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Susceptibility of UK oat (*Avena sativa*) varieties to infection by *Fusarium* species and subsequent HT-2 and T-2 toxin contamination

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Abstract

The aim of this study was to compare the susceptibility of oats to *Fusarium langsethiae* infection, as measured by combined HT-2 and T-2 mycotoxin concentration (HT2 + T2) in harvested oat grain samples. Over 10 years (2004–2013), samples from single replicates of each UK Recommended List oat trial were analyzed for HT-2 and T-2. For spring oats, there were small but statistically significant differences between varieties, whereas for winter oats, they had a broader range and higher mean of HT2 + T2 concentration compared with spring oats. For winter oats, the short-strawed varieties had consistently high HT2 + T2 levels compared with other varieties, whereas naked varieties were at the lower end of the range, and short, naked varieties had intermediate levels. A separate set of harvested oat grain samples of eight common varieties from 17 field experiments were analyzed by modified joint regression analysis. Results showed that environment had the strongest impact on HT-2 and T-2 concentrations but that the varietal susceptibility to HT-2 and T-2 contamination was highly stable across environments. This methodology can be used to calculate a *Fusarium* (HT2 + T2) resistance score for oats to aid grower selection of suitable varieties, as is available for *Fusarium* (DON) resistance for wheat varieties in many countries.

KEYWORDS

Avena sativa, *Fusarium langsethiae*, mycotoxin, oat, T-2 toxin, T-2 toxin, trichothecene

1 | INTRODUCTION

HT-2 and T-2 are related trichothecene fusarium mycotoxins produced by several *Fusarium* species but are predominantly produced by *Fusarium langsethiae* on UK oats (Edwards et al., 2012). This species is not a typical *Fusarium* Head Blight pathogen in that it does not produce symptoms on infected crops or grains (Imathiu et al., 2013). Surveys have determined that high levels of HT-2 and T-2 can occur in UK oats but not wheat and barley (Edwards, 2009a, 2009b, 2009c).

The European Union set legislative limits for the fusarium mycotoxins, deoxynivalenol and zearalenone in 2006 (European Commission, 2006). Since 2006, legislative limits for HT-2 and T-2 have been discussed. In 2013, the European Commission published a recommendation for the continued monitoring of HT-2 and T-2 by Member States in collaboration with industry (European Commission, 2013). The recommendation includes indicative levels for the combined concentration of HT-2 and T-2 (HT2 + T2) in various cereals and cereal products. Where these indicative limits

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(1000 µg/kg 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 µg/kg) varied between 1% and 30% each year, with a mean of 16% (Edwards, 2012). European legislative limits for HT-2 and T-2 are currently in draft. In the absence of UK legislation for HT-2 and T-2, oat processors in the UK will need to ensure that oat and oat products destined for the European Union and Northern Ireland conform to the EU legal limits when set.

The analysis of the impact of agronomic factors on the HT-2 and T-2 concentration of commercial oat crops in previous studies (Edwards, 2007, 2012) identified significant differences between HT2 + T2 concentration across oat varieties. However, as these studies were observational, there was a highly unbalanced distribution of varieties present, and differences between varieties may have been confounded by other agronomic factors (e.g., region and drilling date). The Agriculture and Horticulture Development Board (AHDB) in the UK conducts Recommended List trials on spring and winter oats each year. Typically, five spring and six winter trials are conducted each year in key oat growing areas of the UK. Varieties are categorized by type; conventional hulled oats retain the husk (hull) during harvesting, whereas for naked varieties, the hull is removed during harvest. Most varieties are tall whereas a few varieties possess the dwarfing gene (*Dw6*) and are referred to as short strawed. Quality analysis is conducted on a single replicate from each trial. A second set of replicated samples from oat variety trials were available as part of a large UK collaborative oat project (QUOATS).

The aim of this study was to quantify the HT2 + T2 concentration in UK oat variety trials. This would ensure accurate and complete information on the comparative resistance of UK oat varieties to HT2 + T2-producing *Fusarium* species.

2 | MATERIALS AND METHODS

2.1 | Variety oat samples

Each year (2004–2014 for winter oats and 2006–2014 for spring oats), single replicate samples (1 kg of grain) for each variety were collected from each AHDB Recommended List oat trial from across the UK. As part of the QUOATS project (<https://ahdb.org.uk/harnessing-new-technologies-for-sustainable-oat-production-and-utilisation-quoats>), replicated plot samples from 17 variety trials conducted at multiple locations in the UK from 2009 to 2013 were also available. Samples were couriered to Harper Adams University for analysis. On receipt of samples, they were milled in a ZM200 centrifugal mill (Retsch, Haan, Germany) with a 1 mm screen, mixed in a tumbler mixer before a 200 g laboratory sample was collected. Samples were ‘as harvested’, so that conventional oats still retained a husk (hull), whereas naked oat samples lose the vast majority of husks during harvest. Samples were analyzed using Ridascreen T-2 ELISA kits (R-Biopharm, Glasgow,

UK). Based on the known ratio of HT-2 to T-2 in UK oat samples from a previous project and the known cross-reaction of the T-2 antibody with HT-2, the concentration of HT2 + T2 was estimated as detailed previously (Edwards et al., 2012).

2.2 | Statistical analysis

Varietal differences in HT-2 and T-2 accumulation were tested for AHDB Recommended List winter and spring oats separately using ANOVA with trial site/year as the block factor (Genstat v20, VSN International, Hemel Hempstead). HT2 + T2 concentrations were log₁₀ transformed to achieve normally distributed residuals. As varieties are present on the Recommended List for an unequal number of years, the combined dataset across all years was analyzed by first normalizing the data by adjusting logarithmic mean concentrations to a percentage value compared to the trial mean HT2 + T2 concentration of three control varieties (%log₁₀) to account for the temporal and spatial variation in HT2 + T2 concentration. This minimized any bias that may occur due to a variety only occurring in Recommended List trials for a limited number of years. After analysis, %log₁₀ values were back-transformed to HT2 + T2 concentrations (µg/kg). The control varieties occurred in all trials and were ‘Canyon’, ‘Firth’ and ‘Rozmar’ for spring oats and were ‘Dalguise’, ‘Gerald’ and ‘Mascani’ for the winter oats. Height data from Recommended List trials were also collated from the AHDB archive (<https://ahdb.org.uk/knowledge-library/recommended-lists-for-cereals-and-oilseeds-rl-harvest-results-archive>). Height measurements from the last year a variety appeared in the dataset were collated and normalized as a percentage of the three control varieties (%height) as described for HT2 + T2 above and then back-transformed to height (cm). Height was analyzed as an explanatory variate to log₁₀HT2 + T2 concentration using generalized linear models and simple linear regression using Genstat. For the QUOATS samples, the log₁₀ transformed HT2 + T2 concentration for each replicate plot was analyzed for the eight common varieties present in all 17 environments by Genotype × Environment-modified joint regression analysis (Digby, 1979) using Genstat. The mean HT2 + T2 concentration for the eight varieties within the two datasets was also compared using the Pearson correlation coefficient (Genstat).

3 | RESULTS

All samples had HT2 + T2 concentrations above the limit of quantification of 50 µg/kg. On average, each year there were 4.8 trials for spring oats with 11.1 varieties and 6.0 trials for winter oats with 10.0 varieties with a total 477 spring oat and 662 winter oat samples analyzed for HT2 + T2. Comparison of the individual trials identified that spring oat trials had a mean HT2 + T2 of 228 µg/kg, whereas winter oat trials had a mean of 804 µg/kg.

For both winter and spring trial datasets, variety had a very highly significant effect on HT2 + T2 concentration ($p < .001$). Results

TABLE 1 \log_{10} transformed HT2 + T2 concentration ($\mu\text{g}/\text{kg}$) and height (cm) as a percentage of the control varieties and back-transformed mean HT2 + T2 concentration and height for spring oat varieties from the Agriculture and Horticulture Development Board (AHDB) Recommended List trials from 2006 to 2014

Variety	Year	No. of trials	%height	Height (cm)	% \log_{10} (HT2 + T2)	HT2 + T2 ($\mu\text{g}/\text{kg}$)
Gabby	2013	5	101	95	90	85
Montrose	2013–2014	9	101	95	90	86
Symphony	2014	4	112	106	93	96
Conway	2013–2014	9	100	94	95	109
WPB-Valdez	2014	4	105	99	95	109
Melody	2011	6	94	89	95	110
Circle	2011	6	101	95	95	110
Gandalf	2011	6	103	98	96	114
VOK	2011	5	103	98	96	115
Monaco	2013–2014	9	96	91	96	116
Emotion	2006–2007	10	98	93	97	116
Glamis	2013–2014	9	98	93	97	117
Valene	2011	6	95	90	97	120
Lennon (n)	2008–2013	18	95	90	97	121
Olympic	2011	6	99	93	97	121
Rozmar	2011	30	106	100	98	122
SW Argyle (c)	2006–2013	38	103	97	98	123
Ascot (c)	2006–2013	39	103	97	98	125
Husky	2007–2013	34	101	95	98	125
Atego	2006–2014	43	95	90	98	126
Zuton (n)	2007–2009	14	94	88	99	131
Dominik	2011	6	100	94	99	134
Canyon	2009–2014	30	108	102	100	135
Leven	2006–2011	30	99	93	100	136
Drummer	2006–2009	19	105	99	100	140
Carron	2008	8	92	87	102	153
Aspen	2013–2014	9	98	93	102	154
Cavalcade	2011	6	93	88	103	158
Winston	2006–2007	10	96	91	104	166
Firth (c)	2006–2014	43	98	93	104	169
Capri	2011	6	99	93	105	174
<i>p</i> -Value (DF = 404)					<.001	
Minimum LSD					2.93	
Average LSD					6.32	
Maximum LSD					9.61	
%cv					6.91	

Abbreviations: c, control variety; %cv, percentage coefficient of variation; DF, degrees of freedom; LSD, least significance difference; n, naked.

showed a narrow range of back-transformed HT2 + T2 mean values for spring varieties between 85 and 174 $\mu\text{g}/\text{kg}$ (Table 1). In general, there were higher HT2 + T2 mean values for winter oat varieties compared with spring oats, with a broader range from 171 to 1426 $\mu\text{g}/\text{kg}$ mean HT2 + T2 concentration (Table 2). It should be noted that the HT2 + T2 distribution was highly skewed with a long right-handed tail. Twenty spring oat samples (4.2%) and 158 winter

oat samples (23.9%) exceeded 1000 $\mu\text{g}/\text{kg}$ HT2 + T2 with the highest concentration of 18,206 $\mu\text{g}/\text{kg}$ for a sample of 'Balado' in 2014. Naked oats tended to have a low HT2 + T2 content; the short-strawed varieties were consistently high and naked short-strawed varieties had intermediate levels of HT2 + T2. A generalized linear model of $\log_{10}\text{HT2} + \text{T2}$ using type (straw length * hull presence) identified both factors were significant ($p < .001$ and $p = .009$,

TABLE 2 Log₁₀ transformed HT2 + T2 concentration (µg/kg) and height (cm) as a percentage of the control varieties and back-transformed mean HT2 + T2 concentration and height for winter oat varieties from Agriculture and Horticulture Development Board (AHDB) Recommended List trials from 2004 to 2014

Variety	Years	No. of trials	%height	Height (cm)	%log ₁₀ (HT2 + T2)	HT2 + T2 (µg/kg)
Expression (n)	2006–2007	22	109	132	86	171
Jalna	2004	5	100	122	86	171
Millennium	2004–2006	15	98	120	87	178
Maestro	2014	7	109	109	91	224
Beacon	2013–2014	11	97	108	92	247
Grafton (n)	2004–2014	66	101	115	93	253
Dalguise (c)	2004–2014	66	102	116	94	269
Mason	2011–2012	12	97	111	94	276
Bastion	2010–2012	13	99	118	96	303
RGT Lineout	2014	7	111	111	96	312
Elgar	2013–2014	12	91	101	97	323
Kinross	2004–2009	34	108	129	98	347
Mascani (c)	2004–2014	66	98	112	99	368
Ayr	2004–2005	10	105	131	99	378
Hendon (n,s)	2004–2011	48	71	81	100	399
Selwyn	2013–2014	12	94	104	101	406
Fusion (n,s)	2008–2014	44	77	88	101	421
Rhapsody	2013–2014	12	94	104	102	448
Fergus	2014	7	110	110	102	451
Tardis	2006–2011	38	93	106	103	463
Kinnell	2006–2007	12	na	na	105	527
Gerald (c)	2004–2018	66	99	113	107	592
Brochan	2006–2011	38	100	114	108	619
Penderi (s)	2004	5	94	107	109	688
Balado (s)	2008–2014	44	74	87	113	841
Buffalo (s)	2004	5	83	97	122	1426
<i>p</i> -Value (DF=586)					<.001	
Minimum LSD					2.56	
Average LSD					5.59	
Maximum LSD					9.29	
%cv					7.49	

Abbreviations: c, control variety; %cv, percentage coefficient of variation; DF, degrees of freedom; LSD, least significance difference; n, naked; na, not available; s, short-strawed.

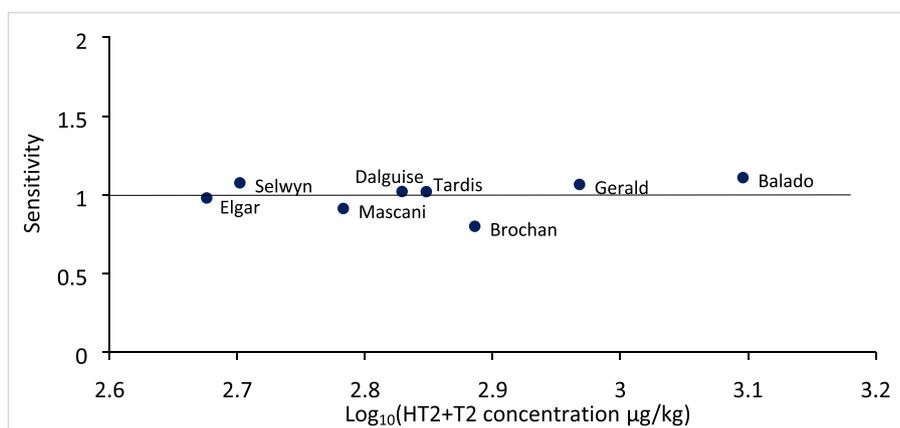
respectively), and there was no significant interaction ($p = .228$). The predicted back-transformed means for tall and short types were 329 and 646 µg/kg HT2 + T2 and were 421 and 264 µg/kg HT2 + T2 for hulled and naked types, respectively.

Data for height showed a range from 104 to 127 cm for spring varieties, a range of 22 cm (22%) (Table 1). For the spring variety dataset, there were no short-strawed varieties and only two naked ones. Analysis of height for spring varieties was therefore performed using a simple linear regression against log₁₀HT2 + T2 after removal of the naked varieties. This analysis was just significant ($p = .046$) and accounted for 11% of the variance in HT2 + T2 concentration with greater height having a lower HT2 + T2 concentration. For the winter

variety dataset, there were both conventional and short-strawed varieties of both hulled and naked oats. There was an overlap in height with short-strawed varieties ranging from 81 to 107 cm and conventional tall varieties ranging from 101 to 131 cm. Despite this range, height was not a significant ($p = .337$) explanatory variate when added to the end of the generalized linear model detailed above.

For the QUOATS samples, 362 winter oat grain samples from eight common varieties across 17 trials were analyzed using a modified joint regression analysis (Digby, 1979). Results showed that both Environment (trial) and Genotype (variety) were very highly significant ($p < .001$) with environment and genotype accounting for 72% and 5% of the total variance, respectively. The Environment × Genotype

FIGURE 1 Sensitivity and susceptibility of eight UK winter oat varieties to HT2 + T2 contamination as calculated by a modified joint regression analysis. A sensitivity value close to one signifies a variety with a phenotypic response that is stable across multiple environments.



interaction was not significant ($p = .382$) resulting in sensitivity values close to one (Figure 1), indicating that all the varieties tested had a stable susceptibility to HT2 + T2 contamination across the environments tested. Comparison of the mean HT2 + T2 for each of the eight varieties from both studies showed a strong correlation ($r = .96$) with lower values for 'Elgar', 'Selwyn', 'Dalguise' and 'Mascani', whereas consistently high values for the short-strawed variety, 'Balado'.

4 | DISCUSSION

This study has confirmed the importance of variety as an agronomic trait that impacts on the HT2 + T2 concentration of oat as indicated in previous observational studies in the UK (Edwards, 2017). Another observational study in Switzerland (Schoneberg et al., 2018) could not test for varietal differences due to multicollinearity within the dataset; they did however identify higher HT2 + T2 in winter sown crops compared with spring sown crops. A more recent observational study in Ireland did not identify a significant effect of variety on HT-2 and T-2, but this study had fewer samples of known variety ($n = 172$) and was dominated by a single variety ('Husky') with all other varieties represented by less than 20 samples (Kolawole et al., 2021). Observational studies are limited due to the highly unbalanced distribution of varieties with high numbers of a few popular varieties and no or few samples of others. The analysis in this study of over 1000 samples from oat variety trials at multiple locations and over multiple years has identified clear differences in oat susceptibility to HT-2- and T-2-producing *Fusarium* species. For the UK, this species is primarily, if not solely *F. langsethiae* (Edwards et al., 2012). The close correlation between *F. langsethiae* DNA and HT2 + T2 concentrations in oat grains from other studies also suggests that this is true for Nordic countries as well (Hofgaard et al., 2022).

Results indicated that winter oats had a broader range of HT2 + T2 and in general were more susceptible to HT2 + T2 contamination than spring oats although this may be due to drilling date rather than genetic background. Naked varieties had lower HT2 + T2

compared with conventional husked oats, and short oat varieties were more susceptible than conventional tall varieties.

There are many studies conducted to determine the sensitivity of wheat varieties to *Fusarium* species and where different *Fusarium* species have been tested, data indicate that resistance is non-species-specific across the *Fusarium* species (Mesterhazy, 2020). This was shown to be true for oats in a study conducted at sites across Germany and Finland inoculated with various type A and type B trichothecene producers either alone or in combination (Herrmann et al., 2020). This study included *F. langsethiae* but the actual infection by individual species was not reported, and as such, the HT-2 and T-2 detected may have been produced by *Fusarium sporotrichioides*. Results indicated that increased plant height was associated with lower concentrations of DON and HT2 + T2. Conflicting results were found by Chrpová et al. (2020), who found no significant correlation between DON and HT2 + T2 in Czech Republic oat varieties after inoculation with *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium poae*. Height was a significant resistance factor for DON although not for HT2 + T2. Both mycotoxins were lower in the naked compared with the husked varieties. Other studies have consistently shown lower HT2 + T2 in naked compared with husked varieties (Gavrilova et al., 2021; Martin et al., 2018).

There are several studies on varietal resistance to DON in oats (Hautsalo et al., 2020; Hietaniemi et al., 2004; Tekle et al., 2018). For Nordic oats, Tekle et al. (2018) showed reduced DON associated with nakedness and increased height. There are much fewer studies of HT2 + T2 resistance in any cereals. Schwake-Anduschus et al. (2010) analyzed four German oat varieties from 10 sites but did not statistically compare the varieties. Subsequent analysis of the log₁₀ transformed data by ANOVA with a post hoc Tukey test (Genstat v.20) showed that the HT2 + T2 concentration of 'Dominik' was significantly higher than that of 'Pergamon'. Recent studies in Norway identified differences in *F. langsethiae* (and HT2 + T2) resistance across a range of spring oat varieties in naturally inoculated field trials (Hofgaard et al., 2022), and they showed a lack of correlation in oat varietal resistance to *F. graminearum* (DON) compared with *F. langsethiae* (HT2 + T2).

Previous studies have shown that naked varieties had less HT-2 and T-2 than conventional husked varieties (Edwards, 2012), and this is thought to occur as the majority of HT-2 and T-2 are present in the husks (Scudamore et al., 2007), which are removed from naked oat varieties during harvest. This is therefore unlikely to be a resistance mechanism rather a difference in the material sampled at harvest, that is, oat grains with or without a husk. To compare actual *Fusarium* resistance across both naked and hulled oats would require analysis of equivalent samples, for example, panicles before harvest or groats (de-hulled oats) after laboratory de-hulling of conventional oats. Short-strawed varieties had higher levels of HT-2 and T-2, and naked short-strawed varieties had intermediate levels. Short-strawed varieties may have higher concentrations of HT-2 and T-2 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 HT-2 and T-2-producing *Fusarium* species. Several studies have shown association between dwarfing genes *Rht B1b* and *Rht D1b* and susceptibility to *Fusarium* Head Blight in wheat (He et al., 2016), whereas no association has been demonstrated for the dwarfing gene, *Rht24b* (Miedaner et al., 2022). Genetic linkage has been demonstrated for *F. graminearum* (DON) susceptibility and *Rht D1b* in wheat (Srinivasachary et al., 2008). Results from this study are inconclusive. For spring oats, where the dwarfing gene is not present in any variety, height was a significant factor but only accounted for 11% of the overall HT2 + T2 variance. For winter oats, the presence of the dwarfing gene, *Dw6*, in short-strawed varieties resulted in higher HT2 + T2; however, height itself was not a significant factor when added later in the model despite there been a similar range of height as spring oats within the short- and tall-strawed varieties. This would suggest that there is some susceptibility linkage to *Dw6* as well as height itself or an associated morphological trait having a minor role in resistance to HT2 + T2-producing *Fusarium* species. Further studies of the impact of *Dw6* within breeding lines with similar genetic background would help elucidate the role of *Dw6* and reduced height in this susceptibility.

This study, due to the inclusion of all varieties across multiple locations and years, has provided robust comparison of all varieties on the UK oat variety Recommended List from 2004 to 2013. The method adopted, to normalize the impact of environment 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 with varieties in trial over many years. Analysis of the QUOATS samples identified that the eight varieties tested have a stable expression of this phenotype across environments, and the strong correlation between the two datasets further supports the validity of such methodology.

The European Commission is currently drafting legislation for HT-2 and T-2 in cereals and cereal products. When legislation is set, then growers will need to minimize 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 oat variety. This methodology can be used to calculate a *Fusarium* (HT2 + T2) resistance score for oats to aid grower selection of suitable varieties, as is available for *Fusarium* (DON) resistance for wheat varieties in many countries.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Simon G Edwards guided all aspects of the research project, provided the analysis and interpretation of data and contributed to the writing and revision of the manuscript. Tijana Stancic was involved in the writing, critical review and editing the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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