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# Project Report No. PR593

## Validation of fusarium infection risk calculator with AHDB mycotoxin risk assessment and actual DON results

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# 1. Abstract

Concerns in the wheat supply chain about the reliability of farmer AHDB Mycotoxin Risk Assessments (MRA) has indicated a need for increased awareness amongst farmers of mycotoxin risk. The Fusarium Infection Risk Calculator (FIRC) was developed to link average daily rainfall for each county with real time anthesis (flowering) progress information provided by agronomists. In order to validate the forecasts provided by the FIRC, grain samples (and associated AHDB MRAs) were collected in 2015, 2016 and 2017 from sites with FIRC risk scores (175 samples in total). These were tested for deoxynivalenol (DON) and compared to the forecast FIRC and AHDB MRA risk scores. Camgrain supplied additional DON test results in 2015 (n=105), 2016 (n=106) and in 2017 (n=159) with associated AHDB MRA, giving a total of 545 samples. The DON results from these were also compared to both the FIRC and AHDB MRA scores.

In total there were six samples in 2016 and 2017 that exceeded the 1250 µg/kg threshold for food safety. The FIRC forecast two of these samples as being high risk, whilst the AHDB MRA forecast one as high risk. The high DON levels in the 2017 samples were thought to be due to showers during anthesis disrupting T3 ear wash fungicide applications. These showers were sufficiently frequent to prevent treatment, but volume of rainfall at the country level did not exceed 40mm. It may also be that rainfall variability within the county meant that these crops actually received more rainfall than the county average and were at greater risk than the country level forecast picked up.

In 2016, there was no obvious reason why the two samples tested high, bar the fact that the farms had large amounts of maize in the rotation (if not in the previous crop) and had maize game strips. All the other samples that were forecast by both tools to be at high risk, had DON levels well below the 1250µg/kg threshold. The low incidence of fusarium in the three test years (97% of samples <500 µg/kg DON) meant that both the FIRC and the AHDB MRA tended to overestimate the risk of DON being present in the harvested grain. The assumptions around rainfall alone used in the FIRC tended to forecast medium risk infection more frequently than the AHDB MRA forecast medium mycotoxin risk, although the difference was not statistically significant.

In conclusion, the approach of linking timing of anthesis with rainfall is possible, however, the ability of this to forecast infection risk is limited, as there are multiple factors that influence overall infection, including the presence of sufficient inoculum. In low infection years, the model based on rainfall alone, tends to over-estimate risk, whilst only occasionally identifying as high risk crops that ended up having mycotoxin levels that exceeded the 1250 µg/kg threshold for human consumption. The rainfall at flowering data used in the FIRC can give an indication whether a season is likely to be higher or lower risk of fusarium infection, but the consistency of the FIRC results in relation to final DON test results indicates that it tends to overestimate risk.

## 2. Introduction

Mycotoxins are a health risk within the food supply chain. The main sources of mycotoxins in UK wheat grain are the fungal pathogens *Fusarium graminarium* and *Fusarium culmorum* – both of which produce deoxynivalenol (DON), one of the more commonly detected mycotoxins in cereals (Edwards, 2009). Legal limits are set for common mycotoxins in the EU, for example for DON, the limit is set at a maximum of 1250 µg/kg in unprocessed cereals (apart from durum wheat), 750 µg/kg in pasta and 500 µg/kg in bread and breakfast cereals (EU 2006).

There are industry initiatives in place to minimise the level of mycotoxin in grain, reducing the risk of the legal limit being exceeded. One example of this is the AHDB Mycotoxin Risk Assessment (MRA) which is submitted with grain consignments. The AHDB MRA helps to raise awareness amongst farmers about factors that affect mycotoxin risk, such as variety choice, previous cropping, cultivation techniques and rainfall at anthesis and pre-harvest, and aims to provide a risk score that identifies which grain loads have a higher risk of mycotoxin contamination and should therefore be tested, or taken out of the food supply chain. The area of the AHDB MRA that was judged to be most challenging to complete by farmers was the self-directed completion of the section relating to rainfall during anthesis as farmers do not often have access to accurate data about rainfall in their area at this time or accurately measure length of flowering.

To address this, the Fusarium Infection Risk Calculator (FIRC) was developed, based on the rainfall during anthesis risk as set out in the AHDB MRA. The FIRC links average daily rainfall (supplied by the Met Office) for specific UK counties (those where wheat is widely grown) with real time anthesis progress information from those counties (provided by agronomists) and uses this information to determine fusarium infection risk throughout the anthesis period.

Cereal crops are most susceptible to fusarium infection during anthesis (Edwards, 2007). Where rainfall during anthesis splashes spores from lower in the canopy up onto the cereal ear; and warm, humid weather which then allows the fusarium spores to germinate and infect the cereal ear. Once infection has occurred, further rainfall and warm humid conditions between anthesis and harvest encourages profuse sporulation and colonisation of developing grains (FSA, 2007). Fusarium infection can only occur if fusarium spores are present in the environment of the crop, combined with appropriate weather conditions. If no inoculum is present, even if the weather conditions are suitable for infection there will be no fusarium infection. It is important to note that fusarium infection does not guarantee mycotoxin development; mycotoxin development is driven by a variety of factors including moisture content of the grain during storage, temperature, storage period, contamination rate, broken grain and impurities, insect presence, oxygen rate, damage during harvest processing and grain and seed transport (Birck *et al.,* 2006).

This is the report from the third and final year of a three-year project. It completes the validation of the FIRC using data on anthesis progress from 26 independent agronomists reporting on anthesis progress in 29 counties, distributed around the main wheat growing areas in GB and daily rainfall data from the Met office to produce a series of weekly reports during wheat flowering, to estimate fusarium infection risk in each region.

The live monitoring of wheat anthesis occurred between 12 May – 21 July 2015, 2 June - 12 July 2016 and 23 May – 18 July 2017. Independent agronomists (predominantly AICC) from 30 counties (one agronomist per county) monitored, on a weekly basis, the proportion of crop at ear emergence complete (GS 59), early anthesis (GS 60-62), mid anthesis (GS 63-67) and anthesis complete (GS 68-69) for crops in their region and reported this information to ADAS. Weekly county anthesis information was then linked to county level rainfall data provided by the Met Office to provide a fusarium infection risk score based on the rainfall categories set out in the AHDB MRA whereby <10mm rain indicates low risk, 10-40mm of rainfall indicates mediumrisk, 40-80mm indicates high risk and over 80mm of rainfall indicates very high risk. The individual weekly reports and final summary reports for each year are available on the AHDB website.

In order to validate the forecasts provided by the FIRC, grain samples were collected in 2016 and 2017 (and associated AHDB MRAs) from sites with a known FIRC risk score (total 175 samples). These grain samples were then tested for DON and the results compared to the forecast FIRC risk and AHDB MRA risk scores. In addition, Camgrain, a grain co-operative supplied additional DON test results from 105 samples in 2015, 106 in 2016, and 159 samples in 2017 with associated AHDB MRA. The DON results from these were also compared to both the FIRC scores and the AHDB MRA score for each sample.

## 2.1. Aim and objectives

The overall aim of this project was to improve the information available on fusarium infection risk during flowering, to raise awareness amongst farmers of mycotoxin risks whilst aiding accurate completion of mycotoxin risk assessments and increasing reliability of information. In addition, this also aims to give an early indication of season regional mycotoxin risks for the supply industry.

The specific objectives of the work were as follows:

1. Develop the Fusarium Infection Risk Calculator (FIRC) and determine whether the model can be used to predict mycotoxin (DON) risk in harvested grain samples.

2. Provide up to six, weekly reports on fusarium infection risk for each of the three years using the data from the FIRC, during the summer flowering season.

3. Use DON testing of grain samples in each of the three years to validate the findings of the FIRC and compare this to the AHDB MRA.

This report briefly summarises the development of the FIRC and the live monitoring, but focuses predominantly on the DON testing of grain samples in order to validate the risk scores produced by the FIRC model.

# 3. Materials and methods

## 3.1. The FIRC Model

The Fusarium Infection Risk Calculator (FIRC) was developed to help farmers more accurately complete the rainfall at anthesis section of the AHDB Mycotoxin Risk Assessment (MRA). The FIRC is Excel based, and uses the rainfall parameters as set out in the AHDB MRA, actual daily rainfall data (country level) and live data on anthesis progress provided by independent agronomists (at country level) and combines the data to provide graphical representations of the fusarium infection risk (based on rainfall at flowering) to help farmers more accurately complete their AHDB MRAs. There are a number of assumptions that are built into the FIRC:

- Anthesis (flowering) (GS 60-69) or the fusarium infection risk period lasts seven days and uses cumulative rainfall over that seven day period to give an indication of fusarium infection risk.

- The risk categories, based on the AHDB MRA categories were rainfall of <10mm rain indicates low risk, 10-40mm of rainfall indicates mediumrisk, 40-80mm indicates high risk and over 80mm of rainfall indicates very high risk.

The FIRC identifies what proportion of the county winter wheat area was at the at risk growth stage (GS60-69) on each day of the week (e.g. if 70% of the crop was at mid-anthesis in week 2 the model assumes that 10% would be at mid-anthesis on day 1 and 10% on day 2 etc.), assuming that every crop will be at risk for a period of 7 days, but that in any one week, some of the crops will be starting anthesis whilst others are finishing. For every day of the anthesis period (late May - early July), the FIRC identifies the area of crop at mid-anthesis on a particular day and then totals the rainfall for the three days before and three days after that day (giving a total of seven days of rainfall). This total rainfall value for the day is used to calculate the rainfall risk. The charts that are presented in the data sets on the AHDB websites then allow the farmer to read of the mid-point of anthesis for his crop, clearly see the pattern of rainfall around that date and, based on the colour of the bar understand the rainfall related infection risk.

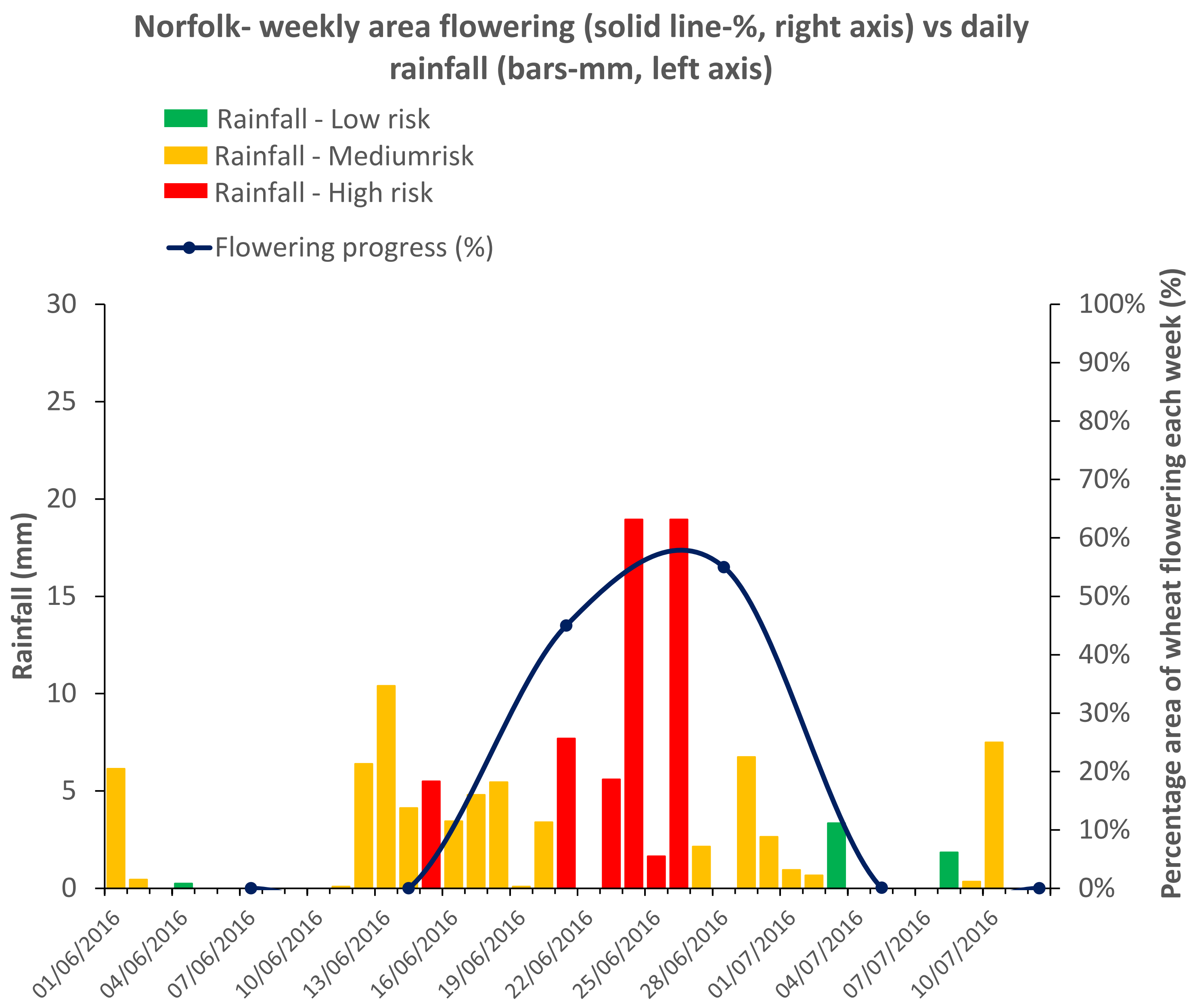


**Figure 1.** **Example county level FIRC chart. The line indicates the proportion of the area of crop at risk in that county on that date, the height of the bars indicates the daily rainfall and the colour of the bars indicates infection risk, green low through to red high.**

### 3.2. Weekly reporting

In order to collect data to populate the FIRC, weekly reports on anthesis progress were provided by 26 agronomists across 29 counties/ reporting areas. Reporters provided the following details:

- Proportion of crop area established by ploughing vs minimum tillage

- Varieties grown (as a proportion of total crop area)
- Proportion of crop area planted with varieties with high fusarium resistance rating
- Anthesis progress (proportion of the winter wheat crop in their area, that had reached each growth stage – pre-GS 59, GS 59, GS 60-62, GS 63-67, GS 68-69, GS 70+)
- Details on fungicide applications around anthesis.

Data was collected from the following sample areas:

| | | | |
|---|---|---|---|
| NE Scotland | Warwickshire/West Midlands | Berks/Oxfordshire | N Yorkshire |
| SE Scotland | Beds/Herts | Kent | S and W Yorkshire |
| SW Scotland | Cambridge | Hampshire | E Yorkshire |
| North West | Norfolk | Cornwall/Devon | Derbyshire/Nottinghamshire |
| Durham | Suffolk | Dorset/Somerset | Leicestershire/Rutland |
| Northumberland | Essex | Wiltshire | Lincolnshire |
| Shropshire/Staffs | Hereford/Worcester | Gloucestershire/Avon | Northamptonshire |
| Wales | | | |

This was then combined with rainfall data provided by the Met Office to calculate a cumulative seven day rainfall figure for each sampling area and complete the FIRC. A fusarium infection risk category was calculated for each day, allowing a farmer to select the day on which their crop was at mid-anthesis and read off an infection risk score. Using the results of the FIRC, a weekly report was submitted to AHDB, outlining anthesis progress across Great Britain and the associated fusarium infection risk. The weekly fusarium infection risk reports, and the season summaries are available on the AHDB webpage here.

In 2015, the live monitoring data and the FIRC model forecast that due to dry weather during early and mid-June, 76% of the UK winter wheat crop was at low risk of fusarium infection, with 23% at mediumrisk – mainly in the Midlands and Scotland - and just 1% at high risk – mainly affecting the small area of crops that flowered late in the East Midlands and in Scotland.

In 2016, the live monitoring data and the FIRC model forecast that 10% of the UK winter wheat crop was at low risk of fusarium infection, 73% was at mediumrisk and 17 % was at high risk of infection and <1% at very high risk of fusarium infection.  The majority of the low risk crops were late anthesis crops in the south and east of the UK, whilst the highest risk crops were those anthesis in Kent, Norfolk, Cambridgeshire, Shropshire/Staffordshire and Derbyshire and Nottinghamshire, with up to 26 mm of rainfall falling in Derbyshire and Nottinghamshire between 14-15 June.

In 2017, the FIRC model forecast that 41% of the winter wheat area was at low risk, with 53% at mediumrisk. 6% of the wheat area was as high risk, concentrated in Wales, Yorkshire & Humber and the North West, with smaller areas also at high risk in the North East and the East Midlands. These tended to be areas with later anthesis crops.

### 3.3. Agronomist sampling

The FIRC was developed to help farmers complete the rainfall at flowering section of the AHDB MRA, and therefore, uses the rainfall risk categories, as set out in the AHDB MRA to drive its infection risk scores. It was designed to help farmers more accurately complete their AHDB MRA, rather than as a standalone tool to calculate mycotoxin risk. However, in order to understand whether infection risk alone can be used to support the farmer in identifying the likelihood of mycotoxin presence in grain samples, a validation exercise was completed where grain samples were taken from known locations, with known FIRC scores and known AHDB MRA scores and sent for DON testing. The aim was to assess 20 low, medium and high risk samples in each of the three crop years the project was running (60 samples per year in total). However, due to the rainfall patterns in each season, it was not always possible to get 20 samples in each risk category every year, instead a representative sample of the risk scores achieved in any one year was selected.

Each year, 12 counties were selected for sampling, with the aim of representing the proportion of crops predicted to be at high, mediumand low fusarium infection risk during anthesis based on the FIRC model with five samples taken per county (see Table 1).

**Table 1 Number of samples taken from each county at each fusarium infection risk category (high, mediumor low) as predicted by the fusarium infection risk calculator (FIRC) – in each project year.**

| FIRC score | 2015 Counties | 2016 Counties | 2017 Counties |
|---|---|---|---|
| **HIGH** | SE Scotland (1), Leicestershire/ Rutland (5) | Derbyshire and Nottinghamshire (5), Warwickshire and West Midlands (5), Shropshire/Staffordshire and Wiltshire (5). | Cheshire/ Lancs/ N Wales (5), Leicestershire/ Rutland (5), North Yorkshire (5), Pembrokeshire & South, Wales (5) |
| **MODERATE** | SE Scotland (4), SW Scotland (3) | Northumberland (5), SE Scotland (5), Gloucester and Avon (5), SW Scotland (5), NE Scotland (5), Bedfordshire (5) and N Yorkshire (5). | Dorset (5), Norfolk (5), Gloucestershire/ Avon (5), Beds/ Herts/ Bucks (5) |
| **LOW** | Leicestershire/Rutland (10), SW Scotland (2), Durham (5), N. Yorkshire (5), Northamptonshire (5), Norfolk (5), Nottinghamshire (5), Suffolk (5) | Kent (5) | North East Scotland (5), Northumberland (5), South East Scotland (5), Hampshire & IOW (5) |

#### 3.3.1. Sampling approach

Each agronomist (representing a county) was given a detailed protocol explaining the methodology for sampling, including approximate location (county) and target anthesis date. For each sample, 35 wheat ears were taken from 10 separate random points along any diagonal traverse of the whole field. In total 350 ears were cut from stems using scissors, to provide enough grain for a 500g grain sample. If it was not possible to take wheat ear samples prior to harvest, agronomists were asked to take grain samples immediately after harvest. This was done by taking ten small grain samples

(50g) from different places within the grain heap/trailer, which were then bulked together in a bag to provide a total of 500g of grain. Once collected, samples were then sent to ADAS Boxworth. At ADAS Boxworth ear samples were dried and threshed to give 500g grain samples.

### 3.3.2. DON analysis

Each sample was split in half, with half retained by ADAS (in case reanalysis was required or samples went missing), and the other half was sent to Sciantec laboratories for DON analysis by ELISA (Enzyme Linked Immunosorbent Assay) to indicate presence or absence of DON. The process involves extracting a sample, binding any DON in the sample from the grain and diluting it to 450nm concentration. This solution is then exposed to to a DON antibody in a specific test well. The level of DON present is indicated by the intensitity of colour present following the reaction. A light of specific wavelength is then shone through the sample and the absorbtion of this light is used to estimate the level of DON present. The minimum limit of detection is is 220 µg/kg, so any samples with DON levels lower than this threshold will be returned as a nil sample. Only a single sample is assessed at the lab, and therefore this approach relies on a representative sample of grain being taken in the first place, the fact that only a single sample is given means that it is not possible to calculate a standard error on individual results, however the indication from other labs is that the ELISA test approach should not overestimate the presence of DON by more than 10% (ELISA Technologies, 2018).

DON results were classed as low risk (<1,000 µg/kg), medium risk (1,000-1,500 µg/kg) or high risk (>1,500 µg/kg) with the legal limit for DON being 1250 µg/kg. For each sample, a completed AHDB MRA was provided by agronomists to give the wider context of the sample, taking into account other factors that affect mycotoxin risk, e.g. varietal resistance, cultivations etc.

Anthesis dates for each sample, as stated in the AHDB MRA, were checked against the live anthesis monitoring data and rainfall using data from the FIRC model in order to check the fusarium infection risk score as calculated using FIRC.

For each of the samples, the DON analysis results, AHDB MRA and FIRC scores were collated into an Excel spreadsheet. The FIRC and AHDB MRA scores assigned were compared with each other and validated against the DON results using correlation probability to show how well each approach (AHDB MRA or FIRC) matched the actual DON levels of the samples and how well AHDB MRA scores and FIRC scores aligned.

### 3.4. Commercial sampling

It is standard practice at Camgrain Co-operative to test all loads of grain that are brought on to site. As part of this project, farmers were also asked to send in fully completed AHDB MRA risk assessments for their loads of wheat. Camgrain provided full AHDB MRAs and deoxynivalenol (DON) test results for 105 crops in 2015, 106 crops in 2016 and 159 crops in 2017. All data provided by farmers was kept anonymous and individual farms/fields cannot be identified. The locations, by county, of the samples are summarised in Table 2 below.

**Table 2 – Commercial sampling – source of samples each project year**

| Year | Counties |
|---|---|
| **2015** | Bedfordshire (2), Cambridgeshire (13), Essex (25), Hertfordshire (14), Norfolk (2), Northamptonshire (10), Oxfordshire (6), Rutland (1), Staffordshire (1), Suffolk (23), Warwickshire (5), Wiltshire (1) |
| **2016** | Bedfordshire (3), Buckinghamshire (2), Cambridgeshire (30), Essex (22), Hertfordshire (10), Norfolk (4), Northamptonshire (6), Oxfordshire (1) and Suffolk (28), |
| **2017** | Bedfordshire (5), Buckinghamshire (1), Cambridgeshire (29), Essex (60), Hertfordshire (8), Lincolnshire (2), Norfolk (3), Northamptonshire (12), Oxfordshire (8), Suffolk (23), Warwickshire (8) |

These data sets did not include anthesis date, therefore direct alignment with the FIRC score was not possible. In order to provide a proxy FIRC score, the live monitoring reports prepared during June and July were used to identify when the majority of crops in each particular county were in flower to estimate likely anthesis date and the cumulative rainfall for that seven day period was taken as the proxy FIRC score, this was cross checked against the anthesis rainfall score given in the AHDB MRA. The AHDB MRA and the FIRC scores for each sample were then compared with the DON results using correlation probability to assess how well both the ADHB MRA score and FIRC scores aligned with the actual DON levels in the grain samples and how well they compared with each other.

# 4. Results

## 4.1. Weekly reporting

### 4.1.1. 2015

Winter wheat crops started anthesis at the end of May in the South East and South West. This coincided with some wet weather that increased the risk of fusarium infection for these early crops. However, the main anthesis period in England and Wales in mid-June was largely dry, so infection risk was low for many crops. Wet weather in Scotland in late June and early July coincided with peak anthesis for these regions, resulting in mediumfusarium infection risk for most late flowering crops. Consequently, the FIRC forecast the infection risk for 2015 to be low-mediumacross GB, with 76% of the winter wheat area at low risk, 23% at mediumrisk and 1 % at high risk. Figure 2 shows the pattern of rainfall risk and flowering progress at a national level.

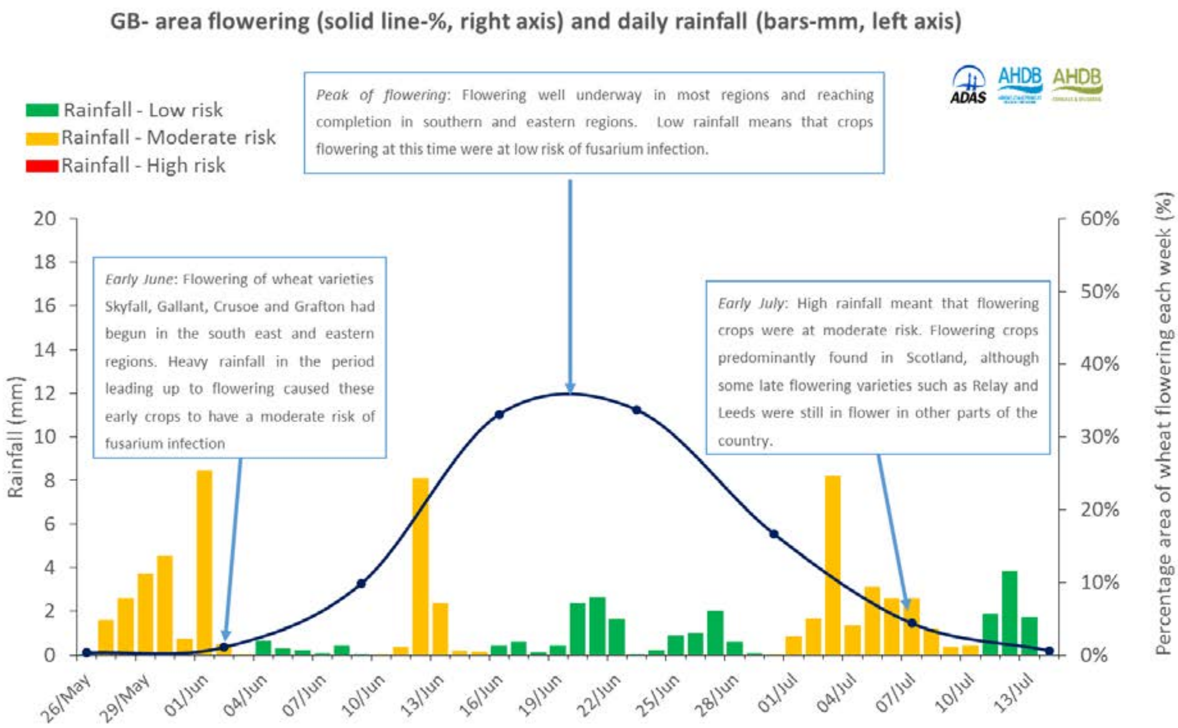


**Figure 2** **Example chart – summarising flowering progress in GB and average GB rainfall. Crops that were at mid-anthesis when the bars were orange were at mediumrisk of fusarium infection (based on rainfall related risk), whilst those at mid-anthesis when the rainfall bars were green were at low risk of infection.**

#### 4.1.2. 2016

Winter wheat crops started anthesis at the beginning of June in southern and eastern regions and was complete in England and Wales by 5 July and virtually complete in Scotland by 12 July. Due to above average rainfall during anthesis in many areas of the country with large wheat crop areas, the FIRC forecast was for an overall moderate-high DON risk season for GB as a whole. This corresponded to 10% of the winter wheat crop at low risk, 73% at mediumrisk, 17% at high risk and ~1% at very high risk.

#### 4.1.3. 2017

Winter wheat crops started anthesis at the end of May 2017 in the southern and eastern regions and anthesis was largely complete across England and Wales by 27 June, with a small area remaining in Scotland. Average GB rainfall was relatively high during the first half of anthesis and the majority of the crops that reached mid anthesis during the first 10 days of June receiving over 10 mm of rainfall, although some received in excess of 40mm, meaning that they were at mediumto high risk of fusarium infection. The second half of anthesis (during mid-late June saw a period of settled weather affecting the whole of GB, reducing the fusarium infection risk to low. Overall the fusarium infection risk was mediumfor GB as a whole, with 41% of the crop area at low risk, 53% at mediumrisk and 6% at high risk.

### 4.2. Validation of FIRC (agronomist & commercial samples)

#### 4.2.1. 2015 results overview

Results from the 55 agronomist grain samples and 105 commercial grain samples showed minimal DON levels in most cases, with just one of the 55 agronomist samples and none of the 105 commercial samples having DON levels in the mediumcategory (500-1000µg/kg ) and no samples with high DON levels (>1000µg/kg ) (see Table 3.). None of the samples had DON levels high enough to necessitate exclusion from the human food chain.

The FIRC overestimated DON risk in the 2014/2015 season in 24% of cases (12.5% of samples to be at mediummycotoxin risk, 11.5% at high risk), whilst the AHDB MRA overestimated DON risk in 41% of cases (31% of samples predicted to be at mediumrisk and 10% at high risk). Importantly though, in the case of the one sample where DON levels were moderate, both FIRC and MRA estimated the fusarium infection and mycotoxin risk to be low. Despite the fact that there appeared to be some alignment between DON results and FIRC and AHDB MRA scores when plotted on a graph (see Figures 3 and 4), this relationship was not statistically significant.

**Table 3. 2015 comparison of number of samples (agronomist & commercial combined) in low, mediumor high mycotoxin risk category based on total number (No=160) FIRC, AHDB MRA scores and DON results. Relative percentages of number of samples in each category compared to total**

| | No. of samples in each category | | | |
|---|---|---|---|---|
| Risk category* | FIRC | AHDB MRA | DON score | DON levels |
| LOW risk | 139 *(87%)* | 79 *(49%)* | <500µg/kg | 159 *(99%)* |
| MEDIUMrisk | 15 *(9%)* | 72 *(45%)* | 500-1,250µg/kg | 1 *(1%)* |
| HIGH risk | 6 *(4%)* | 9 *(6%)* | >1,250µg/kg | 0 *(0%)* |
| TOTAL | 160 | 160 | | 160 |

*No direct alignment between risk categories and DON score

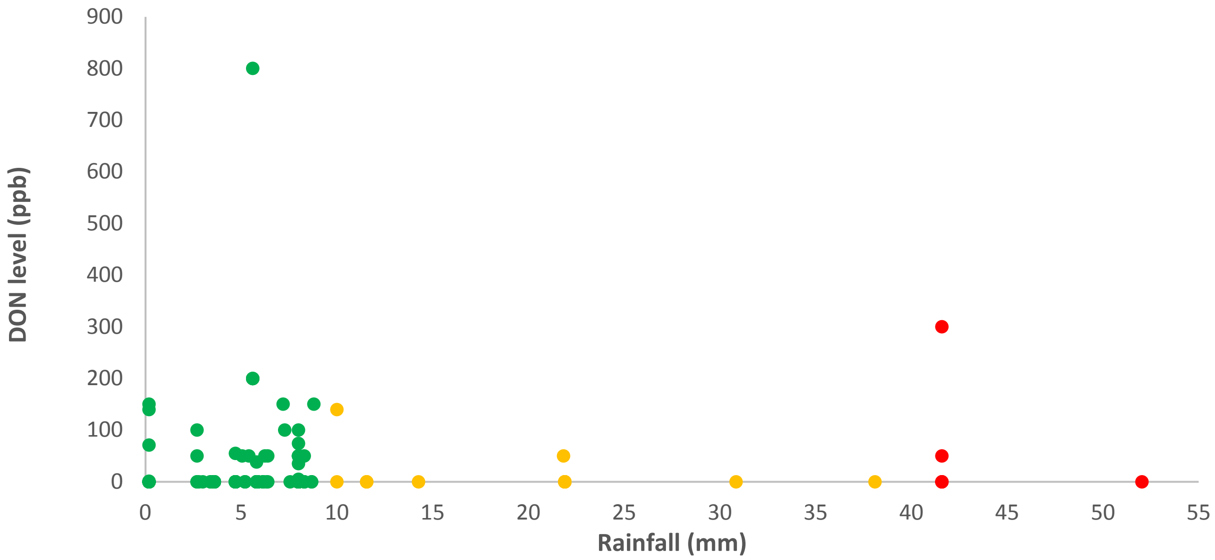


**Figure 3. Rainfall (mm, which corresponds with FIRC score) vs DON result (µg/kg or ppb) for agronomist samples (No. =55) and commercial samples (n=105) in 2015. Points are colour coded according to FIRC score- green= low risk, orange= mediumrisk, red= high risk.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and rainfall – this is particularly the case of the low rainfall (<10mm) nil DON samples.*

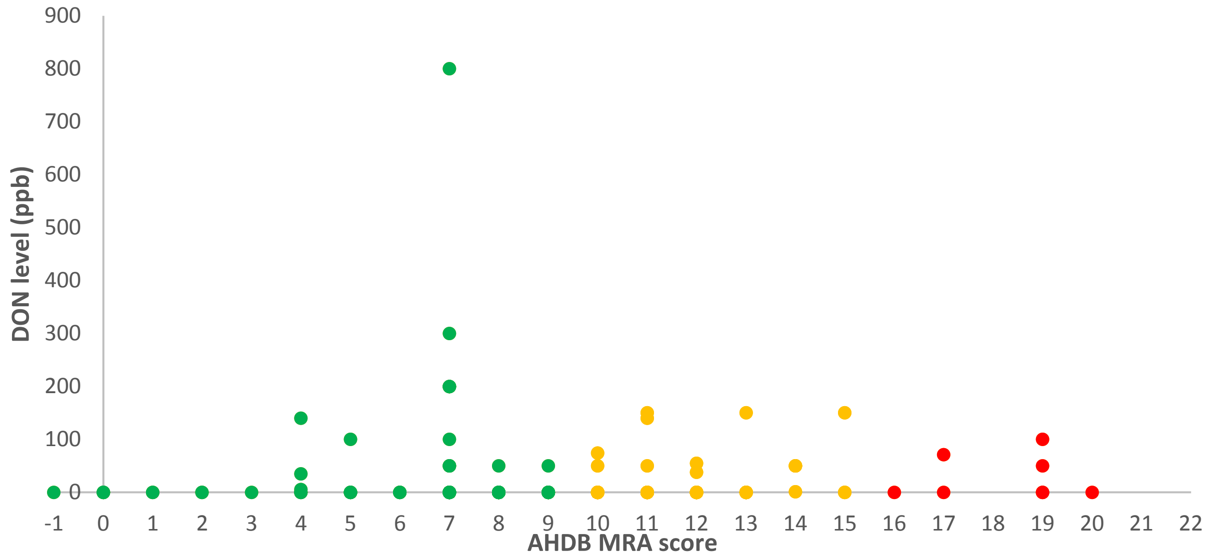


**Figure 4. AHDB risk assessment score vs DON result (µg/kg or ppb) for agronomist samples (No. =58) and commercial samples (No. =106) in 2015. Points are colour coded according to AHDB MRA score- green= low risk, orange= mediumrisk and red= high risk. Each point represents a single sample.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and AHDB MRA score – this is particularly the case of the nil DON samples.*

#### 4.2.2. 2016 results overview

Of the 60 agronomist grain samples and 106 commercial grain samples (provided by Camgrain) there were two samples which had DON levels in excess of 1250 µg/kg (the threshold for rejection from the human food chain) (see Table 4.). The FIRC assessment had identified these as mediumrisk, whist the accompanying AHDB MRA forecast them to be low risk. There were just two samples that had DON levels between 500 µg/kg and 1250 µg/kg, of these the FIRC forecast one to be mediumrisk and one to be high risk, whilst the AHDB MRA forecast one to be at low risk and one to be at high risk.

All other grain samples came back with DON results of less than 500µg/kg. In 2016, the FIRC forecast that the majority of samples (76%) were at mediumrisk of infection, based on rainfall during flowering, and therefore tended to overestimate the risk of mycotoxin development compared to the DON results. The AHDB MRA also forecast a fair proportion of samples (48%) to be at mediumrisk of infection and therefore tended to overestimate the actual DON risk. The low levels of DON detected meant that there were no significant relationships detected between either the DON results and FIRC, or DON and AHDB MRA (see Figures 5 and 6).

**Table 4 2016 comparison of number of samples in low, mediumor high mycotoxin risk category based on total number (agronomist & commercial samples combined) (No=166) FIRC, AHDB MRA scores and DON results. Relative percentages of number of samples in each category compared to total number of samples are shown in brackets.**

| | No. of samples in each category | | | |
|---|---|---|---|---|
| **Risk category*** | **FIRC** | **AHDB MRA** | **DON score** | **DON levels** |
| **LOW risk** | 7 (4%) | 64 (36%) | <500µg/kg | 162 (98%) |
| **MEDIUMrisk** | 126 (76%) | 79 (48%) | 500-1,250µg/kg | 2 (1%) |
| **HIGH risk** | 33 (20%) | 23 (14%) | >1,250µg/kg | 2 (1%) |
| **TOTAL** | 166 | 166 | | 166 |

*No direct alignment between risk categories and DON score

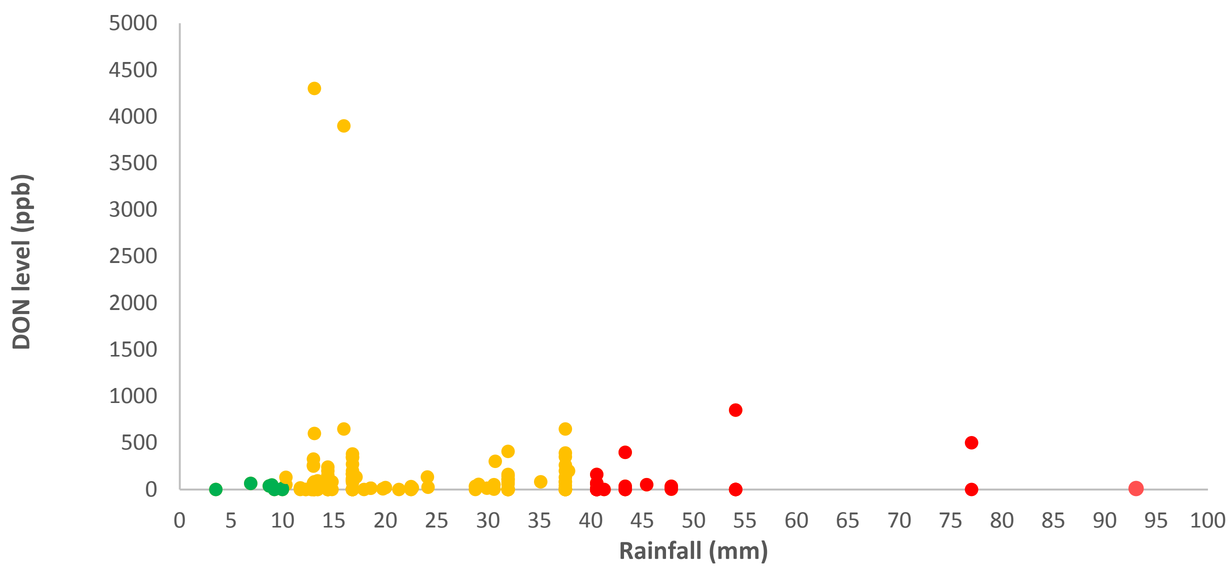


**Figure 5 Rainfall (mm, which corresponds with FIRC score) vs DON result (µg/kg or ppb) for agronomist samples (No. =58) and commercial samples (n=106) in 2016. Points are colour coded according to FIRC score- green= low risk, orange= mediumrisk, red= high risk and dark red= very high risk. Each point represents a single sample.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and rainfall – this is particularly the case of the nil DON samples.*

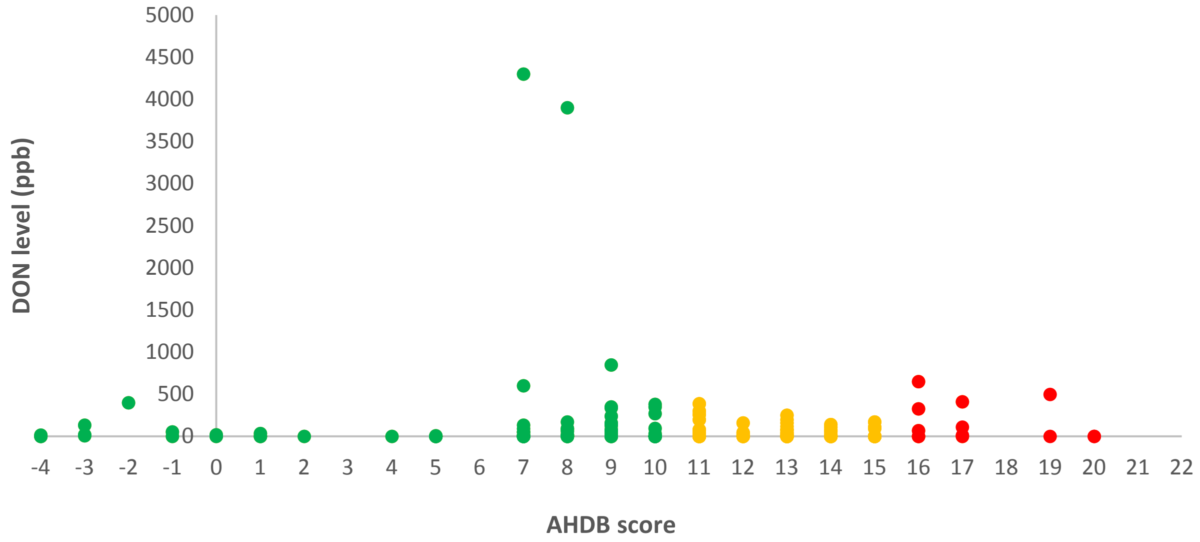


**Figure 6 AHDB MRA score vs DON result (µg/kg or ppb) for agronomist samples (No. =58) and commercial samples (No. =106) in 2016. Points are colour coded according to AHDB MRA score- green= low risk, orange= mediumrisk and red= high risk. Each point represents a single sample.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and rainfall – this is particularly the case of the nil DON samples.*

### 4.2.3. 2017 results overview

When the 159 commercial samples and 60 agronomist grain samples were tested for DON levels, just four samples had levels of DON above 1250µg/kg, which is the threshold for rejection from the human food chain (see Table 5). Of these samples, two were identified as high risk and two were identified as medium risk by the FIRC. The AHDB MRA identified one sample as high risk – although not the same one as the FIRC – with the rest identified as medium risk. Again there was no statistically significant correlation between the results of either tool and DON levels in samples (see Figures 7 and 8).

Importantly, 10 samples registered medium DON levels (between 500µg/kg, but below 1250µg/kg) Despite the FIRC predicting 161 samples to be at medium risk, only three of the samples with medium DON levels were predicted correctly by the FIRC, with six forecast to be low risk and one sample at high risk. In contrast, all of these samples with medium DON levels were correctly forecast to be at medium risk by the MRA. However, both tools greatly overestimated the number of samples at mediumrisk, with the FIRC forecasting 161 samples and the MRA predicting 127 to be at medium risk (table 5). Similarly, although the remaining 206 samples registered DON levels of under 500µg/kg, the FIRC and MRA forecast just 35 and 70 samples to be in this category, respectively.

The FIRC is based on rainfall during flowering, in 2017 anthesis rainfall was mediumwith most crops (74%) receiving 10-40mm during the anthesis period.  However, when used alone, based on the DON results, this has overestimated the risk of mycotoxin development when compared to the DON levels recorded. Likewise, the AHDB MRA also overestimated the likelihood of mycotoxin development, although to a slightly lesser extent than the FIRC. Importantly, although both tools forecast almost the same number of samples to be in the high risk category, in most cases these were not the same group of samples, suggesting that factors other than simply rainfall are driving many of the high MRA scores. Overall though, both tools overestimated the mycotoxin development risk by a considerable amount.

**Table 5 Comparison of number of samples in low, mediumor high MRA category based on 2017 results from FIRC, AHDB MRA scores and DON results. Relative percentages of number of samples in each category compared to total number of sample (n=219)**

| | No. of samples in each category | | | |
|---|---|---|---|---|
| Risk category* | FIRC | AHDB MRA | DON score | DON levels |
| LOW risk | 35(16%) | 70 (32%) | <500µg/kg | 206 (94%) |
| MEDIUMrisk | 161 (74%) | 127 (58%) | 500-1,250µg/kg | 11 (5%) |
| HIGH risk | 23 (11%) | 22 (10%) | >1,250µg/kg | 4 (2%) |
| TOTAL | 219 | 219 | | 219 |

*No direct alignment between risk categories and DON score

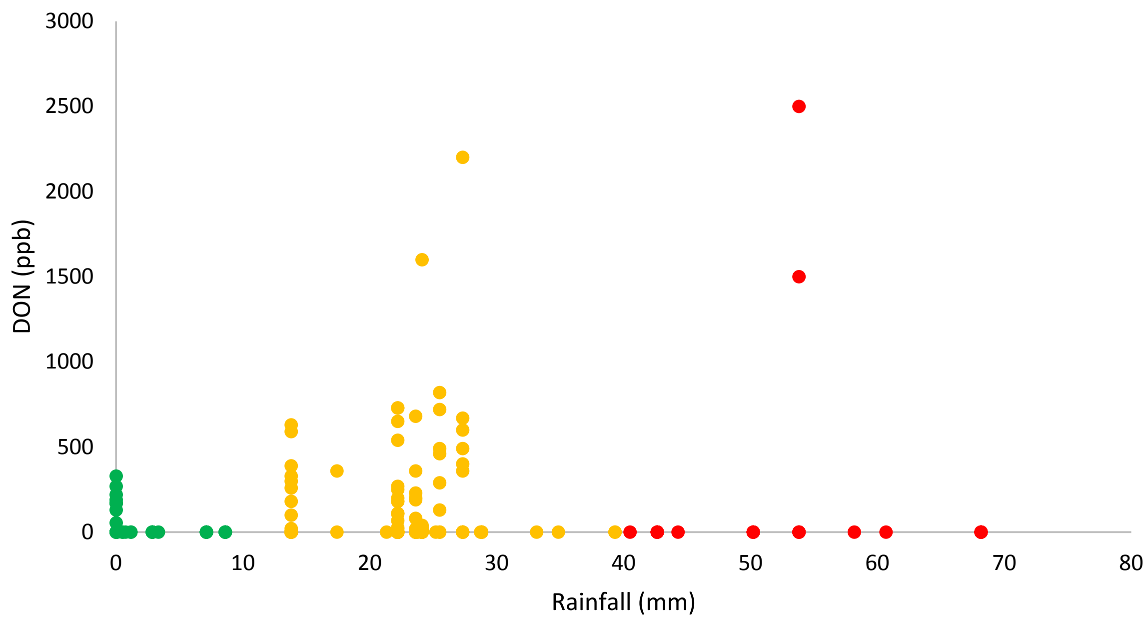

**Figure 7. Rainfall (mm, which corresponds with FIRC score) vs DON result (µg/kg or ppb) for agronomist samples (No. =60) and commercial samples (n=156) in 2017. Points are colour coded according to FIRC score- green= low risk, orange= mediumrisk, red= high risk and dark red= very high risk. Each point represents a single sample.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and rainfall – this is particularly the case of the nil DON samples.*

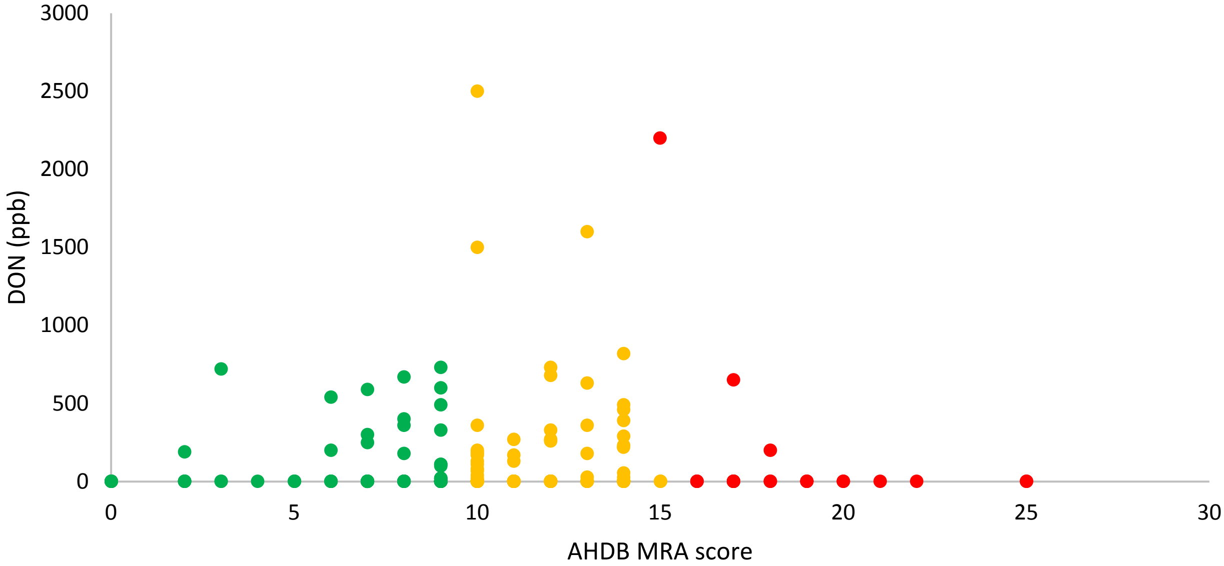


**Figure 8. AHDB risk assessment score vs DON result (µg/kg or ppb) for agronomist samples (No. =60) and commercial samples (No. =156) in 2017. Points are colour coded according to AHDB MRA score- green = low risk, orange = mediumrisk and red = high risk.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and AHDB MRA score – this is particularly the case of the nil DON samples.*

#### 4.2.4. 2015, 2016 and 2017 combined data

When the data for 2015, 2016 and 2017 were combined for both the agronomist samples and the commercial samples (n=545 samples) this confirmed the findings of previous years' reports that the FIRC and AHDB MRA were consistently overestimating the actual risk of mycotoxin in grain and underestimating the number of samples at low risk (see Table 6).

Over the three years, there was minimal difference between the two forecasting methods, with 33% of FIRC samples and 39% of AHDB MRA predicting low fusarium infection risk, 55% of FIRC and 51% of AHDB MRA predicting mediumfusarium infection risk. In the case of high risk, the FIRC predicted high fusarium infection risk in 11% of samples, compared to 10% of samples predicted by the AHDB MRA. DON levels were generally low over all three years (n=545), with 97% of samples having DON levels <500 µg/kg, ~2% with 500-1,250 µg/kg and ~1% with DON >1,250µg/kg (Table 5).

**Table 6 Comparison of number of samples in low, mediumor high mycotoxin risk category based on 2015, 2016 and 2017 results from FIRC, AHDB mycotoxin risk scores and DON results. Relative percentages of number of samples in each category compared to total number of sample (n=545).**

| | No. of samples in each category | | | |
|---|---|---|---|---|
| Risk category* | FIRC | AHDB MRA | DON score | DON |
| LOW risk | 181 (33%) | 213 (39%) | <500µg/kg | 527 (97%) |
| MEDIUMrisk | 302 (55%) | 278 (51%) | 500-1,250µg/kg | 13 (2%) |
| HIGH risk | 62 (11%) | 54 (10%) | >1,250µg/kg | 6 (~1%) |
| TOTAL | 545 | 545 | | 545 |

*No direct alignment between risk categories and DON score

Based on 2015, 2016 and 2017 data there was no statistically significant relationship between DON levels and AHDB MRA and DON levels and FIRC (rainfall during flowering) as shown by Figures 9 and 10 below. For DON and rainfall the $R^2$ value was 0.0416 and for the relationship between AHDB score and DON result the $R^2$ value was 0.0051. This indicates that in low pressure fusarium years that the FIRC and AHDB MRA are not consistently correctly forecasting the risk of infection and subsequent mycotoxin development.

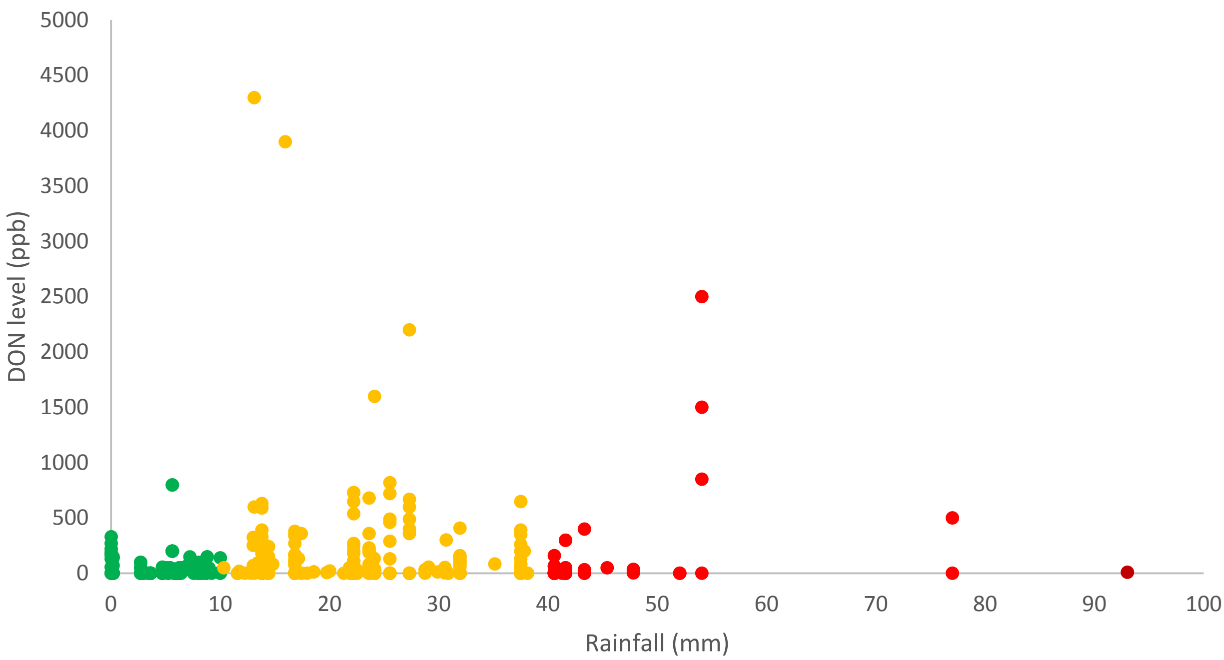


**Figure 9 Rainfall (mm, which corresponds with FIRC score) vs DON result (µg/kg) for agronomist and commercial samples for 2015, 2016 and 2017 (No. =545). Points are colour coded according to FIRC score- green= low risk, orange= mediumrisk, red= high risk and dark red= very high risk. $R^2$=0.0416.**

*Note individual dots may represent more than one sample, if they happen to have a similar DON score and rainfall – this is particularly the case of the nil DON samples.*

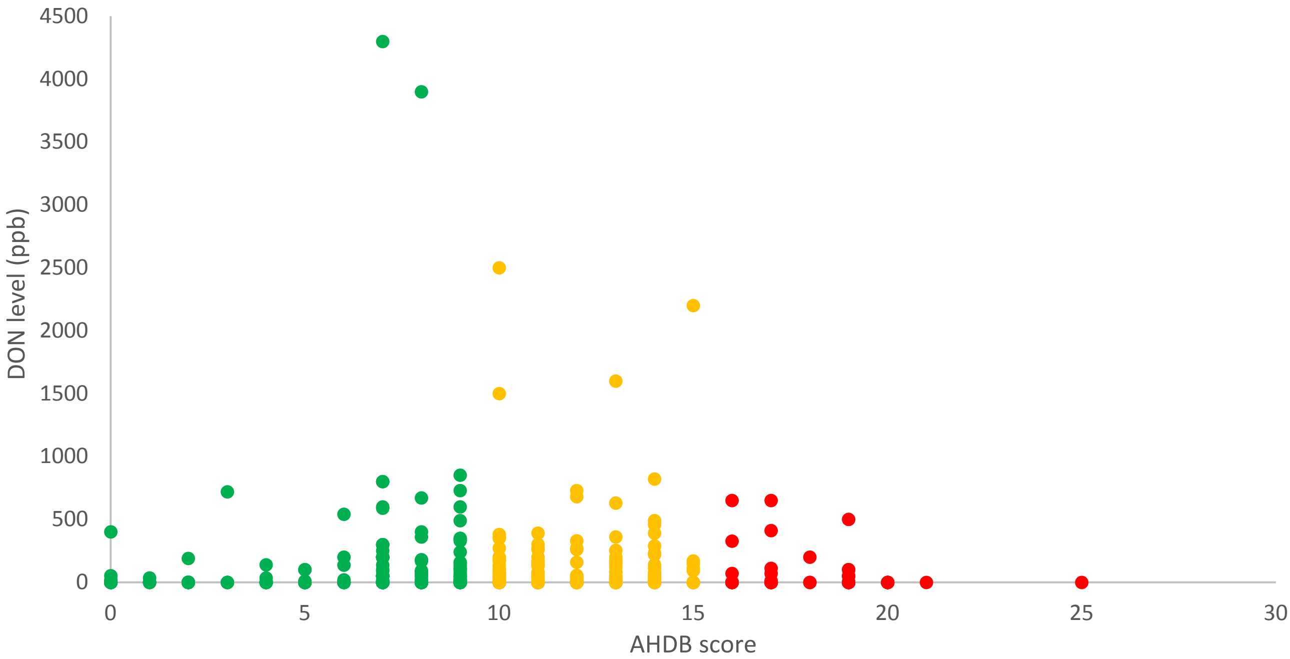


**Figure 10 AHDB risk assessment score vs DON result (µg/kg or ppb) for agronomist and Camgrain samples for 2015, 2016 and 2017 (No. =545). Points are colour coded according to AHDB MRA score- green= low risk, orange= mediumrisk and red= high risk. $R^2$=0.0051.** *Note individual dots may represent more than one sample, if they happen to have a similar DON score and AHDB MRA score – this is particularly the case of the nil DON samples.*

## 5. Discussion

The low levels of DON in the samples tested are not unexpected, as it is well known that in the UK, the risk of exceeding legal limits for fusarium mycotoxins in cereals is low (FSA, 2007). This is supported by other research which shows that between 2001-2008 DON levels typically ranged between <10-750 µg/kg , with most samples having DON levels around 100µg/kg – which is well below the legal limit for DON of 1250 µg/kg (Edwards, 2011). In addition, other factors affect mycotoxin development that are unrelated to risk of fusarium infection. These include use and correct timings of T3 fungicides, varietal resistance to fusarium, whether the crop lodged or not and level of rainfall close to and during harvest. For example, recent research found that warm, wet conditions in the weeks just before harvest can influence mycotoxin development and a delayed, wet harvest after the crop is ripe, further increases the risk of mycotoxin contamination (Edwards, 2013).

The number of samples that were analysed within this project that meant that it was always going to be a challenge to obtain statistically robust data on mycotoxin incidence in relation to the forecasts created by the Fusarium Infection Risk Calculator (FIRC) or the AHDB Mycotoxin Risk Assessment (AHDB MRA). In total across the three years, 175 agronomist samples and 370 commercial samples (545 combined) were sent for DON testing, each with an associated FIRC and AHDB MRA score. Of these samples, 6 (~1%) tested above the 1250 µg/kg threshold and 13 (2%) tested between 500-1250 µg/kg. Of the samples that tested above 1250µg/kg DON, the FIRC forecast that three were at high risk of fusarium infection based on rainfall at flowering, with the other three at mediumrisk. The AHDB MRA forecast that two of these same samples were at high risk, three of these samples were at mediumrisk of developing mycotoxin and one at low risk.

In 2016, the two samples with high DON levels both came from Kent, whereas in 2017, one originated from Warwickshire, one from Suffolk, one from Cardiganshire and one from Pembrokeshire. Although the two Welsh samples were correctly forecast as high risk by the FIRC, the only other correct high risk prediction over both years was made by the AHDB MRA on the 2017 sample from Warwickshire. For the other samples with high DON levels, both tools forecast medium DON levels. In all these areas, cumulative rainfall around flowering was not abnormally high. However, based on information from the agronomists, as well as warm moist conditions, it's likely that heavy showers during the T3 spray window prevented effective disease control and therefore increased the likelihood of mycotoxin development.

Compared to 2016, 2017 saw slightly higher numbers of samples with a medium DON level (500µg/kg -1250µg/kg), with 5% (11) samples in this category compared to zero in 2015 and 1% (two) in 2016. Overall, of the 10 samples in 2017 that had elevated DON levels (but not exceeding the 500-1250µg/kg threshold) the FIRC forecast that all were at mediumrisk of infection, based on rainfall at flowering. For the same 10 samples, the AHDB MRA forecast that six were at low risk of developing mycotoxin, three were at mediumrisk and one was at high risk. This suggests that for these samples, rainfall at flowering was having a consistent influence on the risk.

In general though, the two approaches both tended to overstate the mycotoxin risk over the three years. Based on rainfall during anthesis alone, the FIRC indicated that 33% of the samples were at low risk of infection, with 55% at mediumrisk and 11% at high risk. This was mirrored by the AHDB MRA which forecast 39% of samples at low risk, 51% at mediumrisk and 10% at high risk. Neither approach actually gave a consistently clear indicator that any of the samples would be worse than others with regards to DON level.

There are a number of elements that may have contributed to the disparity between the forecasts and the actual data, these are discussed below:

**DON analysis method |** The DON tests were done using a commercial DON test – ELISA method. This approach uses a single replicate from the sample and has a minimum level of detection of 220ppb, with an accuracy of up to 10% over estimation. There are other testing options available e.g. DONCheck dip sticks that are able to detect done at 1ppm and can be used by inexperienced opperators, it basically gives a cut off and whether the sample is above or below that cut off. There are also HPLC m onoclonal antibody –based affinity chromatography tests that are able to detect DON at levels as low as 100ppb and give precise numerical results.

**Location |** The ADHB MRA identifies that there are different inherent mycotoxin risks associated with geographical location, with crops in Scotland having a lower risk of infection than those further south. The FIRC has no moderation of risk categories based on location, therefore when looked at overall, the FIRC forecast tends to include a proportion of mediumand high results from the wetter north and west, where the actual mycotoxin risk (according to AHDB MRA) is lower overall.

**Risk Categories |** The FIRC uses four different risk categories based on the level of rainfall at flowering. The AHDB MRA uses three categories – high, medium and low based on the score achieved across the whole assessment. There is nowhere that indicates how those risk categories might link to the final DON result, they merely indicate likelihood of infection or mycotoxin development, respectively. Likewise, it is unclear how a medium risk or high risk might actually map across to final DON results. Therefore, the alignment of those risks to the DON level has had to be done slightly arbitrarily.

The threshold for human consumption (1250 µg/kg) was taken as the high risk category, whilst a lower threshold of more than 500µg/kg was taken for mediumrisk. However, if the approaches were providing a good reflection of risk, it is expected that the higher the DON level, the higher the FIRC score or AHDB MRA score, or at least some form or alignment. However, based on the level of data collected in this project it was not possible to detect a statistically significant relationship between the scores and the eventual DON levels. This serves to highlight the complexity of the plant pathogen interaction and the fact that it cannot easily be simplified to one or two key variables that are driving infection and subsequent mycotoxin formation.

**Presence of inoculum |** The FIRC and AHDB MRA approaches both assume that there is inoculum present to cause infection. If there is no inoculum present, then even in the presence of perfect infection conditions and subsequent mycotoxin development conditions there will not be any mycotoxin formed. Spore data was provided by Rothamsted who currently have an ongoing project to monitor *Fusarium* spore numbers at three locations (Hertfordshire, Herefordshire and Lincolnshire) using spore traps (AHDB project RD-2140021). Equipment developed by their plant pathologists allows 10 litres of air to be sampled per minute and the DNA of airborne pathogens to be analysed, to test for Fusarium spores. This data pointed to low levels of *Fusarium* spores present in the air in 2017, which meant that although the weather conditions were ideal for fusarium infection, few inoculum were actually present.

**Weather |** The FIRC forecast uses weather data from the period immediately around anthesis to forecast the risk of fusarium infection, and at present only uses rainfall data. Fusarium infection is influenced by rainfall (accounted for by FIRC and AHDB MRA), but also temperature and humidity (not accounted for by FIRC and AHDB MRA) and all three need to come together to give greatest risk of fusarium infection. For example, temperatures above 25°C have been shown to cause greater disease severity (*F. culmorum)* (Wagacha and Muthomi, 2007) and high humidity (>80%) is also linked to increased fusarium infection risk (Popovski and Celar, 2012).

In 2015, weather conditions were not conducive to fusarium infection as conditions were generally dry during anthesis (average rainfall for England and Wales of 5 mm, Scotland 21 mm), humidity <90% and generally cooler air temperatures than optimal for the DON producing species *culmorum* and *F. graminearum.* This can be seen in the Met Office rainfall and temperature anomaly map below, which shows below average levels of rainfall during June and temperatures close to the long-term average (see figure 10.). In addition, the pattern of rainfall in 2015 meant that rain often fell in short heavy bursts, or where a small amount of rain fell each day and crops quickly dried out. This meant that in some situations even where the crop received over 10mm of rain (and therefore was in the mediumor high fusarium infection risk category) it may not have stayed wet for more than 24 hrs, which can influence fusarium infection risk (Xu et al., 2007), with a minimum wetness duration from 24 to 48 h required for infection, when temperatures range from 15 to 25 ºC.

In 2016, conditions were more conducive to fusarium infection during flowering; rainfall was generally above average in the east during June, but below average in July (and vice versa for northern regions (Figure 11), humidity during anthesis averaged between 83-84% in the UK and temperatures were generally warmer than expected for the time of year (Figure 11). According to research, these type of conditions should have favoured fusarium infection as this is more likely during periods of wet weather, high humidity (<90%) and warm temperatures around anthesis (Wagacha & Muthomi, 2007), with moist periods of over 24 hours increasing the risk of fusarium infection.

Crop year 2017 saw a very dry start to the year with warm temperatures prompting an early anthesis in wheat, starting in late May until early June (see figure 13 – anomaly maps for May 2017 are shown as well as June). However, the first two weeks of anthesis were wet, with regular often heavy showers affecting the whole country. Temperatures for the first half of June typically averaged 16˚C, rising to 21˚C in mid-June in parts of the South East, which was up to 6 ˚C warmer than the long-term average. Temperatures then fell during the last week of June returning to the mid-late teens.

Likewise, relative humidity (RH) ranged from 70-90% during the period, tending to be slightly higher in Wales and Scotland than England. This combination of rainfall, temperature and relative humidity created good conditions for fusarium infection. Despite this, data from spore traps reported low levels of airborne *Fusarium* spores present, so although the conditions were right for infection, there was little inoculum present to infect the plants.

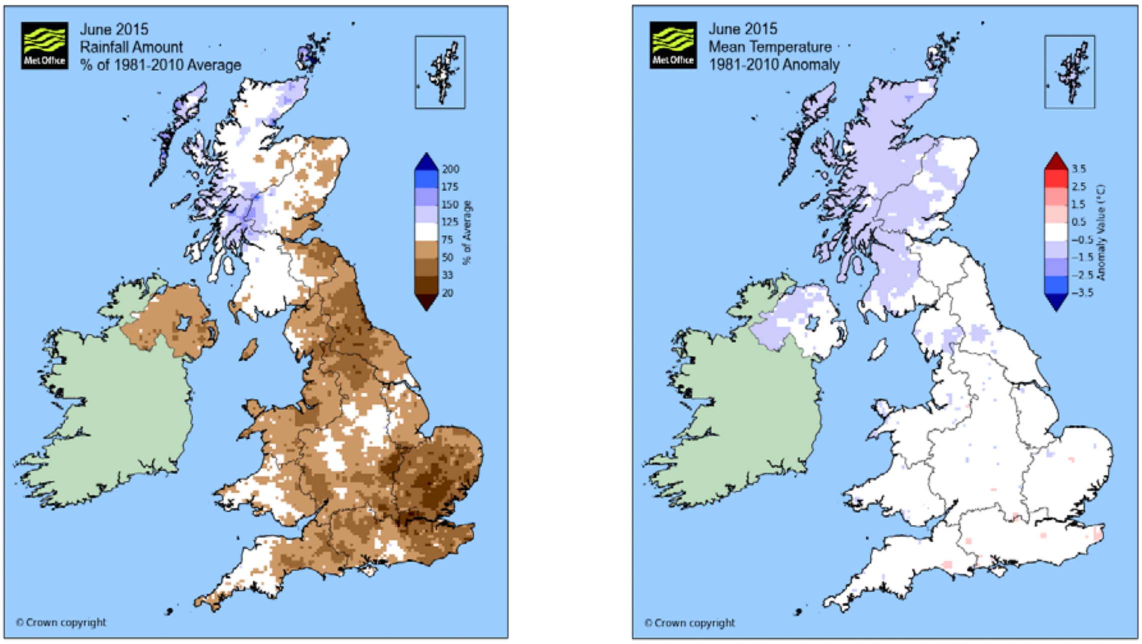


**Figure 11 Met Office rainfall and temperature anomaly maps for June 2015 compared to long term average (1981-2010).**

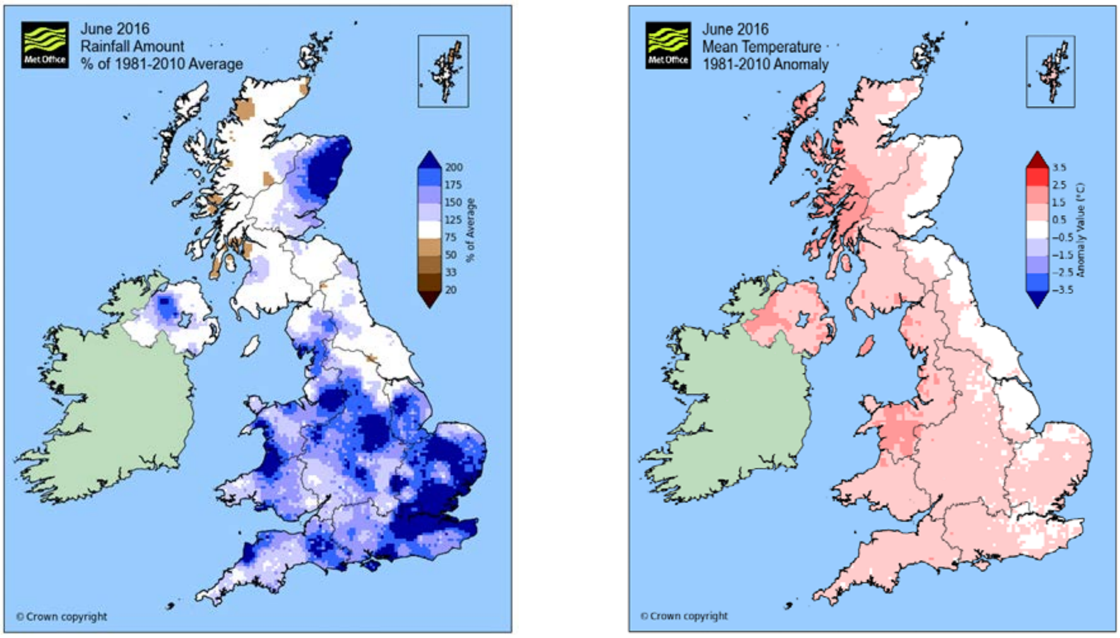


**Figure 12. Met Office rainfall and temperature anomaly maps for June 2016 compared to long term average (1981-2010)**

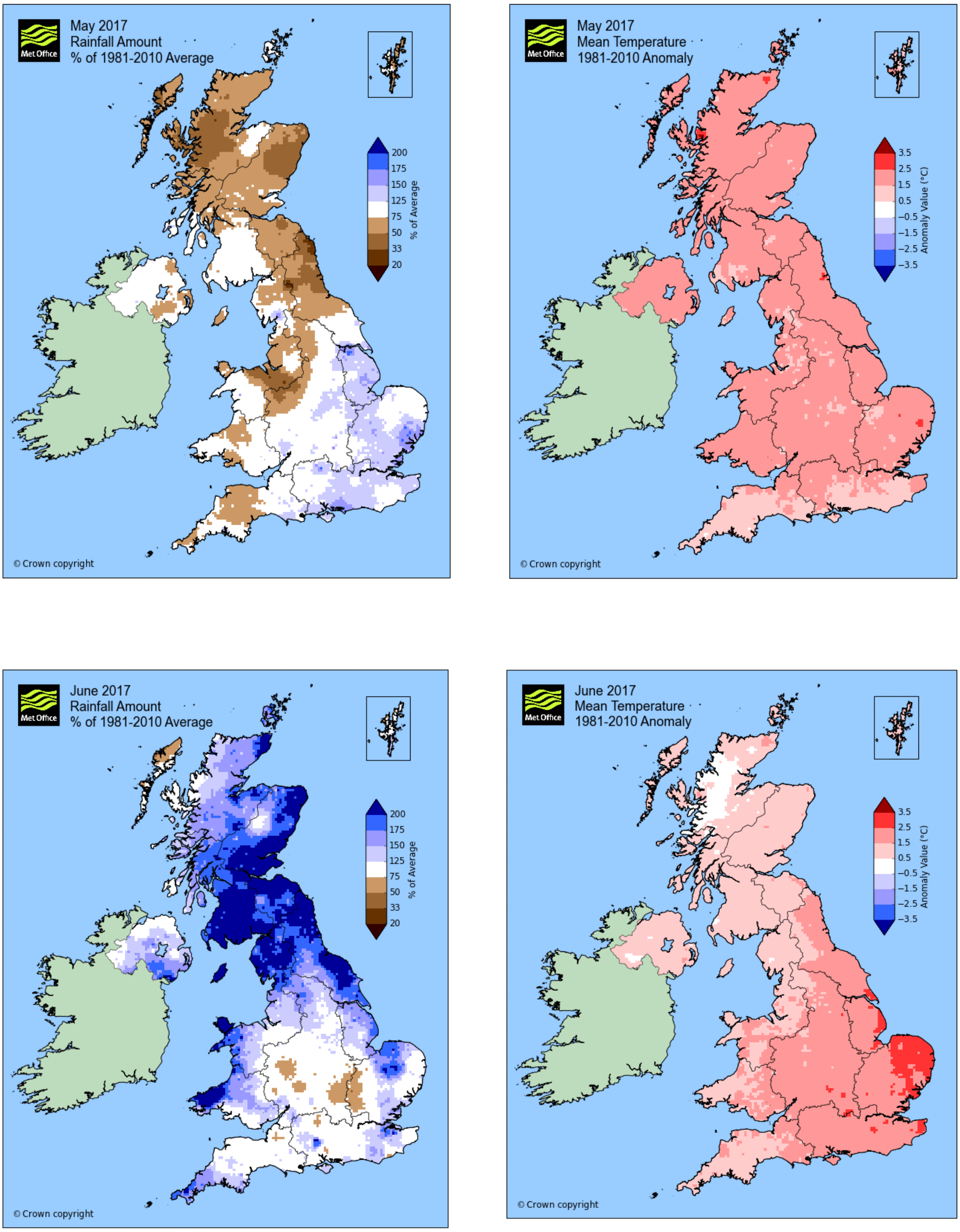


**Figure 13 Met Office rainfall and temperature anomaly maps for May and June 2017 compared to long term average (1981-2010)**

Results from the Defra Winter Wheat Disease Survey from 2016 do show that ear blight symptoms were significantly higher than 2015, with 84% samples and 21% ears affected, compared with 26% of samples and 2% ears affected in 2015. This is the highest incidence of ear blight since 2012, when 96% samples were affected. Likewise, date from the 2017 Wheat Disease Survey shows that although ear blight levels were lower than in 2016 (84% of samples and 21% of ears affected) this is also the second highest figure since 2012. However, it must be noted that the presence of ear blight is not a good indicator of likely mycotoxin risk in UK crops (AHDB, 2008), with the relationship between number of fusarium damaged grains and mycotoxin content not being consistent; grains may appear undamaged, but still contain mycotoxins and vice versa (FSA, 2007).

This work supports previous research that indicates DON levels tend to be low in UK winter wheat crops. In all three years it was not possible to identify a statistically significant relationship between either FIRC or AHDB MRA scores and actual DON levels.  There were only a modest number of grain samples (n=545) assessed in this validation process and full representation across the country was not possible. The results indicate at this stage that neither the AHDB MRA nor the FIRC risk scores correlate with actual DON results in low mycotoxin years, as both tend to overestimate the risk. This is especially the case when inoculum levels are low, as neither tool has a means of assessing this as part of the risk. In the few instances were DON levels were high (over the 1250µg/kg threshold) the FIRC was marginally more accurate at forecasting this correctly and in the case of mediumDON levels (between 500 and 1250µg/kg), the FIRC forecast all samples to be a mediumrisk of infection in 2017.

However, the usefulness of the FIRC in identifying infection risk is limited by the current assumptions (knowledge) available in the AHDB MRA.  At present, based on the relatively small number of grain samples assessed in this project the FIRC and AHDB MRA tend to overestimate risk, especially in years when inoculum levels are low (as they have no way of assessing this part of the risk). In the few instances where elevated or even high (over the 1250µg/kg threshold) DON levels were detected though, the two approaches tended to identify them as mediumrisk.

As mentioned, the FIRC only considers one of the many factors that influence the risk of mycotoxin development. Therefore, although, the FIRC demonstrates that from a practical point of view, it is possible to link live data collection on crop anthesis progress across the country with daily weather data and a more sophisticated model is needed, to allow accurate forecasting of mycotoxin risk. This would need to include wider factors such as associated crop risks, presence of inoculum, and the timeliness of fungicide sprays.  The FIRC could be made more useful to farmers by integrating other know factors such as the location risk, to mediumthe risk in lower-risk areas like the north. Similarly, post-anthesis rainfall could also be added in to increase accuracy, as well as data on relative humidity and temperature. Importantly, data on relative humidity and temperature was collected at the same time as the daily rainfall data meaning it would be possible to retrospectively add this into the FIRC to determine if it improved the accuracy of the forecasting, assuming that suitable parameters had been identified for each type of weather data.

Furthermore, if it were possible to link the FIRC to the spore trap data provided by Rothamsted this would provide even greater robustness. At present the Rothamsted spore trap results are discussed in the reports (i.e. highlighting low levels of spore present, even when rainfall is high), but the results are not included in the FIRC model and therefore, the risk score is not moderated by the data on spore numbers.

In the meantime, the information provided by FIRC helps farmers to recognise whether the rainfall events that they have experienced during anthesis are likely to be sufficient to cause infection and provides another tool to aid them in the decision making process around timing, rate and active to be applied at T3 earwash, to ensure that their crops are protected against infection.

## 6. References

AHDB (2015) Risk assessment for fusarium mycotoxins in wheat [online]. Available at: http://cereals.ahdb.org.uk/media/418930/is40-risk-assessment-for-fusarium-mycotoxins-in-wheat.pdf [accessed 15 December 2016].

Birck, N.M.M, Lorini, I, Scussel, V.M (2006) Fungus and mycotoxins in wheat grain at post-harvest, 9th International Working Conference on Stored Product Protection.

Defra (2016) Defra Winter Wheat Commercial Crops Disease Survey 2016 [online]. Available at: http://www.cropmonitor.co.uk/cmsReport.cfm?id=44 [accessed 15 December 2016].

Edwards, S (2007) Investigation of Fusarium mycotoxins in UK wheat production [online]. Available at: http://cereals.ahdb.org.uk/media/312444/pr413-final-project-report.pdf [accessed 15 December 2016].

Edwards, S.G (2009) Fusarium mycotoxin content of UK organic and conventional wheat. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 26(4):496-506.

Edwards, S.G (2011) Improving risk assessment to minimise fusarium mycotoxins in harvested wheat grain, AHDB Project Report 447 [online]. Available at: http://cereals.ahdb.org.uk/media/308076/pr477-final-project-report.pdf [accessed 15 December 2016].

ELISA Technologies (2018) http://www.elisa-tek.com/diagnostic-testing-kits/mycotoxins/don-eia/

EU (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs [online]. Available at:

http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF [accessed 15 December 2016].

FSA (2007) The UK Code of Good Agricultural Practice to Reduce Fusarium Mycotoxins in Cereals [online]. Available at: http://www.food.gov.uk/sites/default/files/multimedia/pdfs/fusariumcop.pdf [accessed 15 December 2016].

Popovski, S and Celar, F.A (2012). The impact of environmental factors on the infection of cereals with Fusarium species and mycotoxin production – a review. *Acta agriculturae Slovenica*, 101 - 1

Wagacha J.M. and Muthomi J.W. (2007) *Fusarium culmorum*: Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. *Crop Production*. B26 (7): 877-885.

Xu X.M., Monger W., Ritieni A., Nicholson P. (2007) Effect of temperature and duration of wetness during initial infection periods on disease development, fungal biomass and mycotoxin concentrations on wheat inoculated with single, or combinations of, Fusarium species. *Plant Pathology*, 56, 943-956.