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Student Report 60

Investigating fusarium resistance in UK winter oats

Joseph Blackshaw-Crosby[1], Simon Edwards[1] and Catherine Howarth[2]

[1]Harper Adams University, Newport, Shropshire TF10 8NB
[2] Aberystwyth University, Penglais, Aberystwyth, Ceredigion SY23 3FL

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1. Abstract

HT2+T2 mycotoxins are produced by *Fusarium* fungal species. The EU is drafting legislation for maximum limits of these mycotoxins in cereals and cereal products intended for human consumption. Until the discovery of high concentrations of HT2+T2 in UK oats, oats were considered largely resistant to fusarium infection. *Fusarium langsethiae* is the main producer of both mycotoxins in UK oats. The infection of oats by *F. langsethiae* is symptomless and its epidemiology is unknown. Opoku *et al*. (2013) suggested a life cycle for the fungus. Increased growth of the fungus on emerged plant heads and the pathogen's DNA being almost undetectable prior to anthesis are crucial aspects.

To effectively assess control measures, reliable artificial infection of oats with *F. langsethiae* is desirable. Successful artificial inoculation was achieved under glass by using spore suspensions and bagging plants (to increase humidity) at various growth stages. Inoculation made after the emergence of the panicle but before anthesis resulted in higher infection levels. Although it is possible reliably infect oats under glass, there is no evidence that this provides a reliable mimic of natural infection in the field. Furthermore, the ranking of the genotypes, in terms of resistance, did not match that of the naturally infected field-grown plots. In-field artificial inoculation using misting systems (to induce high humidity) failed to achieve higher infection levels than unmisted/uninoculated plots.

Cultivars have varying resistances to HT2+T2 accumulation, with ranking relatively consistent across years. This work further clarified the resistance imparted on oats by the parental origin of quantitative trait loci (QTL). QTL, designated Chr6D, Chr4A, Chr4D and Chr4C, have been previously identified as being associated with *F. langsethiae* DNA and HT2 +T2 concentration in harvested grains. Near isogenic lines (NIL), developed from a mapping population derived from crossing Tardis (a taller earlier cultivar) and Buffalo (a semi-dwarf later cultivar), were used to examine these QTL. Buffalo is the more susceptible of the two cultivars to *F. langsethiae*.

Introgression of the Buffalo-derived Chr6D into the Tardis background resulted in a shorter plant with panicles only partially emerged from the flag leaf boot. The opposite introgression led to plants taller than either parent line. Introgression of the Tardis Chr4D into the Buffalo background resulted in a later plant when sown in autumn, the effect was close to tenfold when sown in spring. The introgression of the Buffalo Chr4D into the Tardis background caused the resultant plant to be earlier in autumn-sown plots and four times as much so in spring-sown plots. Through comparison of the NIL with original parent lines, reductions in HT2+T2 concentrations were seen when Tardis Chr6D and Chr4D alleles were introgressed into the Buffalo background genome. The impact of Chr6D was consistent across all experiments, while the impact of Chr4D was dependant on sowing season. Chr4D had a weaker effect compared to Chr6D, but introgression of the Buffalo alleles into the Tardis background resulted in a reduction of HT2+T2 in autumn-sown plots. Introgression of Tardis-derived Chr4A into Buffalo had no impact on the HT2+T2 concentration, and introgression of Buffalo-derived Chr4A into Tardis had inconsistent effects across years. The ranking of the NIL population after artificial inoculation under glass did not match that of the naturally infected field grown plots.

Plant height and panicle extrusion were correlated to one another, and evidence is presented that either or both could influence plant susceptibility to *F. langsethiae* infection. Dissection of naturally infected panicles and quantification of *F. langsethiae* DNA concentration at the spikelet level demonstrated the independent nature of the infection in each spikelet. This finding reinforces previous work that oats have high type II resistance to fusarium infection. Window-pane analysis of summarised environmental variables, which utilised the NIL field experiments over four years, demonstrated that warm dry conditions post panicle emergence are conducive to higher HT2+T2 concentrations in harvested oats. Grid sampling of the experimental field (at large and small scale) did not identify any consistent patterns in infection but showed high spatial heterogeneity.

2. Introduction

Oats are currently the seventh largest cereal crop in Europe after wheat (soft and durum), barley, maize, rye and triticale in terms of production, but third in the UK after wheat and barley (European Commission, 2021). In 1999, a new type of *Fusarium* was identified as "powdery poae" (Torp and Langseth, 1999). In 2004, *F. langsethiae* was officially described as a distinct species by Torp and Nirenberg and named after their late colleague Dr Wenche Langseth (Torp *and Nirenberg*, 2004). *Fusarium langsethiae* can be differentiated from *F. poae* by its powdery appearance *in vitro* caused by it having little to no aerial mycelium and profuse microconidia, *F. langsethiae* also lacks a fruity odour present with *F. poae*. In terms of each species mycotoxin profile, *F. langsethiae* has been described as being more similar to *F. sporotrichioides* (Torp and Langseth, 1999). The most important aspect of this is the species' ability to produce large amounts of HT2 and T2, a characteristic it shares with *F. sporotrichioides* and not *F. poae* (Thrane *et al.*, 2004).

From 2002 to 2005, a survey was carried out on the effects of agronomic practices on the mycotoxin content and profile of UK oat and barley crops (Edwards, 2009). The survey revealed that although barley had low incidence and concentrations of HT2 and T2 mycotoxins on a par with wheat, quantifiable concentrations (greater than 10 µg/kg) of the HT2 and T2 were found in 92% and 84% of oat samples, respectively (spring and autumn). Across all years, the combined mean concentration was 570 µg/kg for oats as compared to the highest concentration in barley of 138 µg/kg. The maximum combined concentration of HT2 and T2 (HT2+T2) found in oats was 9,990 µg/kg (Edwards, 2009). *Fusarium langsethiae* is now known to be the chief producer of HT2 and T2 mycotoxins in UK oats. In a study conducted at Harper Adams University (Edwards *et al*., 2012), oat samples of known mycotoxin concentration from a previous study (Edwards, 2009) were assayed using real time PCR to quantify the concentration of *F. langsethiae*, *F. poae*, and *F. sporotrichioides* DNA. *Fusarium langsethiae* was found in almost all the samples, and *F. poae* in 90%, whereas *F. sporotrichioides* was absent from all samples. A regression analysis showed no correlation between *F. poae* DNA concentrations and HT2+T2 mycotoxins; however, *F. langsethiae* strongly correlated ($P< 0.001$, $r^2= 0.60$). Although there are other species of *Fusarium* that can synthesise HT2 and T2 mycotoxins (*F. poae*, *F. sibiricum*, *F. sporotrichioides* and *F. armeniacum* (T2 only)), Edwards *et al*. (2012) presents strong evidence from this correlation that the previously seen high levels of HT2+T2 mycotoxins found in oats were a result of *F. langsethiae* infection.

Typical methods for artificial inoculation of a pathogen to a crop include the application of spore suspensions via spray or injection, spreading of heavily infected lab grown material amongst a growing crop, spreading dried naturally infected plant material containing fruiting bodies or viable mycelia, or spreading sclerotia formed by the pathogen. Suitable conditions should then be engineered over the area to encourage the infection of the host crop. Injection and spray application of microconidial suspensions of *F. langsethiae* at various growth stages have been attempted in controlled environments at various growth stages (Divon *et al*., 2012; Opoku, 2012; Mousavi, 2016; Schöneberg *et al*., 2019 and Divon *et al*., 2019). Within experiments, no relationships have been found between growth stage at application and DNA of mycotoxin concentration. Creating very artificial conditions to induce a severe infection has been shown to be successful (Opoku, 2012); however, resultant plants typically display visible mycelial growth, stunting, lesions, bleaching or necrosis. Broadly, spore applications applied by atomised spray during anthesis were the most successful at increasing *F. langsethiae* DNA or HT2+T2 concentration. Divon *et al.* (2019) visualised the infection process after inoculating just after anthesis by microscopic observation; the authors recorded an infection process and observed the preferential growth of the fungus in the presence of oat pollen, they did not measure the HT2+T2

concentration in the resultant grain. Concentrations of *F. langsethiae* DNA or HT2+T2 are highly variable within and between experiments making it difficult to make cross comparisons. These results suggest that anthesis may be optimal in artificial inoculations in part because of the presence of pollen. Such a relationship could have implications for the natural physiological timing of infection in the field. Reliable infection in the glass house of *F. langsethiae* onto oats resulting in infections with similar symptoms to naturally infected oats in the field would aid testing management solutions against *F. langsethiae*.

3. Materials and methods

3.1. Artificial inoculation

Six isolates of *F. langsethiae* were isolated from oat grains grown across the UK in 2015. Each isolate originated from separate grains but not necessarily different batches, these were used in all inoculation experiments excluding the 2016 field inoculation. To isolate, first grains were surface sterilized before being plated onto a semi-selective growth media. Isolates were initially identified by morphology and then confirmed by PCR.

3.1.1. 2017 and 2018 glasshouse inoculation

The 2017 experimental plan was designed as a fully randomised design with seven treatments (including the untreated control) and 15 replicates. A complete randomised design was used to increase the degrees of freedom and the statistical power. Each pot contained three plants and constituted one replication. The variety was Gerald, a tall winter variety popular with millers that was previously demonstrated to be susceptible to *F. langsethiae.*

Inoculum comprised spores from six isolates present in equal proportions at a concentration of 10^6 spores/ml. Potato dextrose broth was added to two solutions at 2.4g/l and 0.24g/l, while a third was unamended. This experiment was run in the glasshouse at Harper Adams University using Gerald oats dressed with a Kento (triticonazole and prochloraz) seed treatment. Seed was sown into 20 cm square pots, in John Innes No. 2 compost on the 10th February 2017. Plants were grown with supplemental lighting, frost protection after vernalisation, and supplemental nitrogen fertiliser.

Due to powdery mildew infection in the plants in early April (10th April 2017), plants were sprayed with Vegas at 0.35 L/ha (cyflufenamid), Hallmark (Lambda-cyhalothrin) at a rate of 50 ml/ha and Gazell (acetamiprid) at 250 g/ha for aphids. In May, 105 pots were selected from the 161 available on the basis of uniformity; these were spaced on the growing table and labelled according to the fully randomised design.

Plants were inoculated at two growth stages: full panicle emergence and late anthesis (GS59 and GS65-9), and on the day of inoculation, tillers at the required growth stages were labelled. Each of the 15 pots was removed from the growing table and sprayed with the relevant spore suspension from four angles at an upward incline, so the spray moved up into the downward facing glumes. Once sprayed, clear non-perforated plastic bags were placed over plants supported by canes and the bags were sealed around the base of the pots. Plants were sprayed between 7pm and 9:30pm to avoid UV damage of the spores and bags were left on the plants for 14 days. The control plants were not sprayed or bagged.

A further glasshouse experiment was conducted to investigate similar hypothesise to the 2017 Gerald experiment; the key differences were the use of a dwarf variety with high susceptibility (Balado), applying the inoculum at earlier growth stages and using panicles from the same plant

but different growth stages to measure the impact of growth stage. Tillers at GS47 and GS51/63 were tagged with coloured tape on the same day as inoculation. Only tagged tillers were harvested at the maturity to be assessed for HT2+T2 concentration.

3.1.2. 2019 experiment

The experiment was grown in the glasshouse with the aims of understanding how selected NIL reacted to inoculation in the glasshouse environment and whether or not manually extruding varieties which naturally retain some of the panicle within the flag leaf boot would change their reaction to the inoculation. Mechanically extruding plants was achieved by peeling back the flag leaf boot and drawing it down the stem of the plant until the panicle was fully exposed. Damage to plants was caused by roughly cutting the flag leaf sheath in a similar manner to the damage caused when mechanically extruding a plant while not exposing the panicle. The inclusion of the damaged treatment was to enable any damage caused by the mechanical extrusion to be differentiated from the mechanical extrusion itself. Selected NIL detailed in Table 3.2 were sown into John Innes No.2 compost in square 25 cm pots, seven plants per pot, five plants per pot were later selected based on uniformity. Mildewcides, insecticides, and fertiliser was applied as appropriate.

Table 3.1: Treatment list for 2019 glasshouse inoculation.

Code	Name	Treatment		Inoculation
		Panicle Extrusion	Flag leaf sheath	
Buffalo	Buffalo	Unextruded	Undamaged	Control
		Unextruded	Undamaged	Inoculated
2012-125/1/26	B NIL	Unextruded	Undamaged	Inoculated
		Unextruded	Damaged	Inoculated
		Extruded	Damaged	Inoculated
2012-125/1/27, 2012-139/6/25	Buffalo + T Chr6D	Extruded	Undamaged	Inoculated
		Extruded	Damaged	Inoculated

3.1.3. Outdoor experiments

Inoculation was attempted in the field in 2016 and again in 2019. The objectives of the 2016 experiment were to determine if spore concentration, crop growth stage at application or targeted irrigation on the same day as inoculation encouraged successful infection of *F. langsethiae.* Isolates were provided by Tijana Stančić of Harper Adams University. All isolates were originally isolated from Gerald oats harvested in the UK in 2012 (Stančić, 2016). Plants were sprayed in the evening using a lunch box plot sprayer (Trials Equipment UK Ltd) with flat fan 02 nozzles. Plants were sprayed on four dates at GS43, GS47, GS59, and GS72, two hours later water was applied to specific plots using the same equipment (after a triple rinse) until run-off.

*Figure 3.1: Oat plants with spore suspension applied to the flag leaf **(a)** at late booting/sheath split (GS49) with no spore suspension applied **(b)** and at fully emerged panicle after spore suspension applied **(c)**.*

2019

A further field inoculation was conducted in 2019 using Near Isogenic Lines from the Buffalo x Tardis mapping population selected on the basis of their variation in panicle emergence times, height and possession of certain QTL of interest. The experiment was in four blocks with each block consisting of 12 different genotype plots of 10 m length. The experiment was a split plot design, one half of the 10 m plot was equipped with three misting heads spaced 1.5 m apart. Within the 5 m of misted plot, one meter had inoculum applied and the remainder was left as a misted control. Figure 3.2 shows the experiment with the irrigation misting running on two of the blocks. The misting was applied during the day in the early afternoon on days the plots were inoculated, but misting was stopped several hours before the inoculant was applied. Spores were sprayed onto the canopy using a pump action killa spray bottle, the application was approximately 40 ml/m^2, the inoculum did not saturate the canopy to the point of run-off. By applying over three days the aim was to make at least one application at full panicle emergence (GS59) for each NIL. The same isolates were used as in the glasshouse experiments.


Figure 3.2: Misting running on two blocks of the 2019 outdoor field inoculation experiment.

3.2. Quantitative trait examination using Near Isogenic Lines

For each harvest year 2017-2020, a collection of NIL developed at Aberystwyth University were sown both in autumn and spring in the experimental field. Experiments were sown in 1 m^2 plots as a randomised block design with four blocks in both autumn and spring. Experiments were treated with a comprehensive programme of fungicides to control foliar pathogens up to flag leaf fully emerged but had no plant growth regulator applied. All experiments were grown in the same field for each of the four years, the field was divided in two, one half growing wheat and the other oats, as had been the case since 2010. For the 2017 and 2018 sown experiments, the seed bed was prepared by discing alone, the rotation within the field caused a build-up of wild oats that were difficult to control. For the 2019 and 2020 experiments, the ground was ploughed to bury the weed bank. Straw was chopped and returned onto the field and the next crop was sown into the previous crop residue, so that plants emerged in contact with the previous crop residue (Figure 3.3a). In the 2019 and 2020 experiments, it was necessary to plough the field prior to drilling for weed control. In these two years straw, from the previous wheat crop was collected and distributed back onto the plots once they were sown (Figure 3.3b). Table 3.2 details the sowing and harvest dates of all the NIL (near isogenic line) experiments. The same experimental plots were used for HT2+T2 quantification.

Figure 3.3: a Crop debris from the previous wheat crop visible amongst young oat plants. b Wheat straw distributed in between plants after sowing.

Table 3.2: Sowing and harvest dates of each NIL experiment.

Experiment	Drilling date	Harvest Date
2017 autumn	11/10/2016	16/08/2017
2017 spring	15/03/2017	07/09/2017
2018 autumn	13/10/2017	03/08/2018
2018 spring	20/04/2018	22/08/2018 (hand sampled)
2019 autumn	02/10/2018	27/08/2019
2019 spring	20/03/2019	13/09/2019
2020 autumn	23/10/2019	27/08/2020
2020 spring	25/03/2020	04/09/2020

The date on which the plants reached early panicle emergence (GS 51) for each plot was recorded; a plot was deemed to have reached GS 51 once half the plot was at GS 51. Panicle emergence was used as a proxy for flowering time as it is difficult and time consuming to assess flowering time for oats. The final height from the ground to the flag leaf ligule, the first whorl of the panicle (not recorded in 2017) and to the top of the plant was measured to an accuracy of 0.5 cm. Four plants per plot were measured for each height and the average used.

Weather data detailing the maximum and minimum temperature per hour was collected from a MET office weather station located 1 km away from the experimental field and growing degree days were calculated based on the average temperature per day with a base temperature of 5°C. Timing of flowering was calculated as degree days to panicle emergence from sowing date and days from January 1st (Julian days).

1.1.1. Harvest and Sampling

Plots were combined using a Winterstieger nursery master combine; the combine was allowed to thresh the plot sample entirely before moving to the next plot. Due to poor weather conditions at harvest in 2020, the spring sown plots were harvested by hand and threshed later after drying in cotton bags in a glasshouse for two weeks to below 12% moisture. A ~200 g sample of grain was milled using a ZM200 centrifugal laboratory mill (Retsch, Leeds, England) using a 1 mm sieve. Milled samples were used for HT2+T2 extraction and analysis. Ridascreen® T-2/HT-2 Toxin ELISA kits (R-Biopharm, AG, Germany) were used to measure the combined concentration of HT2 and T2 in field and glasshouse experiment samples from a 5 g sub-sample of milled grain.

3.3. Window-pane analysis

Window-pane analysis is a means of summarising discrete parcels of environmental data of varying sizes focused around specific times. In the case of this work, for each NIL plot the date of panicle emergence was known and weather data parcels could be summarised for each NIL plot and used to correlate the HT2+T2 concentration at harvest with the environmental conditions - rainfall, humidity and temperature.

3.4. Grids

Samples of oats were collected form grids of either 20 m or 4 m spacings dependant on year, the 2017 and 2019 grids and 2018 and 2020 grids were sampled from the same locations using GPS (Table 3.3).

Table 3.3: Details for grid dimensions, variety and whether or not GPS locations were stored for each year.

Year	Variety	Grid size	GPS positioned
2016	Gerald	20m	No
2017	Mascani	4m	Yes
2018	Mascani	20m	Yes
2019	Mascani	4m	Yes
2020	Balado	20m	Yes

At each sampling point, a 60 cm radius was sampled for threshing, milling and HT2+T2 analysis.

3.5. Panicle Dissection

Four panicles were selected from the highest accumulating year and sowing timing (2018 autumn sown). Entire panicles were selected from the highest and lowest HT2+T2 NIL; one from the lowest accumulating, Buffalo + T Chr6D, and three from the highest accumulating NIL, Tardis + B Chr6D. Each individual spikelet was measured for *F. langsethiae* DNA concentration and its position on the panicle was recorded.

4. Results

4.1. Artificial Inoculation

For the 2017 Gerald glasshouse experiment, the HT2+T2 concentrations were high in the inoculated samples as compared to the control (Figure 4.1). An ANOVA was initially conducted on the square root transformed results; inoculation was significant (P<0.001) as was the growth stage at which inoculation was applied (P<0.001). Visible mycelial growth was seen across the panicles of the inoculated plants, but not on the control plants. The application of inoculum to plants at the earlier growth stage of complete panicle emergence (GS59) resulted in greater concentrations of HT2+T2 in the harvested panicles than spore application at mid to late anthesis (GS65-9). The inclusion of the PDB in the applied spore suspension did not have a statistically significant (P=0.488) effect on the HT2+T2 concentration in harvested panicles.

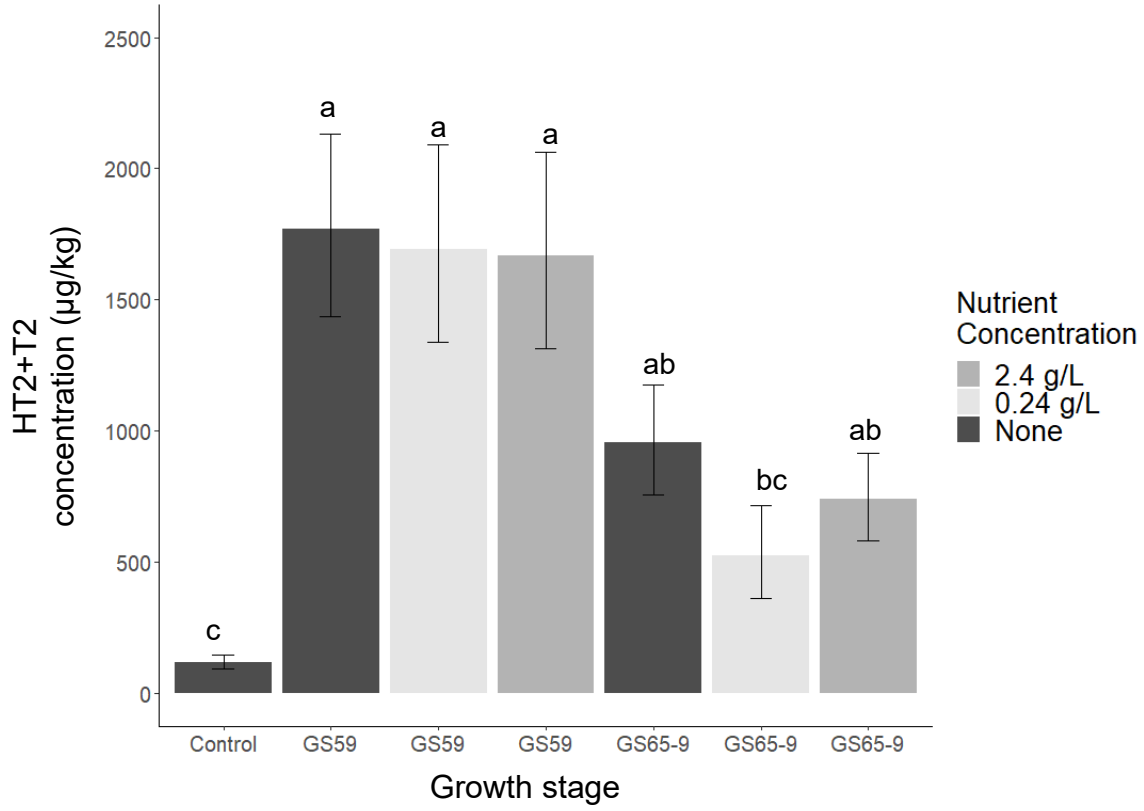

Figure 4.1: Back-transformed concentration of HT+T2 (µg/kg) in panicles of oat (var. Gerald) in the 2017 glasshouse experiment for the Control, GS 59, and GS65-9 F. langsethiae inoculated treatments. Different bar colours represent different concentrations of potato dextrose broth detailed in the legend. Error bars represent one standard error of the mean. Columns headed with the same letter were not statistically different (Tukey, P>0.05).

For the 2018 Balado experiment, the HT2+T2 concentrations were high in some of the inoculated samples as compared to the control, although lower than the Gerald experiment (Figure 4.2). The results were analysed as in the 2017 experiment with Log10 transformation to achieve a Gaussian distribution.

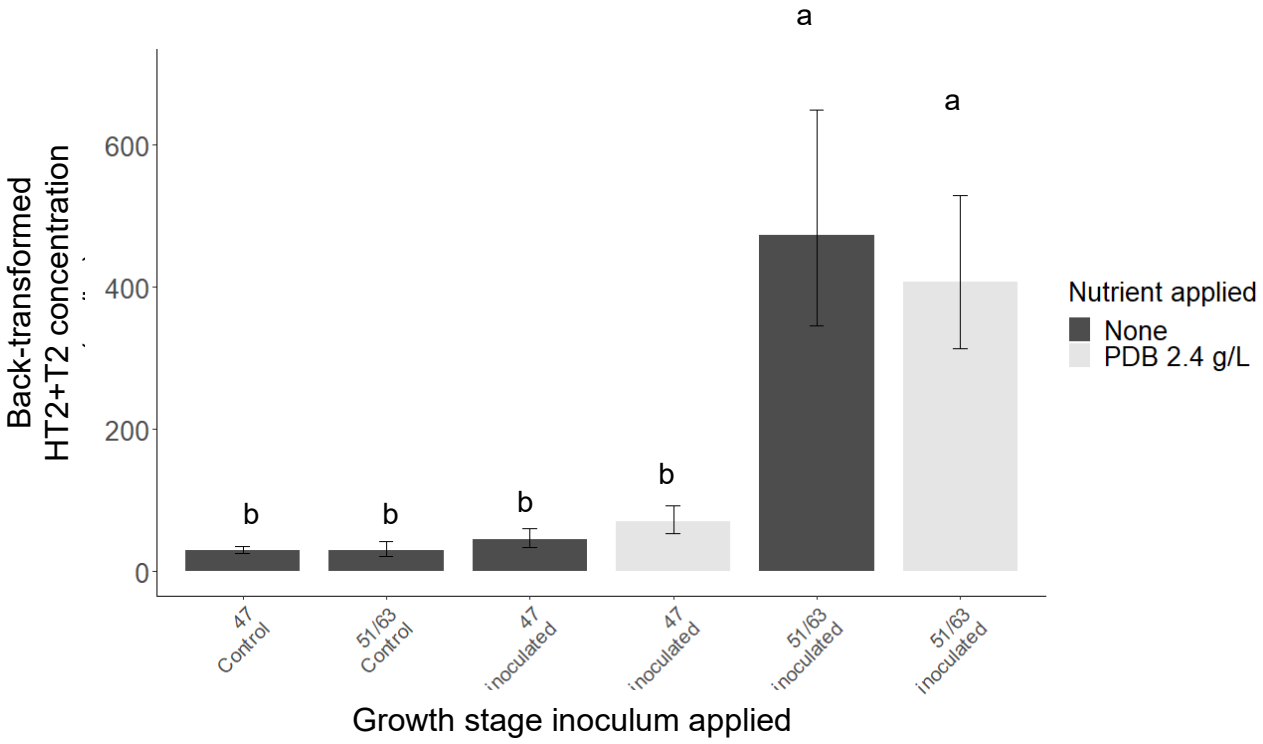

Figure 4.2: Back-transformed concentration of HT+T2 (µg/kg) in panicles of oats (var. Balado) for the 2018 glasshouse experiment inoculated with F. langsethiae. The x axis describes the growth stage at which inoculant or control water sprays were applied. The legend describes the treatments that included PDA amendment at 2.4 g/L. Error bars represent one standard error of the mean. Columns with the same letter are not significantly different (Tukey, P<0.05).

There was no significant interaction between PDB amended inoculum and growth stage at which it was applied (P=0.383). The growth stage at which the inoculum was applied was highly significant (P<0.001); application of inoculum at early panicle emergence/early anthesis (GS51/61) had a large positive impact on the HT2+T2 concentration of the panicle (mean HT2+T2 440.5 µg/kg) whereas application at GS47 (spikes still within the boot) had no significant effect compared to the uninoculated control. The inclusion of PDB had no significant impact on the concentration of HT2+T2 (P= 0.67).

Artificial inoculation was successful in the 2019, increasing the HT2+T2 concentration in the inoculated Buffalo genotype significantly above that of the uninoculated Buffalo (Figure 4.3). The back transformed concentration of the harvested panicle of the inoculated Buffalo was 226 µg/kg and the untreated control 1.0 µg/kg. The two results are statistically distinct from one another (Tukey; P<0.05) showing that the inoculation was the source of the infection and that the untreated control was sufficiently protected from the inoculum.

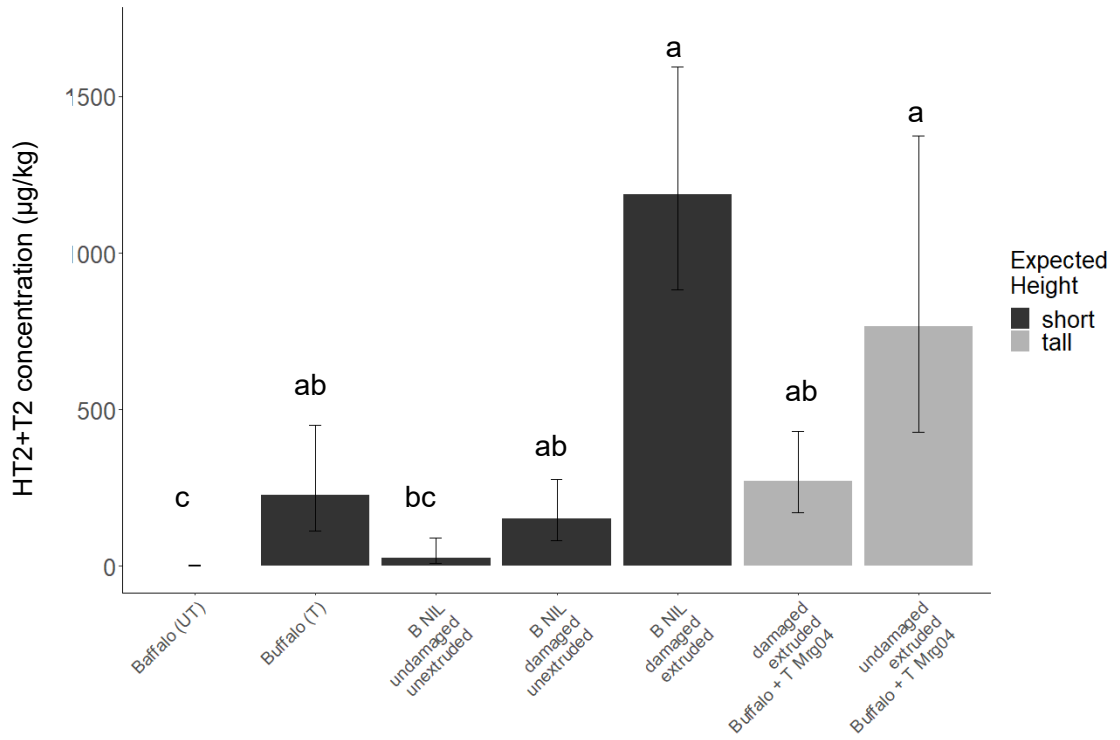

Figure 4.3: Back-transformed concentration of HT+T2 (µg/kg) in panicles of oat for 2019 glasshouse experiment for the variety Buffalo with and without inoculation, the Buffalo NIL (B NIL) undamaged and unextruded, damaged and unextruded, damaged and extruded, Buffalo + T Chr6D damaged and undamaged. The Legend describes the expected heights of the plants predicted from their growth habits in the field. Error bars represent one standard error of the mean. Columns headed with the same letter are not significantly different (Tukey; P<0.05).

For the three B NIL inoculated treatments, the unextruded and undamaged treatment did not have a statistically (P>0.05) higher concentration of HT+T2 than the Buffalo untreated control whereas the extruded and damaged plants had a statistically (P<0.05) higher concentration than both the Buffalo untreated control and the undamaged and unextruded B NIL plants. The damaged but unextruded B NIL plants had a higher concentration than the undamaged and unextruded but not statistically so (P>0.05) and they were also not statistically distinct from the damaged and extruded treatment. The undamaged tall Buffalo + T Chr6D plants had numerically greater HT2+T2 concentrations than the damaged plants, although differences were not significant (P>0.05).

Outdoor inoculation

The outdoor inoculation experiments did not result in any significant uplifts in HT2+T2 concentration; in the 2016 field experiment, the uninoculated control had the highest concentration of HT2+T2 in harvested grain. Figure 4.4 shows all the possible comparisons between the treatments and how the growth stage, the concentration or irrigation had no impact on HT2+T2 concentration.

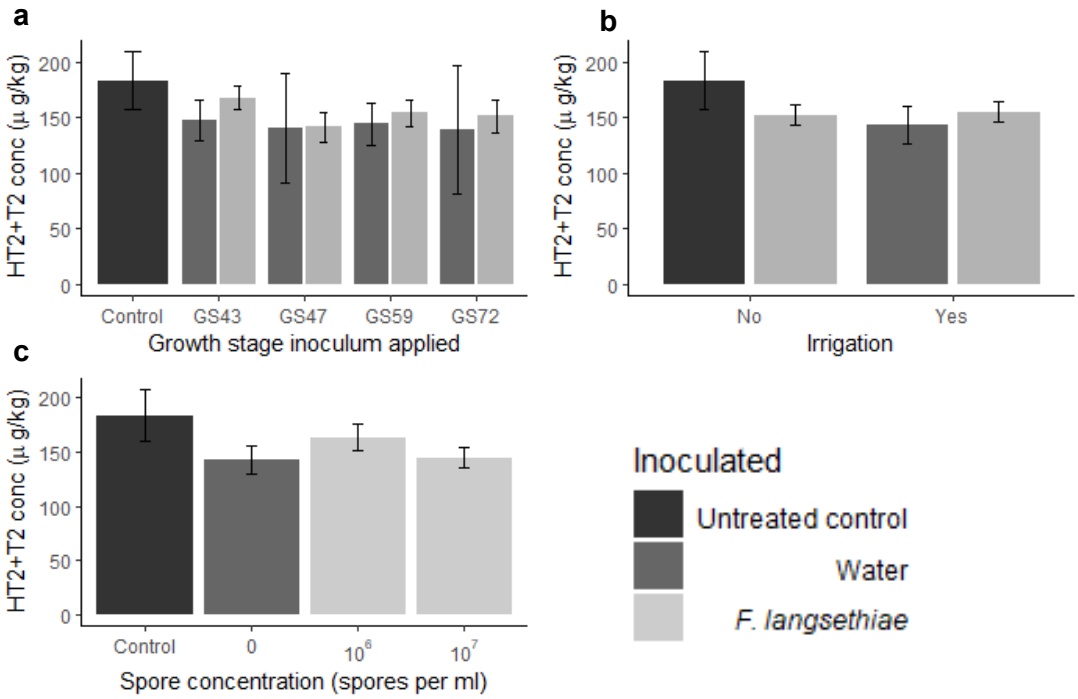

*Figure 4.4: HT2+T2 concentration in harvested oat grain (cultivar Gerald) in the 2016 field experiment after F. langsethiae inoculations **a**: by growth stage of inoculation; **b**: for plots either inoculated or not with and without simulated rain; **c**: for plots inoculated with different spore concentrations. Control not inoculated or irrigated and 0 treatment inoculated with water only. Error bars represent one standard error of the mean.*

The 2019 field experiment was not analysed beyond the Buffalo and Tardis genotypes which represent two ends of the susceptibility spectrum. The HT2+T2 concentrations were not high enough to warrant analysing all samples.

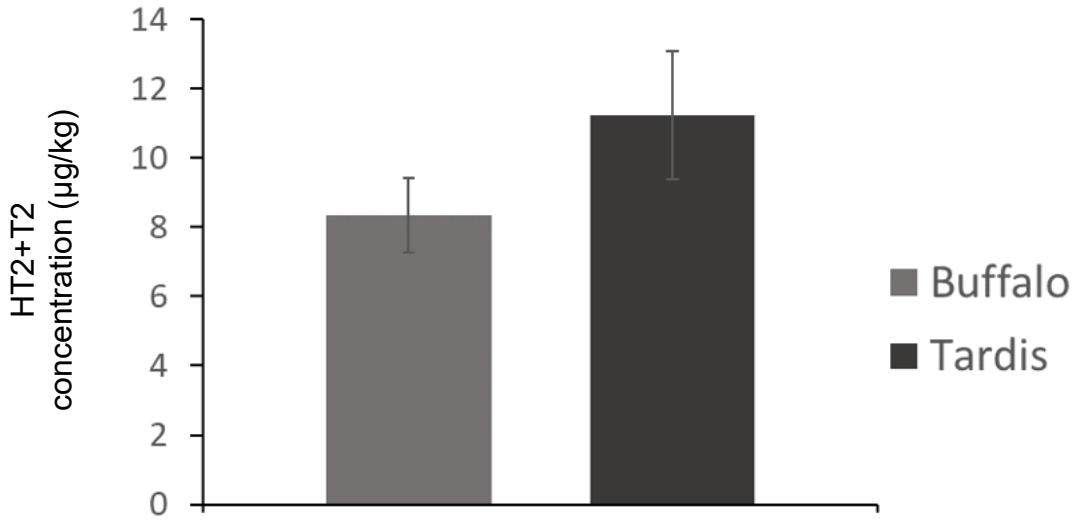

Figure 4.5: Buffalo and Tardis HT2+T2 mycotoxin concentration in harvested grain from F. langsethiae inoculated field plots in 2019. Error bars represent the standard error of the mean calculated for individual means.

4.2. Near isogenic lines

Chr6D

The results for the HT2+T2 concentrations in the harvested oats for the Tardis and Buffalo Chr6D NIL plants are presented in Table 4.1 and 4.2, and Figure 4.6

Table 4.1: Contrast analysis comparing average Log10 transformed HT2+T2 concentrations of the parent line Tardis with the NIL Tardis + B Chr6D. Percentage differences were calculated from the differences between back transformed values.

Year	Contrast	Estimate (Log) (difference between contrasted values)	% Difference	DF	P value
2017	Autumn: Tardis vs Tardis + B Chr6D	0.71	411.0	75	<0.001
	Spring: Tardis vs Tardis + B Chr6D	0.7	360.2	75	<0.001
2018	Autumn: Tardis vs Tardis + B Chr6D	0.8	535.7	101	<0.001
	Spring: Tardis vs Tardis + B Chr6D	0.1	29.4	100	0.4008
2019	Autumn: Tardis vs Tardis + B Chr6D	-0.003	-0.8	80	0.9683
	Spring: Tardis vs Tardis + B Chr6D	0.05	12.8	81	0.4815
2020	Autumn: Tardis vs Tardis + B Chr6D	0.3	123.4	77	0.0231
	Spring: Tardis vs Tardis + B Chr6D	0.4	178.3	77	<0.001

Table 4.2: Contrast analysis comparing average log transformed HT2+T2 concentrations of the parent line Buffalo with Buffalo + T Chr6D. Percentage differences were calculated from the differences between back transformed values.

Year	Contrast	Estimate (Log) (difference between contrasted values)	% Difference	DF	P value
2017	Autumn: Buffalo vs Buffalo + T Chr6D	-0.7	-78.1	75	<0.001
	Spring: Buffalo vs Buffalo + T Chr6D	-0.3	-53.6	75	0.002
2018	Autumn: Buffalo vs Buffalo + T Chr6D	-0.9	-88.5	101	<0.001
	Spring: Buffalo vs Buffalo + T Chr6D	-0.5	-70.5	100	0.0002
2019	Autumn: Buffalo vs Buffalo + T Chr6D	-0.04	-8.4	80	0.7
	Spring: Buffalo vs Buffalo + T Chr6D	-0.3	-44.8	81	0.001
2020	Autumn: Buffalo vs Buffalo + T Chr6D	0.1	31.0	77	0.4
	Spring: Buffalo vs Buffalo + T Chr6D	-0.6	-73.2	77	<0.001

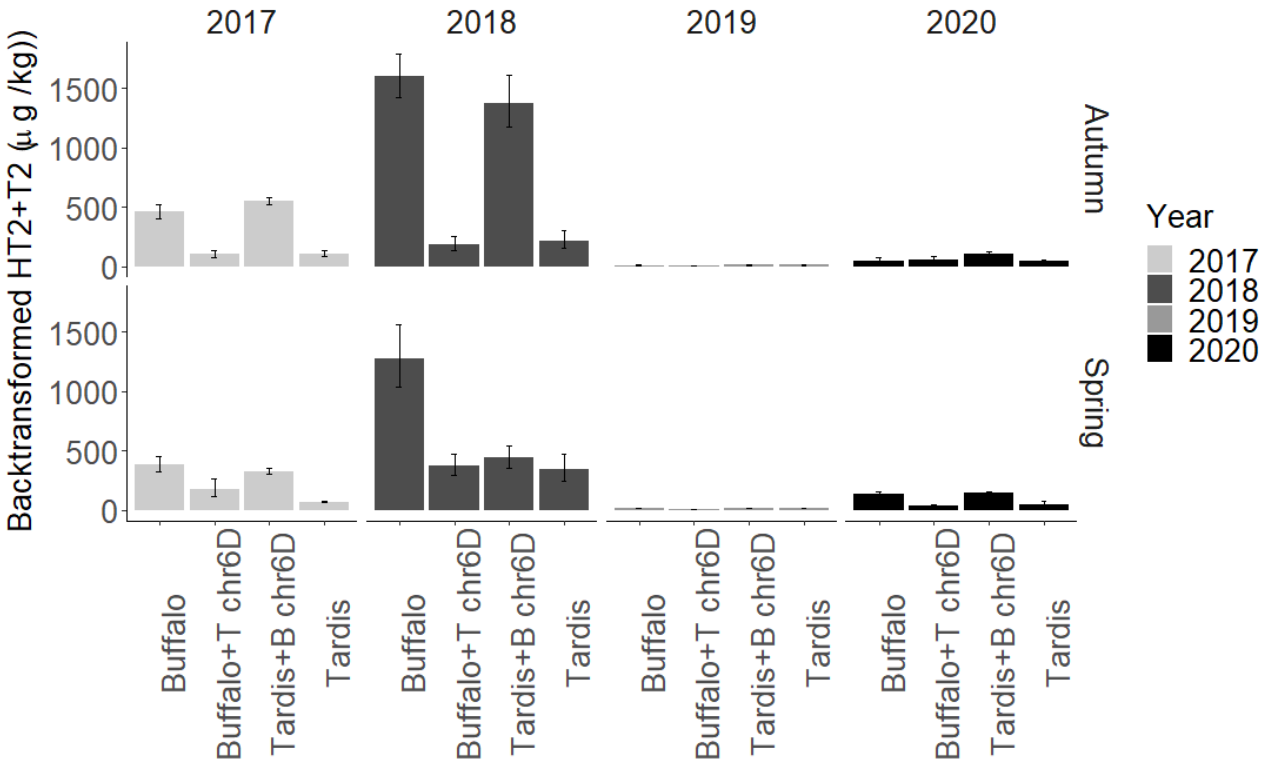

Figure 4.6: Graphical comparison of the parent lines Buffalo and Tardis to their respective Chr6D NIL in terms of HT2+T2 concentration in the harvested grain for each cropping year and sowing season. Data has been back transformed and error bars represent one standard error of the mean.

Chr4D

The results for the HT2+T2 concentrations in the harvested oats for the Tardis and Buffalo Chr4D NIL plants are presented in Tables 4.3 and 4.4 and Figure 4.7. The introgression of the Buffalo Chr4D QTL into the Tardis background caused significant ($P<0.02$) increases in the HT2+T2 concentration in the harvested grain in 2017, 2018, and 2020 spring results, as well as 2020 autumn.

Table 4.3: Contrast analysis comparing average Log transformed HT2+T2 concentrations of the parent line Tardis with the NIL Tardis + B Chr4D. Percentage differences were calculated from the differences between back transformed values.

Year	Contrast	Estimate (Log) (difference between contrasted values)	% Difference	DF	P value
2017	Autumn: Tardis vs Tardis + B Chr4D	0.1296	34.8	75	0.1736
	Spring: Tardis vs Tardis + B Chr4D	0.7	360.2	75	<0.001
2018	Autumn: Tardis vs Tardis + B Chr4D	-0.04	-9.3	101	0.7952
	Spring: Tardis vs Tardis + B Chr4D	0.6	297.4	100	<0.001
2019	Autumn: Tardis vs Tardis + B Chr4D	0.02	4.5	80	0.8209
	Spring: Tardis vs Tardis + B Chr4D	-0.04	-8.8	81	0.5870
2020	Autumn: Tardis vs Tardis + B Chr4D	0.5	228.0	77	0.0231
	Spring: Tardis vs Tardis + B Chr4D	0.4	135.5	77	0.0045

Table 4.4: Contrast analysis comparing average Log transformed HT2+T2 concentrations of the parent line Buffalo with the NIL Buffalo + T Chr4D.

Year	Contrast	Estimate (Log10) (difference between contrasted values)	% Difference	DF	P value
2017	Autumn: Buffalo vs Buffalo + T Chr4D	-0.03	-7.6	75	0.7528
	Spring: Buffalo vs Buffalo + T Chr4D	0.01	3.4	75	0.9052
2018	Autumn: Buffalo vs Buffalo + T Chr4D	-0.2	-35.5	101	0.3142
	Spring: Buffalo vs Buffalo + T Chr4D	-0.7	-81.1	100	<0.001
2019	Autumn: Buffalo vs Buffalo + T Chr4D	-0.03	-5.6	80	0.7965
	Spring: Buffalo vs Buffalo + T Chr4D	-0.07	-14.8	81	0.4169
2020	Autumn: Buffalo vs Buffalo + T Chr4D	-0.1	-20.2	77	0.5756
	Spring: Buffalo vs Buffalo + T Chr4D	-0.3	-47.1	77	0.0503

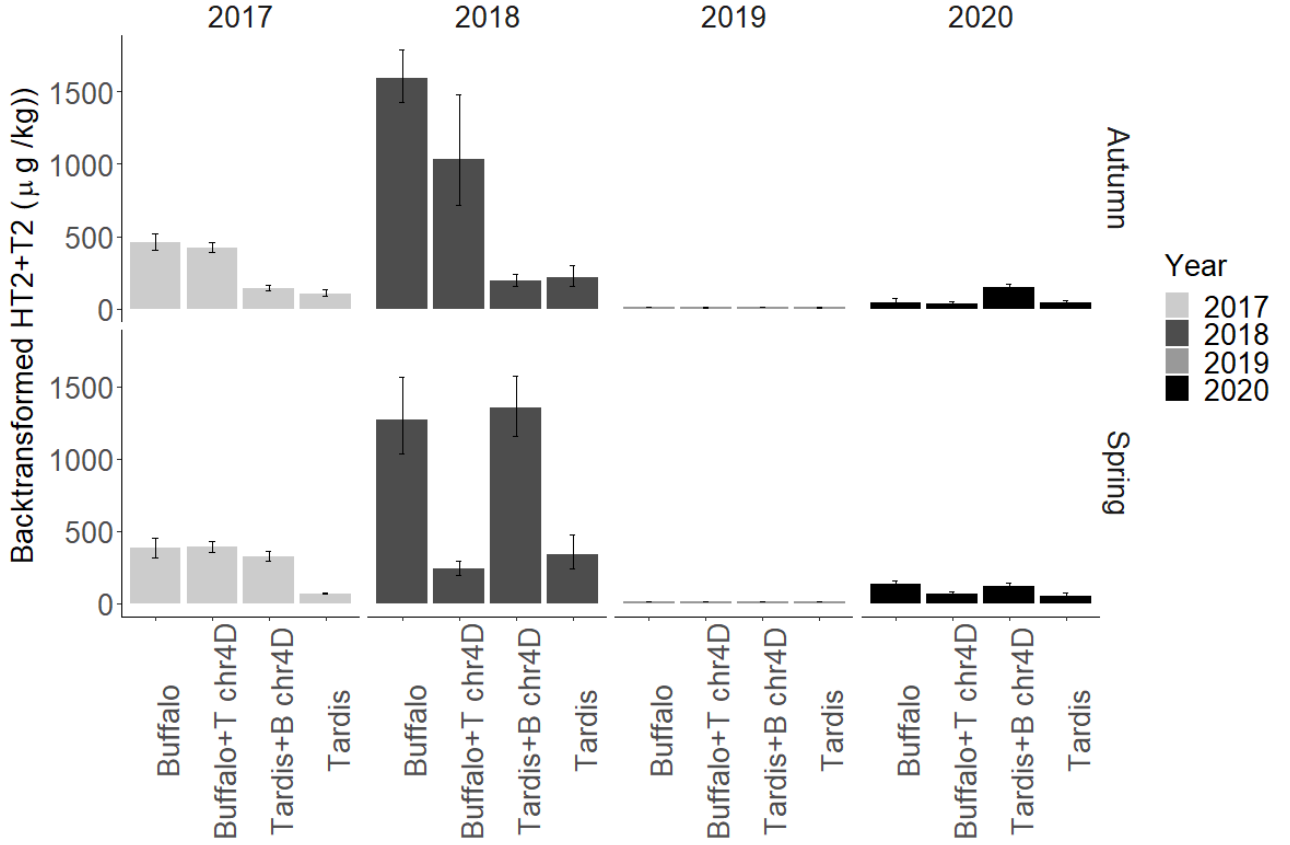

Figure 4.7: Graphical comparison of the parent lines Buffalo and Tardis to their respective Chr4D NIL in terms of HT2+T2 concentration in the harvested grain for each cropping year and sowing season. Data has been back transformed and error bars represent one standard error of the mean.

4.3 Plant height

Table 4.5 and Figure 4.8 show the output of a multiple linear regression height of plants against HT2+T2 split by year and the parental origin of the Chr6D QTL. Year had the largest impact accounting for 54.6% of the variation followed by plant height at 6.1% and then the parental origin of the Chr6D QTL at 4%. The R^2 values for plant height are low although the factor is highly significant (P<0.001).

Table 4.5: Output from the plant height model showing the significance and the percentage variance accounted for by each factor in the model as well as their interactions.

Factor	DF	Sum of squares	Mean sq	F value	P	% Variation accounted for
Year	3	136.6	136.6	576.4	<0.001	54.6
Height	1	15.3	15.3	191.4	<0.001	6.1
Mrg04	1	10.1	10.1	126.3	<0.001	4.0
Height*Year	3	3.3	1.1	13.9	<0.001	1.3
Year*Mrg04	3	9.8	3.2	41.1	<0.001	3.9
Height*Mrg04	1	2.5	2.5	31.8	<0.001	1
Year*Height*Mrg04	3	2.5	0.8	10.3	<0.001	1
Residual	876	70	0.08			

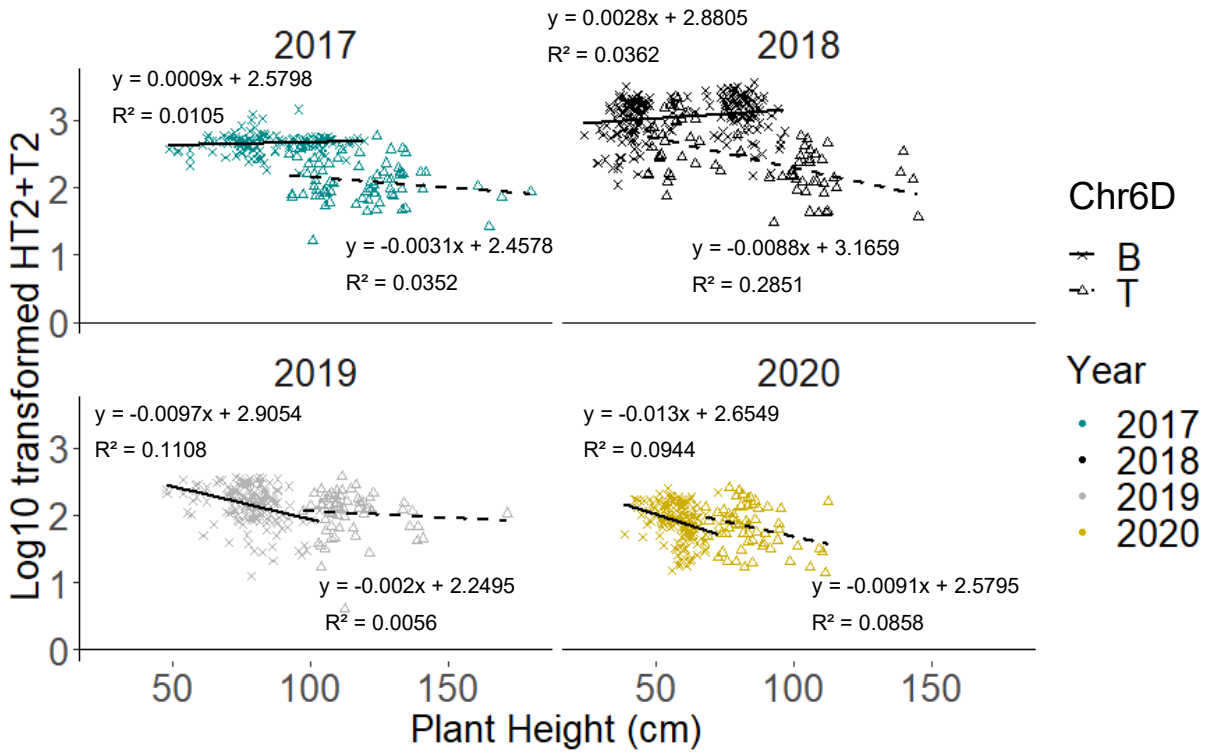

Figure 4.8: A plot of HT2+T2 concentration against plant height split by year and the parental origin of Chr6D. The shape of the data points indicates the parental origin of the Chr6D QTL, the colours indicate the different years described in the legend. Differently textured lines represent the fitted values according to year and the parental origin of the Chr6D QTL

4.4 Earliness

The relationship between the earliness (measured in degree days) of the NIL and the HT2+T2 concentration is described for the NIL in Table 4.5 and Figure 4.9.

Table 4.6: Output from multiple linear model based on degree days from sowing to panicle emergence, year and the parental origin of Chr4D showing the F value, statistical significance and percentage variance accounted for by selected factors and interactions entered into the model. Percentage variation was not calculated for non-significant results.

	DF	Sum of squares	Mean squares	F value	P value	% Variation
Degree days	1	4.0	4.0	43.3	<0.001	1.6
Year	3	138.2	46.1	504.2	<0.001	54.9
Chr4D parental origin	1	6.0	6.0	65.3	<0.001	2.4
Sowing season	1	3	3	31.9	<0.001	1.2
Year*Degree days	3	3.3	1.1	12.0	<0.001	1.3
Year * Chr4D	3	9.4	3.1	34.3	<0.001	3.7
Degree days*Chr4D	1	1.4	1.4	17.6	<0.001	0.5
Year*Sowing season	3	1.8	0.6	7.8	<0.001	0.7
Degree days*Sowing season	1	2.3	2.3	29.9	<0.001	0.9
Chr4D*Sowing season	1	2.2	2.2	28.7	<0.001	0.9
Year*Degree days*Chr4D	3	1.8	0.6	7.6	<0.001	0.7
Degree days * Sowing season * Year	3	11.4	3.8	49.5	<0.001	4.5
Year*Chr4D*sowing season	3	0.06	0.02	0.25	<0.8	0
Degree days*Chr4D*Sowing season	1	0.5	0.5	6.1	0.013	0.2
Year*Degree days*Chr4D*sowing season	3	0.6	0.2	2.6	<0.05	0.2
Residual	860	66.1	0.1			

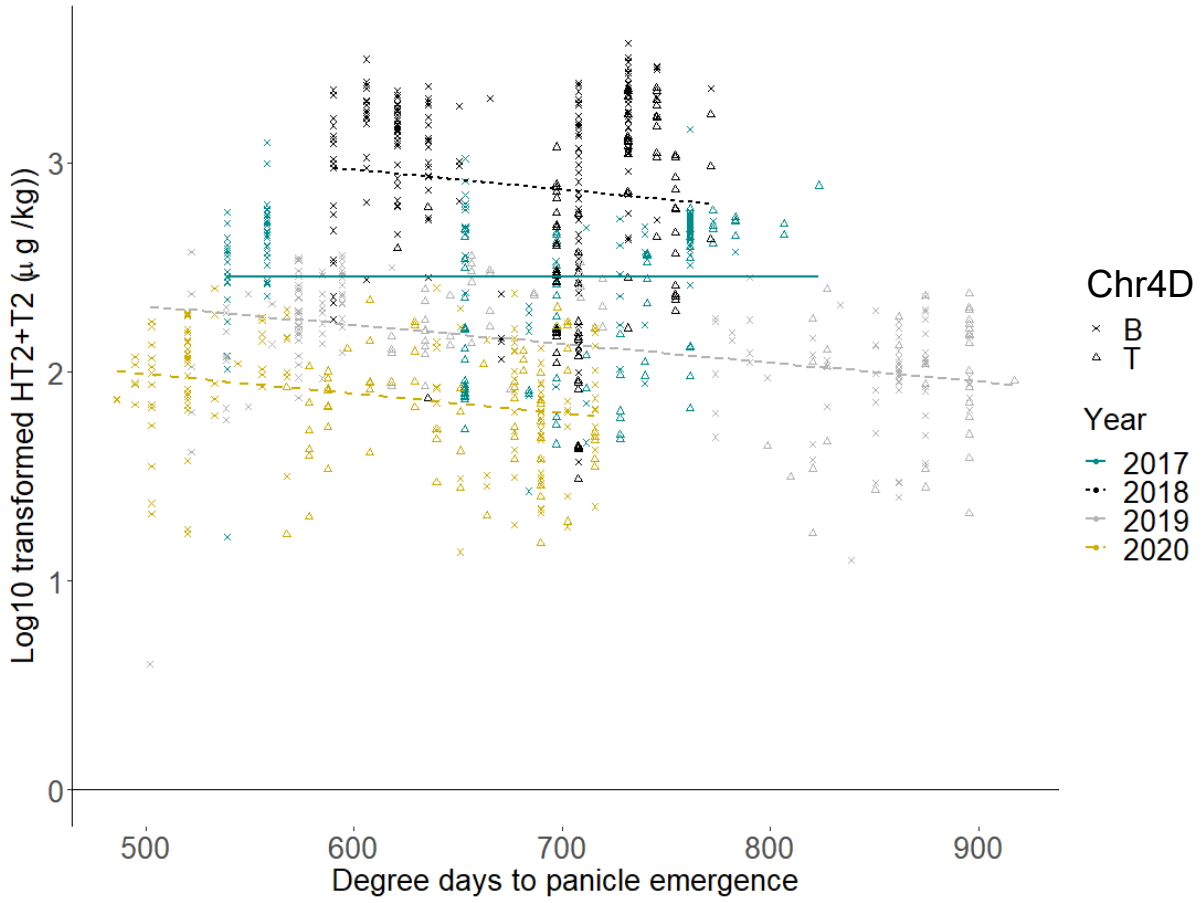

Figure 4.9 A *graphical representation of aspects of the model presented in Table 5.6, plotting log10 transformed HT2+T2 concentration against degree day from sowing to panicle emergence. The shape of the data points indicates the parental origin of the Chr4D QTL, the colours indicate the different years described in the legend. Differently textured and coloured lines represent the fitted values according to year from the model described in Table 5.6, not considering Chr4D origin.*

4.5 Window-pane analysis

Average rainfall did not correlate strongly for any window length pre-emergence of the panicle, with a maximum correlation coefficient of r = -0.24 for a two-day window-pane. Post panicle emergence rainfall negatively correlated with HT2+T2 concentration increasing to r = -0.70 by 30-day length window-panes (Figure 4.11). For both pre and post emergence window-panes temperature was positively correlated with HT2+T2 concentration reaching r = 0.6 by the 30-day window-pane size.

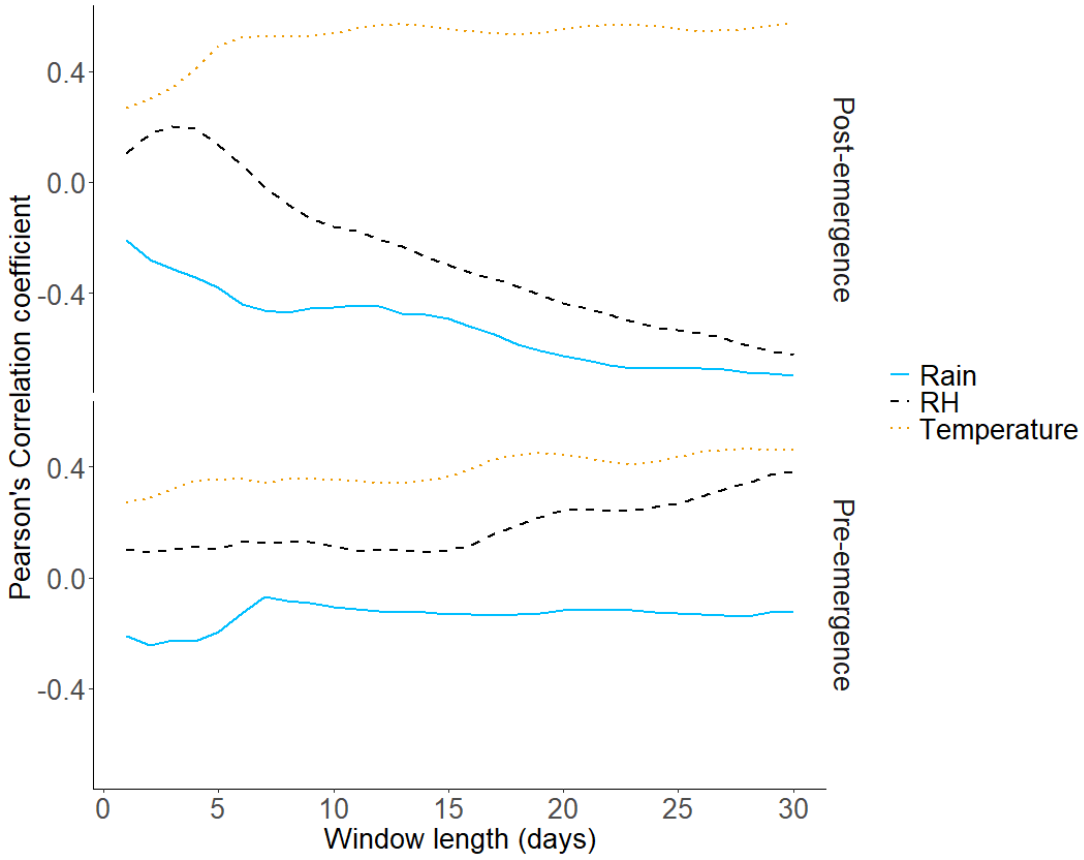

Figure 4.10: Pearson correlation coefficients plotted for average rainfall, average relative humidity, and average air temperature against each window-pane length for pre- and post-panicle emergence.

4.6 Field distribution

The 2017 and 2019 grid results are shown in Figures 4.12 and 4.13 respectively, the colour scales are the same to allow cross comparison. The results were analysed using the Mantel test to detect relationships between distance and HT2+T2 concentration. No relationship was detected in either 2017 (P=0.971) or 2019 (0.133) between distance and HT2+T2 concentration. The test was also applied to compare the differences between concentrations of HT2+T2 at each location within each grid across the two years and again detected no significant relationship (P = 0.78).

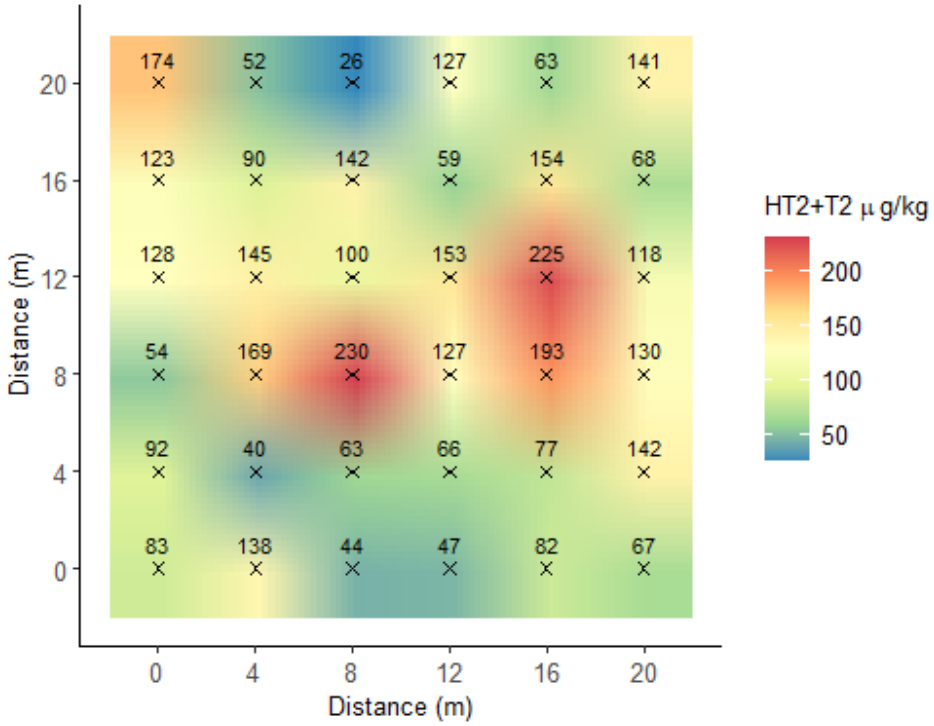

Figure 4.11 A heat map of the HT2+T2 concentrations of grain samples taken in 2017 from the experimental field. Colours on the chart represent linear interpolations of the HT2+T2 concentrations of each sampled location and can be interpreted using the scale in the legend. The relative position of each location is marked by a black x, labelled with the HT2+T2 value for each location.

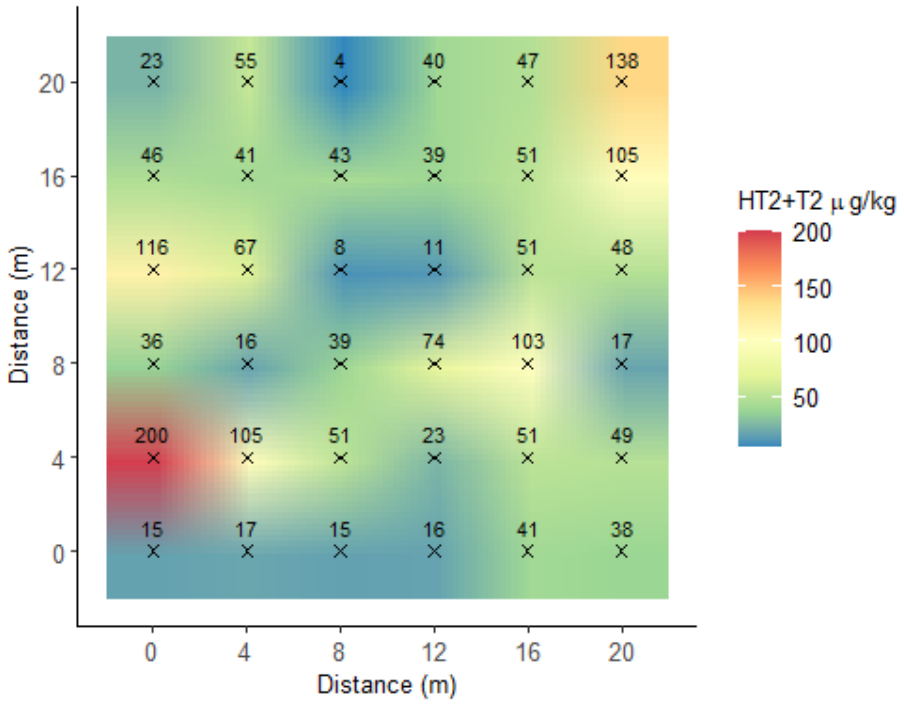

Figure 4.12: A heat map of the HT2+T2 concentrations of grain samples taken in 2019 from the experimental field. Colours on the chart represent linear interpolations of the HT2+T2 concentrations of each sampled location and can be interpreted using the scale in the legend. The relative position of each location is marked by a black x, labelled with the HT2+T2 value for each location.

For the larger grids the Mantel test indicates that the relationship between the distance between locations and the difference in location values for HT2+T2 concentration were not significantly different in 2018 (P = 0.908). However, in 2020 the correlation was found to have a significant (P = 0.003) correlation. The test was also applied to compare the differences between concentrations of HT2+T2 at each location within each grid across the two years and again detected no significant relationship (P=0.38).

Figure 4.14 and Figure 4.15 show the HT2+T2 concentrations (µg/kg) within the 80 x 80 m square sampled in 2018 and 2020 respectively. The values for each location are displayed on the figure. The raster is conditionally formatted to reflect the magnitudes of the HT2+T2 concentrations linearly

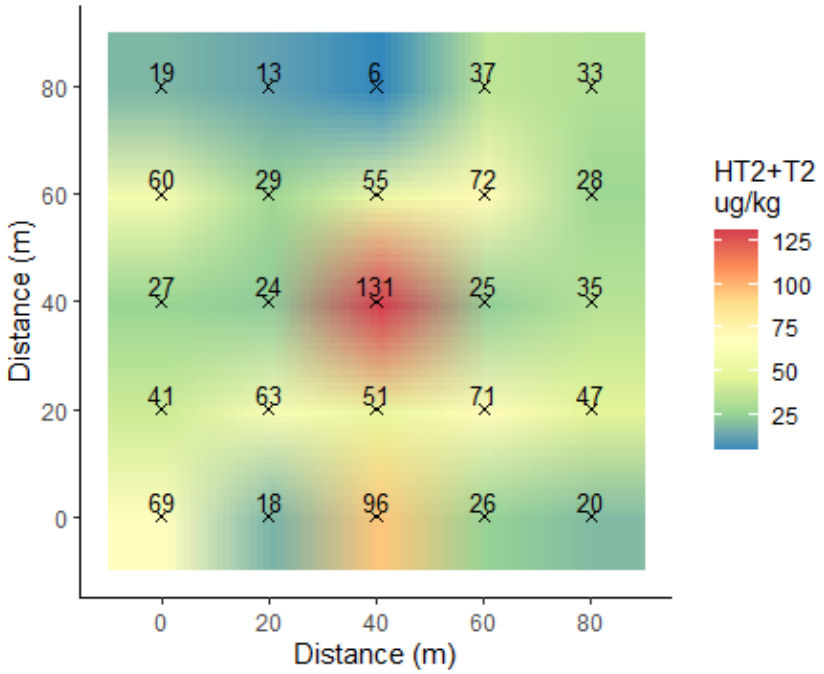

Figure 4.13: A heat map of the HT2+T2 concentrations of grain samples taken in 2018 from the experimental field. Colours on the chart represent linear interpolations of the HT2+T2 concentrations of each sampled location and can be interpreted using the scale in the legend. The relative position of each location is marked by a black x, labelled with the HT2+T2 value for each location.

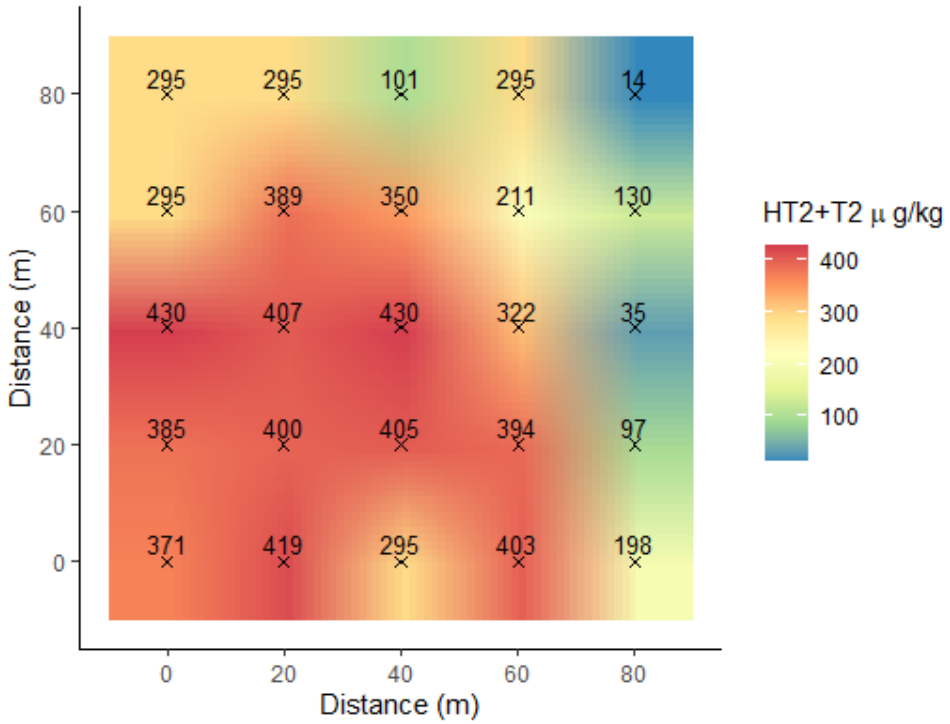

Figure 4.14: A heat map of the HT2+T2 concentrations of grain samples taken in 2020 from the experimental field. Colours on the chart represent linear interpolations of the HT2+T2 concentrations of each sampled location and can be interpreted using the scale in the legend. The relative position of each location is marked by a black x, labelled with the HT2+T2 value for each location

4.7 Panicle dissection

The mapped panicles of Buffalo + T Chr6D and Tardis B CHr6D are displayed in Figures 4.16 to 4.19. There is a range of incidence and concentration of HT2+T2 across each panicle in what appears to be a random distribution.

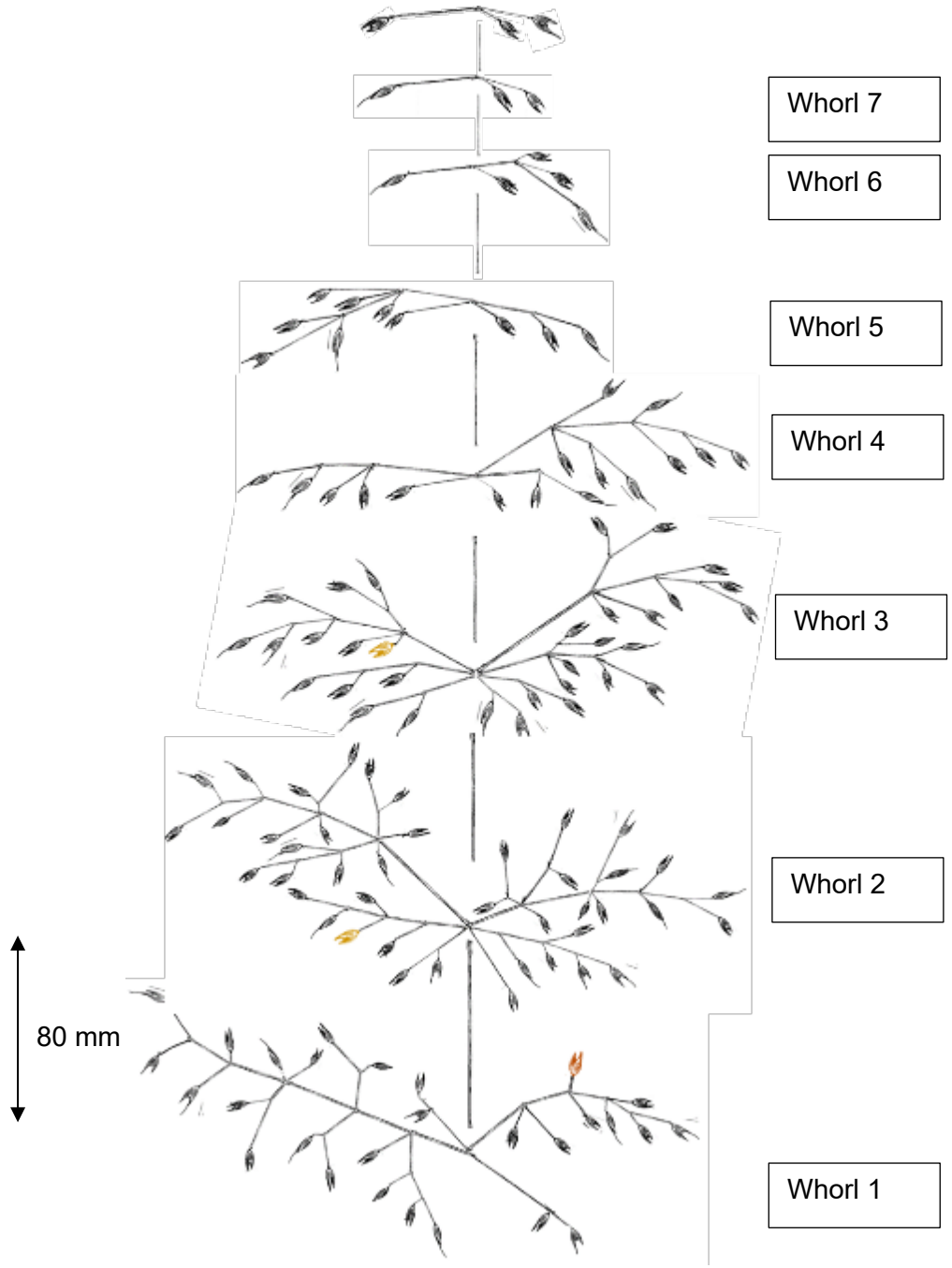

Figure 4.15: Mapped panicle of Buffalo + T Chr6D. Red spikelets showed detectable F. langsethiae *DNA at 5 pg/ng or above; yellow spikelets showed detectable F. langsethiae DNA at 4.9 pg/ng and below; and black spikelets had no detectable* F. langsethiae *DNA. A scale is present in the bottom left of the diagram. Whorls were counted from the bottom up and are labelled on the right of the panicle.*

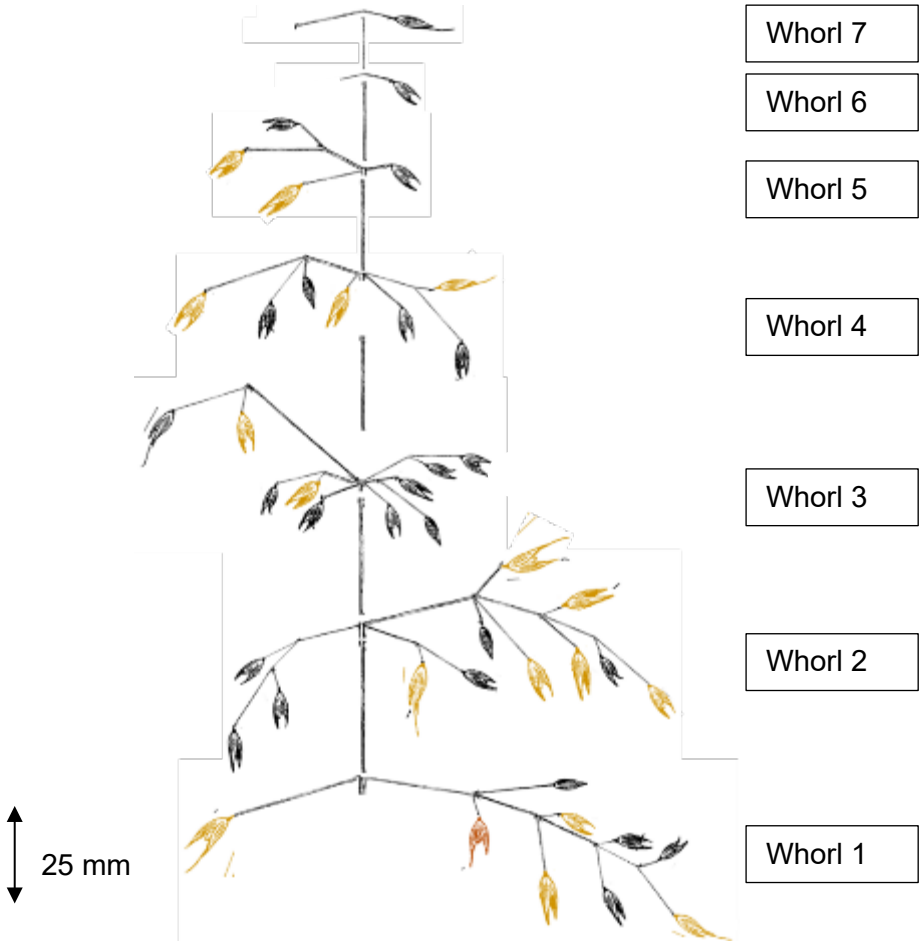

Figure 4.16: Mapped panicle of Tardis + B Chr6D (plant 2). Red spikelets showed detectable F. langsethiae *DNA at 5 pg/ng or above; yellow spikelets showed detectable F. langsethiae DNA at 4.9 pg/ng and below; and black spikelets had not detectable* F. langsethiae *DNA. A scale is present in the bottom left of the diagram. Whorls were counted from the bottom up and are labelled on the right of the diagram.*

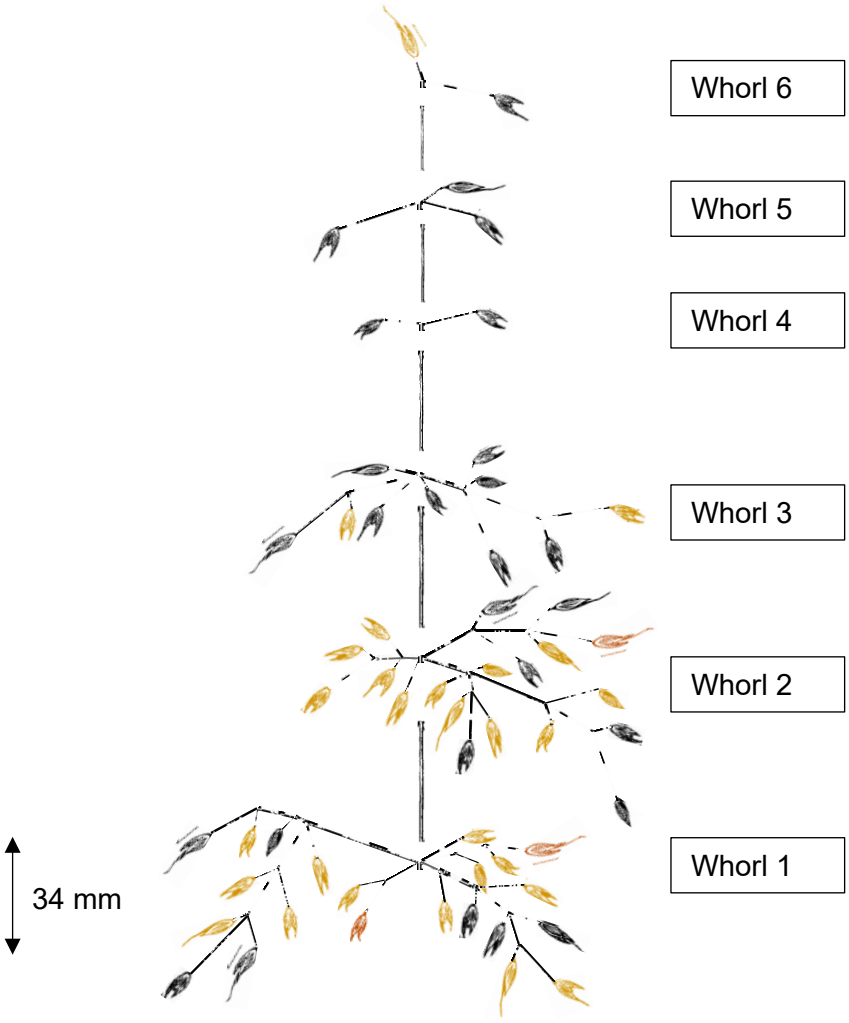

Figure 4.17: Mapped panicle of Tardis + B Chr6D (plant 3). Red spikelets showed detectable F. langsethiae *DNA at 5 pg/ng or above; yellow spikelets showed detectable F. langsethiae DNA at 4.9 pg/ng and below; and black spikelets had no detectable* F. langsethiae *DNA. A scale is present in the bottom left of the diagram. Whorls were counted from the bottom up and are labelled on the right of the panicle.*

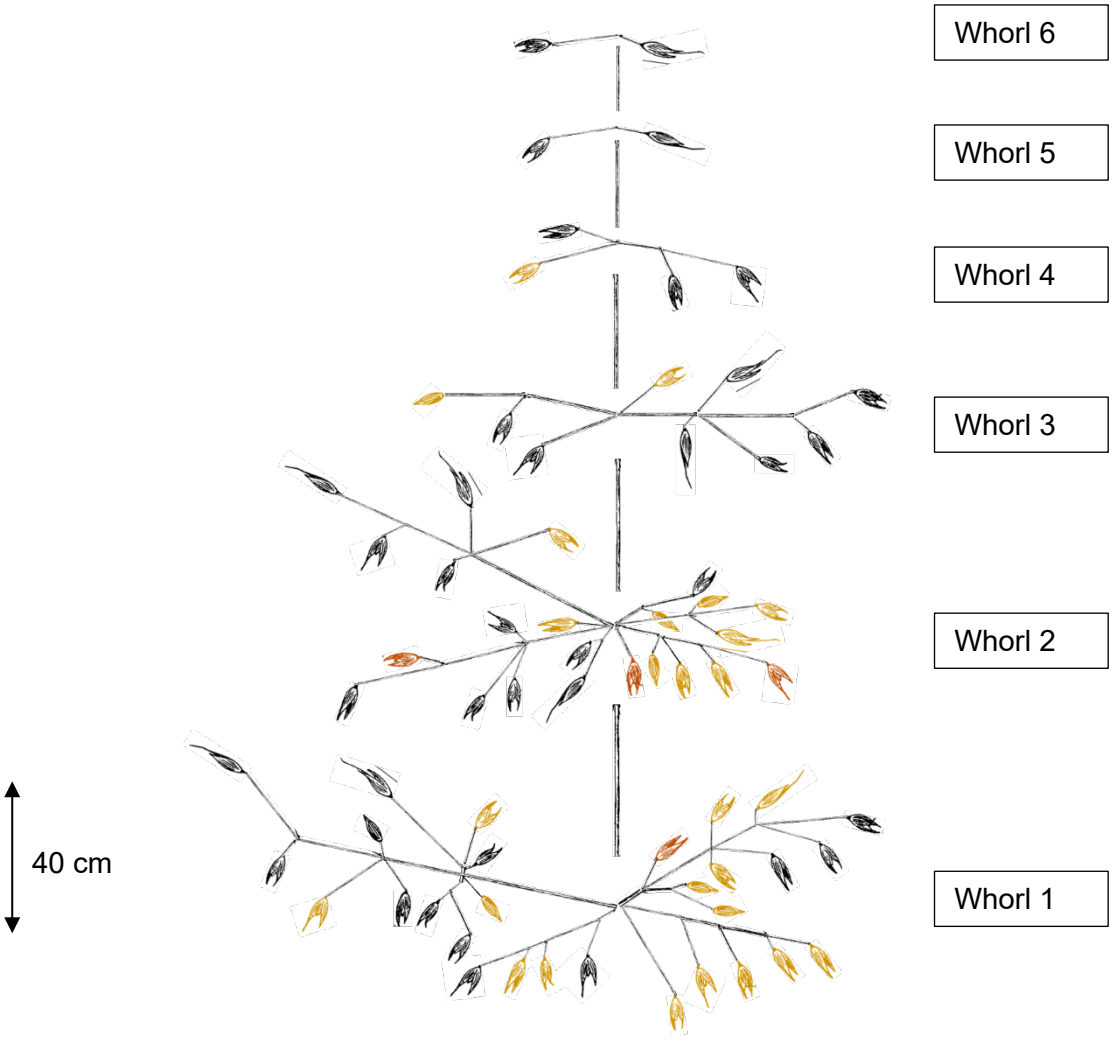

Figure 4.18: Mapped panicle of Tardis + B Chr6D (plant 4). Red spikelets showed detectable F. langsethiae *DNA at 5 pg/ng or above; yellow spikelets showed detectable* F. langsethiae *DNA at 4.9 pg/ng and below; and black spikelets had no detectable F. langsethiae DNA. A scale is present in the bottom left of the diagram. Whorls were counted from the bottom up and are labelled on the right of the panicle.*

5 Discussion

5.1 Artificial inoculation

The 2017 Gerald glasshouse inoculation experiment showed mycelial growth on the outside of the panicles like that seen by Divon *et al*. (2012), Opoku (2012) and Mousavi (2016). The duration of the bagged time was reduced to seven days in subsequent inoculations and mycelial growth was no longer seen. However, HT2+T2 concentrations were typically lower in the two experiments with shorter bagged periods. This reduction occurred even in light of Balado and Buffalo previously being seen to be more susceptible to HT2+T2 accumulation than Gerald in commercial crops (Edwards, 2015).

Growth stage was examined in the Gerald (2017) and Balado (2018) experiments, and in both instances was shown to be an important factor in determining the concentration of HT2+T2 in the panicle. The early panicle emergence/and early anthesis (GS51/63) application in the Balado experiment and the late panicle emergence (GS59) application in the Gerald experiment were physiologically close to one another in growth stage, effectively occurring at the beginning of

flowering, and each had the highest HT2+T2 concentration in their respective experiments. This agrees with other authors who found that applications of inoculum to the plant close to flowering resulted in reliable *F. langsethiae* infections (Schöneberg *et al*., 2019; Mousavi, 2016; Divon *et al*., 2012; Opoku, 2012). In terms of growth stage, the Balado result is especially convincing as the tillers were inoculated at different growth stages on the same plants which were then entered into identical conditions within the bag. The first spikes which emerged from the flag leaf boot in the early panicle emergence/early anthesis (GS51/61) treated tillers had direct contact with the inoculum, whereas the late booting (GS47) tillers could only encounter the inoculum if the fungus penetrated the flag leaf boot or if the spores remained viable until the spikes began to emerge later. The Balado flowered over a short period of time potentially meaning that most of the spikelets had pollen present to encourage infection when inoculum was applied (Divon *et al*., 2019). The large difference in the tillers inoculated at different growth stages provides evidence that the spores need direct contact with the florets. The result also suggests that the pathogen did not cross infect from infected tillers to tillers inoculated at less susceptible growth stages. Inoculants applied at earlier growth stages were not able to infect plants once they later passed through more susceptible growth stages. Within the bag there is no mechanism for spores to be mobile around panicles whereas in the field there could be the opportunity for rain splash, wind dispersal or arthropod vectors. It is also possible that the source of inoculum in the field is constant and not constrained to discrete events.

In the 2017 Gerald and the 2018 Balado experiments, the addition of the PDB did not have a significant effect on HT2+T2 concentration in the panicles. PDB was picked as PDA (potato dextrose agar) provides a functional growth medium for *F. langsethiae* in the laboratory and it was hoped that it would encourage the germination of spores on the plants. Divon *et al*. (2019) showed that the pathogen grew preferentially and faster in the presence of pollen. However, if a pollen based nutrient or pollen analogues were used, given that inoculants are applied to the entire panicle, they could lead to mycelial growth across the exterior of the panicle and therefore not simulate natural infection.

The 2019 NIL inoculation experiment aimed to understand the impact on different levels of panicle extrusion on HT2+T2 concentration in the harvested panicles. The 2019 NIL experiment used full panicle emergence (GS59) as previous inoculation experiments indicated anthesis as the most susceptible growth stage (Divon *et al*., 2012; Divon *et al*., 2019; Drakulic *et al.,* 2016; Opoku *et al*., 2012). Full panicle emergence (GS59) was also the earliest opportunity to differentiate plants in terms of panicle extrusion. This study provided evidence that exposing a panicle that would otherwise have been covered by the flag leaf sheath increases the infection level in the harvested panicle. The Buffalo NIL plants with mechanically extruded panicles accumulated significantly higher concentrations of HT2+T2 than the undamaged unextruded control, and the damaged but unextruded panicles' HT2+T2 concentrations were not statistically different to the undamaged and unextruded plants. Potentially extruded panicles have more exposed spikes for spore suspension to land on and infect, whereas unextruded panicles are protected in the boot. In natural infection conditions in the field the Buffalo NIL would accumulate the highest concentrations of HT2+T2 and the Buffalo + T Chr6D the least. There was no statistical difference between the damaged and the undamaged plants of the same genotype indicating that any impact of the damage was negligible. The Buffalo + T Chr6D had the highest HT2+T2 concentration in the inoculated experiment whereas in the field under natural infection it would typically have the lowest concentration. None of the field inoculations succeeded in inducing a higher level of infection than the untreated controls.

5.2 NIL

5.2.1 Chr6D

For the Buffalo + T Chr6D NIL the contrasts against Buffalo were significant ($P \leq 0.0021$) in every year and sowing season, with the exception of autumn 2019 and 2020 (the lowest HT2+T2 accumulating years). The contrasts remained significant irrespective of sowing season: the magnitudes of the difference were between 44.8% and 88.5% less HT2+T2 in the Buffalo +T Chr6D NIL than Buffalo. Potentially this difference could be attributed to height or panicle extrusion, although the impact of both within the multiple linear models was small. The Buffalo + T Chr6D genotypes differ in panicle emergence date as well as potentially carrying other genetic drag from the introgressed QTL.

The Tardis + B Chr6D NIL grew very short compared to the Tardis parent and was late to initiate panicle emergence; the panicle never fully extruded in any year or sowing season. The HT2+T2 concentration of the Tardis + B Chr6D NIL was often far higher than the Tardis parent. For example, in autumn sown 2018 the Tardis + B Chr6D NIL had an HT2+T2 concentration 535.7% higher than Tardis. The concentrations of HT2+T2 were more similar to Buffalo than Tardis. In the spring sown 2018 plots, however, the HT2+T2 concentration remained similar to the Tardis concentration and the contrast between the two was not significant ($P=0.4$). The Tardis + B Chr6D genotypes were much later to undergo panicle emergence than any other genotype in the experiment in both spring and autumn sowings.

5.2.2 Chr4D

The Chr4D QTL had a significant effect on the time to panicle emergence, the effect was larger when sown in spring, bringing forward panicle emergence in the Tardis + B Chr4D NIL by 7.4 days compared to Tardis and causing the Buffalo + T Chr4D to be 7.3 days later compared to Buffalo. The contrast between Tardis + B Chr4D and Tardis in terms of HT2+T2 concentration was significant in the 2017, 2018 and 2020 spring sowing as well as the 2020 autumn sowing. Height was only increased by 6.3% in autumn and 4.9 % in spring with the introgression of the Buffalo derived Chr4D into Tardis. Such small differences in height are not likely to have caused the relatively large (360.2 %, 297.4 %, 228.0 % and 135.5 % in spring 2017, 2018, 2020 and autumn 2020 respectively) increases seen in HT2+T2 concentration of Tardis + B Chr4D compared to Tardis. The introduction of Buffalo alleles within the Chr4D QTL made the Tardis plants more susceptible, potentially due to bringing forward the panicle emergence date. Introducing the Tardis alleles at Chr4D QTL into the Buffalo background had a less consistent effect on the HT2+T2 concentrations: in spring sown 2018 plots (the year with the highest infection levels) Buffalo + T Chr4D constituted an 80% reduction in HT2+T2. These plants had also reached panicle emergence 7.3 days later than Buffalo. However, regression analysis of the entire data set does not support earliness impacting HT2+T2 concentration to such high degrees.

5.3 Height

Height had a significant ($P<0.001$) negative relationship with HT2+T2 concentration with increasing height leading to lower HT2+T2 concentration. Variation in height was largely achieved with the presence or absence of the *Dw6* dwarfing gene within the Chr6D QTL which also introduces other traits associated with the extrusion of the panicle, the length and number of grains in the panicle and the panicle emergence date. The *Dw6* gene works by shortening the upper internodes which often has the effect of preventing the panicle from fully extruding. Placing the Chr6D QTL within the model in the current work accounted for some of the genetic component and height. Year was the most important factor followed by the origin of the Chr6D QTL; height, although significant, did not contribute greatly to the resistance of the NIL when Chr6D was already considered.

Height has been suggested as an evasion mechanism in wheat against FHB (Yan *et al*., 2011; Mesterhazy, 1995), adding to the plants' type I resistance (resistance to initial infection). The same concept is supported for oats and *F. langsethiae* by evidence that stubble from previous cereal crops can be a source of inoculum (Kaukoranta *et al.,* 2019; Edwards, 2007; Edwards and Jennings, 2016; Edwards, 2017; Schöneberg *et al.,* 2019). From this stubble the spores must reach the panicle at the top of the plant. Previously dwarf oat varieties have been identified as accumulating higher concentrations of HT2+T2 in their grain (Edwards, 2007; Edwards, 2015). However, researchers have struggled to successfully correlate the plant height of oats with resistance to *Fusarium* using plant growth regulators (Stančić, 2016; Edwards, 2011; Edwards and Anderson, 2011; Edwards, 2017) or mapping populations of oat (He *et al*., 2013; Stančić, 2016). Loskutov *et al*. (2017) and Bjørnstad *et al*. (2017) concluded that taller plants resistant to lodging were more resistant to *Fusarium* infection and mycotoxin concentration. However, in work based on various *Avena* species, Gagkaeva *et al*. (2018) found a negative relationship with certain trichothecene producing species of *Fusarium* and plant height and Horsley *et al*. (2006) saw no correlation between height and infection with *F. graminearum* in barley. In wheat, Draeger *et al.* (2007) found several instances in an Arina/Riband population where height QTL did not overlap with FHB QTL and concluded that height itself was not the causal factor in resistance and that the *Rht-D1b* allele was linked to genes inferring susceptibility to FHB rather than the allele itself conferring susceptibility.

5.4 Earliness

In the analysis examining the relationship between earliness and HT2+T2 concentration, degree days to panicle emergence accounted for less than 2% of the variance. The term was statistically significant but its impact was very low. Bjørnstad *et al*. (2017) found negative correlations between days to flowering and FHB (DON producing) scores in an analysis of 424 spring oat lines grown in Norway. However, Hautsalo *et al*. (2020) found later maturing varieties of oat to have higher concentrations of DON when investigating *F*. *graminearum* (spore suspension inoculated) and *F*. *culmorum* (grain spawn inoculated) field experiments. Parry *et al.* (1995) cited Love and Seitz (1987) concluding that there was evidence of resistance independent of maturity factors in wheat to FHB. The conclusion was based on different cultivars maturing at different times and finding that susceptibility was independent of maturity factors as cultivars with similar heading dates differed in the degree of FHB infection. Although the experiment described in this work used a range of genotypes, they were all derived from only two parents, whereas other studies cited have used broader selections and found conflicting results.

Drawing conclusions on the resistance of plants to a pathogen from experiments examining differences in quantitative traits between different genotypes of plants risks erroneously concluding a causal mechanism between those quantitative traits and resistance. The basis of resistance in those instances could be pleiotropy or genetic linkage from closely located genes to those coding the traits being examined.

5.5 Window-pane

The strong negative correlation between rainfall and HT2+T2 concentration by the 30-day length window-pane post panicle emergence and the positive correlation of temperature post panicle emergence for window-panes up to 30 days agrees with the findings of Xu *et al*. (2014) in that dry warm weather after anthesis is correlated to higher HT2+T2 concentrations in oats.

5.6 Field distribution

In all 4 years of study, considerable variation in T2 +HT2 was found across the field. The 2016 grid (20 m intervals, 80 m x 180 m) showed a significant relationship between the distance between locations with the difference in value of each location, leading to the conclusion that similar concentrations are more likely to be located close to one another. No such significant relationship was seen in either of the smaller scale grids sampled in 2017 and 2019. A significant relationship between distance and the difference in concentration was seen in the 2020 (20 m interval, 80 m x 80 m) grid. Significant correlations could be evidence of infection loci within the field, initial points of infection, or discrete locations conducive to infection from which surrounding plants become infected. Schlang *et al*. (2008) performed a similar study on two sites of wheat, samples were collected in a grid pattern spaced 25 m from one another and DON concentration was measured at each point, no relationship was detected between the distance of sampled positions and their respective DON concentrations. Xu *et al* (2008) used quadrate sampling to measure within field variability of *F. avenaceum, F. culmorum, F. graminearum, F. poae, M. majus,* and *M. nivale,* in chaff and grain samples. The distance between quadrants was not measured; however, the researchers concluded that the presence or absence of FHB pathogens within quadrates were independent of one another. In the two instances where it was possible to compare grids across years with one another using the Mantel test, no relationships were found between the two. Therefore, there is no evidence that location at the scale examined within this work influences infection in subsequent years. Location at field scale has effectively been associated with *F. langsethiae* infection (Edwards, 2017) by demonstrating that cereal intensity was influential on the level of infection as measured by HT2+T2 concentration in the grain.

5.7 Panicle dissection

Examination of the Buffalo + T Chr6D panicle, a tall plant with a low natural HT2+T2 concentrations, shows the infected spikelets are on different whorls. Edwards *et al*. (2012) quantified the *F. langsethiae* DNA concentration in 122 oat grains from one sample, *F. langsethiae* DNA ranged between 0.0002 pg/ng and 13.85 pg/ng, with a mean of 0.54 pg/ng from a bulk sample with 8399 µg/kg HT2+T2. The highest concentration of *F. langsethiae* DNA across all three panicles from Tardis + B Chr6D was 18.2 pg/ng, and the lowest value (excluding 0) was 0.0005 pg/ng. Previous work (Stančić, 2016; Imathiu, 2008) has shown high concentrations of HT2+T2 in entire panicles. In this work, entire spikelets were analysed including the glumes, the peduncle connecting primary and secondary spikes and the husks. Divon *et al*. (2019) observed that *F. langsethiae* initially germinated on the glumes of the oat plants potentially leading to high concentrations of *F. langsethiae* DNA and HT2+T2 on the glumes of the plants. The higher HT2+T2 concentrations in whole panicles is potentially derived from glumes.

The distance between the spikelets and the difference in their *F. langsethiae* DNA concentrations did not significantly ($P>0.05$) correlate with one another in any of the three Tardis + B Chr6D panicles when examined within whorls. The concentration of *F. langsethiae* DNA seems independent within each spikelet, supporting the theory that each spikelet is infected independently. Bjørnstad and Skinnes (2008) speculated that oats may have an inherent resistance to *Fusarium* species due to their panicle structure. Infection has never been observed to move out of the initially infected spikelet (Divon *et al*., 2019), the length of branches within the panicle potentially has no direct impact on resistance. Tekle *et al.* (2012) observed that *F. graminearum* moved between florets of oats within spikelets through physical contact rather than through the rachis. The evidence presented in this work supports infection being retained within the originally infected spikelets.

6 Conclusions

- Impact of QTL
    - The introgression of Chr6D QTL had the largest and most consistent effect on HT2+T2 concentration of the QTL studied.
    - Introgression of the Tardis (more resistant) Chr6D QTL into the Buffalo (susceptible) background results in a taller earlier plant in both spring and autumn sowing that is more resistant to the accumulation HT2+T2.
    - The introgression of the Buffalo (susceptible) Chr6D QTL into the Tardis (more resistant) background results in a shorter later plant in both spring and autumn sowing that that accumulates higher HT2+T2 concentrations.
    - The introgression of the Tardis Chr4D QTL into the Buffalo background reduced HT2+T2 concentrations in spring sowings and created a later flowering plant. The opposite was true for the introgression of the Buffalo Chr4D into the Tardis background, an earlier flowering plant was generated that often accumulated higher HT2+T2 concentrations.
    - The introgression of the Buffalo derived Chr4A QTL into the Tardis background genome led to a plant only marginally later than the Tardis parent but with a reduction in HT2+T2 concentration in autumn sown plots.
    - Introgressions of the Tardis Chr4C had no impact on the concentration of HT2+T2, height or earliness.
    - Analysis of earliness through regression suggested a small although significant impact on HT2+T2 concentration where later plants accumulated lower concentrations among the examined lines. Other researchers reported conflicting results in oats infected with various *Fusarium* pathogens suggesting that the trait of earliness could impact differently in different cultivars.
    - Analysis of height through regression suggested a small although significant impact on HT2+T2 concentration where taller plants accumulated lower concentrations among the examined lines.
- This work has provided further evidence that warmer dryer summers are inducive to higher *F. langsethiae* infection.
- The field scale distribution of F*. langsethiae* in oats is similar to that of *F. graminearum* in wheat in that it does not spread from foci and is heterogeneous.
- The distribution of *F. langsethiae* within the panicle is random and not related to the proximity of infected spikelets to one another.
- The discrete manner in which the *F. langsethiae* DNA is distributed across spikelets in the panicle is further evidence that the pathogen and associated mycotoxins are not mobile across the panicle.
- Application of spore suspensions to plants has only been shown to be successful in controlled environments and differences in susceptibility of plants when naturally infected as opposed to artificially infected suggests that artificial inoculation by spore suspension is a poor means of testing for resistant or susceptible lines.

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