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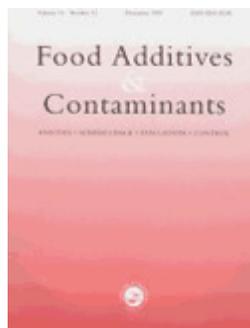
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Fusarium mycotoxin content of UK organic and conventional wheat

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Abstract

Each year (2001-2005), three hundred samples of wheat from fields of known agronomy were analysed for ten trichothecenes by GC/MS including deoxynivalenol (DON), nivalenol, 3-acetyl-DON, 15-acetyl-DON, fusarenone X, T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol and T-2 triol and zearalenone by HPLC. Of the eleven mycotoxins analysed from 1624 harvest samples of wheat only eight were detected, of these only five, deoxynivalenol, 15-acetyl-DON, nivalenol, HT-2 and zearalenone were detected above $100 \mu\text{g kg}^{-1}$. DON was the most frequently detected fusarium mycotoxin, present above the limit of quantification ($10 \mu\text{g kg}^{-1}$) in 86% of samples, and was usually present at the highest concentration. The percentage of samples which would have exceeded the recently introduced legal limits varied between 0.4% and 11.3% over the five-year period. There was a good correlation between DON and zearalenone concentrations although the relative concentration of DON and zearalenone fluctuated between years. Year and region had a significant effect on all mycotoxins analysed. There was no significant difference in the DON concentration of organic and conventional samples. There was also no significant difference in the concentration of zearalenone between organic and conventional samples however organic samples did have a significantly lower concentration of HT-2 and T-2. Overall, the risk of UK wheat exceeding the newly introduced legal limits for fusarium mycotoxins in cereals intended for human consumption is low, but the percentage of samples above these limits will fluctuate between years.

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3 **Keywords:** *trichothecenes, deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin,*
4 *zearalenone, organic, conventional*
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For Peer Review Only

Introduction

The trichothecene mycotoxins are produced by some of the *Fusarium* head blight pathogens and head blight severity is dependant on weather conditions. High humidity during and after flowering is conducive to head blight epidemics and mycotoxin production. Deoxynivalenol (DON) and nivalenol (NIV) are Type B trichothecenes produced predominantly by *F. culmorum* and *F. graminearum*. Isolates of both these species are either DON or NIV producers. DON producers are referred to as Type 1 chemotype, this chemotype is further divided into 1A and 1B depending on the acetylated DON that is produce as a co-contaminant, 3- or 15-acetyl DON respectively (Jennings et al. 2004a, Jennings et al. 2004b). *F. poae* has also been linked to high levels of NIV (Pettersson et al. 1995). HT-2 toxin (HT2) and T-2 toxin (T2) are type A trichothecenes, which are thought to be produced predominantly by *F. sporotrichioides* and *F. langsethiae* (Kosiak et al. 2003, Thrane 2004).

The predominant *fusarium* mycotoxin found in UK wheat grain during previous studies was DON. An HGCA survey of mycotoxins in UK wheat grain harvested in 1999 (Prickett et al. 2000, MacDonald et al. 2004) showed levels of DON were below the previous EC monitoring level of 750 $\mu\text{g kg}^{-1}$ although 58% were above 50 $\mu\text{g kg}^{-1}$ in what was classed as a year with low head blight. Surveys of cereal products have indicated that *fusarium* mycotoxins are a common contaminant of human and animal diets, however they usually occur at low concentrations. DON causes reduced feed intake, reduced weight gain and vomiting in farm animals (Anon. 2004a). Nausea, vomiting, diarrhoea, abdominal pain, headache, dizziness and fever have been reported when high concentrations of DON were consumed by humans (Anon. 1999). Other trichothecenes have the same cellular activity which is disruption of protein synthesis, and have a higher cellular toxicity than DON. NIV and T2 are ca. 20 times more toxic than DON, although the relative differences are dependent on the target cell or animal studied (Desjardins 2006). HT2 and T2 were implicated in Alimentary Toxic Aluekia caused by the consumption of cereals which had overwintered in fields in Russia in the 1940s (Desjardins 2006).

Although, DON is considered the predominant trichothecene mycotoxin within grain, some of the other trichothecenes have greater toxicity, so it is important that they are also monitored. Of the other trichothecenes, only HT-2 and T-2 toxins are currently being considered for legislation within the EC. There was a previous

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3 proposed combined (HT2+T2) maximum limit of 100 $\mu\text{g kg}^{-1}$ for unprocessed wheat
4 grains.
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7 Zearalenone is another mycotoxin produced predominantly by *F. culmorum*
8 and *F. graminearum*. Zearalenone has low cellular toxicity but is problematic as it
9 has high estrogenic activity causing hyperoestrogenism in animals and humans. In
10 animals the mycotoxin causes a range of fertility problems, with young female pigs
11 being particularly susceptible (Anon. 2004b). There are no proven cases of human
12 zearalenone toxicosis but the mycotoxin has been implicated in cases of premature
13 puberty in young females (Anon. 2000).
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17 The European Commission (EC) has set legislative limits for the fusarium
18 mycotoxins including the trichothecene, DON and ZON in cereal grains and cereal-
19 based products intended for human consumption (Anon. 2006b). A combined limit
20 for the trichothecenes HT2 and T2 will be introduced in the near future. The
21 European Commission also set guideline limits in 2006 for fusarium mycotoxins in
22 animal feed (Anon. 2006a).
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26 The presence of relationships between mycotoxin concentrations are important
27 as they allow assumptions for one mycotoxin based on the concentration of another.
28 For example, legislative limits have not been set for NIV as it is considered by the EC
29 to be a co-contaminant of DON and as such can be controlled by controlling DON
30 (Anon. 2006b).
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34 There have been limited studies comparing mycotoxin concentrations in
35 organic and conventional wheat samples. Many studies failed to identify significant
36 differences between the two practices within Europe (Malmauret et al. 2002, Cirillo et
37 al. 2003, Champeil et al. 2004, Jestoi et al. 2004). However, significantly lower
38 levels of DON in organic samples was detected in some regions of Germany (Doll et
39 al. 2002, Schollenberger et al. 2002, Schollenberger et al. 2005) although this was not
40 the case in northern Germany in the mid-1990s (Berleth 1998).
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44 The aims of the present study were: i) to identify the distribution of *fusarium*
45 mycotoxins in UK wheat, ii) to identify relationships between the concentrations of
46 *fusarium* mycotoxins detected, iii) to identify how *fusarium* mycotoxin distributions
47 vary across seasons and regions and iv) to identify if there were differences in the
48 *fusarium* mycotoxin concentrations of organic and conventional wheat in the UK.
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Material and methods

Sample collection

Each year, from 2001-2005, about three hundred grain samples and related agronomic data were collected by agronomists and growers. Samples were collected at harvest from specific fields either from the combine or from trailers leaving the field. Ten approx. 300 g sub-samples were taken from arbitrary points around the field and combined to provide a 3 kg sample. Growers and consultants sent these samples in cotton bags by overnight courier along with agronomic data pertaining to that field sample. A similar number of samples were requested from each of six regions within the UK. These were South, East, Midlands and North of England, Scotland and Northern Ireland. A few samples were collected from South Wales and these were included in the South of England region. A similar number of samples were requested from conventional production using different fungicide sprays at flowering; no spray, azole, strobilurin or azole/strobilurin mixture and from organic samples.

In the first year of the project the target number of samples was not achieved with 283 samples collected (Table I). This was primarily due to the outbreak of foot and mouth disease which resulted in restricted access to farms with livestock in 2001. A lower proportion of samples was collected from organic farms in 2001 as these were predominantly livestock farms (Table II). A total of 1624 samples were analysed over the five year period (2001-2005), including 247 organic samples. Samples were collected from Northern Ireland from 2002 onwards. Numbers of samples collected from Scotland and Northern Ireland were lower than for other regions, this was due to the smaller areas of wheat in these regions (Table I).

On receipt of samples their moisture contents were determined; any samples above 18% moisture content were dried to ca. 15% overnight. A 500 g sub-sample of grain was removed using a ripple divider, dried to 12% moisture content and stored at room temperature for visual assessment. The remaining sample was milled with a 1 mm screen, mixed in a tumbler mixer before three 200 g laboratory samples were collected. One sample was sent to RHM Technology for GC-MS analysis of trichothecenes while a second sample was sent to the Central Science Laboratory for HPLC analysis of ZON. A third sample was held as an archive sample at -20°C .

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7 *Mycotoxin analysis*
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10 The GC-MS analysis of trichothecenes was performed by RHM Technology (High
11 Wycombe) according to the published method (Patel 1996). The trichothecenes
12 analysed for were deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-
13 acetylDON, fusarenone X, T-2 toxin (T2), HT-2 toxin (HT2), diacetoxyscirpenol
14 (DAS), neosolaniol and T-2 triol. Spiked samples were included in each batch to
15 determine extraction recovery. The UKAS accredited method had acceptable
16 recovery range for each trichothecene of 70-110%. Results were adjusted according
17 to recovery. For this study the calculation of the measurement uncertainty was carried
18 out using in-house data, performance in international collaborative trials and Food
19 Analysis Performance Assessment Scheme (www.fapas.co.uk/fapas.cfm) thus
20 incorporating repeatability and reproducibility data. The expanded measurement of
21 uncertainty was calculated using a standard coverage factor of two, equivalent to a
22 confidence of approximately 95% that the actual level of the mycotoxin being
23 measured lies within the quoted range. The expanded measurement of uncertainty
24 was calculated to be $\pm 25\%$. The limit of quantification (LoQ) was determined as six
25 times the baseline noise and calculated to be $10 \mu\text{g kg}^{-1}$.
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38 The HPLC analysis of ZON was performed by Central Science Laboratory
39 (York) according to a previously published method (MacDonald et al. 2005). As for
40 the trichothecenes, the method was UKAS accredited. Spiked samples were included
41 and results adjusted according to recovery. The recovery range for ZON was 70-
42 110%. For this study the calculation of the measurement uncertainty was carried out
43 using performance in an international collaborative trial (MacDonald et al. 2005). The
44 expanded measurement of uncertainty was calculated using a standard coverage factor
45 of two, equivalent to a confidence of approximately 95% that the actual level of the
46 mycotoxin being measured lies within the quoted range. The expanded measurement
47 of uncertainty for wheat flour was calculated to be $\pm 34\%$ at the concentration of 227
48 $\mu\text{g kg}^{-1}$ ZON. Similar measurements of uncertainty were achieved for other cereal
49 matrices with ZON concentration ranging from 67 to $143 \mu\text{g kg}^{-1}$. The limit of
50 quantification (LoQ) was calculated to be $5 \mu\text{g kg}^{-1}$.
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Statistical analysis

Summary statistics for each mycotoxin were calculated using Excel (Microsoft Office 2003). Statistics calculated included the percentage greater than $10 \mu\text{g kg}^{-1}$ (LoQ for trichothecenes), the mean, median, 90th percentile, 95th percentile and the maximum concentration for each mycotoxin detected. For the calculation of the mean, samples below the LoQ were assigned a value of (LoQ)/6, ie 1.667 and $0.833 \mu\text{g kg}^{-1}$ for trichothecenes and ZON respectively. This allows data to be directly compared to recently compiled fusarium mycotoxin occurrence data from EU member states (Anon, 2003)

For regression analysis samples below the LoQ were removed from the dataset and concentrations \log_{10} transformed to normalise the variance. For ANOVA samples below the LoQ were assigned a value of (LoQ)/2, ie $5 \mu\text{g kg}^{-1}$ for trichothecenes and $2.5 \mu\text{g kg}^{-1}$ for ZON, \log_{10} transformed to stabilise the variance and analysed using Genstat (version 10, Lawes Agricultural Trust). For analysis of deviance of incidence data samples below the LoQ were allocated a value of zero, those above the LoQ were allocated a value of one and analysed using a Bernoulli distribution with Genstat.

Results and discussion

Mycotoxin concentration

Of the eleven mycotoxins analysed only eight were detected, of these DON, 15-AcetylDON, NIV, HT2 and ZON were detected above $100 \mu\text{g kg}^{-1}$ and T2, T-2 triol and 3-AcetylDON were detected below $100 \mu\text{g kg}^{-1}$. Table III shows the summary statistics for each mycotoxin detected over the five year period. Combined values are provided for HT2 and T2 (HT2+T2) as these closely related mycotoxins have equivalent toxicity and European legal limits will be based on a combined concentration.

DON was the most frequently detected fusarium mycotoxin and was usually present at the highest concentration. The distribution was lognormal with a left censor truncated by the LoQ. By comparison of the mean and median values for all other mycotoxins detected indicates they follow a similar lognormal distribution, however the degree of truncation of the distribution at the LoQ varies markedly. NIV

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3 was the next most common fusarium mycotoxin detected although it was never
4 detected at a high concentration (maximum = 430 $\mu\text{g kg}^{-1}$). This would support the
5 decision not to set a maximum limit for this mycotoxin, although NIV should not be
6 considered a co-contaminant with DON, as regression analysis shows the relationship
7 between the two is complex as detailed below. Low levels detected in UK wheat
8 would indicate that the production of NIV in UK wheat is limited. It should be noted
9 that the relationship between pathogens and hosts is a dynamic one, and the current
10 situation may change.
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18 HT-2 and T-2 was detected in 31 and 16% of samples respectively, the
19 concentration was usually low but some samples (0.5%) exceeded the EC previously
20 proposed combined limit for consideration and discussion of 100 $\mu\text{g kg}^{-1}$ HT2 and T2
21 (HT2+T2) (Table IV). ZON was quantified in 39% of samples (LoQ = 5 $\mu\text{g kg}^{-1}$),
22 19% of samples exceeded 10 $\mu\text{g kg}^{-1}$. However, due to the lower legal limits for this
23 mycotoxin, more samples exceeded the legal limit for ZON than for DON (Table IV).
24 It should be noted that the legal limits for DON and ZON in unprocessed cereals
25 include a measurement of uncertainty. Therefore for a consignment of unprocessed
26 wheat intended for human consumption to exceed the legal limit for DON and ZON
27 the concentration as determined by the analytical procedures employed in this project
28 would have to exceed 1563 $\mu\text{g kg}^{-1}$ DON or 134 $\mu\text{g kg}^{-1}$ ZON.
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37 T-2 triol, 3-acetylDON and 15-acetylDON were detected in very few samples
38 and always as a low concentration, co-contaminant in the presence of a high
39 concentration of a primary contaminant (HT-2 and DON respectively). Fusarenone
40 X, diacetoxyscirpenol and neosolaniol were not detected in any sample (LoQ = 10 μg
41 kg^{-1}).
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Regression analysis

The relationship between DON and ZON concentration was analysed by regression analysis of logarithmic transformed values, grouped by year. The regression and year were both highly significant ($p < 0.01$). The regression was best fitted by separate, non-parallel lines ($r^2 = 0.56$). Such a relationship could be expected as DON and ZON are produced by the same species, namely *F. culmorum* and *F. graminearum*, but isolates of these species may or may not produce DON and/or ZON. The major difference was the higher ratio of ZON to DON in 2004. ZON is known to be produced as the crop senesces at the end of the growing season (Matthaus et al. 2004). It is likely that the relatively high ratio of ZON to DON in 2004 was a result of the delayed, wet harvest that year. An important point to note in Figure 1 is that samples which exceeded $1250 \mu\text{g kg}^{-1}$ DON may, or may not have exceeded $100 \mu\text{g kg}^{-1}$ ZON, and vice versa. In fact, close to $1000 \mu\text{g kg}^{-1}$ DON or $100 \mu\text{g kg}^{-1}$ ZON could occur in the absence of detectable quantities of the other. The percentage of samples which exceeded $1250 \mu\text{g kg}^{-1}$ DON and $100 \mu\text{g kg}^{-1}$ ZON fluctuated each year and the relationship between the two was not stable between years (Table IV). The consequence for the cereal industry is that a single mycotoxin test can not be used as a screen at cereal intake as samples may exceed the legal limit for DON but not ZON or vice versa. It is therefore important that both DON and ZON are analysed in samples which may exceed legal limits for fusarium mycotoxins.

There were no other positive relationships between the concentrations of other commonly detected fusarium mycotoxins detected in UK wheat. In fact both NIV and HT2+T2 show signs of mutual exclusion with DON, ie when one mycotoxin was present at high concentration then the other was low. For HT2+T2 this appears to be simple mutual exclusion (Figure 2); this is probably because HT2+T2 are produced by *Fusarium* species which appear to have different environmental requirements to *F. culmorum* and *F. graminearum*. As a consequence of this relationship, DON concentration cannot be used to predict HT2+T2 concentration. For NIV the relationship is more complex (Figure 3) as: i) NIV and DON are produced by different chemotypes of the same species (*F. culmorum* and *F. graminearum*), ii) NIV is produced as a low level co-contaminant by DON chemotypes (and hence there is always some NIV present in samples with high DON concentration) and iii) NIV is

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3 also produced by *F. poae*, which has different environmental requirements to *F.*
4 *culmorum* and *F. graminearum* (Xu et al. 2008).
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7 The acetylated versions of DON (3-acetyl and 15-acetyl DON) are co-
8 contaminants of DON which occur at a low percentage of the DON concentration, and
9 as such are only normally detected when DON is present at a high concentration
10 (Placinta et al. 1999). As such legislation that reduces consumer exposure to DON
11 also reduces consumer exposure to acetylated DON in cereals and cereal products
12 intended for human consumption. *F. graminearum* and *F. culmorum* DON
13 chemotypes are either 3-acetyl or 15-acetyl DON producers. The plot of acetylated
14 DON against DON (Figure 4) shows that this relationship fits for the majority of
15 samples with no acetylated DON detected when DON concentration was low and a
16 low concentration of acetylated DON when the DON concentration was high. For
17 these samples, the percentage concentration of acetylated DON to DON was between
18 0.25 and 2.5%. However, Figure 4 shows two anomalies: i) Samples with high DON
19 exist with no detectable acetylated DON suggesting a chemotype of *F. culmorum*
20 and/or *F. graminearum* may exist which does not produce an acetylated DON and ii)
21 in five samples (circled in Figure 4), acetylated DON occurred as a high proportion of
22 the total DON concentration with a percentage acetylated DON to DON concentration
23 of 25 to 150%. All five samples were from the 2005 harvest, four samples were from
24 the Midlands and one from the East of England. This anomaly is of concern as
25 legislation is set for DON alone on the assumption that acetylated forms of DON
26 occur as a co-contaminant at a low percentage of DON concentration. Therefore there
27 is a need to monitor acetylated DON as well as DON in cereals and cereal products to
28 ensure that high concentrations of acetylated DON do not occur.
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46 There was a weak relationship between HT2 and T2 concentration (Figure 5).
47 A relationship between these two mycotoxins would be expected as the two
48 mycotoxins are produced by the same species on the same metabolic pathway. A
49 good relationship over a much higher concentration range was found for HT2 and T2
50 in UK oats over the same period (Edwards, manuscript in preparation).
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Year, region and practice

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5 The DON concentration according to practice was analysed using ANOVA. Year and
6 region were entered into the model first to account for temporal and spatial variation.
7 There was a highly significant interaction ($p < 0.001$) between year and region with an
8 overall trend of lower DON towards the North of the UK (Figure 6). There was no
9 significant difference in the DON concentration between organic and conventional
10 samples ($p = 0.147$). As a consequence of the low incidence of samples with ZON and
11 HT2+T2 above the limit of quantification then the concentration of these samples
12 could not be analysed by ANOVA. Incidence of these two datasets were analysed by
13 analysis of deviance using a Bernoulli distribution. Concentration of ZON and
14 HT2+T2 was then analysed for the subset of samples with concentrations above the
15 limit of quantification using ANOVA. As for DON analysis, year and region were
16 entered into the models first to account for temporal and spatial variation.
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26 For ZON there was a highly significant interaction ($p = 0.004$) between year
27 and region with an overall trend, as for DON concentration, of lower ZON incidence
28 towards the North of the UK (Figure 7A). There was no significant difference in the
29 ZON incidence of organic and conventional samples ($p = 0.961$). For positive samples
30 the ZON concentration was significantly different between years ($p < 0.001$) and
31 regions ($p < 0.001$) although the interaction was not significant ($p = 0.094$) (Figure 7B).
32 There was no also significant difference in the ZON concentration of organic and
33 conventional positive samples ($n = 615$, $p = 0.513$). Consequently there was no
34 significant difference in the overall concentration of ZON between organic and
35 conventional samples of wheat.
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44 For HT2+T2 there was a highly significant interaction ($p < 0.001$) between year
45 and region with no consistent trend across year or region (Figure 8A). There was a
46 significant difference ($p < 0.001$) in the HT2+T2 incidence of organic and conventional
47 samples. The predicted incidence for HT2+T2 in organic samples was 20% compared
48 to 36% in conventional samples. ANOVA of HT2+T2 concentration in positive
49 samples ($n = 534$) indicated a significant ($p = 0.017$) difference between organic and
50 conventional samples. The predicted concentration of positive organic samples was
51 19 compared to 23 $\mu\text{g kg}^{-1}$ HT2+T2 for positive conventional samples. Consequently
52 there was a significant difference in the overall concentration of HT2+T2 between
53 organic and conventional samples of wheat, with lower concentrations present in
54 organic samples.
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7 *General discussion*
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10 This research has clearly identified the extent to which UK organic and conventional
11 wheat contains fusarium mycotoxins at harvest. Overall, levels of fusarium
12 mycotoxins in UK wheat are lower than those frequently found in continental Europe
13 and in North America (Jones and Mirocha 1999, Schaafsma 2002, Anon. 2003). The
14 majority of samples contained fusarium mycotoxins, the predominant one being
15 DON, however the vast majority of samples had a concentration close to the limit of
16 quantification ($10 \mu\text{g kg}^{-1}$) with only a low percentage of samples exceeding the
17 recently introduced legal limits for DON and ZON for unprocessed wheat intended
18 for human consumption. Numbers of samples exceeding $1250 \mu\text{g kg}^{-1}$ DON were
19 between about 0.5 and 5% over the five year period. More samples, between 1 and
20 10% exceeded $100 \mu\text{g kg}^{-1}$ ZON over the five year period
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30 HT2+T2 concentrations were generally lower although until legislative limits
31 are set the percentage of samples exceeding new limits cannot be determined. The
32 number of samples exceeding $100 \mu\text{g kg}^{-1}$ HT2+T2 varied from 0 to 1% between
33 years. The poor relationship between HT2 and T2 is important as legislation will be
34 introduced as a combined limit for HT2+T2. ELISA assays exist for T2 but these kits
35 cross-react to a low percentage (ca. 10%) with HT2. Consequently, with no
36 consistent relationship between HT2 and T2 in wheat, ELISA assays for T2 are of
37 limited use. Consequently there is no quick or cheap method to allow the industry to
38 monitor these mycotoxins at intake or other critical control points during processing.
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46 NIV was not detected at high concentrations. This may be because NIV-
47 producing isolates and species of *Fusarium* are less virulent on wheat than DON-
48 producing isolates (Wong et al. 1995, Desjardins et al. 2004). Consequently, without
49 very high levels of initial inoculum, infection can not cause high NIV concentrations
50 in harvested grains. However, NIV is not a co-contaminant of DON, either based on
51 the known chemotaxonomy or based on observational data such as this study, and as
52 such NIV should not be treated as a co-contaminant within the legislation.
53 Consequently the lack of a requirement for specific legislation for NIV should be
54 based on the estimated low exposure rather than as a co-contaminant. High NIV may
55 occur in different cereals and/or countries and should be monitored accordingly.
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Acetylated versions of DON did occur as low level co-contaminants of DON in most samples, however five samples were analysed in 2005 with equivalent levels of DON and acetyl DON. These were not of concern as the combined concentration of these mycotoxins was not high. However, it is important to be aware that samples may occasionally contain equivalent concentrations of acetyl DON compared to DON and as such the acetylated versions of DON should be monitored as well as DON itself.

Highest concentrations of DON and ZON occurred in 2004. In 2004, most of the UK was drier than average during June, when most wheat were in flower. However, a wetter than average summer followed for the whole of the UK resulting in high levels of head blight reported at harvest, particularly where harvest was delayed due to continued wet weather. This indicated the importance of weather in late summer, as well as early summer on the final mycotoxin content of grain at harvest. It is also important to note that the impact of weather late in the season varied between mycotoxins, with relatively higher concentrations of ZON in 2004 compared to DON as shown in Figure 1.

Spatial variation of DON across the UK showed that higher DON occurred in the South and East of the UK. There was a consistent decline in DON towards the North in all years, which can be attributed to cooler temperatures. *Fusarium graminearum*, which is the most potent DON producer, is known to have an optimum temperature for sporulation, growth and infection of ca. 28°C (Xu 2003). The higher DON identified in the East may be attributable to the higher intensity of cereals in rotation (Anon. 2007a). The distribution of high DON concentrations closely matches the distribution of *F. graminearum* across the UK (Anon. 2007b). The interaction of year and region is probably due to differences in rainfall distribution across the regions in different years resulting in higher average concentrations in the East in some years and in the South in others. The ZON distribution was a similar pattern to DON which is to be expected as they are produced by the same *Fusarium* species. The distribution of HT2 and T2 was very different with similar levels across all regions although levels fluctuated inconsistently between seasons and regions.

Previous studies comparing organic to conventional wheat have either failed to identify significant differences between the two practices (Berleth 1998, Malmauret et al. 2002, Cirillo et al. 2003, Champeil et al. 2004, Jestoi et al. 2004) or have detected

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3 significantly lower levels of DON in organic samples in some regions of Germany
4 (Doll et al. 2002, Schollenberger et al. 2002, Schollenberger et al. 2005). Many
5 previous studies have been limited due to low sample number. This study with 247
6 organic and 1377 conventional samples distributed across the whole of the UK and
7 over five years is the most robust study to date. Results from this study identified no
8 significant difference in DON and ZON concentration between organic and
9 conventional samples. There are several reasons why organic samples may have
10 lower (eg less cereal intense rotations and less minimum tillage) or higher (eg no
11 fungicides) DON compared to conventional samples based on known impacts of
12 agronomic factors on DON concentration in harvested grain (Edwards, 2004). It is
13 likely that these factors balance one another resulting in a similar concentration of
14 DON in the two practices. In Germany, there is a higher acreage of maize in
15 conventional rotations which is largely absent from organic rotations. Maize, as a
16 previous crop to wheat, is known to be a major risk factor for high DON
17 concentrations (Edwards, 2004). This maybe one reason why differences were
18 detected in previous German studies but not in this UK study. The incidence and
19 concentration of positive samples for HT2+T2 were both significantly lower for
20 organic samples. This would suggest that factors that can result in lower HT2+T2 in
21 organic practice out balance any possible negative impacts of organic practice.
22 However, it is important to note that the differences, although significant, were
23 unlikely to have any relevance regarding the relative food safety of organic compared
24 to conventional wheat and wheat products as the overall incidence and concentration
25 of these mycotoxins were low.

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28 Overall, the risk of UK wheat exceeding the newly introduced legal limits for
29 fusarium mycotoxins in cereals intended for human consumption is low, but the
30 percentage of samples above these limits will fluctuate each season.
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Table I. Sample distribution by year and region.

Year	Region						Total
	South	East	Midlands	North	Scotland	N.Ireland	
2001	50	79	63	58	33	0	283
2002	64	62	93	67	38	19	343
2003	75	62	69	77	29	16	328
2004	60	73	93	72	33	13	344
2005	61	67	81	73	31	13	326
Total	310	343	399	347	61	164	1624

Table II. Sample distribution by year and practice.

Year	Practice		Total
	Organic	Conventional	
2001	17	266	283
2002	57	286	343
2003	61	267	328
2004	54	290	344
2005	58	268	326
Total	247	1377	1624

Table III. Concentrations of all fusarium mycotoxins detected in UK wheat in 2001-2005 (1624 samples).

	% > 10 $\mu\text{g kg}^{-1}$	Mycotoxin concentration ($\mu\text{g kg}^{-1}$)				
		Mean ^a	Median	90th%	95th%	Max
DON	86	230	42	368	722	20333
15AcDON	2.7	<10	<10	<10	<10	217
3AcDON	1.2	<10	<10	<10	<10	44
NIV	67	27	16	64	95	430
T-2 triol	0.4	<10	<10	<10	<10	45
T-2	16	<10	<10	13	17	52
HT-2	31	<10	<10	19	28	193
HT2+T2	34	<20	<20	30	43	214
ZON	19	17	<5	27	61	1292

^aBased on a value of 1.667 (0.833 for ZON) for all samples below the limit of quantification ($10 \mu\text{g kg}^{-1}$; $5 \mu\text{g kg}^{-1}$ for ZON).

Table IV. Percentage of samples exceeding 1250 $\mu\text{g kg}^{-1}$ DON, 100 $\mu\text{g kg}^{-1}$ ZON and 100 $\mu\text{g kg}^{-1}$ HT2+T2.

Year	% > 1250 $\mu\text{g kg}^{-1}$ DON	% > 100 $\mu\text{g kg}^{-1}$ ZON	% > 100 $\mu\text{g kg}^{-1}$ HT2+T2
2001	0.4	0.7	0.7
2002	1.7	0.6	0.0
2003	2.4	1.2	0.6
2004	5.5	11.3	0.9
2005	1.5	1.2	0.3
Overall	2.4	3.1	0.5

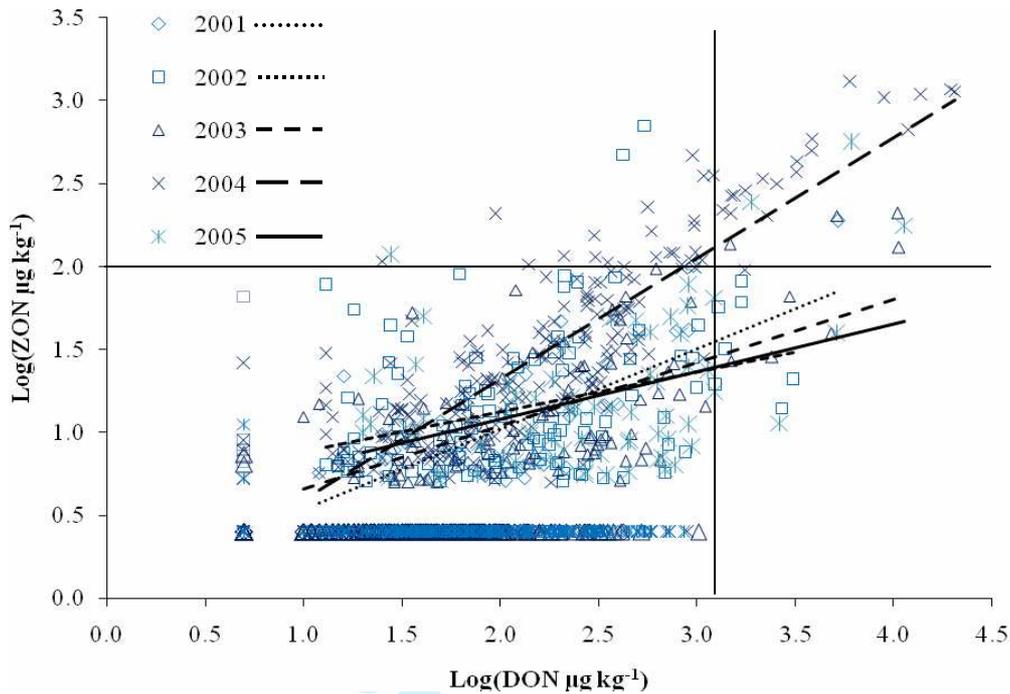


Figure 1. Regressions of ZON against DON concentration (log log plot) for UK wheat for each year from 2001 to 2005 (n = 1624). Regressions fitted for all samples above LoQ. Vertical line represents 1250 $\mu\text{g kg}^{-1}$ DON and horizontal line represents 100 $\mu\text{g kg}^{-1}$ ZON

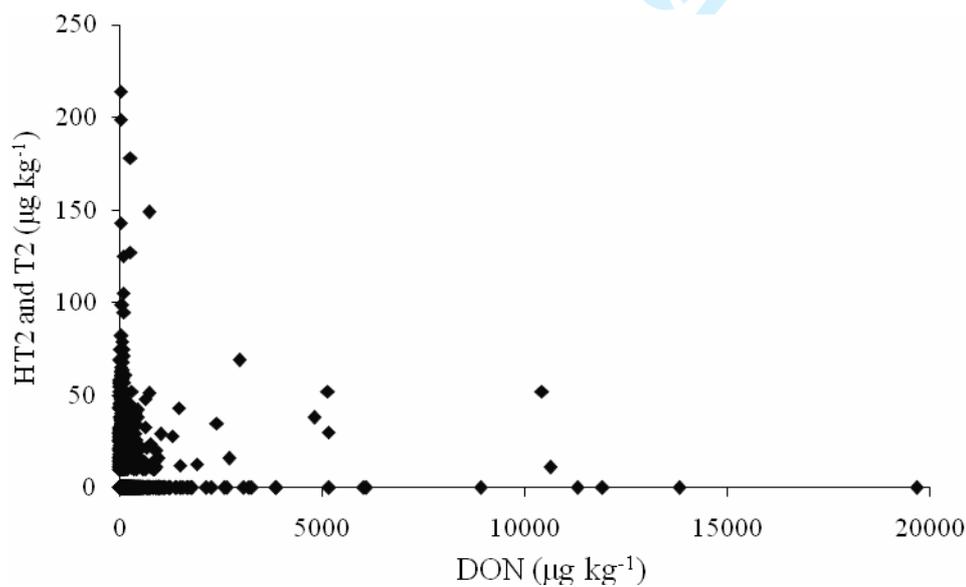


Figure 2. Relationship between HT2+T2 and DON concentration for UK wheat 2001-2005 (n=1624).

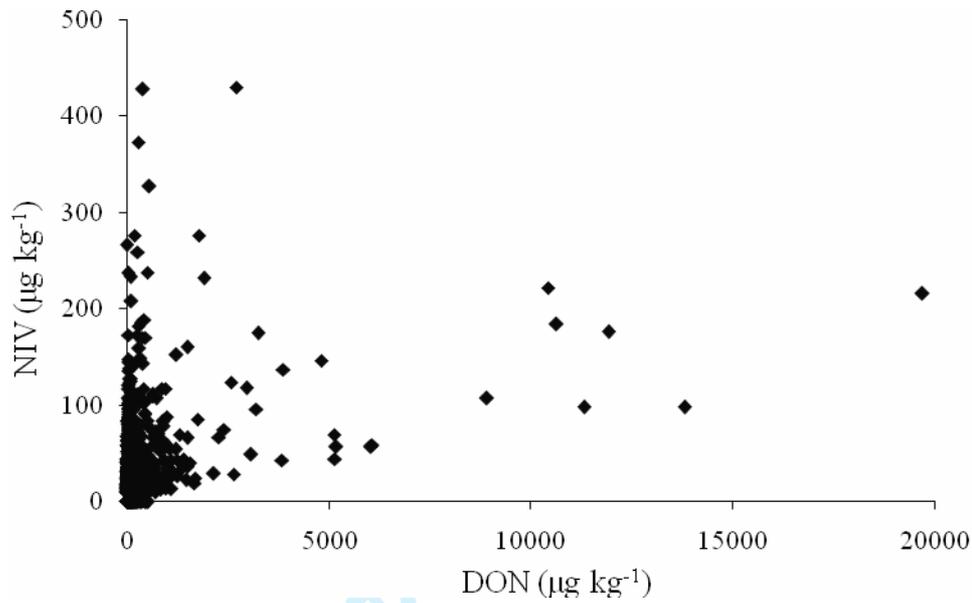


Figure 3. Relationship between NIV and DON concentration for UK wheat 2001-2005 (n=1624).

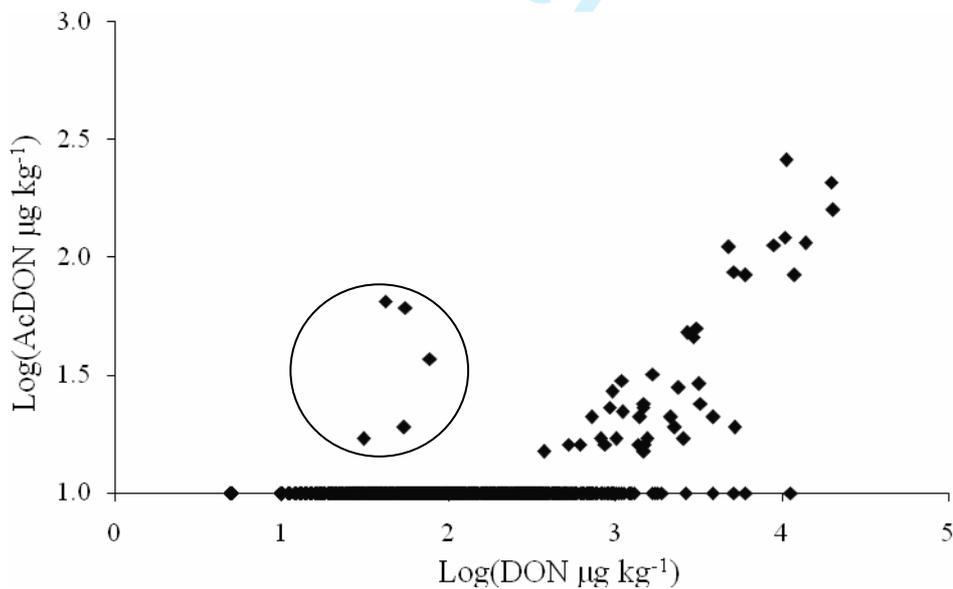


Figure 4. Relationship between AcDON (3- and 15-acetyl DON) and DON concentration for UK wheat 2001-2005 (n=1624). Samples with high ratio of acetylated DON to DON are circled.

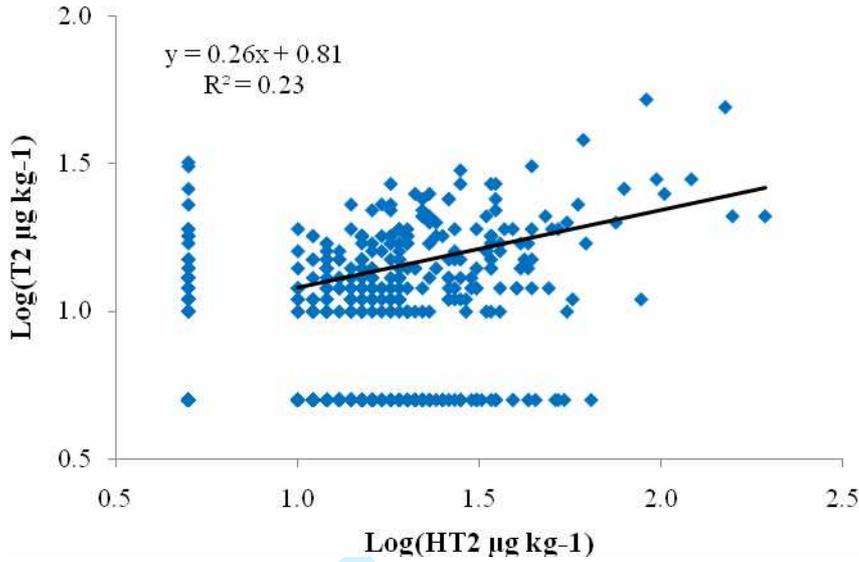


Figure 5. Relationship between T2 and HT2 concentration for UK wheat 2001-2005 (n=1624). Regression fitted for all samples above LoQ.

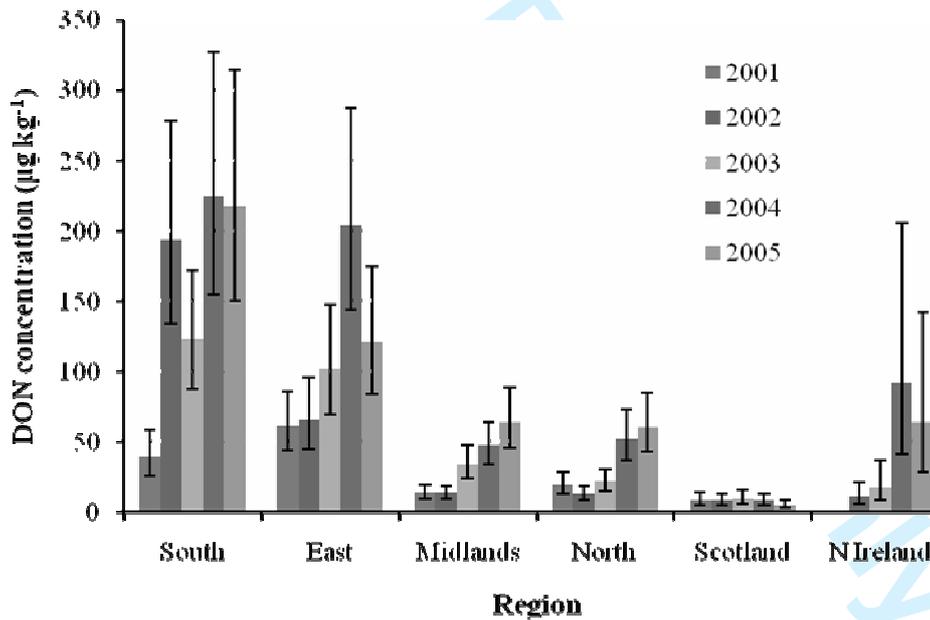
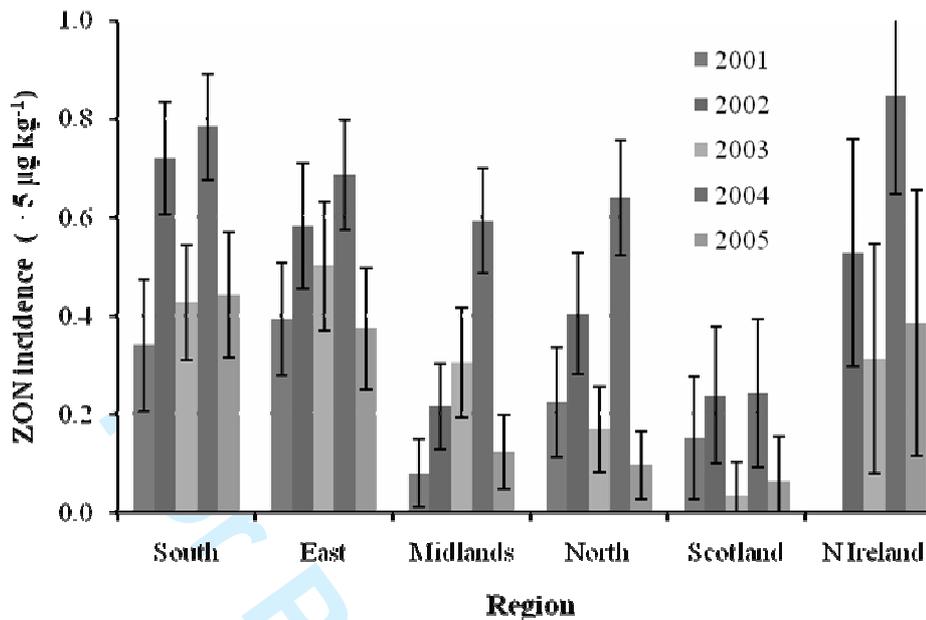
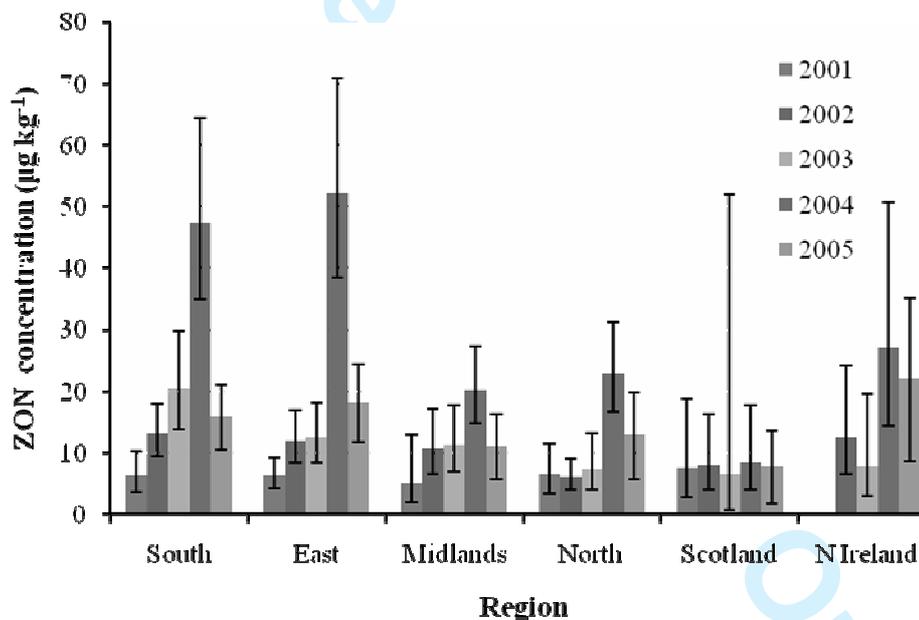


Figure 6. Predicted mean DON concentration for UK wheat from each region in each year. Bars represent 95% confidence limits for predictions.



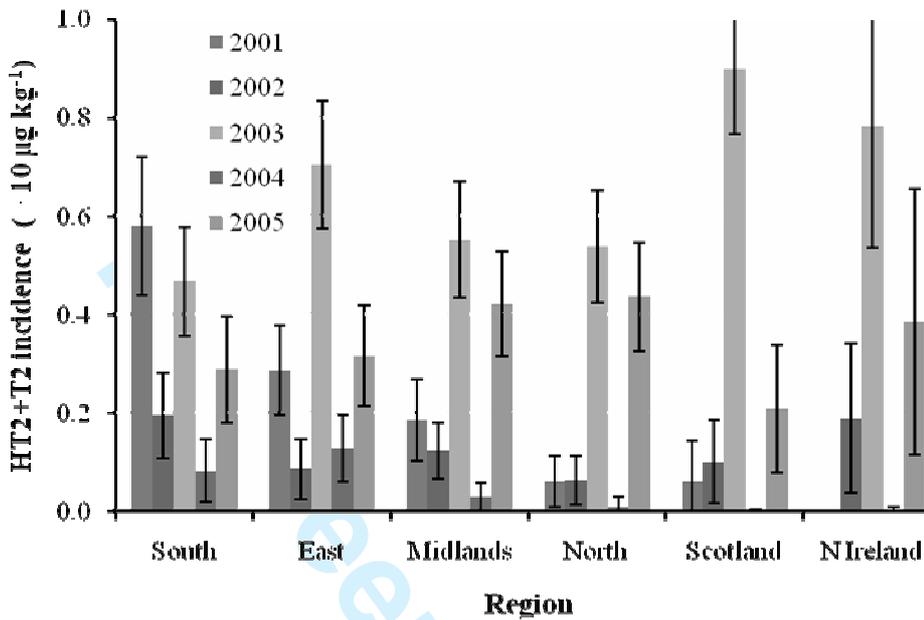
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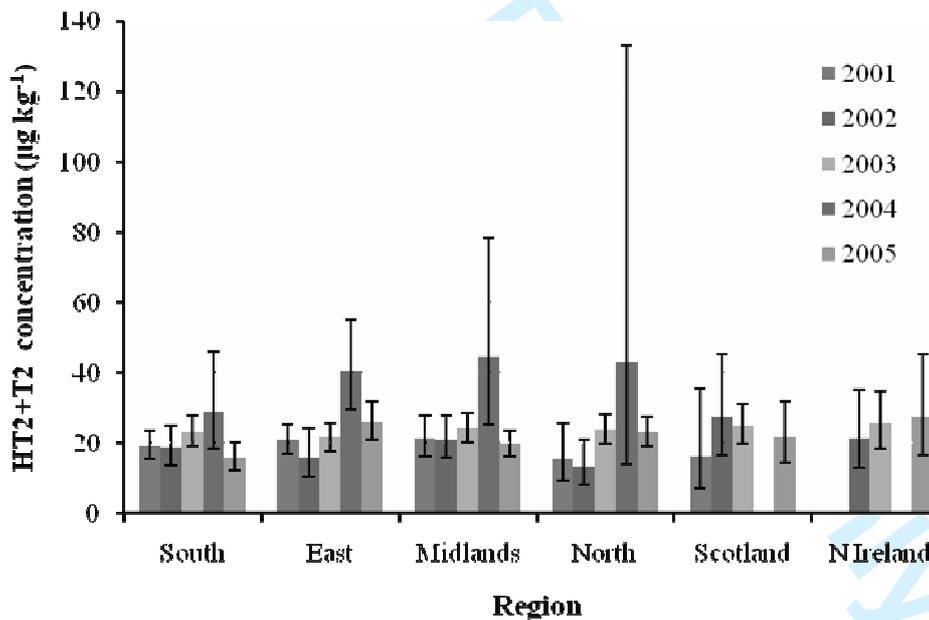
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Figure 7. Predicted ZON incidence (A) and mean concentration of positive samples (B) for UK wheat from each region in each year. Bars represent 95% confidence limits for predictions.



A.



B.

Figure 8. Predicted HT2+T2 incidence (A) and mean concentration of positive samples (B) for UK wheat from each region in each year. Bars represent 95% confidence limits for predictions.