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1 # Susceptibility of cereal species to *Fusarium langsethiae* under identical field conditions

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7

8 ## Abstract

9 Experiments were conducted to determine the extent of *Fusarium langsethiae* infection in
10 wheat, barley and oats grown under identical experimental conditions. In total, four
11 experiments were conducted with both winter and spring sown experiments at two locations.
12 The amount of *F. langsethiae* infection was determined by quantifying *F. langsethiae* DNA
13 and quantifying the combined concentration of the trichothecene mycotoxins HT-2 and T-2
14 (HT-2+T-2) in cereal head fractions (grain and rest of the head) after threshing at harvest.
15 Results of the study showed that under identical experimental conditions, oats had the highest
16 *F. langsethiae* DNA and HT-2+T-2 concentrations compared to wheat and barley. This
17 indicates that the high levels detected on UK oats compared to wheat and barley from surveys
18 of commercial crops is a consequence of genetic differences rather than differences in
19 agronomy applied to the cereal species. The concentration of HT-2 and T-2 per unit of *F.*
20 *langsethiae* DNA in oats compared to wheat and barley was also significantly higher indicating
21 host differences in either the stimulation of HT-2 and T-2 production or in the metabolism of
22 HT-2 and T-2. The study also showed that the proportion of *F. langsethiae* DNA in threshed
23 grains was significantly lower than that in the rest of the cereal head.

24 **Key words:** cereal, wheat, barley, oats, HT-2, T-2, trichothecene, mycotoxin

25

26

27 **Introduction**

28 *Fusarium* species can infect cereal crops resulting in the disease fusarium head blight and the
29 contamination of cereal grains with fusarium mycotoxins. *Fusarium* species can produce
30 numerous mycotoxins including several trichothecenes. The type A trichothecenes include the
31 closely related mycotoxins HT-2 and T-2. A survey of commercial crops identified a high
32 proportion of UK oats contained high concentrations of the mycotoxins HT-2 and T-2
33 (Edwards, 2007). It was subsequently determined that *Fusarium langsethiae* is the primary, if
34 not sole, species responsible for this type of mycotoxin contamination (Edwards et al, 2009).
35 There are currently no legal limits for these toxins but the European Commission has published
36 a recommendation (Anon 2013) stating that these toxins should be monitored by Member
37 States and where they exceed indicative levels, investigations should be performed as to why
38 these high levels occur and what methods can be used to reduce the occurrence of these
39 exceedances. The indicative level for unprocessed oats is 1000 $\mu g\ kg^{-1}$ for the combined
40 concentration of HT-2 and T-2 (HT-2+T-2). Surveys conducted from 2001-2008 identified
41 that on average 16% of UK oats exceeded the indicative level of 1000 $\mu g\ kg^{-1}$ HT-2+T-2, whilst
42 levels detected in wheat and barley were much lower (Edwards, 2009a, b, c; Edwards, 2012).
43 Data from a three year commercial field survey (2009-2011) showed that *F. langsethiae* DNA
44 as well as HT-2 and T-2 was highest in oats compared to wheat and barley (Opoku et al., 2013).
45 In this field survey, the authors indicated that fields sampled, while in close proximity to one
46 another (within 30 km), they were grown under differing agronomy (eg sowing dates, rotations
47 and chemical inputs). Agronomic factors are known to influence mycotoxins such as
48 deoxynivalenol (DON), HT-2 and T-2 concentrations in cereal grains. For example oats sown
49 after a cereal have a higher average concentration of HT-2 and T-2 in grains compared to oats

50 sown after a non-cereal in a rotation (Edwards et al., 2009). The greater *F. langsethiae* infection
51 (as indicated by high *F. langsethiae* DNA and HT-2+T-2 levels) in oats as compared to wheat
52 and barley under commercial field conditions (Opoku et al., 2013) is not properly understood.
53 Field experiments were therefore performed to determine if, under the same agronomic
54 conditions, *F. langsethiae* DNA would be higher in oats than wheat and barley. Higher *F.*
55 *langsethiae* DNA and subsequent high HT-2 and T-2 levels in oats compared to wheat and
56 barley under identical agronomic conditions would suggest that the high infection rate of *F.*
57 *langsethiae* and subsequent HT-2 and T-2 production in oats is more of an inherent host
58 character and under the influence of genetic control rather than difference in the agronomy
59 applied to the different cereal species. There are currently no known methods to successfully
60 inoculate *F. langsethiae* under field conditions (Imathiu et al. 2013; Opoku 2012).
61 Consequently field experiments were conducted using natural inoculum using fields that had
62 intensive cereal rotations to increase the level of inoculum present. In a previous field survey
63 (Opoku et al, 2013) where *F. langsethiae* infection was found to be higher in oats than in wheat
64 and barley, DNA and mycotoxin analyses were performed on whole heads. For most
65 agricultural systems however, cereals are threshed and the grains used as food or feed. It is
66 important, therefore, to determine if threshed cereal grains are analysed a similar trend of
67 results as in the field survey will be obtained.

68 The objectives of these experiments were therefore to determine the susceptibility of wheat,
69 barley and oats to *F. langsethiae* infection under similar agronomic conditions and to compare
70 the amount of *F. langsethiae* biomass (as measured by DNA) and HT-2+T-2 contained in
71 threshed cereal grain and the rest of the cereal head.

72

73 **Materials and Methods**

74 Experiments were carried out on two different locations; Woodseaves located in Staffordshire,
75 UK and Harper Adams University located in Shropshire, UK. Experimental sites were about
76 12 km apart. At each location two experiments were conducted within the same field, one for
77 winter cereal varieties and the other for spring cereal varieties. Three varieties each of winter
78 and spring wheat, barley and oats were selected based on their popularity within UK cereal
79 production for use in the experiments (Table. 1). The experiment was laid out in a split plot
80 design with cereal as the whole plot and the variety as the sub-plot. Each experiment comprised
81 of four blocks with three whole plots which were divided into three sub-plots. Cereal species
82 were randomized within each whole plot and the cereal variety randomised within each sub-
83 plot.

84 Experiments were conducted after a crop of winter wheat in cereal intense rotations. Fields
85 were ploughed and power harrowed just prior to sowing. Winter cereal varieties were sown in
86 mid-October and spring varieties in mid-March using a plot drill (Plotman, Wintersteiger
87 Austria). For winter cereal varieties a rate of 250 seeds m^{-2} was used for wheat and oats and
88 300 m^{-2} for barley. All spring cereal varieties were sown at 350 seeds m^{-2}. The different seed
89 rates were used to achieve a similar number of stems in late spring. Standard agronomic inputs
90 were carried out on both fields based on a balance of the optimum agronomy for each cereal
91 species. At maturity cereal heads were harvested manually and air dried after which they were
92 threshed using a mini-threshing machine (F. Walter-H. Wintersteiger, Austria). Grains and the
93 rest of the cereal heads (comprising of rachis, lemma and glume in wheat, the awns and the
94 rachis in barley and rachis, rachis branches and the glumes in oats) were collected and milled
95 in a sample mill (Cyclotec 1093).

96 *DNA extraction*

97 Milled samples were mixed thoroughly by hand and 5 and 3 g of milled grain and rest of cereal
98 head samples, respectively, were weighed into 50 ml centrifuge tubes for DNA extraction. To
99 each 50 ml centrifuge tube, 30 ml of CTAB buffer (87.7 g NaCl, 23 g sorbitol, 10 g N-lauryl
100 sarcosine, 8 g hexadecyl trimethylammonium bromide, 7.5 g ethylenediamine tetraacetic acid
101 and 10 g polyvinylpolypyrolidone, made up to 1 L with distilled water) were added. Tube
102 contents were mixed thoroughly by hand and then with an Hs501 digital shaker (IKA
103 Labortechnik) for 20 minutes and incubated at 65ºC for 1 hour. Tubes were then shaken again
104 by hand and centrifuged at 3,000 x g for 15 minutes after which 0.9 ml of the supernatant was
105 removed and added to 0.3 ml potassium acetate (5M) in a sterile 1.9 ml Eppendorf tube, mixed
106 for 1 minute and frozen at -20ºC for 1 hour. Tube contents were thawed at room temperature
107 before 0.6 ml chloroform was added. The contents of the tubes were then mixed for 1 min and
108 centrifuged at 12,000 x g for 15 min. One milliliter of the aqueous phase was removed and
109 added to a sterile 1.9 ml Eppendorf tube containing 0.8 ml of 100% isopropanol and mixed
110 for 1 min before centrifuging at 12,000 x g for 15 min. Resulting DNA pellets were washed
111 twice with 1 ml 44% isopropanol. Pellets were air dried before re-suspending in 0.2 ml TE
112 buffer and incubating at 65ºC for 25 min. Tube contents were vortexed and left at room
113 temperature overnight before spinning at 12,000 x g for 5 min. DNA concentrations were
114 determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Basingstoke, UK).
115 DNA extractions were diluted to 40 ng $\mu l^{-1.}$ After dilution, DNA concentrations were
116 measured again to determine the final working concentration and stored at 4°C.

117

118 *Control PCR to determine the presence of amplifiable DNA in samples*

119 An initial control PCR was carried out on all DNA samples prior to quantitative PCR to ensure
120 the presence and quality of DNA in samples. This involved amplification with ITS4 and ITS5

121 primers (TCC TCC GCT TAT TGA TAT GC and GGA AGT AAA AGT CGTAAC AAG G.
122 These primers amplify both fungal and plant DNA present in a sample at an anneal temperature
123 of 50°C. PCR was carried out using a 25 µl reaction mixture made up of 100 µM of each
124 nucleotide, 100 nM of each primer, 20 U of *Taq* polymerase (ABgene, Epsom, UK) ml^{-1}, 10
125 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 50mM KCl, 100 µg of gelatine ml^{-1}, 0.5 mg ml^{-1} of
126 Tween 20, 0.5 mg ml^{-1} of Nonidet P-40 and 5 µl of DNA sample. PCR grade water was used
127 as negative control. Samples were amplified using a PTC-100 thermal cycler (MJ Research
128 Inc., Minnesota, USA) programmed for initial denaturation at 94°C for 75 s followed by 35
129 cycles of 15 s at 94°C, 15 s at 50°C and 45 s at 72°C. There was a final extension step at 72°C
130 for 4 min 25 s before cooling to 4°C until sample recovery. Amplicon gel electrophoresis was
131 carried out on 2% agarose gels stained with ethidium bromide (0.05%). PCR products (ca. 650
132 bp) were viewed on a Gel Doc 1000 system (Bio-Rad, Buckinghamshire, UK) under UV light.
133
134 *Quantitative PCR to quantify* Fusarium langsethiae *DNA*
135 DNA samples were amplified with a quantitative PCR instrument (iCycler Bio-Rad, UK) with
136 *F. langsethiae* primers; FlangF3 5'-CAAAGTTCAGGGCGAAAACT-3'.and LanspoR1 5'-
137 TACAAGAAGACGTGGCGATAT-3' (Wilson et al., 2004) as detailed previously (Edwards
138 et al., 2012). qPCR MasterMix Plus for SYBR® Green I with fluorescein (Eurogentec, USA)
139 reagent was used according to manufacturer's instructions with a 25 µl reaction volume which
140 included 5 µl template DNA. PCR water (5 µl) was used instead of template as negative control.
141 A 10-fold dilution series of *F. langsethiae* DNA (10^0 – 10^{-4} ng $µl^{-1}$) was included in each PCR
142 run to provide a standard curve. Concentrations of *F. langsethiae* DNA were divided by the
143 total DNA concentration within a sample to give values per ng of total DNA.
144 The quantitative PCR conditions included an initial activation step (UNG) of 50°C for 2:30
145 min and an initial melt of 10 min at 95°C followed by 45 cycles with a melting step of 95°C

146 for 10 s, annealing temperature of 65°C for 10 s, extension at 72°C for 30 s, and a hold at 82°C
147 for 10 s during which fluorescence was measured. Melting curve fluorescence was determined
148 by holding at 95°C for 1 min, cooling to 55°C for 1 min and then raising the temperature to
149 95°C at a ramp rate of 0.05°C s^{-1}.

150

151 *HT-2+T-2 estimation*

152 T-2 in milled cereal head sub-samples was measured using Ridascreen® T-2 ELISA assay (R-
153 Biopharm AG Darmstadt, Germany) following manufacturer's instructions. Total HT-2+T-2
154 was estimated based on the known ratio of HT-2 and T-2 in UK oats and the known cross-
155 reactivity of the T-2 antibody with HT-2 (Edwards et al., 2012).

156

157 *Data analysis*

158 Data was $\log_{10}$ transformed before analysis. Initial analysis of the data determined the winter
159 sown experiments from the two locations had equivalent variance for both the DNA and
160 mycotoxin concentrations and as such the data could be combined and analysed together. The
161 same result was determined for the spring sown experiments which were also combined. Split
162 plot ANOVA were carried out with block nested within location in the design structure and
163 significant differences between means determined by LSD ($p = 0.05$) using Genstat (V.13 VSN
164 International Ltd.) for both the winter and spring sown experiments. Regression analysis with
165 groups (Genstat) was used to compare the relationship between *F. langsethiae* DNA and HT-
166 2+T-2 concentration between cereal species and cereal parts (grain and rest of head).

167

168

169

Results

Fusarium langsethiae DNA recovered from cereal heads

In general, levels of *F. langsethiae* DNA in oat heads were higher than in wheat and barley for both spring and winter varieties with levels ca. three times higher in winter oats compared to spring oats and only small differences between winter and spring sown wheat and barley (Table 2).

Winter cereal species

In winter cereal species, there was no significant interaction between cereal and cereal part ($P = 0.165$). The interaction between cereal, variety and cereal part was also not significant ($P = 0.778$). It was observed that *F. langsethiae* DNA recovered from the rest of the cereal heads was significantly higher than that recovered from the grains ($P < 0.001$). *F. langsethiae* DNA in the rest of the cereal heads was about fifteen times higher than that in the grains only with overall mean of 0.029 and 0.002 pg ng^{-1} respectively.

Fusarium langsethiae DNA recovered from the different varieties of wheat, barley and oat differed with a significant interaction between cereal and variety ($P < 0.001$). For winter wheat, Alchemy had the highest *F. langsethiae* DNA which was significantly different from that recovered from Robigus but not Solstice (Fig. 1). Flagon had the highest *F. langsethiae* DNA among the winter barley varieties with a mean of 0.032 pg ng^{-1} and was significantly ($P < 0.05$) different from that recovered from Cassia.(Fig 1). In winter oats, *F. langsethiae* DNA in heads of the different varieties were significantly ($p < 0.05$) different from each other (Fig 1) with that recovered from Gerald having the highest mean of 0.104 pg ng^{-1}.

Spring cereal species

192 In spring species, the interaction between cereal and cereal part was significant ($P < 0.001$). In
193 all three cereals *F. langsethiae* DNA in the rest of the head was significantly ($P < 0.05$) higher
194 than that in the grains. Log_{10} transformed mean *F. langsethiae* DNA in the rest of the head was
195 about 38 times higher than in the grains alone for wheat, eight times higher for barley and 17
196 times higher for oat (Fig. 2). The interaction between cereal and variety was significant ($P <$
197 0.001).

198 In wheat, *F. langsethiae* DNA recovered from the different varieties were not significantly (P
199 > 0.05) different from each other (Fig. 3). For barley, Quench had a mean *F. langsethiae* DNA
200 of 0.004 pg ng^{-1}. This was the highest *F. langsethiae* DNA recovered from the spring barley
201 varieties and was significantly ($P < 0.05$) higher than that recovered from Propino which had
202 the lowest mean *F. langsethiae* DNA of 0.0017 pg ng^{-1} (Fig 3). In oats, the highest *F.*
203 *langsethiae* DNA was recovered from Firth with a mean *F. langsethiae* DNA of 0.23 pg ng^{-1}
204 followed by that from Atego and Husky with mean *F. langsethiae* DNA of 0.004 and 0.002 pg
205 ng^{-1} respectively. These means were all significantly ($p < 0.05$) different from each other (Fig.
206 3).

207 ***HT-2+T-2 concentration in cereal heads***

208 Levels of HT-2+T-2 in cereals followed similar patterns as for *F. langsethiae* DNA (Table 3).

209 *Winter cereal species*

210 In winter cereal species there was no significant interaction between cereal and cereal part ($P=$
211 0.059) and the interaction between cereal, variety and part was not significant ($P = 0.25$). HT-
212 2+T-2 levels in different cereal parts analysed were different with the grains having
213 significantly lower levels than that in the rest of the head ($P < 0.001$). HT-2+T-2 levels in the

214 rest of the head was about two times higher than that found in the grains with overall mean
215 values of 206 and 115 μg kg^{-1} respectively.

216 Levels of HT-2+T-2 in the different winter cereal varieties differed with a statistically
217 significant interaction between cereal and variety ($P = 0.001$). Significant HT-2+T-2
218 difference was observed in winter oat varieties with mean values for Gerald, Mascani and
219 Dalguise of 1956, 841 and 237 μg kg^{-1} respectively (Fig. 4). There were no significant ($P >$
220 0.05) differences in HT-2+T-2 concentration in the winter wheat or barley varieties (Fig. 4)
221 with an overall mean HT-2+T-2 concentration of 159 and 154 μg kg^{-1} respectively.

222 A simple linear regression between *F. langsethiae* DNA recovered from winter cereal heads
223 and their corresponding HT-2+T-2 grouped by cereal species showed a highly significant ($P <$
224 0.001) regression best fitted by separate non-parallel lines. However for wheat and barley alone
225 a single line accounted for 68% of the variance and parallel lines although significant accounted
226 for only an additional 1.5% of the total variance observed. Thus two lines where fitted, one for
227 oat ($P < 0.001$, $r^2 = 0.79$) and another for wheat and barley ($P < 0.001$, $r^2 = 0.68$) (Fig.5).

228 *Spring cereal species*

229 For HT-2+T-2 in spring cereal species, a significant ($P < 0.001$) interaction was found between
230 cereal species and cereal part. In spring wheat, HT-2+T-2 in the rest of the head was about
231 three times higher than that found within the grains, whilst it was about one and half times
232 higher in barley and about five times higher in oats (Fig. 6).

233 HT-2+T-2 levels in the different spring cereal varieties differed with a significant interaction
234 between cereal and variety ($P < 0.001$). In oats, the highest HT-2+T-2 concentration was
235 recorded in Firth with a back-transformed mean of 433 μg kg^{-1}. This was significantly ($p <$
236 0.05) higher than for Atego and Husky with a mean of 141 and 117 μg kg^{-1} respectively (Fig.

237 7). HT-2+T-2 concentration in Quench was the highest among the three spring barley varieties
238 (71 μg kg^{-1}). This was significantly ($P < 0.05$) higher than that for Propino (55 μg kg^{-1}) which
239 had the least HT-2+T-2 concentration among the three varieties (Fig.7). HT-2+T-2
240 concentration for the three spring wheat varieties were not significantly ($P > 0.05$) different
241 from each other (Fig. 7) with an overall back-transformed mean of 87 μg kg^{-1}.

242 A simple linear regression between *F. langsethiae* DNA recovered from spring cereal heads
243 and their corresponding HT-2+T-2 grouped by cereal species was highly significant ($P < 0.001$)
244 and was best fitted by separate non-parallel lines. However for wheat and barley alone, a single
245 line account for 73% of the variance and parallel lines although significant accounted for only
246 an additional 5.3% of the total variance observed. Thus two lines were fitted, one for oat ($P <$
247 0.001, $R^2 = 0.78$) and another for wheat and barley ($P < 0.001$, $R^2 = 0.73$) (Fig.8). A visual
248 comparison of Fig. 5 and 8 shows that the regressions for winter and spring oats were similar
249 and the regression for winter and spring wheat and barley were nearly identical. Regression
250 analysis was repeated for all oats grouped by sowing date (winter and spring). The data was
251 best fitted by separate parallel lines (Fig. 9) which accounted for 84% of the observed variance.
252 The regression plots shows that for a fixed concentration of *F. langsethiae* DNA there is a
253 higher concentration of HT-2+T-2 on winter oats compared to spring. Regression analysis was
254 also repeated for all barley and wheat samples grouped by sowing date. This regression showed
255 sowing date had no significant effect on the relationship of HT-2+T2 to *F. langsethiae* DNA
256 concentration and was best fitted by a single line ($y = 0.024x + 2.45$). The regression accounted
257 for 64% of the observed variance.

258

259 **Discussion**

260 This study has identified greater *F. langsethiae* infection and subsequent HT-2+T-2
261 contamination in oats compared to wheat and barley in both winter and spring sown cereals
262 under controlled experimental conditions. This is consistent with observations from field
263 surveys (Edwards et al., 2009; Opoku et al., 2013) and indicates that the differences observed
264 during field surveys were primarily due to genetic differences between the cereal species rather
265 than differences in the agronomy applied to these crops.

266 There was higher *F. langsethiae* DNA and HT-2+T-2 in winter sown compared to spring sown
267 oats. This is in agreement with previous surveys (Edwards, 2012), however, as different
268 varieties were sown in different experiments on different dates this can not be tested
269 statistically, and it can not be determined if these differences are due to variety or sowing date.
270 Only small differences were observed between winter and spring sown wheat and barley. This
271 agrees with the three year UK survey data for winter and spring barley (Opoku et al., 2013)
272 which showed in the year of this experiment there was little difference in the *F. langsethiae*
273 levels between survey samples of winter and spring sown barley. Within the three years of the
274 survey the difference between spring and winter barley were highly variable between seasons
275 with higher levels in winter barley in 2009 and higher levels in spring barley in 2010 indicating
276 weather during a key phenological period, such as flowering, maybe important. A three year
277 survey of French barley found higher levels of HT-2+T-2 in spring compared to winter sown
278 barley (Orlando et al., 2010) although it was not reported how consistent this was across the
279 three years of the survey.

280 Results of this study have shown that a significant difference existed between *F. langsethiae*
281 DNA and HT-2+T-2 levels in grains and the rest of the head with higher concentrations
282 occurring in the rest of the cereal head compared to the grains. This would be expected as

283 higher mycotoxin concentrations tend to occur in the outer layers/structures of cereal spikes
284 compared to the grains (Edwards et al., 2009; Cowger and Arellano, 2013) and indicates that
285 infection and mycotoxins production primarily occurs in the outer layers of the cereal head.
286 There were significant differences for both DNA and HT-2+T-2 between the concentration in
287 grains and the rest of the head for spring cereals but not winter cereals and the reasons for this
288 are not clear. In general, the ratio was much higher for DNA (above 5-fold) compared to HT-
289 2+T-2 (ca. 2-fold). This shows there is not a consistent relationship between *F. langsethiae*
290 DNA and HT-2+T-2 concentration at harvest across plant structures and this may be due to
291 differences in production rates, translocation and or metabolism of the mycotoxins.

292 It is important to note that not only does *F. langsethiae* have a greater ability to infect oats, but
293 the concentration of HT-2 and T-2 per unit fungal biomass is far higher in oats than the other
294 two cereals. For example, in winter cereal varieties when the concentration of *F. langsethiae*
295 DNA recovered from cereal heads is about 0.01 pg ng^{-1} the corresponding HT-2+T-2
296 concentration is about 100 µg kg^{-1} in both wheat and barley heads and about 250 µg kg^{-1} in oat
297 heads. A ten-fold increase in *F. langsethiae* DNA concentration (0.1 pg ng^{-1}) in cereal heads
298 resulted in about a 50% increase in HT-2+T-2 concentration (150 µg kg^{-1}) in wheat and barley
299 and a 400% increase (1000 µg kg^{-1}) in winter oat heads. The reasons for these observed
300 differences are not clear, however, there is evidence that some plant species are able to
301 metabolise trichothecenes. For example Lemmens et al. (2005) reported that the resistance of
302 a wheat genotype (CM82036) to FHB and DON was due to its ability to convert DON to DON-
303 3-glucoside, a less toxic derivative. The possibility thus exists that the observed relatively lower
304 HT-2+T-2 concentration in wheat and barley heads per unit *F. langsethiae* DNA compared to
305 oats could be due to a greater ability of wheat and barley to metabolise HT-2 and T-2. Recently
306 researchers have identified numerous plant metabolites of HT-2 and T-2 in wheat, barley and
307 oats (Nathanail et al., 2015; Meng-Reiterer et al., 2016; Veprikova et al., 2012). A greater

308 proportion of glucoside metabolites compared to the parent mycotoxins were found in wheat
309 compared to oats (Lattanzio et al., 2012). Indicating wheat may have a greater ability to
310 metabolise HT-2 and T-2 compared to oats. Nathanail et al. (2015) showed that HT-2 and T-
311 2 are rapidly metabolised by wheat with less than 15% of the original parent molecule
312 remaining at harvest after addition of the mycotoxins during flowering. It could also be that
313 the oat head provides certain conditions that stimulate greater HT-2 and T-2 production. The
314 higher HT-2+T-2 concentration per unit of *F. langsethiae* in winter compared to spring oats
315 may be due to either the genetic differences between varieties or due to the fact that winter oats
316 develop earlier with heads emerging earlier than spring oats. This means that panicles of winter
317 sown oats are exposed to *F. langsethiae* infection earlier and for a longer period allowing for
318 more HT-2 and T-2 production compared to spring oats. This assumption however, does not
319 hold for wheat and barley since little differences were seen between winter and spring sown
320 varieties for these species.

321 This study has confirmed the preference of *F. langsethiae* for oats and the subsequent
322 production of high HT-2 and T-2 in oat heads even when wheat, barley and oats are cultivated
323 under the same agronomic conditions. Not only are oats more susceptible to *F. langsethiae*
324 compared with the other cereal species but they also accumulate more HT-2+T-2 compared to
325 other cereal species per unit of *F. langsethiae* biomass.

326

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329

330 **Compliance with ethical standards**

331 **Conflict of interest** The authors declare that they have no conflict of interest.

332

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386 **Tables and Figures**

387 **Table 1**. Winter and spring varieties of wheat, barley and oats used within the autumn and
388 spring sown experiments respectively

	Variety (sub-plot)	
Cereal (whole plot)	Winter varieties	Spring varieties
Wheat variety 1	Alchemy	Belvoir
Wheat variety 2	Robigus	Paragon
Wheat variety 3	Solstice	Granary
Barley variety 1	Cassia	Propino
Barley variety 2	Flagon	Quench
Barley variety 3	Suzuka	Tipple
Oats variety 1	Dalguise	Firth
Oats variety 2	Gerald	Atego
Oats variety 3	Mascani	Husky

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391 **Table 2.** Whole plot (cereal species) ANOVA mean values and statistical values for $\log_{10}$
392 transformed *F. langsethiae* DNA concentrations (pg ng^{-1}) for heads of wheat, barley and oat at
393 harvest. Back transformed data in parentheses**.**

	F. langsethiae DNA (pg ng^{-1})	
	Winter varieties	Spring varieties
Wheat	-2.371 (0.0043)	-2.420 (0.0037)
Barley	-2.762 (0.0017)	-2.597 (0.0025)
Oat	-1.754 (0.0176)	-2.239 (0.0058)
P-value	<0.001	0.01
%CV	7.1	8.4
LSD (5%)	0.176	0.234

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398 **Table 3.** Whole plot (cereal species) ANOVA mean values and statistical values for log_{10}-

399 transformed HT-2+T-2 concentrations (µg kg^{-1}) in heads of wheat, barley and oats at harvest**.**

400 Back-transformed data in parentheses.

	HT-2+T-2 (µg kg^{-1})	
	Winter cereals	Spring cereals
Wheat	1.910 (81)	1.865 (73)
Barley	1.790 (62)	1.792 (62)
Oat	2.905 (804)	2.285 (193)
P-value	< 0.001	0.04
%CV	2.5	3.4
LSD	0.24	0.29

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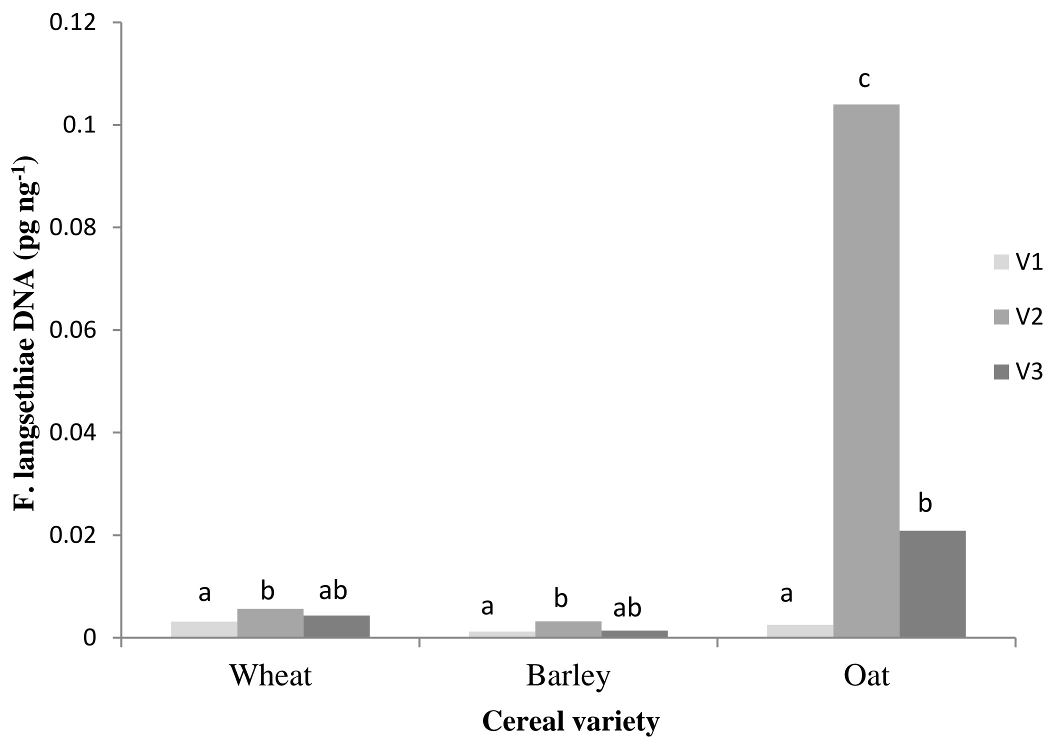

404

405 **Figure 1.** Back-transformed *F. langsethiae* DNA recovered from the different winter cereal
406 varieties. For wheat V1 = Alchemy, V2 = Robigus and V3 = Solstice. For barley V1 = Cassia,
407 V2 = Flagon and V3 = Suzuka. For oat V1 = Dalguise, V2 = Gerald and V3 = Mascani.
408 Varieties of the same cereal species with the same letter are not statistically different from each
409 other LSD, P=0.05).

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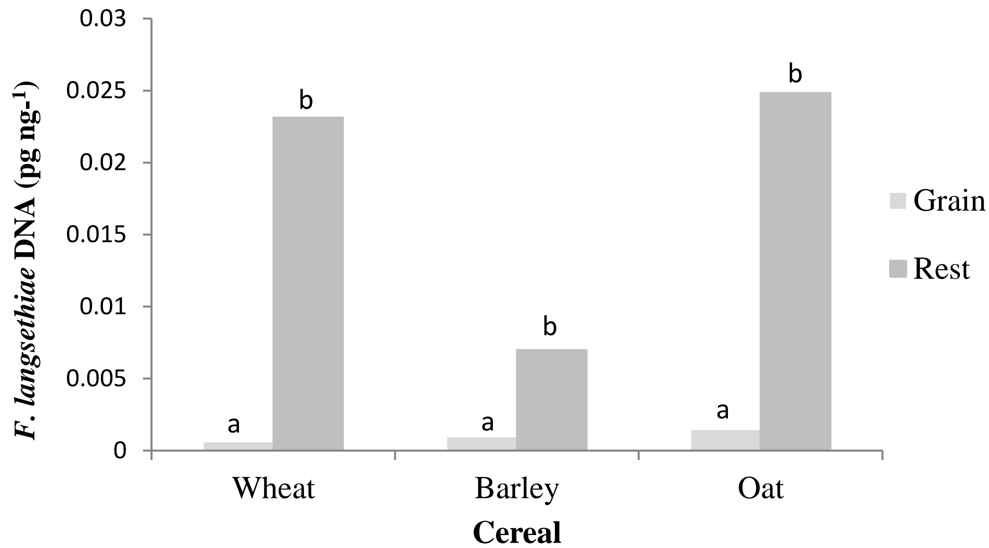

414

415 **Figure 2**. *F. langsethiae* DNA recovered from grains and the rest of the heads of spring wheat
416 barley and oats. Parts of cereal for each species with the same letter are not statistically different
417 from each other (LSD, P = 0.05).

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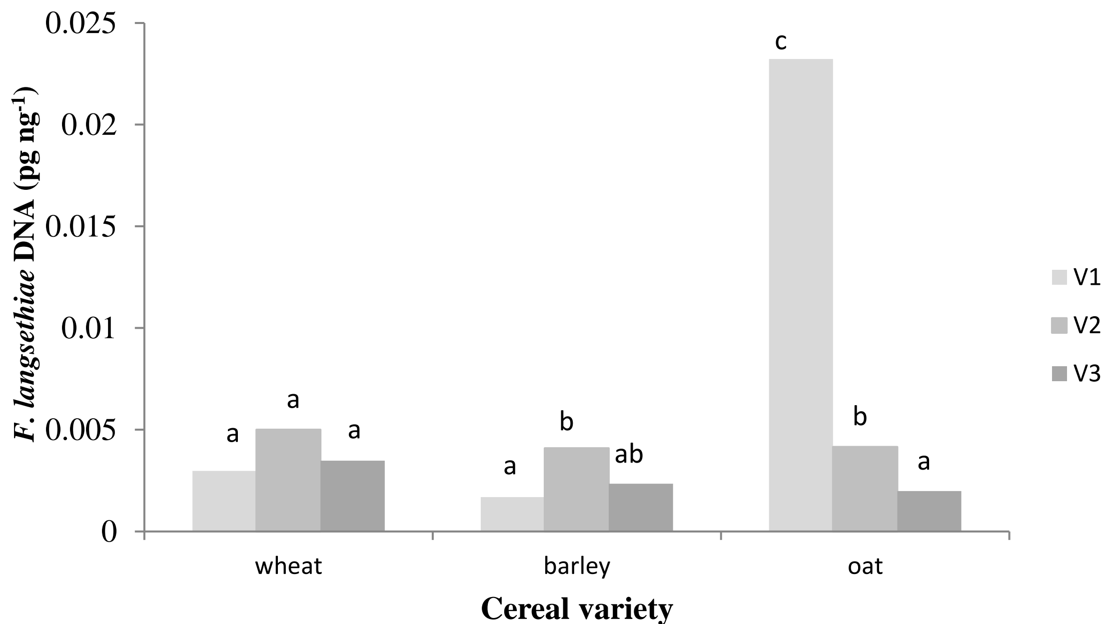

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421 **Figure 3.** Back-transformed mean *F. langsethiae* DNA recovered from the different spring
422 cereal varieties. For wheat V1 = Belvoir, V2 = Paragon and V3 = Granary. For barley V1 =
423 Propino, V2 = Quench and V3 = Tipple. For oat V1 = Firth, V2 = Atego and V3 = Husky.
424 Varieties of the same cereal species with same letter are not statistically different from each
425 other (LSD, P= 0.05)

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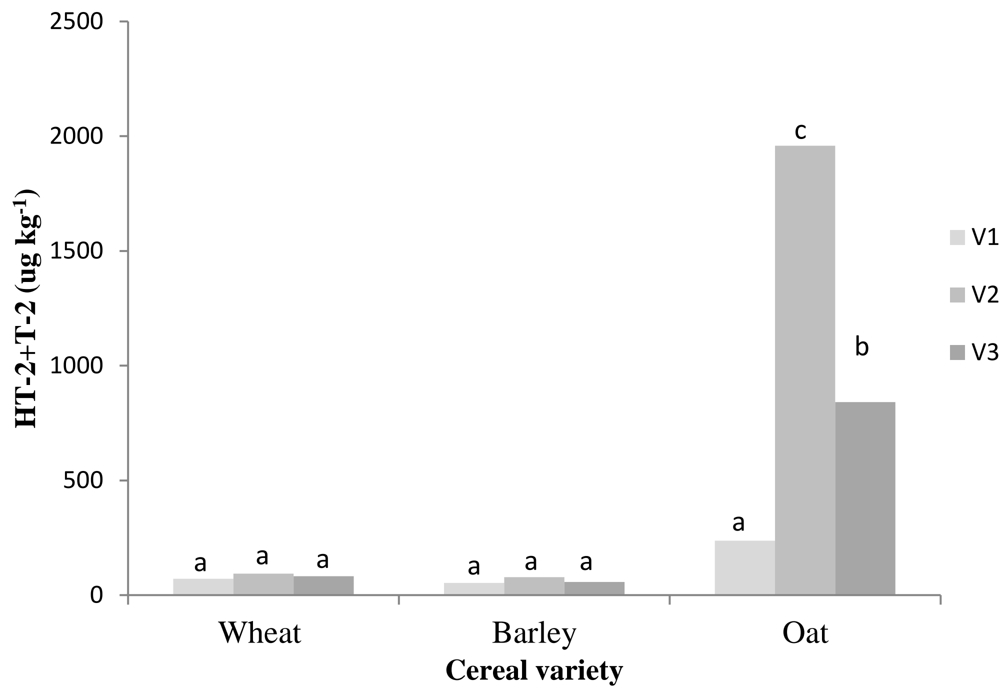

428 **Figure 4.** HT-2+T-2 levels in the different winter cereal varieties. For wheat V1 = Alchemy,
429 V2 = Robigus and V3 = Solstice. For barley V1 = Cassia, V2 = Flagon and V3 = Suzaka. For
430 oat V1 = Dalguise, V2 = Gerald and V3 = Mascani. Varieties of the same cereal species with
431 same letter are not statistically different from each other (LSD, p=0.05)

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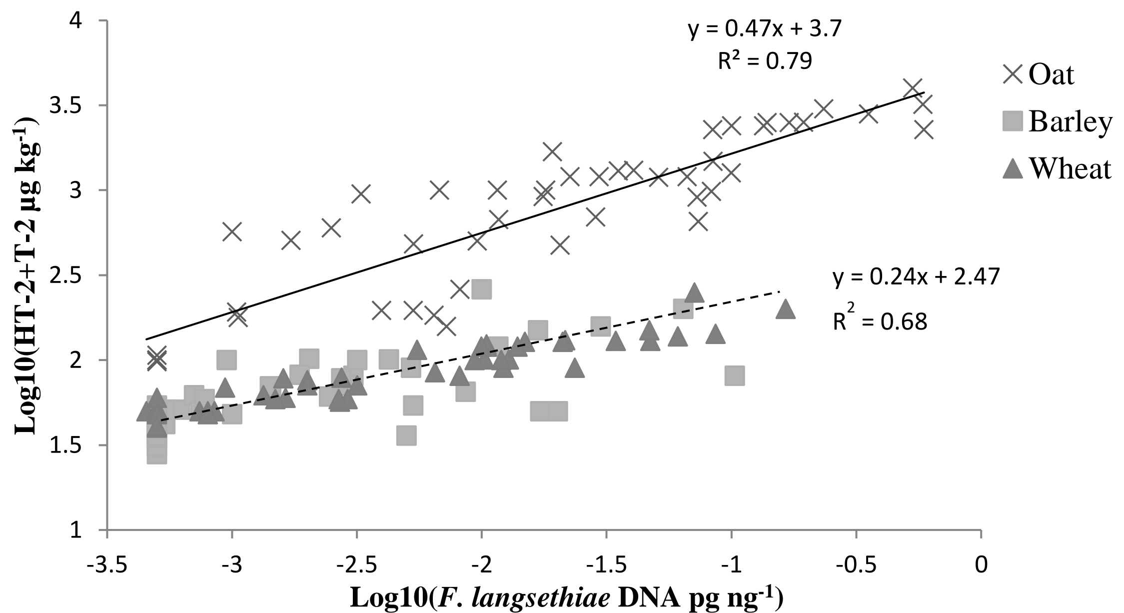

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435 **Figure 5.** The relationship between *F. langsethiae* DNA and HT-2+T-2 concentration in winter
436 cereal heads.

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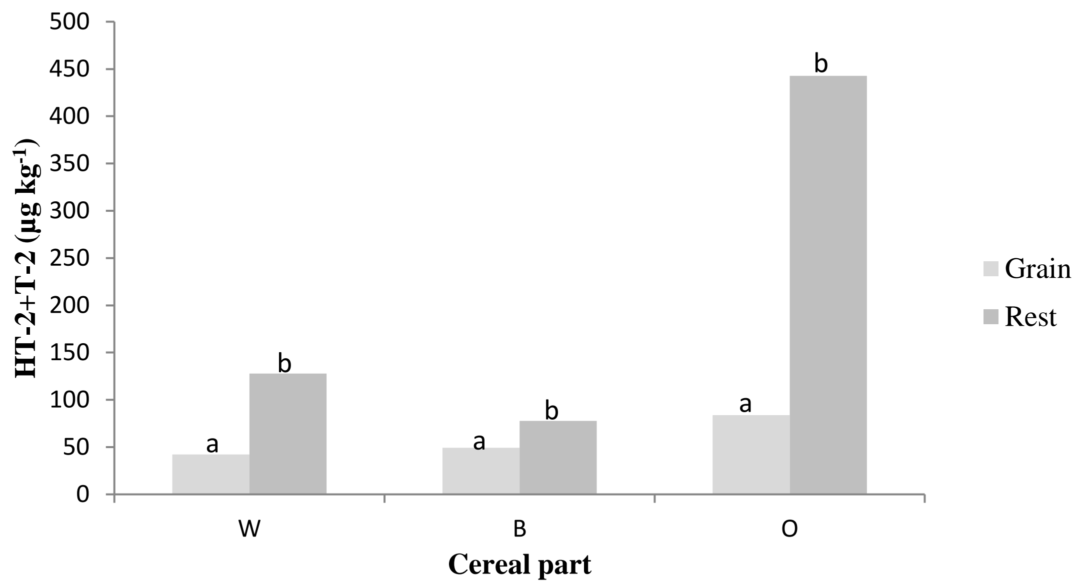

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440 **Figure 6.** Back-transformed HT-2+T-2 concentration for grains and the rest of the heads of
441 spring wheat, barley and oats. For each cereal species parts of cereal with the same letter are
442 not statistically different from each other (LSD, p=0.05))

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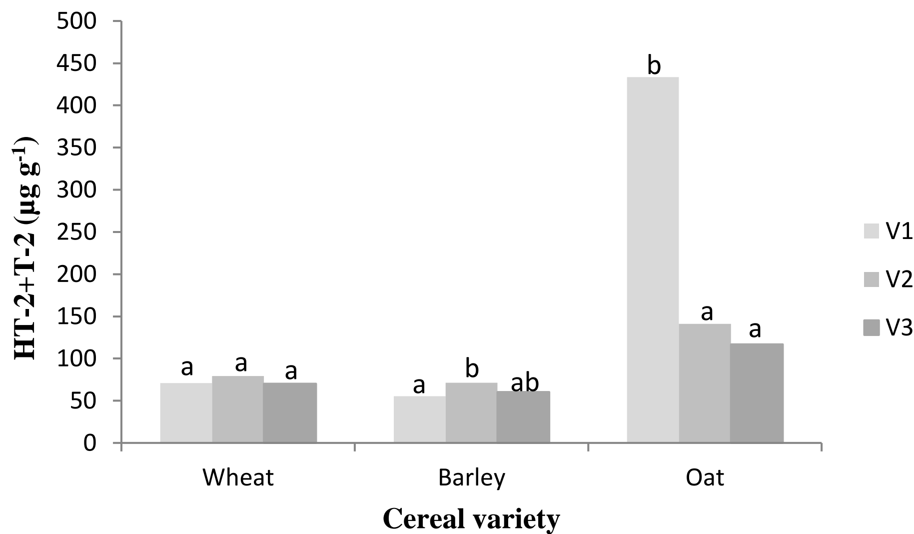

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447 **Figure 7.** Back-transformed HT-2+T-2 levels in the different spring cereal varieties. For wheat,
448 V1 = Belvoir, V2 = Paragon and V3 = Granary. For barley V1 = Propino, V2 = Quench and
449 V3 = Tipple. For oat V1 = Firth, V2 = Atego and V3 = Husky. Varieties of the same cereal
450 species with same letter are not statistically different from each other (LSD, P = 0.05).

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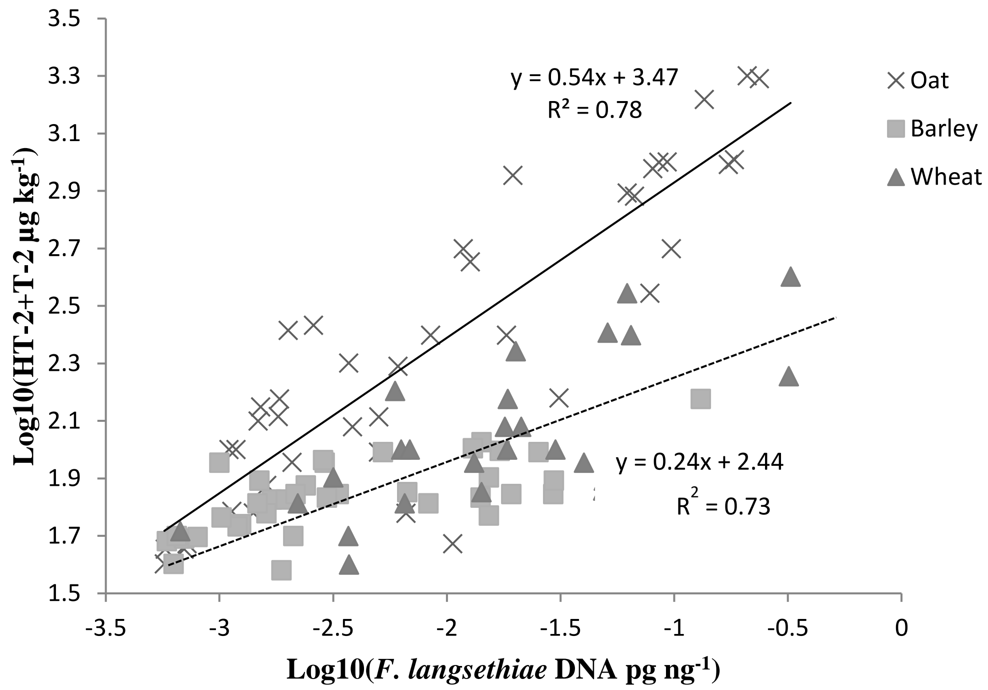

453

454 **Figure 8.** Relationship between *F. langsethiae* DNA and HT-2+T-2 concentration in spring
455 cereal heads**.**

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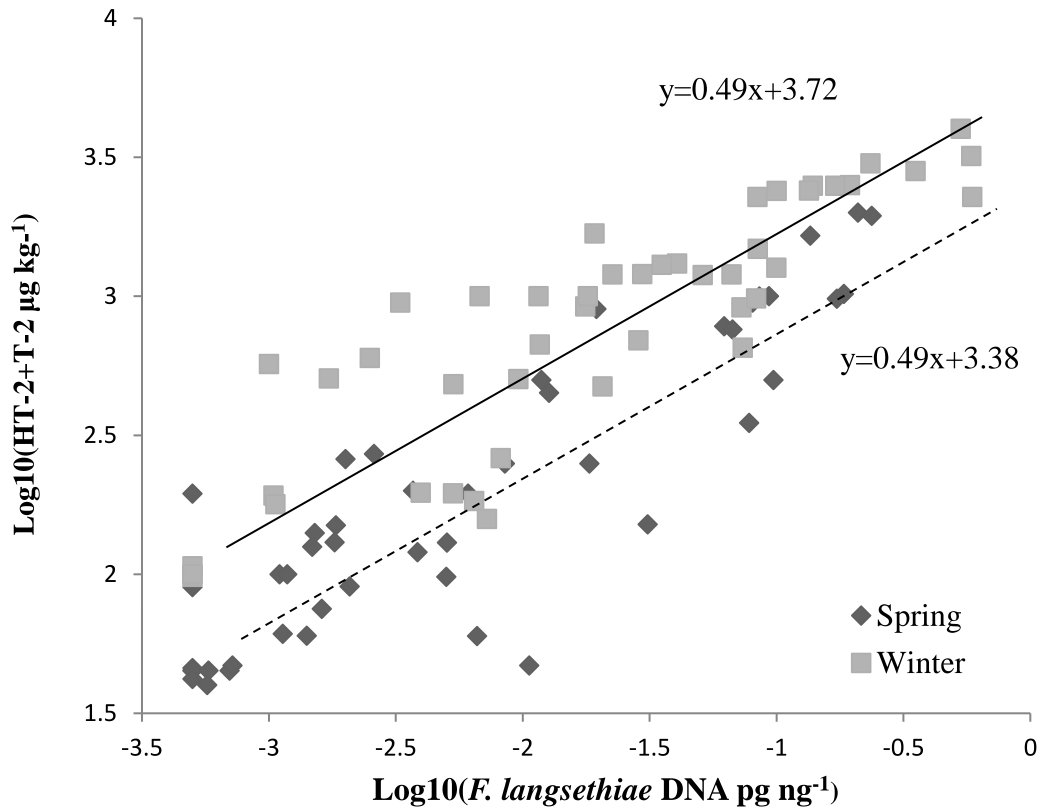

458

459 **Figure 9.** Relationship between *F. langsethiae* DNA and HT-2+T-2 concentration in winter
460 and spring sown oats.