



Genotype and time of inoculation effects on DON per grain and grain weight of winter wheat under two environmental conditions

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ABSTRACT

Experiment was conducted using near isogenic lines of Mercia background in a controlled environment to evaluate the mean effect of timing of inoculation and subsequent increase in temperature on deoxynivalenol (DON) concentration, amount of DON per grain and grain weight. The experiment was completely randomised consisting of three genotypes differing in semi-dwarfing alleles; Mercia 0 {Rht-B1a + Rht-D1a (wild type)}, Mercia 1 (Rht-B1b) and Mercia 2 (Rht-D1b) and four inoculation timings. The experiment was a complete factorial combination with four randomised replicates. Data showed that genotype differed in DON concentration ($P<0.001$), DON per grain ($P=0.006$) and mean grain weight ($P=0.001$) while time of inoculation influenced mean grain weight ($P<0.001$) and DON Concentration ($P<0.001$) but not Don per grain ($P=0.23$). Temperature influenced mean grain weight ($P=0.002$) with high temperature adversely affecting the size of the wheat grains.

Indexing terms/Keywords:

DON; dwarfing alleles; Fusarium; genotype; Mercia, timing.

Academic Discipline and Sub-Discipline

Crop Science; Plant disease

Subject Classification

Plant pathology

Type (Method/Approach)

Research article

INTRODUCTION

Two major dwarfing alleles Rht-B1b and Rht-D1b formerly known as Rht1 and Rht2 respectively, derived from Norin 10 have been used in over half the World's wheat crop (Miedaner and Voss, 2008). In the UK, the majority of recommended wheat cultivars contain Rht-D1b after its first introduction in 1974 (Gosman *et al.*, 2007). Short cultivars, the majority of which carry the semi-dwarfing allele Rht-D1b (Rht2), are preferred because of higher achievable grain yields and lower risk of lodging in fertile and humid conditions (Voss *et al.*, 2008; Gooding, 2009). According to Youssefian *et al.* (1992), the strong competition for resources by genotypes linked with Rht alleles due to faster growth rate either delays floret abortion and/or reduces the rate at which florets die resulting in more competent florets per spikelets at anthesis. These alleles confer short plants with stiff straw that allows for the utilization of more intensive agronomic measures, such as high doses of nitrogen, pesticides and irrigation (Miedaner and Voss, 2008), leading to increased spikelet fertility, higher grains per spike and grain yield depending on genetic background and environment (Flintham *et al.*, 1997; Worland *et al.*, 2001). Borner *et al.*, (1993) found that increased grain number observed in GA insensitive semi-dwarfing alleles counterbalances the reduced grain size. However, wheat cultivars carrying Rht-D1b have been linked to higher susceptibility to Fusarium head blight (FHB) possibly due to the shortened distance from the spike to infected crop debris (Mesterházy, 1995). The resistance of wheat to FHB is a complex phenomenon due to different factors that are involved in the infection (Stack, 2003). Of most importance in susceptible genotypes is the likelihood of mycotoxin production of which deoxynivalenol (DON) is of most concern and in highly resistant genotypes resistance is the major factor in suppressing disease development and DON accumulation. Authors have observed low disease symptoms and DON production for inoculation performed between spike emergence and the start of anthesis. Both disease severity and DON content sharply decreased for inoculations performed after mid-anthesis. With high temperature stress predicted to be important in the UK as climate changes, maximum daily temperatures in major wheat-growing areas of UK could reach between 28°C and 30°C during flowering (Lukac *et al.*, 2012). Exposure to such temperatures which are optimum for FHB pathogens and which also favour economic viability of grain maize over a larger area in the UK (West *et al.*, 2012); FHB infection could be more severe. This risk combined with the significant reductions in grain weight and grain yield loss could have direct implications in the resultant level of mycotoxin in the grains. This study was therefore aimed at determining whether time of Fusarium inoculation, temperature and wheat genotypes mean effect could influence the amount toxin in each grain.



MATERIALS AND METHODS

Three near isogenic lines (NIL) namely: Mercia 0 (Rht-B1a + Rht-D1a) wild type, Mercia 1 (Rht-B1b); and Mercia 2 (Rht-D1b) used in the experiment was supplied by John Innes Centre, Norwich, UK and sown on 13th December, 2011. The experimental design was a complete factorial combination of 3 x 5 x 2 x 4 {3 genotypes, 5 inoculation treatments (inoculation at GL+0, GL+4, GL+8, GL+10 and sterile distilled water (SDW), 2 temperature regimes and 4 randomised replicates}. For inoculation, the main stems were sprayed with 1ml of 1×10^5 /ml spore suspension per spike using a hand sprayer. The corresponding control plants were sprayed with sterile distilled water. After inoculation, both the inoculated and controls plants were enclosed for 24 hours using clear polythene bags to increase humidity and promote disease development. The plants were watered and left overnight in the glasshouse at a day/night temperature of 20/12°C and 84 - 99% relative humidity. At GL+9 and GL+11 for inoculation at GL+0, GL+4 and GL+8 and inoculation at GL+10 respectively, pots were randomly placed in the growth cabinets allowing the imposition of two temperatures, 23/15°C (cool) and 28/20°C (hot) under 16 hours light at 88 – 93% relative humidity for 14 days. Plants were then carefully taken outside until maturity. Harvesting was done when the plants were fully senesced and the grain below 15% moisture content. Spikes were hand thrashed carefully and the grains bulked into two replicates for the determination of DON and the calculation of amount of DON per grain. All statistical analyses were carried out using GenStat 13th Edition, VSN international Ltd, United Kingdom.

RESULTS

Genotype differed in DON concentration ($P < 0.001$), grain DON content ($P < 0.006$) and grain weight ($P = 0.001$), while time of inoculation ($P < 0.001$) only influenced the DON concentration (Table 1). Mercia 2 had higher DON accumulation than the other genotypes and grains from pots inoculated at GL+4 and GL+10 accumulated high levels of DON. No temperature effect was observed in both DON concentration and amount of DON per grain. Mean grain weight showed a significant main effect of time of inoculation ($P < 0.001$), genotype ($P < 0.001$), temperature ($P = 0.002$) (Table 1). On average, high temperature reduced grain weight by 9%, and Mercia 2 was the most susceptible having the least mean grain weight. Generally, control pots had significantly higher grain weights when compared to *Fusarium* inoculated pots in all genotypes.

DISCUSSION

There is clear evidence from the study that inoculation timing is very important in FHB infection and subsequent DON production. The length of time of fungal growth leading to early production of DON and/or adjustment to the environment before stress could affect the quality of wheat grains. The acceleration of rate of grain filling under high temperature as reported by Farooq *et al.* (2011) may have resulted in lighter grains observed at higher temperature. Van der Fels-Klerx *et al.* (2013) reported reduced DON concentration due to shorter duration of grain filling at high temperature and this may have contributed to the non significant effect found at both temperatures. Although, there is lack of agreement on the exact size of time of vulnerability to DON accumulation, some authors have identified that infection occurring mainly at anthesis (Del Ponte *et al.*, 2007, Cowger and Arrellano, 2010, Wegulo, 2012) would greatly impact on the grains. This makes visual assessment of disease on wheat spikes a poor estimator of the actual infection level (Edwards *et al.* 2001; Schaafsma *et al.* 2004). Another point could be that confusing natural spike maturation with desiccation at higher temperatures could lead to an overestimation of FHB severity in most cases (Siou *et al.*, 2013). FHB infection after grain development would be expected to have lesser impact on grain weight (Schwarz and Horsley 2006) but not on DON accumulation as relatively high levels of DON were found on grains with weights close to the control. Apparently Rht-B1a + Rht-D1a even in the presence of DON were able to fill kernels, it is speculated that the genotype may have had more deposits of DON in severely damaged grains and/or aborted the infected grains. Therefore, accurate prediction models for DON concentration especially under the changing climate should consider *Fusarium* infection at different stages of flowering. This confounds the already difficult task of timely prediction and control of FHB and DON contamination especially in the changing climate where higher temperatures during and after anthesis are predicted to negatively affect the quality of wheat grains (Semenov and Shewry, 2011).



Table 1: Main effect of genotype, temperature and time of inoculation on DON concentration ($\mu\text{g/g}$), grain DON content (μg) and mean grain weight (mg) at controlled environment.

Treatment	DON Conc. ($\mu\text{g/g}$)*	Grain DON (μg)*	Grain weight (mg)*
Time of inoculation			
GL+0	1.38	0.04	29.0
GL+4	1.71	0.042	24.8
GL+8	1.59	0.042	27.0
GL+10	1.62	0.049	30.3
Control	-	-	35.8
SED	0.057	0.002	1.34
P value	<0.001	0.23	<0.001
Cultivar			
Mercia 0	1.56	0.047	31.6
Mercia 1	1.36	0.037	30.3
Mercia 2	1.81	0.043	26.2
SED	0.049	0.002	1.04
P value	<0.001	0.006	0.001
Temperature			
23/15°C	1.55	0.044	30.7
28/20°C	1.60	0.042	28.0
SED	0.04	0.002	0.85
P value	0.18	0.23	0.002

*Data are mean of four replicate spikes. – Not applicable {Data from control were negligible (>2%)}

CONCLUSIONS

There was evidence from the study that time of inoculation and not temperature could affect DON concentration in the infected kernels. Grain weight of the winter wheat genotypes were adversely affected by increase in temperature thus a concern in the changing climate. *Fusarium* infection occurring at different growth stages and subsequent increase in temperature during grain filling could influence the quality of harvested wheat grains.

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