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Investigation of HT2 and T2 mycotoxins in oats from the 2014 harvest

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1. Abstract

The European Commission published a Recommendation in 2013 on the fusarium mycotoxins HT2 and T2. The Recommendation states that Member States, in collaboration with industry, should monitor the levels of these mycotoxins in cereals and cereal products and where levels are detected above published indicative limits of the combined concentration of HT2 and T2 (HT2+T2), investigations should be conducted to determine why the indicative limits were exceeded and to determine the measures to be taken to avoid or reduce such exceedances in the future.

In 2014, oat millers detected exceedances of the indicative limit for unprocessed oats (1000 ppb HT2+T2) in oat samples analysed at mill intake. In collaboration with the Food Standards Agency (FSA), AHDB Cereals & Oilseeds and the British Oat and Barley Millers Association (BOBMA) funded the investigation detailed in this report. The aim of the investigation was to identify why exceedances had occurred and what mitigation could be used to avoid such exceedances occurring in the future.

BOBMA sent details of 113 oat consignments for which HT2 and T2 analysis had been completed. Details included the growers' location and the variety, if reported. Growers were contacted for further agronomic information and details were provided for a subset of 38 samples. There was limited traceability of the associated agronomy for these oat samples due to the storing of oats from multiple fields in unsegregated stores. This reduces the ability of a retrospective investigation, such as this study, to determine the factors that influenced the concentration of HT2 and T2 in harvested oats.

The average HT2+T2 level of samples was 804 ppb and 26% of samples exceeded the indicative limit of 1000 ppb. The low number of samples limited the statistical strength of the investigation, however statistical differences in location (defined at country or regional level), oat type (winter/spring), variety and crop rotation were detected. For regional differences there were higher concentrations in Scotland and in particular in northern Scotland. Fluctuations between years and regions indicate that weather is a key determining factor of HT2+T2 levels in UK cereals. There was a higher concentration in winter oats compared to spring oats and a significant effect of rotation with an increasing mean HT2+T2 with increasing numbers of cereals in the previous 4 years of the rotation.

Overall, results are in agreement with previous studies on the impact of agronomy on the HT2+T2 content of UK oats. Another study that considered the economic implications of mitigation strategies to reduce HT2+T2 content of UK oats by reducing the intensity of cereals in a rotation and/or a switch from winter to spring oats identified that these were currently not realistic or viable options for wide spread adoption. There should be careful consideration as to what is reasonably achievable as a strategy for the mitigation of HT2 and T2 risk in UK oat production.

2. Introduction

Mycotoxins are secondary metabolites of fungi that can be present on cereals as a result of plant diseases in the field or crop spoilage in store. The fusarium mycotoxins are produced during infections of cereal crops in field. *Fusarium langsethiae* has been identified as the primary producer of the mycotoxins HT2 and T2. These mycotoxins are the predominant fusarium mycotoxins that occur on UK oats.

The European Commission published a Recommendation in 2013 on the presence of HT2 and T2 in food and feed (Anon, 2013). This Recommendation set indicative levels for the combined concentration of HT2 and T2 (HT2+T2) in unprocessed cereals, intermediate and finished products with a requirement that Member States, in collaboration with industry, monitor the presence of these mycotoxins and where indicative levels are exceeded conduct an investigation to determine why these exceedances occurred and what mitigation measures can be used to avoid future exceedances. The indicative limit for HT2+T2 in unprocessed oats is 1000 parts per billion (ppb, µg/kg).

After the harvest of 2014, members of the British Oat and Barley Millers Association (BOBMA) reported exceedances of the indicative level of 1000 ppb HT2+T2 in a number of samples of oats during random sampling of oat consignments at mill intake. Consequently, it was decided by a stakeholder group to instigate an investigation of the cause of these exceedances with funding from BOBMA, the Food Standards Agency and AHDB Cereals & Oilseeds which was conducted by Harper Adams University in collaboration with Fera.

3. Materials and methods

Members of BOBMA were asked to provide contact details of suppliers of all oat samples that had been analysed for HT2 and T2 at mill intake in 2014/15. A questionnaire was prepared to determine all the agronomic information pertaining to each sample (Appendix 1). Growers were contacted by telephone, e-mail and/or letter and advised of the investigation and asked to cooperate with the provision of agronomic details pertaining to the oats delivered to mill intake.

On receipt of agronomic data, it was inputted into an Excel spreadsheet and additional factors calculated, such as the number of continuous cereals previous to the oat crop, the number of oat crops grown in the previous four years and the number of cereals grown in the previous four years. The Limit of Quantification (LoQ) of the analysis was 10 ppb for each toxin; samples below the LoQ were reported as <20 ppb. For calculation of summary statistics and regression analysis samples below the LoQ were inputted as 20 ppb.

The data was analysed as two datasets. Firstly location and type (winter or spring) and location*type interaction was analysed for all 113 samples using Generalized Linear Models after log10 transformation (Genstat v.17). Location was analysed at two levels – country (2 levels) and region (9 levels).

Secondly, the subset of 38 samples for which completed questionnaires were provided was analysed using Generalized Linear Models after log10 transformation (Genstat v.17) with stepwise addition of each agronomic factor. Based on the geographic distribution of samples and the results of the 113 sample dataset, this subset was split into 3 regions: North England and South Scotland combined, North Scotland and East Scotland. Agronomic factors were added to the model in chronological order of when the agronomy would occur. Region was placed at the front of the model to account for spatial variation first. Host was analysed at two levels; firstly by type (winter or spring) and secondly by variety for all varieties that were represented by more than 5 samples.

For all factors that were determined to be significant from the Generalized Linear Models the predicted mean and 95% confidence limits were calculated and presented. The predicted mean is the mean value that would be expected to occur from the model if there were an equal number of levels in the other factors within the dataset eg the predicted mean for each variety is weighted as if there was an equal number of each variety from each region. This reduces the biases within the dataset due to its unbalanced structure.

Rainfall data was collected from the nearest meteorological station for all samples with completed questionnaires. The start of flowering was predicted based on the limited dates on which growth stages close to flowering were recorded. Total mm of rainfall was determined for the 2 weeks prior to this date for winter and spring oat samples, when the crops would have been in the panicle emergence stage and for the 2 week period starting 4 weeks prior to flowering, when the crops are predicted to have been in the booting stage. HT2+T2 concentration was correlated to total rainfall for these 2 week periods prior to flowering and prior to panicle emergence for the winter and spring oat samples using linear regression after log10 transformation (Genstat v.17).

4. Results

BOBMA supplied mycotoxin results and suppliers details for 113 oat consignments delivered to their mills. Grower's location was detailed for all samples; 46 samples were of a known variety, 8 were of mixed variety and 56 had no recorded variety. Summary statistics for the complete dataset are detailed in Table 1. The mean HT2+T2 concentration was 804 ppb with a maximum of 5813 ppb; 26% of samples exceeded the indicative level of 1000 ppb HT2+T2. The frequency distribution (Figure 1) shows a peak just above the LoQ (20–125 ppb), a left-sided truncation at the LoQ and a highly skewed right-handed tail extending beyond 4000 ppb with a second peak at the 500–1000 ppb range.

Table 1. Summary statistics of HT2+T2 concentration (ppb) of oat samples from England and Scotland 2014 harvest.

Country	Number	Mean	Median	Min	90%ile	95%ile	Max	%>1000
All	113	804	304	<20	2135	3056	5813	26
England	39	440	150	<20	1086	1567	3060	13
Scotland	74	996	534	<20	2388	4652	5813	32

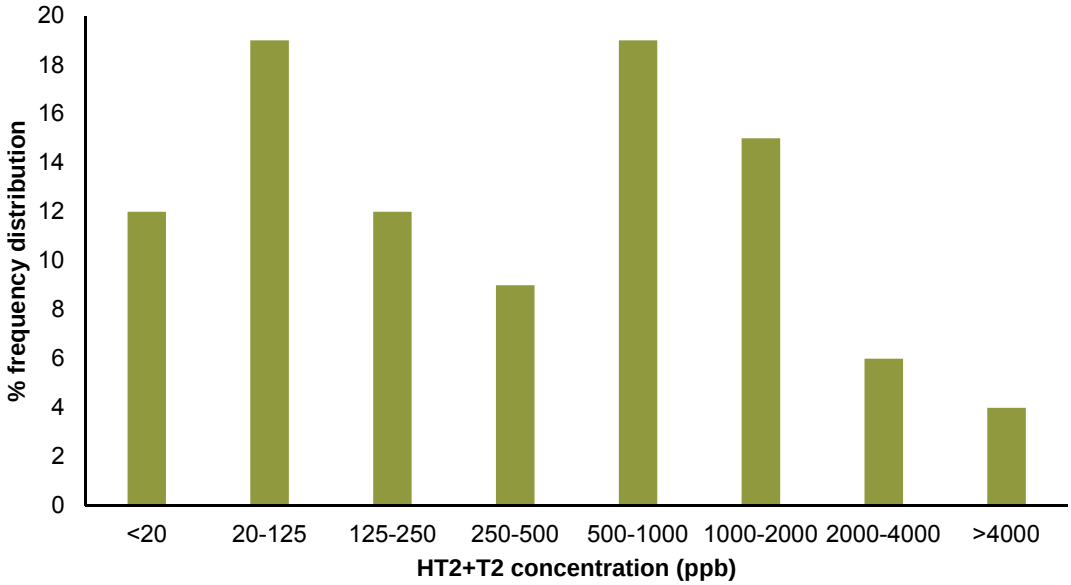

Figure 1. Frequency distribution of HT2+T2 concentration for 113 samples of oats analysed at mill intake from the 2014 harvest.

From the complete dataset of 113 samples, 17 samples were delivered via grain traders. The information for these samples were not pursued due to potential issues of trader/grower confidentiality. Growers who supplied the remaining 96 samples were contacted directly either by telephone or by letter. Thirty eight questionnaires were completed (40% return). This return is poorer than the targeted response (60%); this is likely due to the timing of the project which started in April 2015. This resulted in the questionnaires being requested when growers were busy with

spring field work. One query from a grower when contacted by telephone was “Why you asking me now when I am busy and not two months ago?”

One issue with traceability of samples is that most samples (35) were delivered from a store containing oats from two or more fields which had a similar but different agronomy. For example, some fields had different soil types and/or different cropping histories. Such samples were reported as “MIXED” for any agronomic factor which differed between two or more fields.

Of the 38 samples for which agronomy data was received, 6 were from North England and 32 were from Scotland. There were 24 spring variety samples, 13 winter variety samples and one sample from a store with a mixture of a winter and a spring variety.

Growth stages: Dates for oat growth stages (Tottman, 1987) close to flowering were provided for 8 winter oat samples and 3 spring oat samples. From these the average start of flowering (GS61) was predicted to be on the 17th and 30th June 2014, respectively. These dates were compared to dates recorded for early panicle emergence (growth stage 51–55) for Scottish Agronomy field trials performed in the Borders and Fife in 2014 (Adam Christie, Scottish Agronomy, pers. comm.) and appeared to be consistent.

Location: For the complete dataset of 113 samples, the location and type (winter or spring) were significant but there was no location*type interaction ($p=0.21$). Using country as the location factor was significant ($p=0.004$) and accounted for 11% of the variance within the dataset with Scotland having a higher predicted mean than England. Summary statistics for the English and Scottish samples are detailed in Table 1.

Using region as the location factor was highly significant ($p<0.001$) and accounted for 43% of the variance within the dataset. Figure 2 shows the predicted mean HT2+T2 concentration for each region. The figure is on a log scale as the large range of predicted means (28 – 6998 ppb) are not visible together on a linear scale. Due to the uneven distribution of samples across the regions there is a large difference in the confidence limits with the extremes been South and East Scotland with 4 and 58 samples, respectively. As can be seen in Figure 2 there is variation in the mean HT2+T2 concentration across the regions within each country with higher levels in South England compared to the Midlands and North East England. The South Scotland mean is similar to North East England with ever increasing predicted means in a northern direction through Scotland and the North West of England mean is similar to West Scotland.

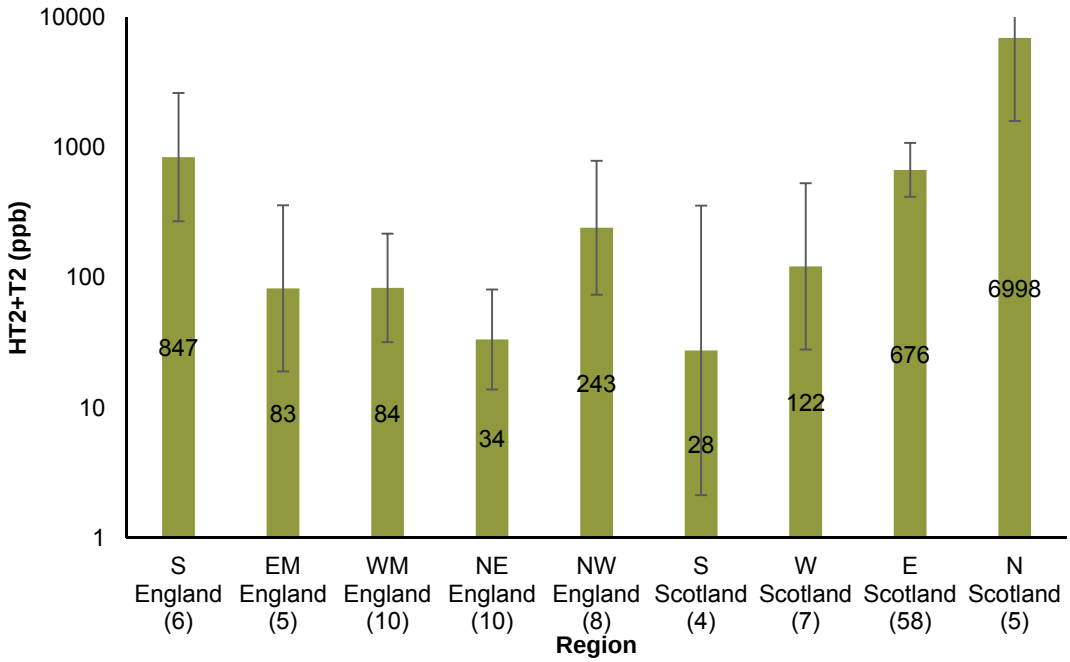

Figure 2. Predicted mean HT2+T2 concentration for each region. Values above each column are the predicted means. Bars represent the 95% confidence limits for the predictions. EM and WM are East and West Midlands, respectively. Numbers in parenthesis are the number of samples from each region.

Oat type and variety: The mean concentration of HT2+T2 in winter oats was significantly ($p<0.001$) higher than for spring oats with the type of oat grown accounting for an additional 12% of the variance. Table 2 gives the summary statistics for winter and spring oats. If variety was used as the host factor rather than type it was significant ($p=0.014$) and accounted for an additional 14% of the variance. Figure 3 shows the predicted means for varieties represented by more than five samples within the dataset. As can be seen from Figure 3, all varieties have high confidence limits but the spring varieties, Atego, Canyon and Firth, have lower mean HT2+T2 concentrations than the winter oat Gerald, and the winter oat Mascani has an intermediate level of HT2+T2.

Table 2. Summary statistics of HT2+T2 concentration (ppb) of winter and spring oat samples from the 2014 harvest.

Type	Number	Mean	Median	Min	90%ile	95%ile	Max	%>1000
Winter	35	1046	881	<20	3030	3900	4770	40
Spring	32	919	261	<20	2901	5461	5813	25

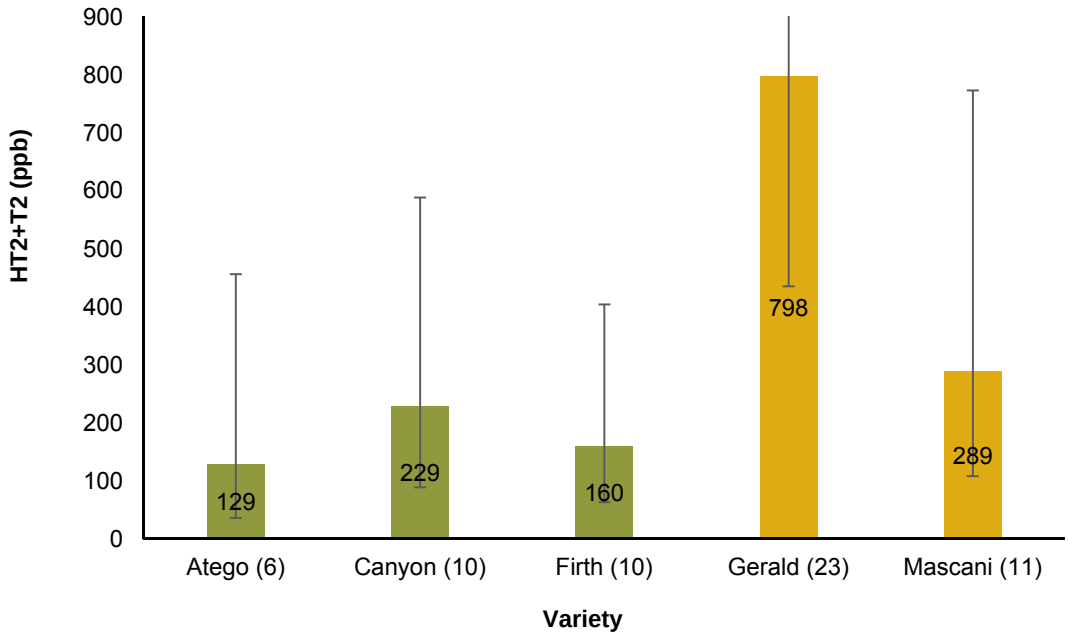

Figure 3. Predicted mean HT2+T2 concentration (ppb) for each variety of oat from the 2014. Only varieties represented by more than 5 samples are included. Green and yellow columns represent spring and winter varieties, respectively. Values above each column are the predicted means. Error bars indicate the 95% confidence limits. Numbers in parenthesis are the number of samples within each category.

Rotation: For the subset of samples with completed agronomy questionnaires, the only additional factors after region and type that were significant were the “number of previous cereal crops in the last 4 years” and “number of consecutive cereals in last 4 years” ($p<0.01$). As these two factors were confounding, the “number of previous cereals in last 4 years” was used alone as a polynomial sub-model as it accounted for the greatest additional variance (20%). Figure 4 below shows the predicted mean HT2+T2 concentration for samples grouped by the number of cereal crops in the previous 4 years of the rotation. As only 4 years of previous crop data was collated, the category with 4 years of previous cereal cropping includes samples with more than 4 years of cereals as the previous crop. Figure 4 shows a linear increase in HT2+T2 concentration with each increasing number of cereals in the rotation prior to the oat crop.

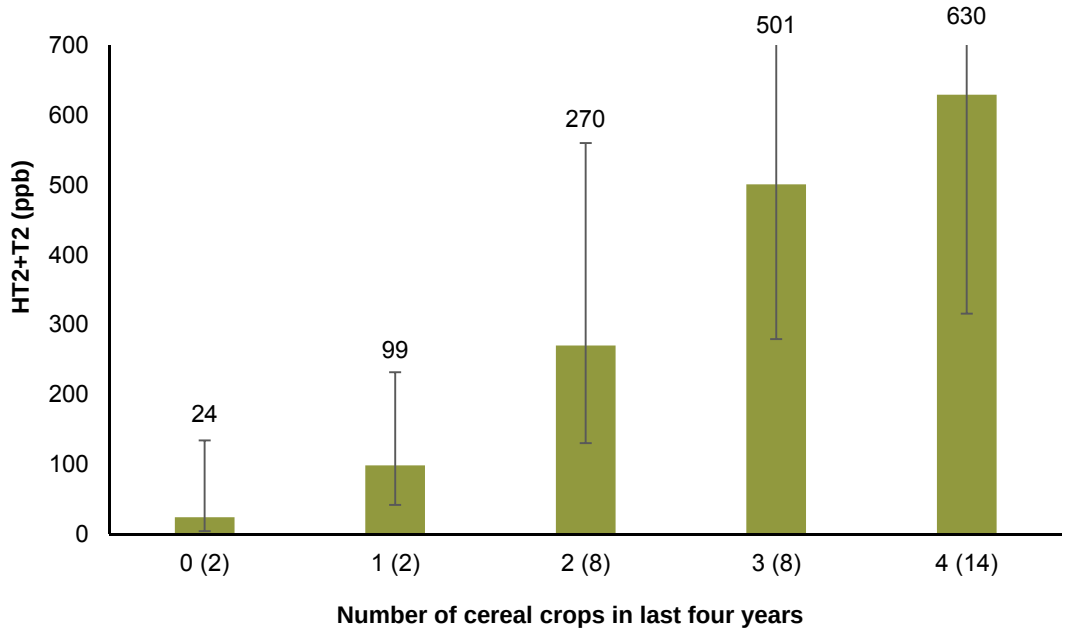

Figure 4. Predicted mean HT2+T2 concentration (ppb) of oat crops from the 2014 harvest grouped by the number of previous cereal crops in the preceding 4 years. Values above each column are the predicted means. Error bars indicate the 95% confidence limits. Numbers in parenthesis are the number of samples within each category.

Weather data: Correlation of HT2+T2 concentration (log10 transformed) against total rainfall for the 2 weeks during the predicted booting and panicle emergence growth stages showed a significant positive relationship for the booting stage for winter varieties ($p=0.025$; $r^2=0.35$; Figure 5). No other correlation was significant ($p>0.2$; $r^2<0.1$).

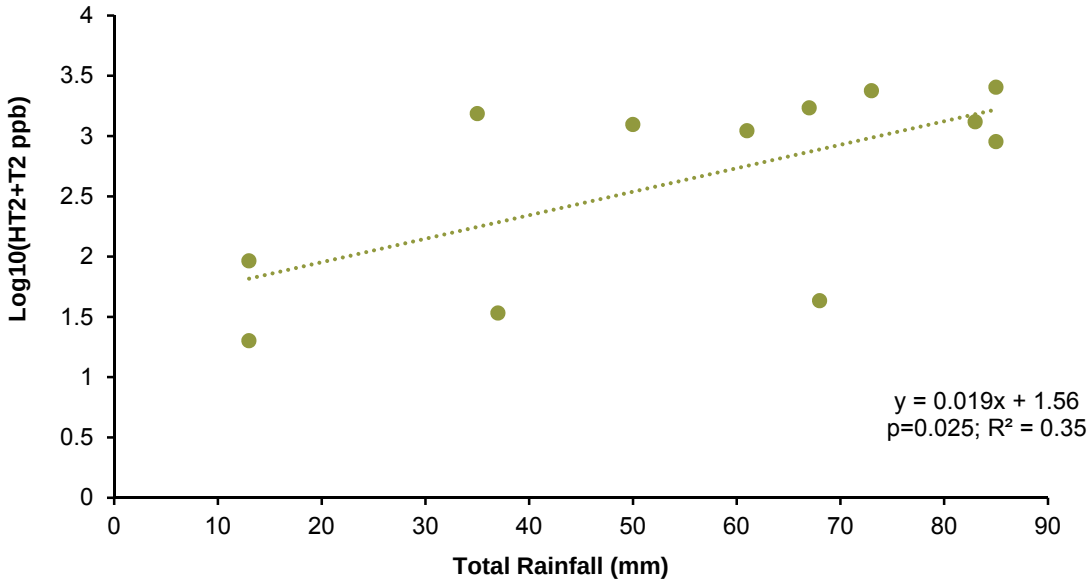

Figure 5. Correlation between total rainfall (mm) for the predicted panicle emergence growth stage period (2–16th June 2014) and log10 transformed HT2+T2 concentration (ppb) for winter oat samples harvested in 2014.

5. Discussion

This investigation is based on a limited number of samples (n=113 for all samples analysed and n=38 for the subset with agronomy data) and as such, the statistical power of any analysis to determine the impact of agronomy is limited. All samples were analysed at mill intake, and as there is no ability to assess the risk of high HT2+T2 prior to analysis, the samples were not biased towards high risk samples. The overall mean concentration of HT2+T2 was 804 ppb and 26% of samples exceeded 1000 ppb. In comparison to previous surveys of commercial oat crops (2002–2008) (Edwards, 2012), this level of HT2+T2 is similar to 2006 and was exceeded in 2003 and 2005. There has been no large scale survey of commercial oat samples in the UK since 2008. The HT2+T2 concentration in oats appears to be highly seasonal with samples exceeding 1000 ppb ranging from 1–30% from 2002–2008 (Edwards, 2012).

This investigation has also highlighted the limited traceability of cereal consignments delivered to mills. For some oat mills the variety is not reported and although the growers' details are known, due to the storing of oats from multiple fields in unsegregated stores, it is frequently not possible to determine all the associated agronomy applied to an oat consignment once it has entered a store. This reduces the ability of retrospective investigations to determine the factors that have influenced the concentration of HT2 and T2 in harvested oats.

Region was highly significant and showed a higher risk of HT2+T2 in South England and in North Scotland. Previous studies have shown a significant region*year interaction for samples collected between 2002 and 2005 (Edwards, 2007) and significant differences between year and region but no year*region interaction for samples collected 2006–2008 (Edwards, 2012). Results indicate that the relative concentration of HT2+T2 across regions in the UK is not static, they vary between years, and samples exceeding 1000 ppb HT2+T2 have occurred in all regions across the UK in some years. This temporal and spatial variation would indicate that weather is a key determinant of the HT2+T2 concentration in harvested oat samples. The impact of weather on *Fusarium langsethiae* infection and subsequent HT2+T2 production is not clearly understood and much less is known compared to *F. graminearum* infection and subsequent deoxynivalenol production in wheat. A window pane analysis of weather parameters identified that HT2+T2 content of UK oats was positively correlated to rainfall in May and drier weather later in the season (Xu *et al.*, 2014). In this previous study, samples were predominantly from England and this time period would correspond to booting and panicle emergence (growth stages 41–59). Within the current study, correlation analysis was attempted for the total rainfall during these predicted growth stages within Scotland (as Scottish samples dominated the dataset). The only significant correlation identified was for rainfall in the 2-week period that was predicted to be the panicle emergence growth stage for winter oats. The lack of other correlations is likely to be due to the poor estimate of when growth stages occurred, due to the lack of recording of growth stages for oats during this time

period and the range of growth stages that occur across the spatial range of samples (North England to North Scotland). Observation of the weather data collated showed a day of heavy rainfall in early June (ca. 50 mm) followed by a wet period of ca. 10 days. This may well have allowed a large level of splash dispersal of spores followed by spore germination and infection at a critical, as yet determined, susceptible growth stage for oats.

The host factor of either oat type or oat variety were significant factors with spring varieties having lower HT2+T2 compared to winter varieties. This matches the previous surveys (Edwards, 2007; Edwards, 2012) and although there were limited significant differences between the individual varieties due to the low number of samples, the trends observed, with little difference between the spring varieties and Gerald having a higher mean HT2+T2 compared to Mascani for the winter varieties, match the differences observed in AHDB Recommended List trials (Edwards, 2015). One difference in 2014 compared to previous studies, is that the spring oat samples had a greater skewed distribution with a higher maximum HT2+T2, resulting in much lower median value but a similar mean concentration to winter oats of ca. 1000 ppb HT2+T2.

Due to the small number of samples for which additional agronomic data was collated (38) there was limited statistical strength in the analysis. Of the agronomic factors that were collated, only the intensity of cereal crops in the rotation were significant and this factor accounted for 20% of the additional variance. With an increasing number of cereal crops in the previous rotation there was a linear increase in the mean HT2+T2 concentration. This trend is similar to that observed previously (Edwards, 2012). It is hypothesised that long continuous cereal rotations has resulted in a build-up of *Fusarium langsethiae* inoculum, resulting in particular fields are at high risk of high HT2 and T2 concentrations.

Weather is a key risk parameter and modification of agronomic practices can only reduce the risk rather than eliminate the risk. Based on the results obtained, it can be recommended that to reduce the risk of exceeding the indicative level of 1000 ppb HT2 and T2 in UK oat crops, farmers can be advised to grow spring oats instead of winter oats and to grow oats in rotation with non-cereal crops. Unfortunately, there are large economic barriers to such a strategy. A previous study identified that the additional yield obtained from winter oats compared to spring oats is ca. 1 tonne/ha (Edwards & Wilkinson, 2012). Oats are primarily grown as a break crop for the more profitable cereals of wheat and barley. Farmers are highly unlikely to grow oats in rotation with non-cereals unless the value of oats increased considerably. Therefore it is important to carefully consider what is reasonable achievable as a strategy for the mitigation of HT2 and T2 risk in UK oat production.

In summary:

- Indicative levels for the combined concentration of HT2 and T2 (HT2+T2) for cereals and cereal products were set by the European Commission in 2013.
- The indicative level for unprocessed oats is 1000 ppb HT2+T2.
- Where the indicative level is repeatedly exceeded an investigation should be conducted to identify why the exceedances occurred and what mitigation can be used to reduce such exceedances in the future.
- Repeated exceedances of 1000 ppb HT2+T2 were detected in UK unprocessed oats from the 2014 harvest.
- There were regional differences with higher mean concentrations of HT2+T2 detected in the South of England and the North of Scotland.
- There was a higher mean concentration of HT2+ T2 in winter compared to spring oats although both types had samples which exceeded the indicative level for unprocessed oats.
- There was a stepwise increase in the mean concentration of HT2+T2 with the increasing number of cereals in rotation prior to growing the oat crop.
- There was limited data to align weather data to oat crop growth stages.
- There was a weak positive correlation with rainfall during the predicted panicle emergence growth stage of winter oats and HT2+T2 concentration.
- No correlation could be determined between rainfall and HT2+T2 concentration for spring oats.
- Proposed mitigation strategies to reduce the risk of exceedances of HT2+T2 based on this investigation are a switch from production of winter to spring oats and the production of oats in less cereal intense rotations.
- There are large economic barriers to growers adopting the suggested mitigation strategies.

6. References

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Appendix 1 – Grower Questionnaire

HT2+T2 Mycotoxin 2014 Harvest Investigation

(circle answer where applicable. If more than one field then detail answers for field A., B. etc.)

Sample Code:

1. Is this oat consignment traceable to: ☐ **a single field**
   ☐ **multiple fields with same agronomy**
   ☐ **multiple fields with different agronomy**

2. Is farm postcode appropriate to identify field(s) location? **Yes** **No**

If no, then please provide location (postcode or village) _____

3. Variety: _____

4. Soil type: _____

5. Previous crop:	**2012/2013**	**2011/2012**	**2010/2011**	**2009/2010**
Wheat	_____	_____	_____	_____
Barley	_____	_____	_____	_____
Oats	_____	_____	_____	_____
Sugar Beet	_____	_____	_____	_____
Potatoes	_____	_____	_____	_____
Oil seed rape	_____	_____	_____	_____
Peas	_____	_____	_____	_____
Beans	_____	_____	_____	_____
Grass	_____	_____	_____	_____
Maize	_____	_____	_____	_____
Set-aside	_____	_____	_____	_____
Other (please state)	_____	_____	_____	_____

6. Previous crop debris treatment: **baled** **chopped** **nothing**

7. Cultivation technique: **ploughed** **minimum cultivation** **direct drilled**

Is cultivation technique same across rotation? **Yes** **No**
if no, record between previous crops in answer 5. Above (p, mc or dd)

8. Sowing Date (D/M/Y): _____ (estimate?)

9. Seed treatment: _____

10. Field Margin Management:

What management: _____

What width: _____

11. Any game cover crops on boundary? **Yes** **No**

If yes, what crop mix: _____

12. Fungicides used	Rate	Date(D/M/Y)	Growth Stage

13. Any recorded growth stage in May or June? GS: _____ Date: _____

14. Do you remember any specific issues with this crop? **Yes** **No**

If yes please give details

Disease issues:

Pest Issues:

Weed issues:

Lodging issues:

15. Harvest date (D/M/Y): _____ (Estimate?)

16. Crop % Moisture content at Harvest: _____ (Estimate?)

17. What date grain dried to below 15%MC (D/M/Y): _____ (Estimate?)

18. Do you remember any specific storage issues? **Yes** **No**

If yes please give details:

19. Contact details for additional information if required (mobile or e-mail?):

_____