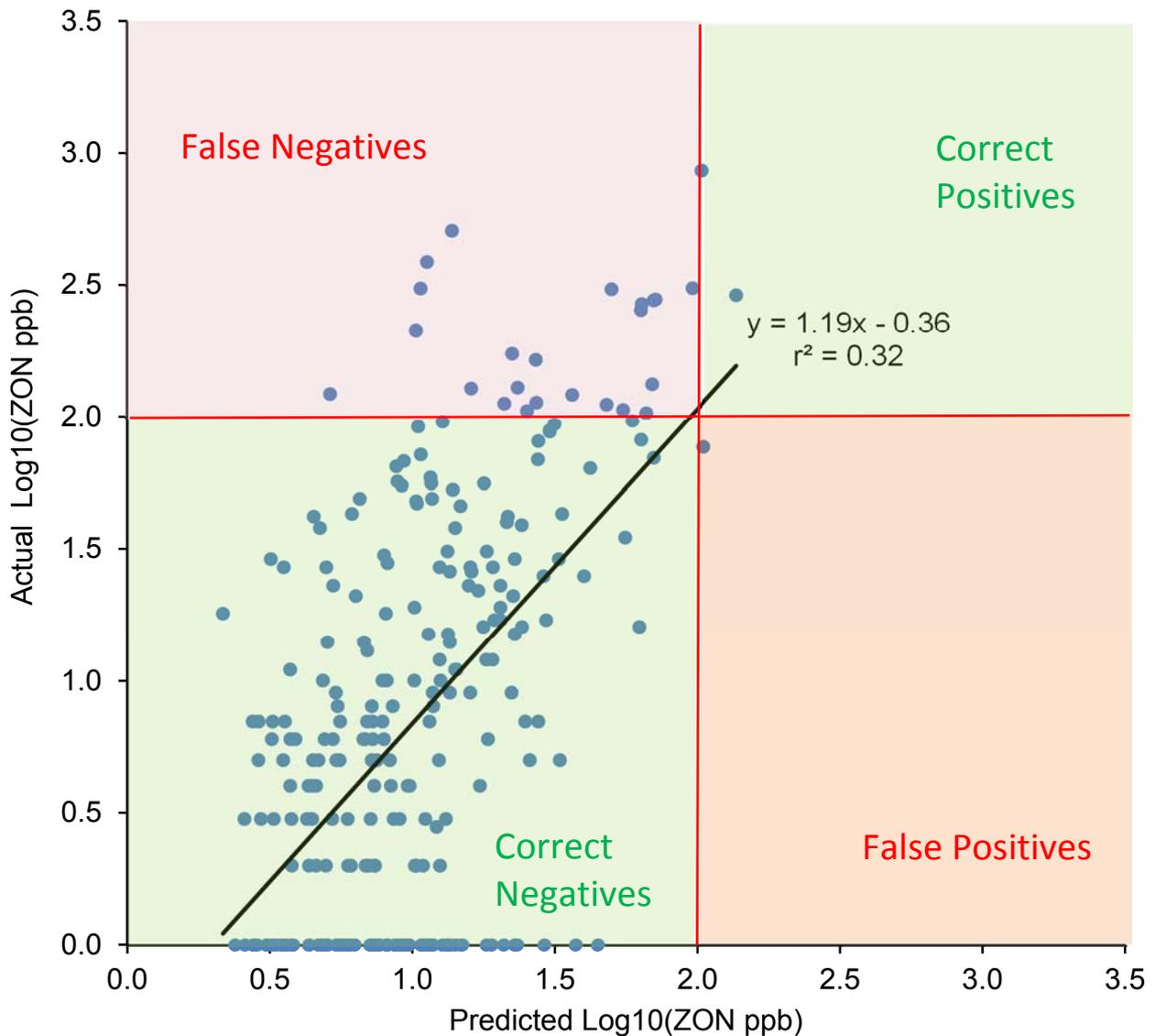


Figure 58. Log plot of actual ZON concentration of samples from 2012 and 2013 against predicted ZON content based on the model generated from the 2006-2011 dataset.

The matrix in Table 15a shows that the DON prediction model correctly predicted 88% of samples as above or below the threshold but had 7% of false positives and 5% of false negatives. False negatives and false positives for the prediction of mycotoxin exceedances at harvest have different levels of importance and as such can be weighted differently. A consignment of wheat deemed positive by the prediction model would require a sample to be collected and tested for mycotoxins, if the consignment was found to be actually below the legal limit, then it would be a false positive and could enter the human food chain having incurred the additional cost of a test. If a consignment of wheat was deemed negative by the prediction model it would not require a mycotoxin test and would enter the food chain. If it was subsequently found to be above the legal limit then the consignment was a false negative and considerable costs may be incurred as well as

a potential risk to human health. As false negatives have a greater consequence for the industry (consignments of wheat exceeding legal limits entering the food chain) then the probability of exceeding the legal limit can be calculated and this can be used to determine a lower risk threshold for predicting false negatives. As examples, the 10% and 5% probability thresholds are presented. If a threshold of 10% is set, then a sample is deemed to be below the legal limit if the probability that the actual concentration will exceed 1250 ppb DON is below 10%. When we do this, the number of correct predictions is reduced to 66% and the false positives is increased to 32% but the number of false positives is reduced to only 1%. If the threshold is lowered to a 5% probability then the number of correct predictions is reduced to 55% and the false positives is increased to 46% but there are no false positives. In this scenario, 54% of samples would need to be tested to find the 8% that exceeded the legal limit of 1250 ppb DON but no samples that were not tested would have exceeded the limit. Such a prediction system would approximately halve the number of tests that were required by the industry compared to sampling every consignment of wheat. The testing would not be required in equal numbers in each year with low risk years requiring limited testing and higher risk years close to all samples would require testing.

Table 15. Matrix of predicted against actual DON concentration based on the legislative limit of 1250 ppb. a) Equal weighting (50% probability); b) weighted against false negatives (10% probability); c) weighted against false negatives (5% probability). Correct predictions are shown in green, false positives in orange and false negatives in red.

50% Probability		Actual		
		<1250	>1250	
Predicted	<1250	85	5	89
	>1250	7	3	11
		92	8	100

a)

10% Probability		Actual		
		<1250	>1250	
Predicted	<1250	60	1	61
	>1250	32	6	39
		92	8	100

b)

5% Probability		Actual		
		<1250	>1250	
Predicted	<1250	47	0	47
	>1250	46	8	53
		92	8	100

c)

For the ZON prediction model the percentage of correct predictions is similar to the DON prediction (92%) with fewer false positives (0.4%) but more false negatives (8%) (Table 16). However, by reducing the probability of exceeding the ZON limit of 100 ppb to either 10 or 5% causes a small increase in false positives and only reduces the number of false negatives to 3 and 2%, respectively. As a result of the poorer prediction ability of the ZON model the DON model was assessed for its ability to identify samples that exceed either the legislative limit for DON and/or ZON. This data is presented in Table 17. The data shows that the DON model is also good at predicting the exceedance of either the DON or ZON legislative limits with 87% correct predictions and 5% false positives and 7% false negatives. When the probability of exceeding 1250 ppb DON is reduced to 10% the correct predictions drop to 71%, the false positives increase to 28% and the false negatives drop to 2%. When the probability of exceeding 1250 ppb DON is reduced to 5% the correct predictions drop to 58%, the false positives increase to 41% and the false negatives drop to 0.4%. So within the 2012 and 2013 validation years, of the 275 samples analysed, one sample exceeded the legal limit for ZON when the model predicted it would not at the 5% probability level.

Table 16. Matrix of predicted against actual ZON concentration based on the legislative limit of 100 ppb. a) Equal weighting (50% probability); b) weighted against false negatives (10% probability); c) weighted against false negatives (5% probability). Correct predictions are shown in green, false positives in orange and false negatives in red.

50% Probability		Actual		
		<100	>100	
Predicted	<100	91	8	99
	>100	0.4	0.7	1
		91	9	100

a)

10% Probability		Actual		
		<100	>100	
Predicted	<100	79	3	82
	>100	12	7	18
		91	9	100

b)

5% Probability		Actual		
		<100	>100	
Predicted	<100	69	2	71
	>100	22	7	29
		91	9	100

c)

Table 17. Matrix of predicted against actual exceedances of the DON and/or ZON legislative limits (1250 and 100 ppb respectively) based on the probability of exceeding 1250 ppb DON. a) Equal weighting (50% probability); b) weighted against false negatives (10% probability); c) weighted against false negatives (5% probability). Correct predictions are shown in green, false positives in orange and false negatives in red.

50% Probability		Actual		
		<LL	>LL	
Predicted	<LL	82	7	89
	>LL	5	5	11
		87	13	100

a)

10% Probability		Actual		
		<LL	>LL	
Predicted	<LL	60	2	61
	>LL	28	11	39
		87	13	100

b)

5% Probability		Actual		
		<LL	>LL	
Predicted	<LL	46	0.4	47
	>LL	41	12	53
		87	13	100

c)

As the risk from fusarium mycotoxins is highly seasonal, the number of consignments that require testing would vary. It may be simpler and safer to set a threshold for the number of samples that required testing so that in a season when the system predicted a high percentage of samples would require testing that all samples were tested, so a safer system of “test all samples” approach was applied in high risk years.

The prediction models were re-run using the full dataset (2006-2013) and validated using the PRESS statistic. This calculates the predictive ability of a model generated using n-1 dataset to predict the concentration of the missing dataset. The closer the predicted r^2 is to the actual r^2 is an indicator that the model may not be a bad prediction model. PRESS analysis was performed to test the predictive ability of the DON and ZON model. For the DON model the predicted r^2 was 0.64 compared to the r^2 of 0.67. As the predicted r^2 is only slightly lower than the actual r^2 , this indicates that the model may be a good predictor of future events. For the ZON model the predicted r^2 was 0.48 compared to the r^2 of 0.58. As the predicted r^2 is markedly lower than the actual r^2 , this indicates that the model may be a poor predictor of future events.

5 Discussion

Differences in frequency of isolations of *F. culmorum*, *F. graminearum* and the *Microdochium* species from each of the leaf layers were seen throughout the three years of monitoring. Generally, *F. culmorum* was isolated throughout the season whereas *F. graminearum* and the *Microdochium* species were rarely isolated at the start of the season with the first detection on leaf layers generally occurring between GS55 and 65. The difference in timing of isolation probably relates to the main spore type produced, with *F. culmorum* producing only macroconidia whereas *F. graminearum* and the *Microdochium* species are more reliant on ascospores produced in perithecia on crop debris and at the stem base, respectively (Manstretta and Rossi, 2015, Doohan et al., 2003). The maturation of perithecia each year seems to occur just as the crop is at its most susceptible to infection.

Comparing the leaf layer monitoring data obtained during this project with those in 2008 suggested that the levels of infection by *F. culmorum*, *F. graminearum* and the *Microdochium* species during flowering would be lower than in 2008. Isolations from the Defra funded survey confirmed this with levels of *F. graminearum* and *Microdochium* lower in all three years. The predominant pathogen isolated from the surveyed crops between 2009 and 2011 was *F. poae*. This pathogen prefers drier conditions for infection which suggested that in addition to lower levels of inoculum, the conditions during flowering were too dry for infection by *F. graminearum* and the *Microdochium* species.

Monitoring the spatial distribution of *F. graminearum* at GS73 over a number of years showed the progression of this pathogen from its original “hotspot” in the South West of England up the East coast of England. *F. graminearum* is now routinely detected in the North East of England and the current “hotspot” is in the East Midlands. The incidence of *F. graminearum* across England has been mirrored by the subsequent DON contamination of harvested grain with exceedances of the legal limits for DON detected across England and up the East of the country to Cleveland but with a higher risk in the East Midlands in recent years. It is not clear why the East Midlands has become a hotspot for DON in recent years but is likely in part to the high incidence of cereals and min-till in rotations within this region. Region was used in the late-season prediction models based on artificial spatial boundaries (i.e. county boundaries). Cluster analysis identified the spatial distribution of *F. graminearum* and fusarium mycotoxins without artificial boundaries. Such cluster analysis could provide a more accurate assessment of the spatial distribution of fusarium mycotoxin risk across England compared to regions based on county boundaries, however such clusters would need to be based on several years to minimise impacts of single year/region weather events. Such cluster maps would also need to be updated to account for future long-term shifts in *F. graminearum* distribution as was seen to occur recently.

One aim of the project was to derive predictive models for *F. graminearum* incidence and the two main mycotoxins found in wheat (DON and ZON). Models were derived based on historical disease and mycotoxin data together with local weather data. The modelling was via two main approaches, one at the regional level and the second at the individual site level. At the regional level, the fusarium model produced did not fit the data very well even though the parameters used in the model appeared to match the current understanding of the conditions required for inoculum development and infection. The expected lack of predictive power of the fusarium model was confirmed when it was validated on data collected in 2012 and 2013. In contrast, the models produced for both DON and ZON were a good fit to the data. However, when the DON and ZON models were tested on independent data collected in 2012 and 2013 the predictive power of both models was shown to be poor, with the DON model generally overestimating levels of contamination, whereas the ZON model underestimated level.

The models produced at the site level were worse than those at the regional level. This was not surprising as looking at individual sites introduced site-to-site variability for sites which are close to one another and therefore, have similar overall weather patterns. The introduction of agronomic factors (such as the previous crop, the harvesting period and the cultivation technique) did not increase the predictive power of the models. As well as being a possible indication that the model (e.g. linear) was not appropriate, it may also indicate that other relevant factors were not included in the models.

Similar modelling approaches have been looked at in the past and, although rarely tested on independent sets of data, some have shown better results when fitting the relationship between weather and disease data. Using the approach used here has successfully produced models for diseases such *Septoria tritici* (te Beest et al., 2009). This disease is driven by 'general' weather patterns (e.g. temperature in the winter months and rainfall around GS37, this is very different to the situation for fusarium where specific weather conditions are required at very specific growth stages. The main difference between the successes of the modelling for the two diseases comes from the meteorological data. For *S. tritici* the disease being driven by general weather patterns means that the use of weather stations are as far as 20 kilometres away from the field does not influence the analyses. However, for fusarium if individual showers at critical periods are important; the current resolution of weather observations is clearly not good enough and only on-site weather observations would provide sufficient information. The other difference in the impact of weather on the two diseases of wheat is that FHB pathogens are believed to have a narrow window of infection i.e. flowering (Hooker et al., 2002) and therefore, weather parameters within this narrow window is critical and as such it is critical to the model to accurately determine when flowering has occurred. Currently, growth stages around flowering are not routinely monitored/recorded and results from this study showed that the ability to predict flowering is not sufficiently accurate. One

outcome from this project is identification of the need for greater recording of flowering growth stages so improvements can be made in the estimation of flowering growth stages to aid future fusarium mycotoxin prediction model.

Analysis of the impact of agronomic factors identified similar results as determined in previous projects (Edwards, 2007, Edwards, 2011). One new significant factor added to the model was harvest timing with a calculation for a field harvest date in respect of a county's six-year average harvest date. The data was categorised into harvest weeks with week 0 been harvested within +/- 3 days of the long-term average and a minus week harvested a week earlier and a positive week a week later. This new factor was highly significant for both DON and ZON ($P < 0.01$) and accounted for 0.6 and 5.4% additional variance within the models, respectively. The impact of a one month delay in harvest was a 25-fold increase for ZON and 10-fold for DON. This observational data fits with recent experimental data that showed that delayed harvests and wet conditions during crop ripening increase both DON and ZON but have a greater effect on ZON (Kharbikar et al., 2015). In the experimental study, the importance of rainfall during the ripening phase was shown to be critical for ZON production. When plots with high levels of FHB and DON were protected from rainfall during the ripening phase then ZON levels remained very low. This can be explained by the timing of production of DON and ZON. DON is produced during infection and can be detected to increase from flowering onwards, whereas ZON remains at low levels until the crop ripens, after this time the level of ZON increase rapidly (Matthaus et al., 2004). Based on this information, a better prediction of ZON would require an accurate recording or prediction of the start of crop ripening, collection of rainfall data from ripening to harvest and modelling of ZON against the rainfall data.

Late season prediction models based on the incidence of FHB pathogens assessed at early milky ripe (GS73) as part of the Defra funded winter wheat disease survey were much more successful, particularly at the national and site level. At the national level, the assessment of FHB pathogen incidence provided an accurate assessment of the seasonal disease pressure caused by FHB pathogens at a national scale. The majority of the variance within the mycotoxin datasets are as a result of seasonal variation in weather parameters. Results indicate that the variation in FHB pathogen incidence is a good indirect measure of the influence of seasonal weather prior to assessment at GS73 on the mycotoxin concentration. The DON model at the national level was a good predictor of new observations and therefore, FHB pathogen national incidence data in conjunction with harvest rainfall can be used to predict the national situation with regards DON risk. The same data was not as good a predictor for ZON and this may be due to the greater importance of pre-harvest rainfall and harvest delays which are only crudely assessed by monthly rainfall data. If this is the case, then it may be better to assess DON risk and use local information regarding

pre-harvest rainfall and harvest delays, or indirect indicators of harvest delays (e.g. Hagberg Falling Number) to suggest that ZON may be a greater risk than the calculated DON risk.

A national prediction at harvest of the percentage of samples that will exceed the legal limits for DON and ZON is useful for the cereal chain industry to consider the necessary degree of due diligence testing that will be required and the availability of grain for processing for human consumption on a national basis. The national late season risk prediction showed a good relationship between the predicted legal limit exceedances for DON and ZON and the actual exceedances within the years of this study.

A regional prediction at harvest of the percentage of samples that will exceed the legal limits for DON and ZON would benefit the cereal chain industry to consider the necessary degree of due diligence testing that will be required and the availability of grain for processing for human consumption on a regional basis. Unfortunately, the results from this study showed that the prediction of average mycotoxin concentrations at the regional level were not as good as the prediction of exceedances at the national level.

An accurate field-scale prediction of DON and ZON will provide growers and processors with a screen to determine which samples require a mycotoxin test before sale for human consumption. The prediction model developed based on the national percentage incidence of FHB pathogens at milky ripe and the field agronomy data allowed for a reasonably accurate prediction of DON and ZON. Regional and site FHB pathogen incidence data did not improve the accuracy of the models despite the data having a smaller spatial scale. This may be due to the fact that for a disease with a low in-field percentage incidence (typically less than 1%), then the relatively low number of ears assessed (50) and the lower number of sites per region results in poorer accuracy of assessment that is greater than the increase in spatial detail. Due to the large number of sites assessed in the Defra winter wheat disease survey (300), an increase in the number of ears assessed at GS73 would have large financial and logistical implications. The validation of the DON and ZON models based on the 2012 and 2013 harvests showed the DON model performed well but the ZON model was not as good. Again, the ZON model may not have been as good due to the greater importance of pre-harvest rainfall. The measure of harvest delays by the timing of harvest to the nearest week, compared to the long-term harvest, is crude and a better assessment of pre-harvest rainfall based on better recording/prediction of pre-harvest crop growth stages and recording of in-field rainfall over this time period is likely to improve the ZON prediction models. However, an assessment of the DON model as a predictor of either DON and/or ZON exceeding legal limits was a reasonable predictor. Increasing the weighting of false negatives by reducing the probability of samples exceeding legislative limits in the models increased the number of false positives but

reduced the number of false negatives. For the DON model, the number of false negatives could be reduced to zero percent for DON and 0.4% for ZON.

5.1 Future work/recommendations:

- The data used for the modelling are very skewed and, in particular, they contain a very large proportion of zeroes. It may be possible that mixed models would provide a better outcome.
- The models considered in this project are all linear models and not necessarily based on real processes. It would be useful to investigate the relationships in greater detail to try and better understand the biological mechanisms behind them in order to derive mechanistic models.
- The weather data were not collected on-site and may therefore, not be representative of what happened to the crop (e.g. localised rain showers may not be identified). This may be very important if the disease incidence is linked to very specific events, rather than general trends, as is believed to be true for fusarium head blight. Improved modelling could be achieved with increased availability of in-field weather data or at least a larger network of weather stations with complete datasets for the relevant meteorological parameters.
- It was not possible to develop an accurate predictor of wheat growth stages at flowering based on the data available. Previous evidence suggests that growth stages over a narrow time period before and during flowering are critical for head blight infection. Therefore, to model head blight incidence and subsequent fusarium mycotoxins in harvested grain, it is important to be able to accurately record or predict flowering growth stages. Greater recording of flowering growth stages within wheat trial programs and collection of in-field weather data would allow better prediction of these growth stages in the future.
- Results on the impact of harvest timing on mycotoxin content of harvested wheat is new data and indicates the importance of harvest timing on the final mycotoxin content of harvested wheat. Recent related results also identified the importance of pre-harvest rainfall on the mycotoxin content of harvested grain. Together this would indicate that greater recording of pre-harvest growth stages within wheat programs and collection of in-field weather data during this period would allow better prediction of pre-harvest growth stages in the future and more accurate assessment of this parameter within future models.
- The impact of pre-harvest rainfall and delayed harvests on the mycotoxin content of the harvested grain could be determined by an indirect measure of these parameters. For example the Hagberg Falling Number may be useful as a predictor of pre-harvest rainfall and harvest delays.
- The incidence of fusarium head blight pathogens as determined by Fera from the Defra funded winter wheat disease survey proved to be useful parameters in the late season prediction of both national, regional and field mycotoxin risk. The continuity of funding for

this program of work should be promoted and the development of a national and field scale risk assessment based on the model developed using the pathogen incidence data should be considered.

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