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Deoxynivalenol Concentration and Grain Quality of *Fusarium* Infected Winter Wheat Genotypes under Restricted Water

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

A field experiment after maize factorially combined shelters and six wheat genotypes varying both in the presence of reduced height (Rht) alleles, known to vary for linked Type II resistance to Fusarium infection, photoperiod sensitivity (Ppd-D1) and baking quality: Oakley (Rht1-B1b + Ppd-D1b), Soissons (Rht1-B1b + Ppd-D1a), SR 53 (Rht1-D1b + Ppd-D1a), SR 94 (Rht1-D1b + Ppd-D1b), Maris Widgeon1 (Rht1-B1b + Ppd-D1b) and Maris Widgeon 2 (Rht1-D1b + Ppd-D1b) in four completely randomised blocks. Shelters were applied from the end of anthesis until 28 days later. Plots were sampled after shelter removal (hand harvest) and also at maturity (combine harvest). Analysis was carried out on the harvested grains which included deoxynivalenol (DON) concentration and grain quality parameters such as crude protein, Hagberg falling number (HFN) and sedimentation volume (SDS). Data show that in hand harvested grains, there was significant (P=0.01) genotype x shelter effect where shelter reduced DON concentration by 48% in Soissons. Crude protein (P=0.08), HFN (P=0.08) and SDS (P=0.19) showed no genotype x shelter effect. In combined harvested grains, DON concentration showed no clear pattern. However, crude protein (P=0.005) and HFN (P=0.034) showed significant genotype x shelter effect where shelter reduced

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the crude protein in Oakley and increased HFN in Maris Widgeon genotypes. This study therefore, reveals that data on the level of DON contamination may not be deduced from results of HFN, SDS volume or protein content, thus these parameters may not be influenced even when wheat kernels are heavily infected by *Fusarium* even under restricted water during grain filling.

Keywords: Deoxynivalenol; genotype; shelter; crude protein; Hagberg falling number; sedimentation volume.

1. INTRODUCTION

Summer drought and reduced rainfall are expected to increase in Europe during the coming decades [1] and this could have an adverse effect on wheat production. Approximately 30% of the wheat area in the UK is on drought-prone soils [2] and most of the estimated 15% wheat yield loss occurs after anthesis [3]. With climate change scenarios predicting more frequent summer droughts these losses are likely to increase [4]. Authors like [5] predicted that with an increasing cropping frequency with grain maize expected under climate change, *Fusarium* head blight (FHB) disease is projected to be more severe. Environment, cultivar and preceding crop account for 48%, 27% and 14-28%, respectively of the variation in FHB infection in wheat [6]. Most UK winter wheat cultivars possess the semi-dwarfing (Rht-D1b) allele on chromosome 4DS which is linked with FHB susceptibility [7]. These alleles confer insensitivity to gibberellic acid (GA) and have pleiotropic effects on plant growth by causing a reduction in plant height and seedling leaf area [8].

Temperature, moisture and relative humidity are the major factors that influence the FHB disease [9] and these factors could be greatly influenced under the climate change conditions [10]. Some authors have reported a more extreme rainfall and drought events under climate change which could influence mycotoxin production [1,5,9,10]. The effect of post-anthesis weather variables such as water shortage on disease and DON levels is also still not well understood and results in some studies have varied considerably [11]. DON was found to have leached from early dough through to harvest under long irrigation [12] while [13] observed higher disease severity but lower DON for non-irrigated treatments for a 26 day period of *Fusarium* inoculation. An increased DON concentration in maize kernel when the plants were grown under long term water deficit conditions has been reported by [1]. However, *Fusarium* head blight (FHB) infection on grain quality of harvested wheat grains is still not well understood [14].

Little is known with regards to effects of water shortage and the time of stress during grain filling on wheat quality parameters. Hagberg falling number decreased with FHB infection, but the protein content and wet gluten content showed no effect of *Fusarium* infection in winter wheat [15]. Environmental conditions preceding crop and/or the choice of genotype also influences protein concentration under FHB epidemics [16]. Under conditions of high FHB infection, kernel resistance could be defined by low DON accumulation and stability of several baking quality parameters [17]. However, there are still contradictory reports on the impact of FHB infection on wheat baking quality [18]. With wheat protein concentration, specific weight and HFN (associated with activity of the α-amylase enzyme) being significant quality criteria for the bread making premium in UK wheat market [19], there is need to assess the possible effect of stress on these qualities in different cultivars under severe FHB infection. The prevalence of GA-insensitive dwarfing alleles in the world's wheat production and the linkage of Rht-D1b to FHB susceptibility [7] also necessitated this research. The aim of this study was, therefore, to evaluate the effect of withholding water during grain filling on *Fusarium* head blight infection and grain quality of wheat in terms of DON concentration, % crude protein, Hagberg falling number (HFN) and sedimentation volume (SDS) in UK winter wheat cultivars.

2. MATERIALS AND METHODS

2.1 Crop Husbandry

The experiment was conducted in the 2012/2013 cropping season at the Crops Research Unit, Sonning, University of Reading, UK (0^{o} 56'W, 51° 29'N), on a free-draining sandy loam. The experimental field was prepared by planting wheat after maize as the previous crop. The maize was topped off, power harrowed twice (second time at 90^{o}) to spread trash.

Table 1. Major genes and GA sensitivity alleles associated with the genotypes used in the experiment

Genotype	Semi-dwarfing allele	Photo periodism allele	Baking quality
Soissons	Rht-B1b	Ppd-D1a	Moderate
Oakley	Rht-B1b	Ppd-D1b	Poor
SR53	Rht-D1b	Ppd-D1a	No data
SR94	Rht-D1b	Ppd-D1b	No data
Maris Widgeon 1	Rht-B1b	Ppd-D1b	Good
Maris Widgeon 2	Rht-D1b	Ppd-D1b	Good

The field was power harrowed again followed by rain and then harrowed for seed drilling. Six wheat genotypes evaluated varied in alleles for reduced height (Rht), photoperiod insensitivity (Ppd-D1a) and also in their baking quality (Table 1). Genotypes were near-isogenic lines back-crossed to Maris Widgeon carrying Rht-B1b or Rht-D1b; Soissons; Oakley and two lines from a double-haploid cross between Savannah and Renaissance (SR 53 and SR 94).

The untreated seeds were drilled at a nominal depth of 50 mm, on 120 mm rows in 9 m x 1.4 m plots separated by 0.5 m double-width track wheeling at the seed rate of 300/m^2. The field had four blocks and each genotype is replicated twice within each block giving a total of forty eight plots. Quantum SX at 30 g/ha in 220 litres of water was applied at GS 19 and Atlantis at 400 g/ha + Biopower adjuvant at 1 litre/ha in 220 litres of water was also applied at growth stage (GS 32). 100 kg N/ha + 40 kg S/ha was applied as a mixture of ammonium nitrate and ammonium sulphate between GS 30-31. A further 100 kg N/ha was applied as ammonium nitrate between GS 34-39. No fungicide was used during the experiment. Rain shelters were applied to the plots a day after the end of flowering for each genotype in turn and this was randomly assigned to the plots.

A total of twenty four plots had shelters, while the other twenty four were without shelters. Soissons, which has Ppd-D1a, started flowering at 200 days after sowing, while the other genotypes flowered at 210 days after sowing, so erection of shelters for Soissons was done 10 days before the other genotypes. SR 53, which also had Ppd–D1a, started flowering just three days before the other genotypes so its shelters were erected the same time as the other genotypes. The shelters were removed after 28 days. This period of sheltering was intended to broadly coincide with the duration of grain filling. After the removal of shelters, 150 spikes per plot were randomly selected and hand threshed and adjusted to 15% moisture content. The grains were then ground into flour and used for deoxynivalenol (DON) analysis and grain quality parameters. At maturity, when the plants were fully senesced, the wheat spikes were harvested using a plot combine harvester for all genotypes. A 2 kg representative sample of grain from each plot was obtained from the lot and was used in the grain analysis. The seeds were partially cleaned and then a sub-sample taken and cleaned further by hand.

2.2 Grain Quality and DON Analysis

Grain samples (300 g per plot) were milled using a Laboratory Mill 31 and used for grain quality tests and DON analysis. DON concentration was analysed using Enzyme Linked Immunosorbent Assay (ELISA) DON kits according to the manufacturer's instructions. The DON range of quantification was between 0.25 – 5.0 µg/g. The treatments were completely randomised in the micro titre plates. Absorbance was measured at 450 nm and a differential filter of 630 nm using a Multiskan Ascent plate reader. DON concentration was calculated by reference to a standard curve generated using the DON kit. Using .

7 g of wholemeal flour adjusted for moisture content to 15%, HFN was determined using a Perten Instruments Falling Number 1500 machine assessed to ISO 3039:1982. The sample was suspended in 25 ml distilled water by shaking in a viscometer tube.

The viscometer tube was placed in boiling water and after 5 seconds a plunger started agitation automatically for 55 second. After 60 seconds, the plunger is released and time taken from the start of agitation for it to fall to a predetermined height was recorded in seconds as the falling number. For SDS-sedimentation volume test, wholemeal flour equivalent to 6 g at 15%

moisture content from each plot was suspended in 50 ml of distilled water in 100 ml cylinder before the addition of 50 ml SDS reagent (20 g SDS + 20 ml diluted lactic acid in a litre of distilled water) and further suspension. Sediment volume was recorded after 20 minutes of settling. Grain nitrogen was determined from a 0.2 g sample of dried wholemeal flour using oxidative combustion with a LECO FP–528. The protein content was derived by multiplying the N result by 5.7.

2.3 Statistical Analysis

Data were analysed as a randomised complete block design with four replicates, using the general ANOVA (Block structure = Block; Treatment structure = cultivar x shelter). Data were considered significantly different at 5% probability level. All the analyses were done using Genstat 13 (VSN International Ltd, UK).

3. RESULTS

3.1 Hand Harvest

All samples were contaminated with DON and the level of concentration was higher than the internationally accepted level of 1.25 µg/g for bread wheat for human consumption in all genotypes. The genotypes showed varying degrees of DON concentration and this only approached significance (P=0.052). There was a significant (P=0.01) genotype x shelter effect (Table 2). Shelter significantly reduced DON concentration by 48% in Soissons and increased DON concentration by 40% in Maris Widgeon 2. The crude protein content showed no significant (P=0.08) shelter x genotype effect but the genotype effect was significant (P<0.001) (Table 3). Maris Widgeon genotypes had the highest % crude protein. Sheltering during grain filling increased HFN and the shelter x genotype effect only approached significance (P=0.08) in the grains. High HFN was observed in Oakley, SR 53, Maris Widgeon genotypes (Table 3) regardless of the level of FHB infection. Shelter increased SDS-sedimentation volume and the difference only approached significance (P=0.06) (Table 3). Genotypes varied significantly (P<0.001) i.e. the highest of the Maris Widgeon lines contrasted with the low values of Oakley, but there was no significant (P>0.05) shelter x genotype interaction.

3.2 Combine Harvest

There was no clear pattern to the profile of DON concentrations in the grains. For example, in hand harvested grains, Soissons had reduced DON when compared with higher DON levels in combine harvested grains in shelter plots (Table 2). In crude protein, genotype x shelter (P=0.005) effect arose mostly because shelter reduced the crude protein of Oakley (Table 4). HFN showed significant (P=0.034) shelter x genotype effect where shelter only increased HFN in Maris Widgeon genotypes, but no significant (P=0.3) shelter x genotype interaction was observed in SDS.

Table 2. Effects of withholding water during grain filling on DON concentration (mg/kg) of winter wheat genotypes grown on a plot previously cropped with maize

Genotype	Dwarfing allele	Photoperiod sensitivity allele	Hand harvest		Combine harvest	
			NS	S	NS	S
Oakley	Rht-B1b	Ppd-D1b	3.77	3.47	2.54	2.47
Soissons	Rht-B1b	Ppd-D1a	3.23	1.67	3.07	2.69
SR 53	Rht-D1b	Ppd-D1a	3.04	2.39	2.03	2.17
SR 94	Rht-D1b	Ppd-D1b	2.74	3.14	2.32	1.99
M. Wid. Rht1	Rht-B1b	Ppd-D1b	2.53	3.18	1.83	2.30
M. Wid. Rht2	Rht-D1b	Ppd-D1b	2.49	3.49	3.28	2.49
P (Genotype)			*0.05*		*0.23*	
P (Shelter)			*0.71*		*0.50*	
P (GxS)			*0.01*		*0.77*	
SED (Genotype)			*0.35*		*0.44*	
SED (Shelter)			*0.20*		*0.26*	
SED (GxS)			*0.50*		*0.63*	

NS = non-shelter and S= shelter

Table 3. Effect of withholding water during grain filling on % crude protein, Hagberg falling number (HFN) and Sedimentation volume (SDS) of winter wheat genotypes grown on a plot previously cropped with maize. Assessment was done on hand harvested grains

Genotype	Dwarfing allele	Photoperiod sensitivity allele	Crude protein (%)		HFN		SDS	
			NS	S	NS	S	NS	S
Oakley	Rht-B1b	Ppd-D1b	10.5	10.1	229	286	34.0	34.0
Soissons	Rht-B1b	Ppd-D1a	11.3	10.9	295	295	58.2	69.8
SR 53	Rht-D1b	Ppd-D1a	11.0	11.4	268	317	47.5	54.5
SR 94	Rht-D1b	Ppd-D1b	11.5	11.6	234	242	44.2	49.0
M. Wid. Rht1	Rht-B1b	Ppd-D1b	12.2	12.4	236	288	64.5	74.2
M. Wid. Rht2	Rht-D1b	Ppd-D1b	12.3	12.7	220	308	64.5	57.5
P (Genotype)			*<0.001*		*0.007*		*<0.001*	
P (Shelter)			*0.85*		*<0.001*		*0.06*	
P (GxS)			*0.08*		*0.08*		*0.19*	
SED (Genotype)			*0.17*		*15.8*		*3.86*	
SED (Shelter)			*0.1*		*9.13*		*2.23*	
SED (GxS)			*0.25*		*22.4*		*5.46*	

NNS = non-shelter and S= shelter

Table 4. Effect of withholding water during grain filling on % crude protein, Hagberg falling number (HFN) and Sedimentation volume (SDS) of winter wheat genotypes grown on a plot previously cropped with maize. Assessment was done on combine harvested grains

Genotype	Dwarfing allele	Photoperiod sensitivity allele	Crude protein (%)		HFN		SDS	
			NS	S	NS	S	NS	S
Oakley	Rht-B1b	Ppd-D1b	11.6	10.7	253	260	40.3	45.3
Soissons	Rht-B1b	Ppd-D1a	12.2	11.8	291	298	60.5	64.5
SR 53	Rht-D1b	Ppd-D1a	12.0	12.0	308	322	55.3	53.8
SR 94	Rht-D1b	Ppd-D1b	12.6	12.4	234	215	47.3	47.0
M. Wid. Rht1	Rht-B1b	Ppd-D1b	13.2	13.5	252	290	74.3	77.5
M. Wid. Rht2	Rht-D1b	Ppd-D1b	13.4	13.8	234	298	72.5	73.3
P (Genotype)			*<0.001*		*<0.001*		*<0.001*	
P (Shelter)			*0.18*		*0.014*		*0.053*	
P (GxS)			*0.005*		*0.034*		*0.3*	
SED (Genotype)			*0.16*		*12.3*		*1.62*	
SED (Shelter)			*0.09*		*7.09*		*0.94*	
SED (GxS)			*0.22*		*17.0*		*2.3*	

NS = non-shelter and S = shelter

4. DISCUSSION

Mimicking a climate change effect using shelters which reduced water and possibly increased temperature during grain filling was employed in the study to evaluate its impact on Fusarium head blight infection in the field. Results of the experiment showed varying effects of withholding water during grain filling on *Fusarium* infection and some grain quality parameters among wheat cultivars. Understanding the basis of these effects is not straightforward considering the unpredictable weather conditions. The mean relative humidity of 88% and temperature of 17.6 ^{o} C for shelter plots and 15.1 ^{o} C for non-shelter plots (data not shown) during grain-filling contrasted the expected hot summer predicted under climate change. Shelter would have increased water deficit and [1] found increased DON concentration in maize under conditions of long-term water deficit. A decrease in DON levels in wheat grains subjected to extended irrigation from anthesis to harvest compared to grain which had some form of water stress has been observed by [12] and the results as presented here are not in sharp contrast to the report of the authors above as the main effect of shelter only reduced visual symptoms (data not shown) but had no effect on DON contamination of the grains. Reduction in rain splashes which aid the dispersal of fungal

inoculum to wheat spike might partly explain the reason for the reduced effect of shelter on visual symptoms as [20] had associated the dispersal of *Fusarium* spores with rainfall events. Increasing early biomass [21] and shorter grain filling period occasioned by the presence Ppd-D1a may have aided in the reduction of DON concentrations in Soissons possibly by shortening the time for fungal growth and toxin formation [5]. Increased early biomass aid the breakdown of DON by plant enzymes [22]. In limited moisture, Ppd-D1a may reduce the effect of water deficit at the most critical stage of development in the host tissue thereby decreasing spike colonisation and DON accumulation by the pathogen. It could also be that higher grains per spike associated with Soissons aided in the reduced DON concentration. [23] advocated the use of cultivars with high tillers in breeding programs to develop cultivars with high yield and low DON. Water and temperature stress during grain filling is more beneficial to HFN [24] while [17] observed that HFN is influenced more by the environment than by FHB infection. A strong genotype x environment interaction on HFN has been observed such that warm environment and restricted water availability reduce α-amylase activity and increase HFN under no *Fusarium* infection [25]. Under FHB infection, there might have been an increased degradation of starch by the fungus under stress which counterbalanced the activities of α-amylase [26] and effectively improved HFN genotypes with higher HFN.

It is a general view that negative trend exists between DON concentration and specific parameters in wheat quality such as protein concentration, HFN and SDS. Grain protein concentration reduction attributed to Rht alleles has been observed regardless of whether the grain yield was increased [27] or reduced [28]. Many authors have proposed that protein concentration can increase, decrease or be unaffected in *Fusarium* infected kernels [17,29]. An increase in protein content after *Fusarium* infection has been reported by [29], while [17] reported a slight decrease in protein content. It could be deduced from the current study that the significant effects of genotypes on grain quality parameters were mainly due the effects by the individual genotypes and not the associated alleles per se. Near isogenic lines of Maris Widgeon still maintained their good baking quality in both environments and across harvests regardless of the effects the associated Rht allele had on FHB rating. This agrees with the findings of [18] that wheat grain quality parameters especially protein content and baking qualities are mostly not influenced by FHB infection. The authors, therefore, opined that good baking quality results can still be obtained even when grains are heavily contaminated with *Fusarium*. Similarly, FHB did not noticeably influence the protein concentration and this coincided with other authors [17, 30] where slight increase in protein was observed. These authors attributed this to the loss of water in grains affected by fungal infection and/or the fungal protein detected as wheat protein.

5. CONCLUSION

The current study therefore concludes that: no evidence that withholding water using shelter which could increase the mean temperature during grain filling will impact negatively on DON concentration. Early flowering genotypes should be harvested shortly after grain filling in order to reduce DON contamination. Data on the level of DON contamination cannot be deduced from results of HFN, SDS volume or protein content. In other words, these parameters might not be influenced even when wheat kernels are heavily infected by *Fusarium*. The different effects of the genotypes to FHB infection cannot be linked entirely to the associated alleles.

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COMPETING INTERESTS

Author has declared that no conflicting interests exist.

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