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IMPACT OF AGRONOMY ON HT-2 AND T-2 TOXIN CONTENT OF OATS

ABSTRACT

Surveys of oats in the UK and Nordic countries have identified high concentrations of HT-2 and T-2 can occur in unprocessed oats. HT-2 and T-2 are two closely related type A trichothecenes and two of the most toxic fusarium mycotoxins. There is currently no legislation on HT-2 and T-2, however, there is a discussion limit of 500 µg kg-1 in unprocessed oats. A previous survey identified that variety, previous crop, cultivation, fungicide use and some other factor(s) within organic oat production, which was not within the model, were all significant agronomic factors in the determination of HT-2 and T-2 concentrations of UK oats. Possible agronomy within conventional compared to organic agriculture would include the use of inorganic fertilisers and plant growth regulators (PGR). Oats harvested from two series of agronomic field experiments were analysed for the combined concentration of HT-2 and T-2 (HT2+T2) using ELISA. Experiments were repeated for both winter and spring varieties over two years. The first experiments were of a factorial design with three varieties, three nitrogen rates and plus/minus a PGR (chlormequat). The second series had twelve fungicide regimes. The results identified that there were no significant differences in HT-2+T-2 between samples from oat plots that received different rates of inorganic nitrogen, a PGR, or a range of different fungicide regimes. There was however a significant difference between varieties for both winter and spring variety experiments.

Key words: agronomy, fungicide, HT-2, nitrogen, PGR, T-2, variety

INTRODUCTION

HT-2 toxin (HT2) and T-2 toxin (T2) are two closely related type A trichothecenes produced by several Fusarium species. These mycotoxins have a high cellular toxicity and as T2 is readily metabolised into HT2 after

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ingestion they have equivalent mammalian toxicity (Anon 2001). Based on limited experimental data HT2 and T2 have a combined temporary Tolerable Daily Intake of 0.06 μg/kg body weight/day (Anon 2001). The European Commission set legislation for several fusarium mycotoxins in 2006; legislative limits for HT2 and T2 are currently under discussion.

F. langsethiae has recently been implicated in the high levels of HT2 and T2 reported in European cereals (Edwards *et al.* 2009). This species is newly identified (Torp and Nirenberg 2004) and appears to be a weak pathogen although little is known of its epidemiology and to date attempts to mimic infection using artificial inoculation has failed (Imathiu 2008), so as yet Koch's postulates have not been satisfied. There have been extensive studies of the impact of agronomy on the development of fusarium head blight of wheat and the resulting contamination of deoxynivalenol (DON) in grain (Edwards 2004; Schaafsma *et al.* 2005), but very little is known regarding the impact of agronomy for HT2 and T2 contamination of oats.

A previous observational study of UK oats identified relatively high concentrations of HT2 and T2 in UK oats (Edwards 2009). The mean and maximum combined concentration of HT2 and T2 (HT2+T2) was 570 and 9990 μg/kg. There was a five-fold higher concentration of HT2+T2 in conventional compared to organic samples. Analysis of the agronomic inputs identified several factors which had an impact on the HT2+T2 concentration (Edwards in press). These included variety, previous crop, cultivation, fungicide use and practice. As a consequence of the multicolinearity within the dataset it was not possible to weight the impact of the individual agronomic factors identified. By moving practice (organic or conventional) from the front to the end of the model identified that there was one or more other factors not included within the model that, in part, explained for the difference between organic and conventional oats.

Several chemical inputs that are not permitted in organic agriculture may have an impact on HT2 and T2 content. These could include inorganic fertilisers, plant growth regulators (PGR) and/or fungicides. A series of agronomic field experiments were conducted in 2007/08 and 2008/09 to identify benefits of various agronomic inputs on yield and milling quality of both winter and spring oats. These experiments were designed to test nitrogen rates, use of a PGR (chlormequat) and a range of fungicides. Quantification of HT2 and T2 from the harvested oats from these replicated field experiments allowed the impact of these agronomic factors on HT2 and T2 content to be measured.

MATERIALS AND METHODS

Field experiments were conducted in 2007/2008 and 2008/2009 in Fife, East Scotland. Each experiment was drilled as a fully randomised block design with three replicate blocks of plots (12 × 2 m). Oats were grown according to stan-

dard farm practice apart from the specific treatments applied as detailed below. Growth stages at which treatments were applied are based on the Zadoks scale (Zadoks *et al.* 1974).At harvest, a 500 g sample of each plot was collected for analysis. Samples were milled with a ZM100 mill (Retsch UK Ltd, Leeds) fitted with a 1 mm screen. Samples were then mixed in a tumbler mixer for 5 minutes before a laboratory sample of 100 g was removed. Samples were analysed using the Ridascreen T-2 ELISA assay (R-Biopharm Rhone, Glasgow) according to the manufacturer's instructions.

The ELISA used was developed for T2, but is known to cross react with HT2. Based on the known ratio of HT2 to T2 in UK oats (Edwards 2009) and the known cross reaction of the assay the combined HT2+T2 content of oat samples was estimated by multiplying the signal obtained from the assay by 3.152. To validate the method thirty oat samples of known HT2 and T2 concentration ranging from 20-3500 µg/kg as determined by GC/MS analysis during a previous study (Edwards 2009) were quantified by the ELISA method. Concentrations of HT2+T2 were log10 transformed to normalise the variance and analysed by regression analysis using Genstat v12 (VSN International Ltd, Hemel Hempstead, UK). For field experiments the HT2+T2 concentrations were log10 transformed to normalise the variance and analysed using factorial ANOVA (Experiments 1 and 2) and general ANOVA with blocks (Experiments 3 and 4) using Genstat v12.

Field experiment 1

- A three factorial design with variety (3) × nitrogen rate (3) × PGR (2)
- Winter oat varieties: Gerald, Mascani and Brochan
- Nitrogen rates: 96, 108 and 120 kg/ha N (applied in three stages :40 kg at GS22-24, 40 kg at GS31-32 and remainder at GS33-39)
- PGR: +/- 1.7 l/ha 3C chlormequat (BASF, Cheadle Hulme, UK) applied at GS31-32

Field experiment 2

- A three factorial design with variety (3) × nitrogen rate (3) × PGR (2)
- Spring oat varieties: Firth, Leven, Husky
- Nitrogen rates: 96, 108 and 120 kg/ha N (all applied pre-emergence)
- PGR: +/- 1.7 l/ha 3C chlormequat (BASF, Cheadle Hulme, UK) applied at GS31-32

Field experiment 3

Winter oat variety, Gerald was treated with a range of fungicides at three timings as detailed in Table 1.

Table 1

Fungicide treatments used in oat agronomy field experiments 3 and 4. Rates quoted are per ha.

Treatment	Growth Stage		
	GS 31-32	GS 39	GS 59-61
1	Untreated	Untreated	Untreated
2	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Opus0.4l Amistar0.75l	Untreated
3	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Fandango0.8l	Untreated
4	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Untreated	Opus0.4l Amistar0.75l
5	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Untreated	Fandango0.8l
6	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Opus0.4l Amistar0.75l	Fandango0.8l
7	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Fandango0.8l	Opus0.4l Amistar0.75l
8	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Fandango0.8l	Opus0.4l Amistar0.75l Flexity0.2l
9	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Untreated	Opus0.4l Folicur0.4l
10	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Opus0.4l Folicur0.4l	Untreated
11	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Untreated	Proline0.3l Comet0.3l
12	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Opus0.75l Folicur0.75l	Untreated
13	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Untreated	Opus0.75l Folicur0.75l
14	Opus 0.4 l Flexity 0.2 l 3C 1.75 l	Opus0.75l Folicur0.75l	Opus0.75l Folicur0.75l

3C; 720 g/l chlormequat, BASF; Amistar; 250 g/l azoxystrobin, Syngenta; Fandango; 100 g/l fluoxastrobin + 100 g/l prothioconazole, Bayer CropScience; Flexity; 300 g/l metrafenone, BASF; Folicur; 250 g/l tebuconazole, Bayer CropScience; Opus; 125 g/l epoxiconazole, BASF; Proline; 250 g/l prothioconazole, Bayer CropScience.

Field experiment 4

Spring oat variety, Firth was treated with a range of fungicides at three timings as detailed in Table 1.

RESULTS

The concentration of HT2+T2 as determined by ELISA and GS/MS were log10 transformed before linear regression. Results for five samples below 50 µg/kg HT2+T2 had a poor correlation. This is probably due to being close to the Limit of Quantification for both methods of analysis (11 and 20 µg/kg for ELISA and GC/MS respectively) and therefore more likely to be variable. There was a good linear regression for 25 samples with a HT2+T2 concentrations between 50 and 3500 µg/kg (Fig. 1). The linear regression, when forced through the intersect, accounted for 90% of the variance and had a gradient close to one (1.03). The expanded measurement of uncertainty for the GC/MS method was previously determined to be 25% using a standard coverage factor of two, equivalent to a confidence of approximately 95% that the actual level of HT2 and T2 measured lies within the quoted range (Edwards 2009).

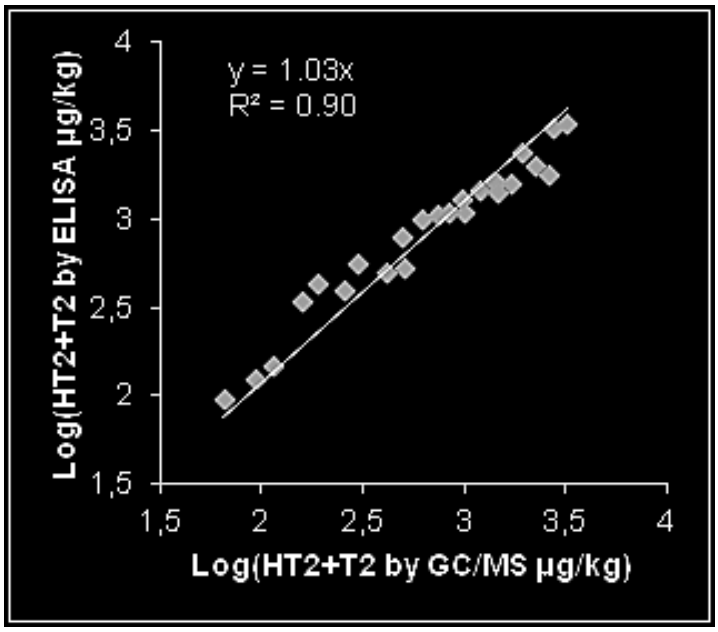

Fig. 1. Linear regression of $\log_{10}$ transformed HT2+T2 (µg/kg) concentration as determined by GC/MS and ELISA.

HT2 and T2 could be quantified in all samples from the field experiments. There were large differences between experiments/years but most differences between treat-

ments were consistent between years. For both the winter and spring varieties (experiment 1 and 2 respectively) there were no significant differences in HT2+T2 concentration between samples from oat plots which had different nitrogen rates or presence/absence of chlormequat (P>0.05) and there were no significant interactions between each factor (P>0.05). There were significant differences between both winter and spring varieties in both years (Table 2). Differences in varieties were not consistent for experiment 1 with Gerald and Brochan having a significantly higher HT2+T2 content compared to Mascani in 2008 and 2009 harvest respectively. For the spring varieties the differences were consistent with Firth have higher HT2+T2 content than Leven and Husky in both years.

Table 2

Statistical analysis of varieties for oat agronomy field experiments 1 and 2

Experiment	Variety	Year			
		2007/08		2008/09	
		P-value	Mean HT2+T2 (µg/kg)	P-value	Mean HT2+T2 (µg/kg)
One (Winter varieties)	Gerald	<0.001	228a	<0.001	1222b
	Brochan		157b		1538a
	Mascani		143b		1156b
Two (Spring varieties)	Firth	<0.011	1648a	<0.001	323a
	Leven		1321ab		109b
	Husky		902b		113b

Means with the same letter are not significantly different according to LSD (P=0.05)

There was no significant difference in the HT2+T2 content of the winter variety, Gerald (experiment 3) or the spring variety, Firth (experiment 4) which received a range of fungicide treatments including the fully untreated control plots in either year (P>0.05).

DISCUSSION

The good linear regression between the T2 ELISA assay and the established GC/MS method indicates the ELISA assay is suitable for quantification of HT2+T2 in UK oats. Its suitability for use with other matrices will depend on the stability of the ratio of HT2 and T2 in those matrices. In UK oats the regression between the two toxins had an r2 of 0.9.

HT2 and T2 was only recently detected at high frequency and high concentrations in some European barley and oat crops (Edwards *et al.* 2009) and to date there are few studies on the impact of agronomy on the HT2 and T2 concentration of harvested cereals. A survey of 451 French malting barley samples

identified that the HT2 and T2 content of harvested grain were significantly higher in:

i. spring compared to winter compared to autumn sown crops
ii. crops that followed a small grain cereal (Orlando *et al.* 2010).

No differences were identified for cultivation. An observational study of 458 UK oat samples implicated several factors were involved, although due to multicolinearity of the dataset their importance could not be weighted (Edwards in press). There was five-fold higher HT2+T2 in conventional compared to organic oats. The opportunity to assay HT2 and T2 content from replicated oat agronomy experiments allowed the impact of varieties and several agronomic factors that are unique to conventional oat cultivation, namely, inorganic nitrogen rates and the use of PGR and fungicides to be determined.

There were significant differences between varieties for both spring and winter varieties. Higher concentrations were detected in the spring variety experiment in 2007/08 and in the winter variety experiment in 2008/09. Experiments were on different sites with different cropping histories, which may explain these differences. Differences between Swedish oat varieties have also been reported (Pettersson *et al.* 2008).

There is no data on the impact of inorganic nutrients or PGR on HT2 and T2 contamination of cereals. A previous study of wheat identified that at commercial rates, as used in this study, the rate or type of inorganic nitrogen had no impact on DON (Lemmens 2004). The rate of inorganic nitrogen also had no impact on the DON content of harvested oats in Finland (Hietaniemi *et al.* 2004). The potential impact of a PGR on fusarium mycotoxin contamination of cereals can be complex due to opposing indirect effects. PGR use results in shorter plants and reduced plant height is implicated in increased DON due to increased splash dispersal of spores (Oldenburg 2004), however, lack of PGR can result in lodging which can also result in increased mycotoxin as the crop remains wet for longer pre-harvest (Langseth and Stabbetorp 1996).

There are several studies on the impact of fungicides on fusarium head blight and resulting DON contamination of wheat. Many studies have shown a range of azole fungicides (eg prothioconazole, and tebuconazole) are effective at reducing DON in wheat (Blandino *et al.* 2006; Edwards *et al.* 2001; Paul *et al.* 2008) and studies have shown some strobilurin fungicides (eg azoxystrobin) can have a negative impact resulting in an increase in DON (Ellner 2005; Simpson *et al.* 2001). There was no significant difference in the HT2 and T2 content of oats harvested from the fungicide experiments conducted for both spring and winter varieties in both years. This is despite the experiments controlling several fungicides known to have an impact of DON in wheat. Similar results were reported from fungicide experiments on oats in Norway; where some most fungicides had no effect and prothioconazole had inconsistent effects (Pettersson *et al.* 2008).

Results from this study have identified that there are differences in UK winter and spring oat varieties in their susceptibility to fusarium infection resulting in HT2 and T2 contamination. Variety trials need to be conducted with large number of oat varieties to identify the breadth of these differences and to determine if they are stable over different seasons and environments. Results have failed to identify any other agronomic factor which may explain the difference in HT2 and T2 content of conventional and organic oats. Other long term studies on crop rotation and cultivation need to be conducted to identify if these factors are important and if they interact with one another. These studies also provide further evidence that the epidemiology and control of HT2/T2-producing *Fusarium* is different to that known for DON-producing *Fusarium* species.

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