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Project Report No. 547

Fusarium mycotoxins in UK oat varieties – monitoring in preparation for legislation

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CONTENTS

1. Abstract

The aim of this project was to monitor the concentration of the fusarium mycotoxins HT2 and T2 in oat samples collected from the AHDB Recommended List trials from 2012, 2013 and 2014. The European Commission will consider legislation for these mycotoxins in 2015. If legislative limits are set then it will be necessary for growers to reduce the levels of these mycotoxins in harvested cereal grains intended for human consumption. Previous studies have identified that high concentrations of these mycotoxins can occur in harvested oat grains in the UK and that one of the limited mechanisms to reduce the levels of HT2 and T2 is through the use of more resistant varieties.

Results showed that the current indicative level of 1000 ppb for HT2 and T2 combined (HT2+T2) was exceeded in one of 14 spring oat trials and in nine of the 18 winter oat trials conducted between 2012 and 2014. The trends were similar to previously reported results with spring oats routinely lower than winter oats although some high levels occurred in one spring oat trial in 2014. For spring oats there were small but statistically significant differences between varieties whilst for winter oats they had a broader and higher range of HT2+T2 levels compared to spring oat trials. For winter oats, the short-strawed variety, Balado had consistently high HT2+T2 levels compared to other varieties and naked varieties, which were consistently low. Several new varieties were at the low end of the HT2+T2 range with Maestro having the lowest mean of 218 ppb HT2+T2, which was ca. four-fold lower than the mean for Balado.

The method adopted within this project to normalise the dataset by using the varietal value as a percentage of the average of standard “control” varieties is used for other Recommended List parameters and allows for varieties that are only present in a limited number of years to be compared to varieties in trial in other years.

This method has allowed the host resistance against HT2+T2 producing *Fusarium* species of new oat varieties entering Recommended List to be determined and ensure accurate and complete information on the comparative resistance of UK Recommended List oat varieties is available if or when legislation is set.

2. Introduction

HT2 and T2 are related trichothecene fusarium mycotoxins produced by *Fusarium langsethiae* (Edwards *et al.*, 2012). This pathogen is not a typical Fusarium Head Blight pathogen in that it does not produce symptoms on infected crops or grains (Imathiu *et al.*, 2013). Studies have determined that this recently identified species can result in high levels of HT2 and T2 in UK oats but not wheat and barley (Edwards, 2009c; Edwards, 2009b; Edwards, 2009a).

The European Union set legislative limits for the fusarium mycotoxins, deoxynivalenol and zearalenone in 2006 (European Commission, 2006). Since 2006, legislative limits for HT2 and T2 have been discussed. In 2013 the European Commission published a recommendation for the continued monitoring of HT2 and T2 by Member States in collaboration with industry (European Commission, 2013). The recommendation includes indicative levels for the combined concentration of HT2 and T2 (HT2+T2) in various cereals and cereal products. Where these indicative limits (1000 ppb for unprocessed oats) are exceeded then investigations should be performed to identify why they have occurred and what mitigation can be implemented to avoid such exceedances in the future. Based on previous surveys of UK commercial oat crops, the proportion of samples exceeding the indicative limit for unprocessed oats (1000 ppb) varied between 1% and 30% each year, with a mean of 16% (Edwards, 2012).

The analysis of the impact of agronomic factors on the HT2 and T2 concentration of commercial oat fields in the previous AHDB Cereals & Oilseeds-funded projects (Edwards, 2007; Edwards, 2012) identified significant differences between HT2+T2 concentration in oat varieties and these differences have been consistent over time and consistent with results from AHDB Recommended List (RL) trials. However, RL trials allow more varieties to be compared under uniform experimental conditions across several years and locations. Previous analysis of HT2 and T2 from RL trial samples identified that spring varieties had small but statistically significant differences between them (e.g. a range of 77–104 ppb HT2+T2 for 2009–2011 RL trials). For winter varieties, the overall mean was higher than spring varieties and they had a wider range (e.g. a range of 261–1312 ppb HT2+T2 for 2009–2011 RL trials). Naked varieties had less HT2 and T2 than conventional husked varieties. This has been reported previously (Edwards, 2012) and is thought to occur as most of the HT2 and T2 are present on the husk (Scudamore *et al.*, 2007), which is removed from naked oat varieties during harvest. Short-strawed varieties had higher levels of HT2 and T2 and naked short-strawed varieties had intermediate levels. Short-strawed varieties may have higher concentrations of HT2 and T2 as they are nearer to the source of *Fusarium* inoculum at ground level, or there may be some genetic linkage between dwarfing genes and susceptibility to HT2 and T2-producing *Fusarium* species. Such linkage has been shown for some dwarfing genes in wheat (Srinivasachary *et al.*, 2008). Of the current conventional husked varieties, Gerald and Balado have had consistently high levels of HT2 and T2 and Dalguise has had consistently low levels (Edwards, 2012).

The aim of this project was to monitor the HT2+T2 concentration in UK oat Recommended List trials from the 2012, 2013 and 2014 harvests. This would allow the host resistance against HT2+T2 producing *Fusarium* species of new oat varieties entering RL to be determined and ensure accurate and complete information on the comparative resistance of UK RL oat varieties was available if or when legislation is set.

3. Materials and methods

3.1. AHDB Recommended List oat samples

Each year (2012-2014), single block samples (1 kg) from each plot were collected from each AHDB Recommended List treated (+fungicide, +PGR) oat variety trial from across the UK. On receipt of samples they were milled with a 1 mm screen, mixed in a tumbler mixer before a 200 g laboratory sample was collected. Samples were analysed using Ridascreen T2 ELISA kits (R-Biopharm, Glasgow). Based on the known ratio of HT2 to T2 in UK oat samples from a previous project and the known cross-reaction of the T2 antibody with HT2, the concentration of HT2+T2 was estimated (Edwards *et al.*, 2012).

3.2. Statistical analysis

The effect of variety was tested for winter and spring oats separately using ANOVA with trial site as a block factor. HT2+T2 concentrations were log10 transformed to achieve normally distributed residuals. For both winter and spring trials the datasets for each year were analysed individually. Predicted mean HT2+T2 concentrations were compared using LSD (p=0.05). To analyse all years together the HT2+T2 concentration variation between trials was normalised by adjusting to a percentage value compared to the mean log10 HT2+T2 concentration of the three control varieties. This removed any bias that may occur due to a variety only occurring in RL trials in a high or low HT2+T2 year. After analysis %log10 values were back transformed to HT2+T2 concentrations (ppb). The control varieties for spring oats were Canyon, Firth and Rozmar, and for the winter oats were Dalguise, Gerald and Mascani.

4. Results

Table 1 details the number of sites and varieties supplied from RL spring and winter oat trial sites from the 2012 to 2014 harvest.

Table 1. Number of sites and varieties for winter and spring oat RL samples in 2012-2014

Year	Spring Oats		Winter Oats	
	Sites	Varieties	Sites	Varieties
2012	5	8	6	8
2013	5	14	5	10
2014	4	11	7	12

As there are few sites each year the concentration of HT2+T2 across sites can only give a crude indication of the seasonal differences in HT2+T2 within the UK. In spring oats the median value for each site in 2012 and 2013 ranged from 32 to 112 ppb and was noticeable higher across all sites in 2014 with an average site median of 379 ppb. Only one of the 14 spring oat sites had samples exceeding the current EU indicative level for unprocessed oats (1000 ppb) with a median of 1185 ppb. For winter oats there were highly variable levels across all sites in all years. Medians fluctuated between 79 and 3572 ppb across all sites. The indicative level of 1000 ppb was exceeded in 9 out of the 18 winter oat sites over the three years.

Figures 1 shows the predicted mean for each spring variety in 2012, 2013 and 2014. As the results show there were significant differences in 2013 but not in 2012 and 2014. The rankings of the varieties are similar across multiple years. Table 2 and Figure 2 show the analysis of all sites from across the three years. For this dataset the variation across years was normalised by calculating the log10 transformed HT2+T2 values as a percentage of the mean control variety samples before analysis. This removed bias due to the unbalanced design of the analysis and the large variation in actual HT2+T2 concentrations between trials. Analysed data were then back-transformed to present data as predicted HT2+T2 concentrations for each variety. Results showed a narrow range of HT2+T2 mean values for spring varieties between 68 and 169 ppb. Despite the narrow range, several new varieties had significantly lower mean HT2+T2 concentrations compared to Firth.

It should be noted that the distribution is highly skewed and some spring oat samples exceeded 1000 ppb HT2+T2.

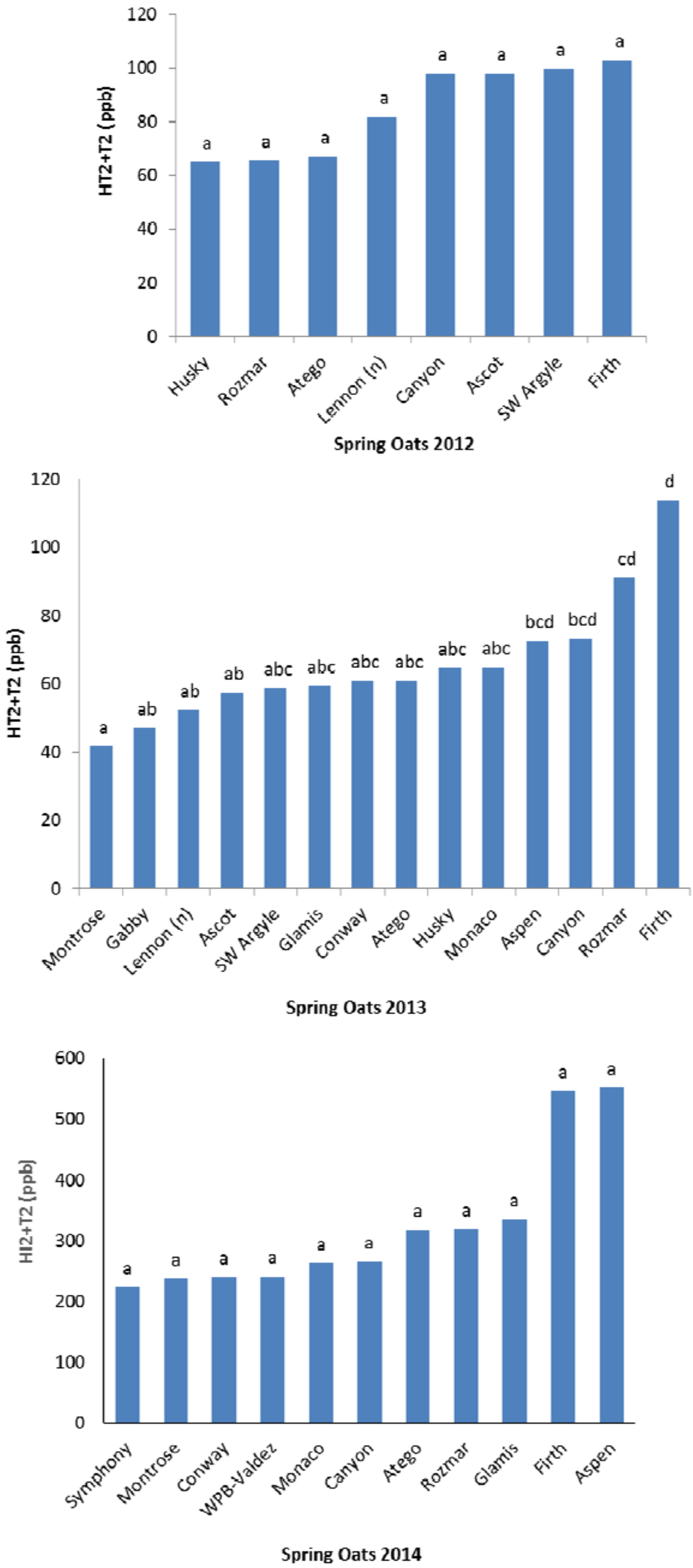

Figure 1. Back-transformed mean HT2+T2 concentration of spring oat varieties included in the AHDB Recommended List trials in 2012, 2013 and 2014. For each year, varieties with the same letter are not significantly different (LSD; p=0.05)

Table 2. Log10 transformed HT2+T2 concentration (ppb) as a percentage of the control varieties (Canyon, Firth and Rozmar), back-transformed mean HT2+T2 concentration and LSD (varieties with the same letter are not significantly different (p=0.05)) for spring oat varieties for RL trials from 2011 to 2013.

Variety	%Log10(HT2+T2)	HT2+T2 (ppb)	LSD
Gabby	86	68	a
Montrose	88	75	ab
Ascot	92	91	abc
Lennon (n)	92	92	abc
Husky	92	93	abc
Conway	92	94	abc
Symphony	93	95	abc
Monaco	94	100	abcd
Atego	94	101	abcd
Glamis	94	101	abcd
SW Argyle	95	108	abcd
WPB-Valdez	95	108	abcd
Rozmar	97	117	bcd
Canyon	97	120	bcd
Aspen	99	133	cd
Firth	104	169	d

(n, naked; s, short strawed)

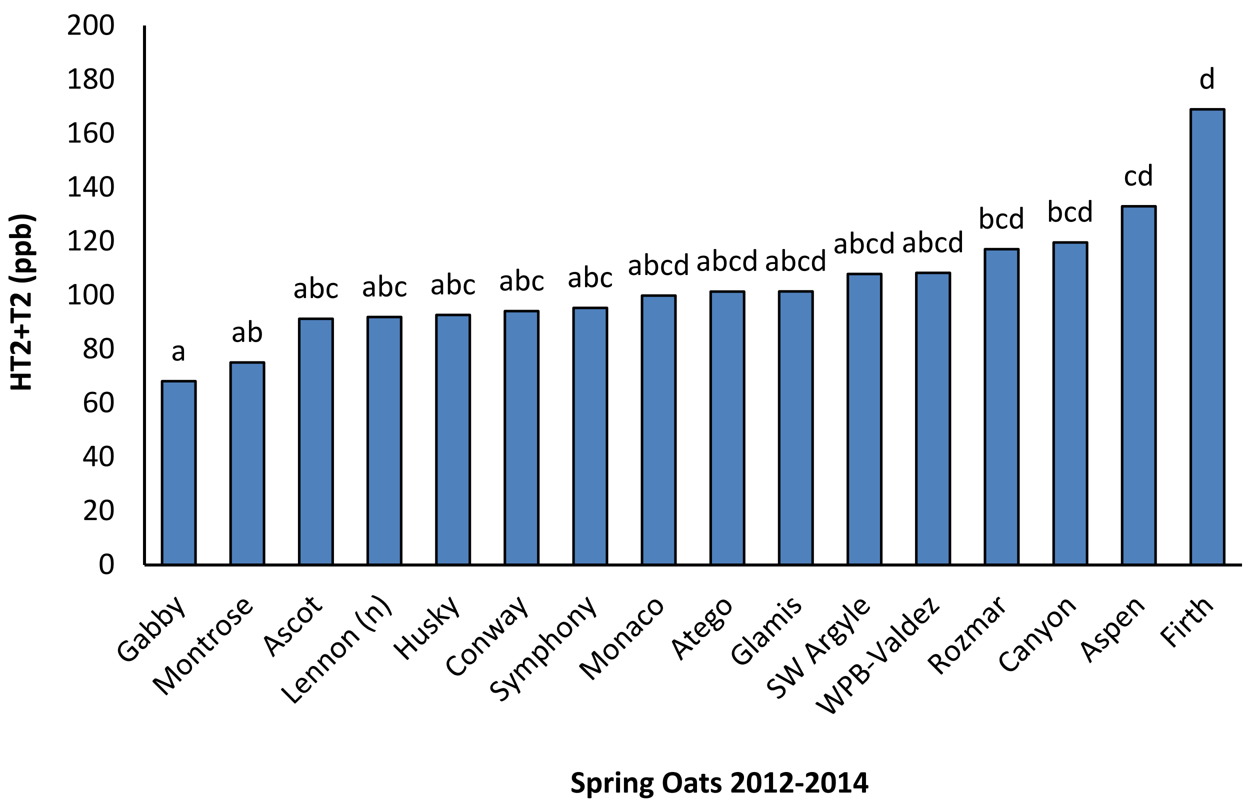

Figure 2. Back-transformed mean HT2+T2 concentration of spring oat varieties from the combined analysis of the AHDB Recommended List trials in 2012, 2013 and 2014. Varieties with the same letter are not significantly different (LSD; p=0.05)

Figure 3 shows the predicted mean for each winter variety in 2012, 2013 and 2014. As the results show there were significant differences in all years with rankings of the varieties similar across multiple years. Table 3 and Figure 4 show the analysis of all sites from across the three years. As for the spring oat dataset, the variation across years was normalised by calculating the log10 transformed HT2+T2 values as a percentage of the mean control variety samples before analysis. Analysed data were then back-transformed to present data as predicted HT2+T2 concentrations for each variety. There were higher HT2+T2 average values for winter oat varieties compared to spring oats, with a broader range from 218 to 848 ppb HT2+T2. Naked oats tended to have a low HT2+T2 content and the short-strawed variety Balado was consistently high. Several new varieties were at the low end of the range with Maestro having the lowest mean of 218 ppb HT2+T2. This is four-fold lower than the mean HT2+T2 concentration for the most susceptible variety, Balado.

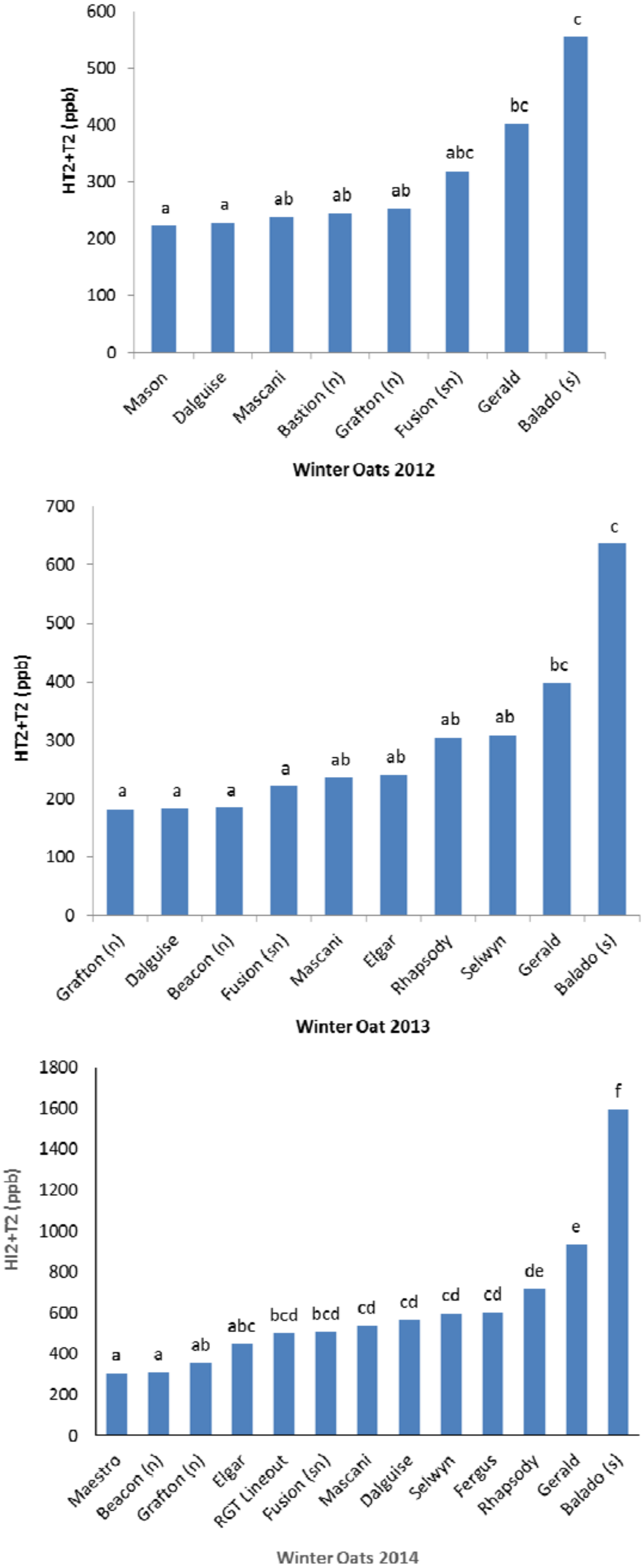

Figure 3. Back-transformed mean HT2+T2 concentration of winter oat varieties included in the AHDB Recommended List trials in 2012, 2013 and 2014. For each year, varieties with the same letter are not significantly different (LSD; p=0.05)

Table 3. Log10 transformed HT2+T2 concentration (ppb) as a percentage of the control varieties (Dalguise, Gerald and Mascani), back-transformed mean HT2+T2 concentration and LSD (varieties with the same letter are not significantly different (p=0.05)) for winter oat varieties for RL trials from 2011 to 2013.

Variety	%Log10(HT2+T2)	HT2+T2 (ppb)	LSD
Maestro	91	218	a
Beacon (n)	92	241	ab
Grafton (n)	94	272	abc
Dalguise	96	295	abc
Mason (n)	96	299	abc
RGT Lineout	96	303	abc
Elgar	97	314	abc
Mascani	98	341	abcd
Bastion (n)	98	344	abcd
Fusion (sn)	99	360	abcd
Selwyn	101	394	bcd
Rhapsody	102	434	cd
Fergus	102	438	cd
Gerald	106	530	de
Balado (s)	113	848	e

(n, naked; s, short strawed)

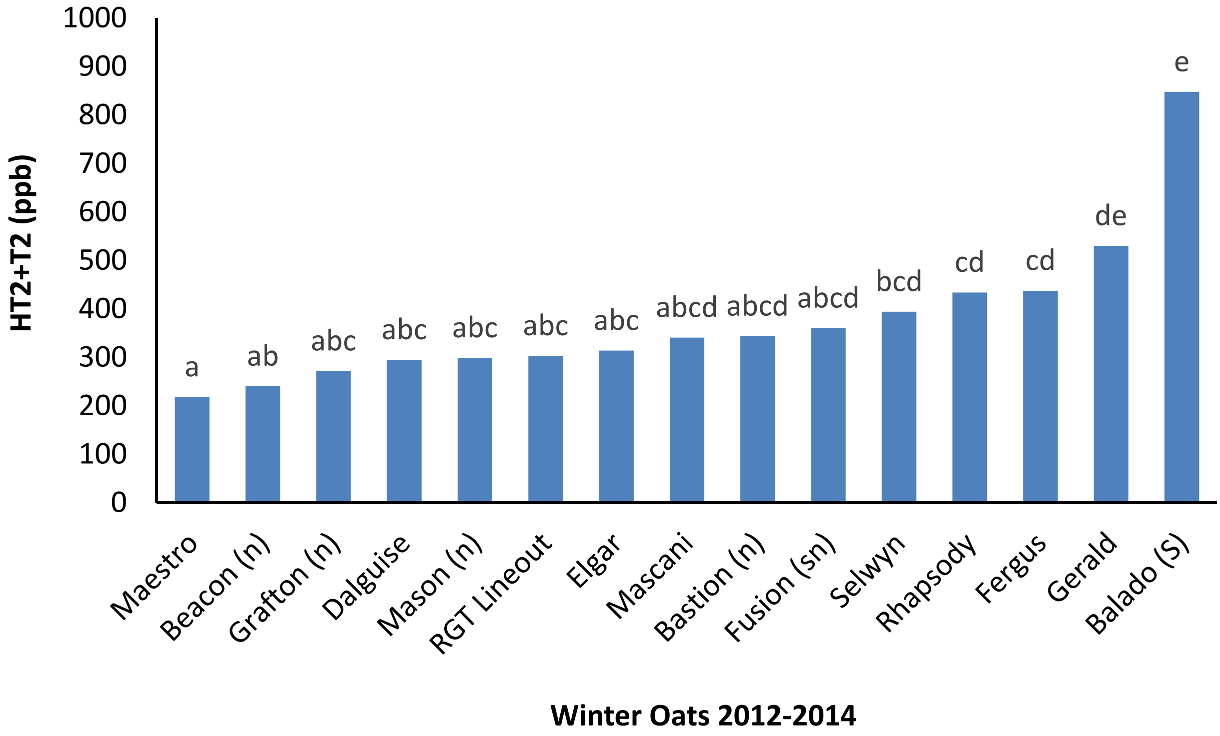

Figure 4. Back-transformed mean HT2+T2 concentration of winter oat varieties from the combined analysis of the AHDB Recommended List trials in 2012, 2013 and 2014. Varieties with the same letter are not significantly different (LSD; p=0.05)

5. Discussion

AHDB Cereals & Oilseeds has monitored HT2+T2 content of RL oat variety trials since 2004 (Edwards, 2007). Results have clearly identified consistent differences in varieties and allowed new varieties to be compared to current RL material.

The European Commission (2013) Recommendation states that the need to set legislation for HT2+T2 in cereals and cereal products intended for human consumption will be reviewed in 2015. If legislation were to be set then growers would need to minimise the risk of exceeding limits. Based on the known impact of agronomy on the HT2+T2 content of oats there are few economically viable options to reduce these mycotoxins. The most readily available option for growers is the change to a more resistant variety with lower average HT2+T2

The method adopted within this project to normalise the dataset by using the varietal value as a percentage of the average of standard “control” varieties is used for other RL parameter and allows for varieties that are only present in a limited number of years to be compared to varieties in trial in other years. This will also allow long term trends to be monitored. Such a method could also be used to determine a Fusarium resistance score for oats and to set a threshold of acceptance for Fusarium resistance for new varieties to be included on the Recommended List if deemed necessary.

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