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Time of Fusarium inoculation and post-anthesis temperature stress affect FHB severity and DON concentration in winter wheat

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ABSTRACT

Two pot experiments were conducted using elite lines and near isogenic lines (NILs) of winter wheat in a controlled environment to evaluate the effect of time of inoculation and subsequent increase in temperature during grain-filling on Fusarium head blight (FHB) and deoxynivalenol (DON) on the infected wheat grains. The experiments were a complete factorial combination with randomised replicates. *Fusarium graminearum* was used to spray inoculate wheat spikes at glume loose (GL). GL was established when the lower glume could be opened with a thumbnail. Fusarium inoculation was done at glume loose (GL+0), 4 and 8 days after (GL+4 and GL+8), respectively. Pots transferred to controlled environment cabinets set at 23/15^{o}C or 28/20^{o}C. Results reveal that FHB severity showed a significant ($P<0.05$) cultivar and temperature interaction in both elite and near isogenic lines. High temperature increased FHB severity by 35% in the NILs. DON concentration showed cultivar sensitive in both sets of cultivars. Inoculation at GL+4 which corresponded with the mid-anthesis gave the highest FHB severity and DON concentration in NILs, while the elite cultivars showed cultivar sensitive to DON accumulation regardless of the time of Fusarium inoculation.

Indexing terms/Keywords

Near Isogenic Lines; Elite Cultivars; Glume Loose; *Fusarium Graminearum*; Deoxynivalenol; Fusarium Head Blight;

Academic Discipline and Sub-Discipline

Agriculture

Subject Classification

Plant pathology

Type (Method/Approach)

Screen house/ pot experiment

INTRODUCTION

Fusarium head blight (FHB), which is caused by some species of *Fusarium* of which *F. graminearum* and *F. culmorum* are of particularly interest in the UK is regarded as one of the most serious fungal disease in wheat worldwide (Xu and Nicholson, 2009). This disease has received significant attention because of its effect on yield and a range of grain quality criteria (Brennan *et al*., 2005; Brennan *et al*., 2007 and Yoshida *et al*., 2007). In addition to loss in yield and grain quality, the contamination of grains with mycotoxin such as deoxynivalenol (DON) is of primary importance in the safety of wheat products for both humans and livestock (Voss *et al*., 2008). Two key periods are important for FHB infection; (i) production and dispersal of sexual ascospores before and up to anthesis and (ii) spike infection during anthesis, and these are favoured by high temperature and high relative humidity (Hooker *et al*., 2002). Mentewab *et al*. (2000) observed that temperatures above 25^{o}C and moist periods of more than 24 hours increase infection by *F. graminearum*. However, optimum temperature for mycotoxin production tends to be specific to the host plant and the strain of pathogen as suggested by some authors (Brennan *et al*., 2005; Cowger *et al*., 2009). Xu *et al*. (2007) also observed that FHB symptoms and mycotoxin concentrations increased with increasing duration of wetness (6 - 48 h) and temperature (10 - 30^{o}C). *Fusarium* susceptibility in most cultivars is higher during anthesis, has been linked to either the initial infection commonly occurring via extended anthers (Yoshida *et al*., 2007) or on dead anthers after anthesis (Pritsch *et al*., 2000). Wegulo (2012) observed that within seven days of anthesis, wheat spikes were highly infected when drops of inoculum were placed on anthers rather that glumes. Disease transmission through the release of spores from infected debris under field conditions could occur any time before and after flowering, extending the receptive period of plants to *Fusarium* infection (Siou *et al*., 2013). This explains the higher DON content often found in wheat spikes with relatively few visible symptoms (Hallen-Adam, 2011). Cowger *et al*. (2009) also reported that under favourable environmental conditions, wheat spikes could be infected after flowering resulting in healthy-appearing kernels containing toxins. Cowger and Arrellano (2013) explored the impact of late infection on disease development and toxin production when inoculation was done from 0 to 20 days after mid-anthesis with four *F. graminearum* isolates. Their result showed that the period of maximum reception was 10-14 days after anthesis, while the occurrence of rain after anthesis extended the level of *Fusarium* susceptibility and increased the proportion of low symptoms and grains containing high DON. These have resulted to lack of agreement about the precise length of the phenological window of vulnerability to infection and DON accumulation in wheat grains (Del Ponte *et al*., 2007).

Furthermore, it has been noted that wheat sensitivity to high temperature stress is cultivar dependent (Farooq *et al*., 2011). Castro *et al*. (2007) observed greater reduction in thousand kernel weight when heat stress was applied early in

grain-filling and this was attributed to lower numbers of endosperm cells (Nicolas *et al*., 1985), while at later phases of grain filling a decrease in starch synthesis could be due to limited supplies of grain assimilates (Blum, 1998).

With climate change scenarios predicting an increase in cropping frequency with grain maize (Van der Fels-Klerx *et al*., 2013), the frequency of unusually high temperature episodes (Chakraborty and Newton, 2011), which could result in the problem of poor grain set which is negatively related with DON concentration (Bechtel *et al*., 1985), Snijders and Perkowski, 1990; Dowell *et al*., 1999 and Del Ponte *et al*., 2007), interaction of these factors is central to understanding climate change impacts on the quality and safety of wheat products. Although few studies have examined the influence of timing of infection on FHB disease and levels of DON accumulation (Yoshida *et al*., 2007; Yoshida and Nakajima, 2010), reports under climate change scenarios is scanty. For the development of highly effective strategies for controlling FHB, particularly for individual grain toxin reduction in infected wheat grains, it is important to understand the resultant effect of sudden increases in temperature when infection occurs at different stages during flowering. Culler *et al*., (2007) had advocated studies aimed at investigating the role of environment on DON accumulation following infection of wheat spikes at different growth stages in a range of environments. However, lack of knowledge on infection timing has also led to the problems associated with the variable efficacy of fungicide sprays (Parry *et al*., 1995).

The objective of this study was to therefore, to evaluate cultivar responses to the timing of Fusarium inoculation on wheat spikes during flowering and subsequent increase in temperature during grain filling on the severity of FHB infection and DON concentration of some UK wheat cultivars.

MATERIALS AND METHODS

Two pot experiments were conducted at the Plant Environment Laboratory (PEL) of the University of Reading, UK (51^{o} 27' N latitude, 00^{o} 56' W longitude) to determine the effect of timing of Fusarium inoculation during flowering and subsequent increase in temperature on FHB severity and DON concentration.

Crop husbandry

Experiment 1

Eight commercial UK winter wheat cultivars namely; Alchemy (Rht-D1b), Claire (Rht-D1b), Gallant (Rht-D1b), Kingdom (Rht-D1b), Oakley (Rht-B1b), Soissons (Rht-B1b) Solstice (Rht-D1b) and Xi19 (Rht-D1b) were used in the experiment. These cultivars which are in Home Grown Cereals Authority (HGCA) recommended list are high-yielding. The experimental design was a complete factorial combination of 6 x 8 x 2 x 3 consisting of: six inoculation times (inoculation with fungal spores and corresponding inoculation with sterile distilled water; eight cultivars; two temperature regimes (23/15^{o}C and 28/20^{o}C) and three randomised replicates.

Experiment 2

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, United Kingdom. The experimental design was a complete factorial combination of 4 x 3 x 2 x 4 {4 inoculation treatments (inoculation at GL+0, GL+4 and GL+8, and sterile distilled water (SDW), 3 genotypes, 2 temperature regimes and 4 randomised replicates}.

In both experiments, seeds were grown in 12.5-cm-diameter pots filled with a 4: 4: 2: 1 mixture of steam-sterilized 6mm gravel, medium vermiculite, and 3-mm sharp sand and peat-based potting compost. To supplement plant nutrition, 2kg of Osmocote Pro 3-4 months (Scotts, UK) was added per cubic metre of planting mixture. Pots filled with planting medium were soaked overnight and five seeds of the different cultivars were sown on each pot at a depth of 2-2.5cm and then thinned to three per pot at the three leaf stage. Pots were then transferred outside and raised to a height of approximately 10cm on bricks to allow free water drainage. Pots were irrigated automatically through a drip irrigation system twice daily. At the start of booting {Growth Stage (GS); Zadoks *et al*., 1974}, first tillers were tagged so that they could be identified for spore inoculation. The plants were treated for powdery mildew 82 days after sowing with Flexity (300g/litre (25.2% w/w) metrafenone; BASF Plc, UK) at 0.5l/ha. Flowers were then monitored for progress in the growth from GS 40 for the first tillers (main stem) and glume loose was established when the lower glume could be opened with a thumbnail. Based on this fungal inoculation was carried out on each plant at glume loose (GL+0), 4 days and 8 days after (GL+4) and (GL+8) respectively, on main stems.

Pot inoculation

Pots were taken to a glasshouse and each main stem spray inoculated with 1 ml of 1 x 10^{5}/ml spore suspension using a hand sprayer. The spray inoculation was done at glume loose (GL+0), 4 and 8 days after, (GL+4) and (GL+8) respectively and control plants sprayed with sterile distilled water. After inoculation, both the inoculated and control 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^{o}C and 84 - 99% relative humidity. The next day pots transferred into the growth cabinets and were randomly placed allowing the imposition of two temperatures, 23/15^{o}C (cool) and 28/20^{o}C (hot) under 16 hours light at 88 – 93% relative humidity for 14 days. After 14 days of temperature treatment, the plants were taken out from the cabinets and FHB severity was assessed. Disease severity was measured as the percentage of infected spikelets within an ear. The plants were then carefully taken back outside until maturity. Harvesting was done when the plants were fully senesced and the grain below 15% moisture content. The spikes were hand threshed carefully to avoid the loss of infected and shrivelled kernels.

Deoxynivalenol analysis

Grains were milled to fine flour in the laboratory using a Laboratory Mill 31 and the flour stored at - 20^{o}C prior to DON extraction. Ground samples were weighed into a flask and distilled water was added and blended for 3 minutes. The solution was allowed to settle and the top layer of extract was filtered through No 1 filter paper. The filtrate was diluted at the ratio of 1:4 (1 ml of extract to 4 ml of distilled water). Although the amount of filtrate varied among samples, a minimum of 100 µL DON was required for DON analysis. DON concentration of the extract was analysed using Enzyme Linked Immunosorbent Assay (ELISA) DON kits (Romer Labs, Singapore Pte Ltd) according to the manufacturer's instructions. The DON range of quantification was between 0.25 – 5.0µg/g. The treatments were completely randomised in the micro titre plates. Absorbance was measured at 450nm and a differential filter of 630nm using a Multiskan Ascent plate reader. DON concentration was calculated by reference to a standard curve generated using the DON kit.

Statistical analysis

All statistical analyses were carried out using GenStat (GenStat® 13th Edition, VSN International Ltd., UK). An analysis of variance (ANOVA) was carried out to determine the effects of the treatments on FHB severity and DON concentration No data transformation was done as the data were normally distributed.

RESULTS

Cultivar x temperature effect

FHB severity

Harvested grains from the pots sprayed with sterile distilled water did not show any *Fusarium* infection. On average higher temperature significantly (P=0.01) increased FHB severity by 8%. Oakley and Solstice had the highest FHB severity values (Fig.1) contrasting with Soissons which had the least FHB severity value. The temperature x cultivar effect approached significance (P=0.075) because the main temperature effect was not apparent on Alchemy, Kingdom and Solstice. In NIL of Mercia, the temperature x cultivar effect was significant (P<0.05) with high temperature increasing FHB severity in both Mercia 0 and Mercia 1 by approximately 35% but had no effect was observed in Mercia 2 (Fig. 2).

DON concentration

Relative increase in DON concentration at high temperature was observed in Solstice and Xi19 while low temperature tends to have increased the DON concentration in Claire and Oakley (Fig. 3). No significant temperature effect was observed in Alchemy, Gallant and Kingdom. In NILs, as was observed in the FHB severity, high temperature increased DON concentration in Mercia 0 by 14% (Fig. 4). On average, Mercia 2 had the highest DON concentration at both temperatures when compared with the other cultivars.

Cultivar x Time of inoculation effect

FHB severity

Inoculation at GL+4 gave the highest FHB severity on average and the inoculation time x cultivar interaction was significant (P<0.001) partly because FHB infection in Claire, Gallant, Kingdom, Oakley, Solstice and Xi19 were significantly higher at GL+4, while that of Alchemy was significantly higher at GL+0 (Fig. 5). Soissons showed no effect of time of inoculation. In NILs of Mercia, only Mercia 2 showed effect of time of inoculation. Inoculation at GL+4 had the highest FHB severity value when compared with the other inoculation times (Fig. 6). No effect of time of *Fusarium* inoculation was observed in Mercia 0.

DON concentration

In *Fusarium* infected grains, there was cultivar sensitivity to DON accumulation regardless of the time of *Fusarium* inoculation. The individual cultivars behaved differently to DON accumulation. Only Gallant and Kingdom had the highest DON concentration at GL+4 reflecting the effect of the fungus on FHB severity (Fig. 7). However, cultivar sensitivity to later inoculation (GL+8) was observed in Oakley, Solstice and Xi19 with increased DON concentration. In NILs, higher DON concentration was observed in Mercia 0 and Mercia 2 at inoculation at GL+4, but Mercia 1 showed no effect of time of inoculation (Fig. 8).

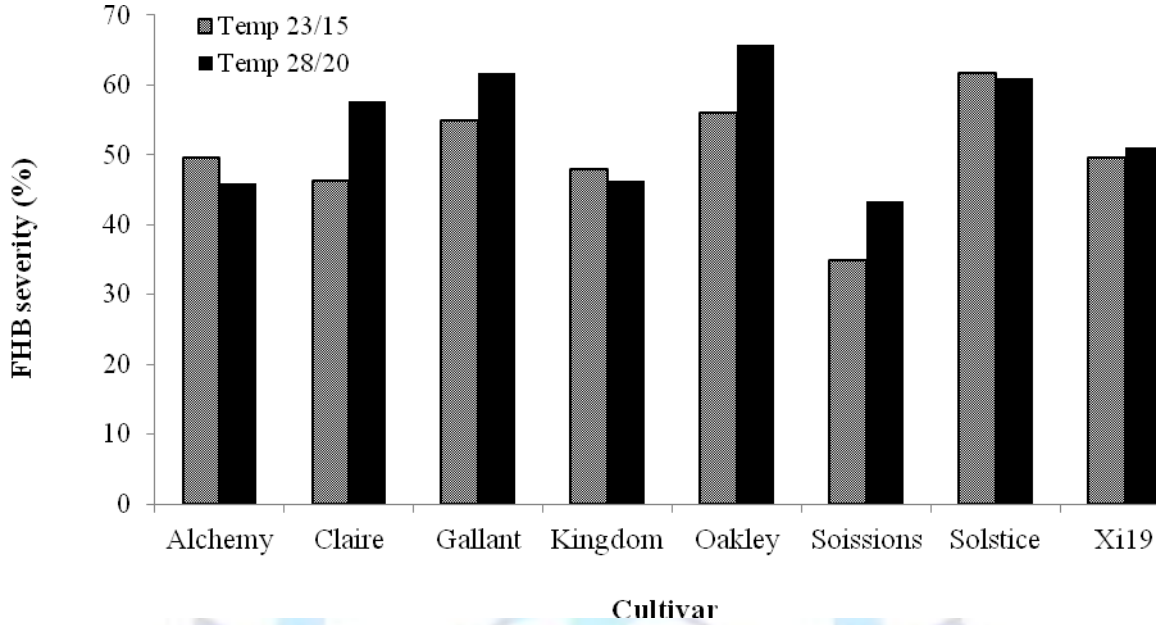

Figure 1: Effect of temperature on FHB severity of wheat cultivars: (a) Alchemy (b) Claire (c) Gallant (d) Kingdom (e) Oakley (f) Soissons (g) Solstice and (h) Xi19 inoculated with *F. graminearum* at different stages. SED = 4.19.

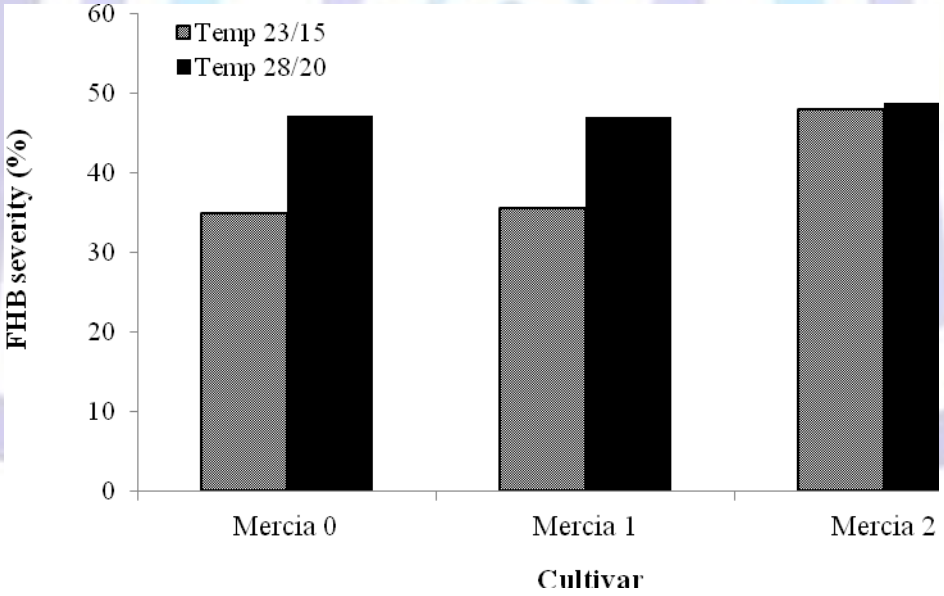

Figure 1: Effect of temperature on FHB severity of wheat cultivars: (a) Mercia 0 (b) Mercia 1 (c) Mercia 2 inoculated with *F. graminearum* at different stages. SED = 3.91.

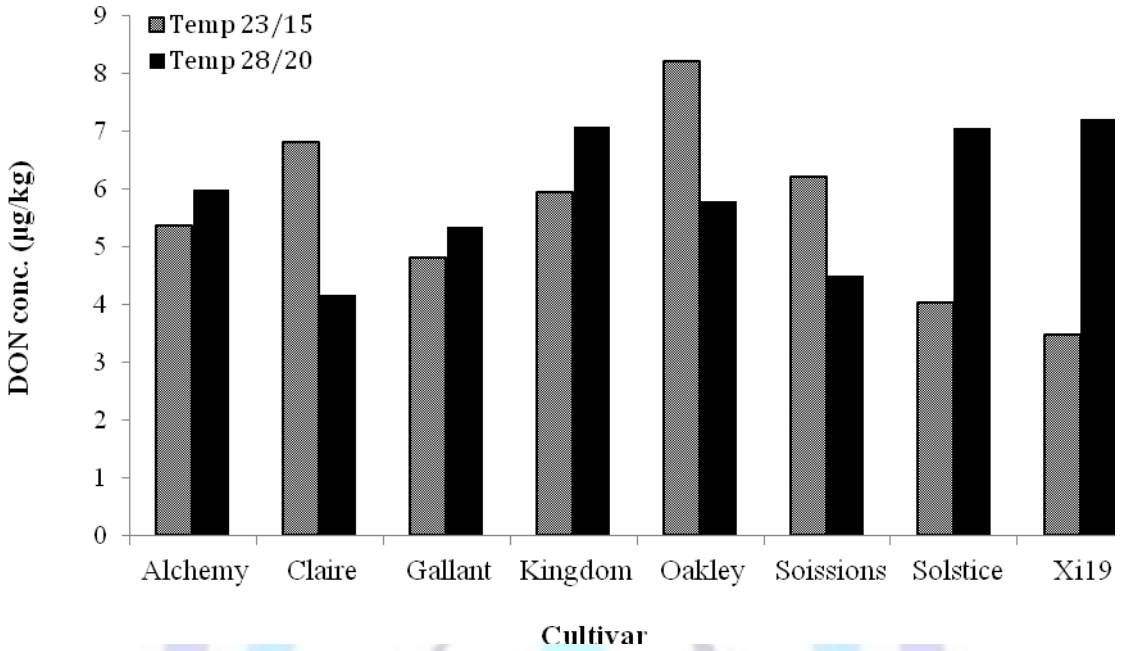

Figure 3: Effect of temperature on DON concentration of wheat cultivars: (a) Alchemy (b) Claire (c) Gallant (d) Kingdom (e) Oakley (f) Soissons (g) Solstice and (h) Xi19 inoculated with *F. graminearum* at different stages. SED = 0.78.

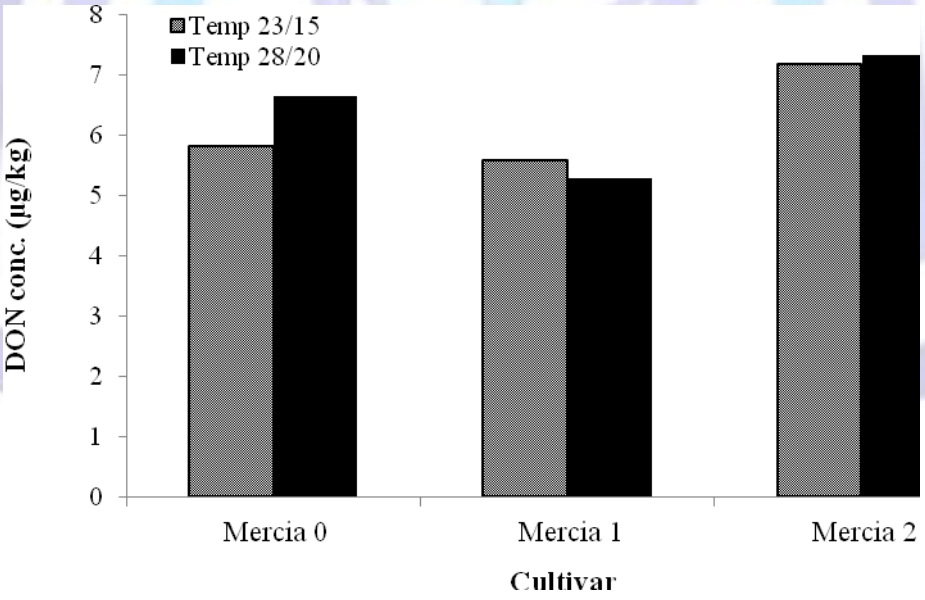

Figure 4: Effect of temperature on DON concentration of wheat cultivars: (a) Mercia 0 (b) Mercia 1 (c) Mercia 2 inoculated with *F. graminearum* at different stages. SED = 0.28.

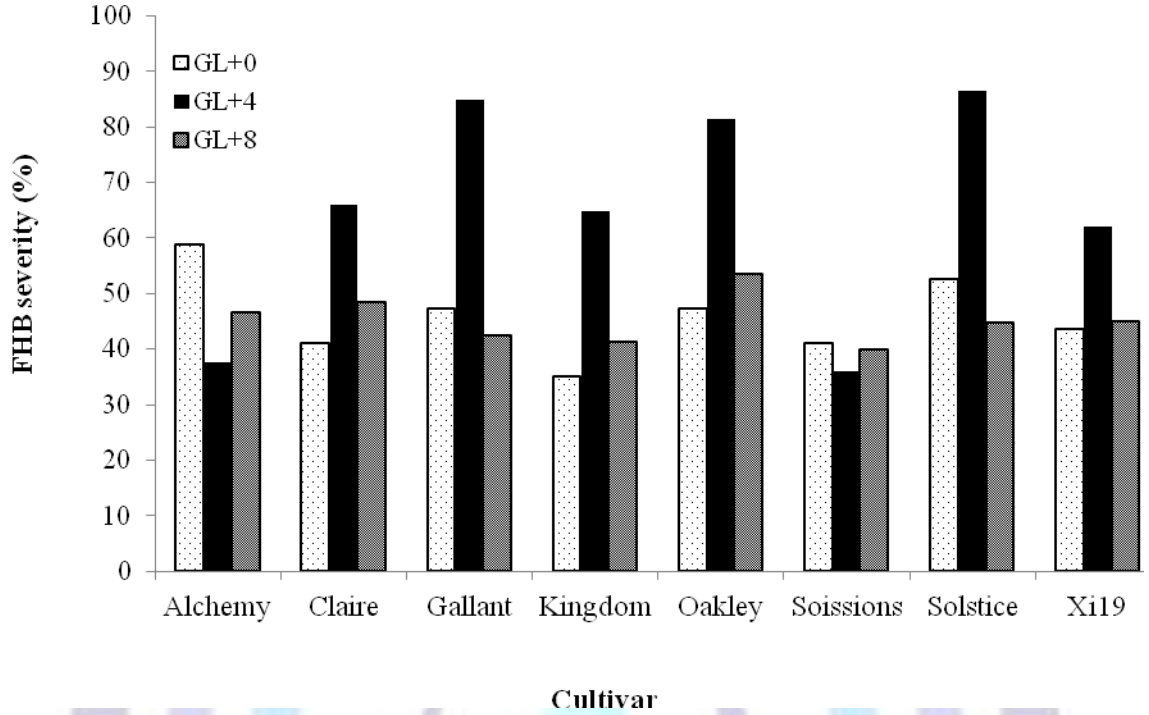

Figure 5: Effect of time of inoculation on FHB severity of wheat cultivars: (a) Alchemy (b) Claire (c) Gallant (d) Kingdom (e) Oakley (f) Soissons (g) Solstice and (h) Xi19 inoculated with *F. graminearum* and maintained at two temperatures. SED = 5.13.

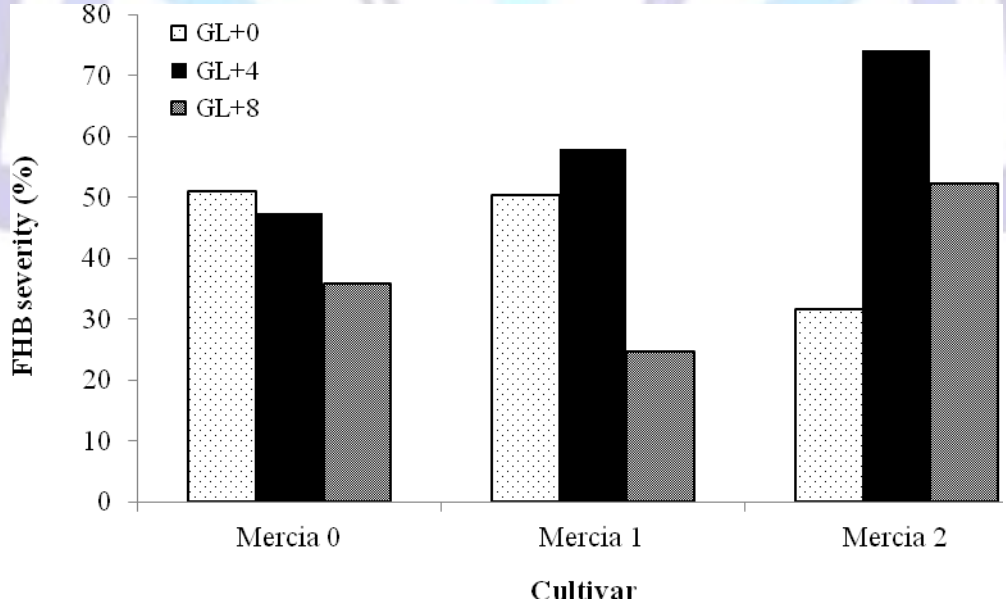

Figure 6: Effect of time of inoculation on FHB severity of wheat cultivars: (a) Mercia 0 (b) Mercia 1 (c) Mercia 2 inoculated with *F. graminearum* and maintained at two temperatures. SED = 5.53.

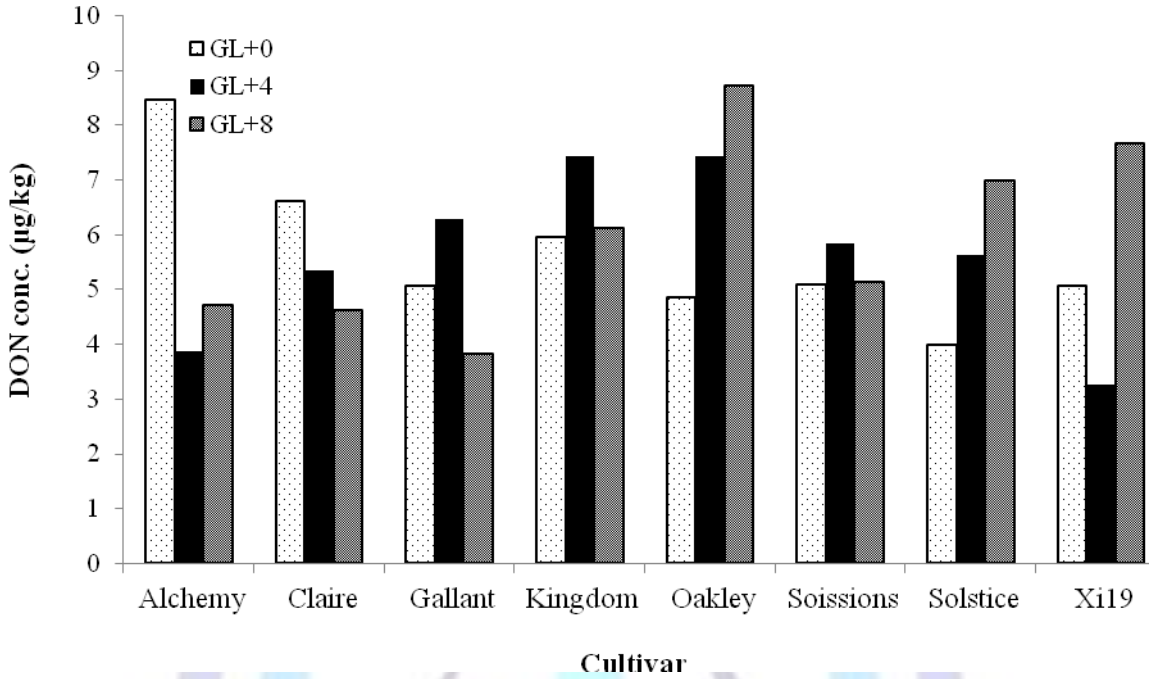

Figure 7: Effect of time of inoculation on DON concentration of wheat cultivars: (a) Alchemy (b) Claire (c) Gallant (d) Kingdom (e) Oakley (f) Soissons (g) Solstice and (h) Xi19 inoculated with *F. graminearum* and maintained at two temperatures. SED = 0.95.

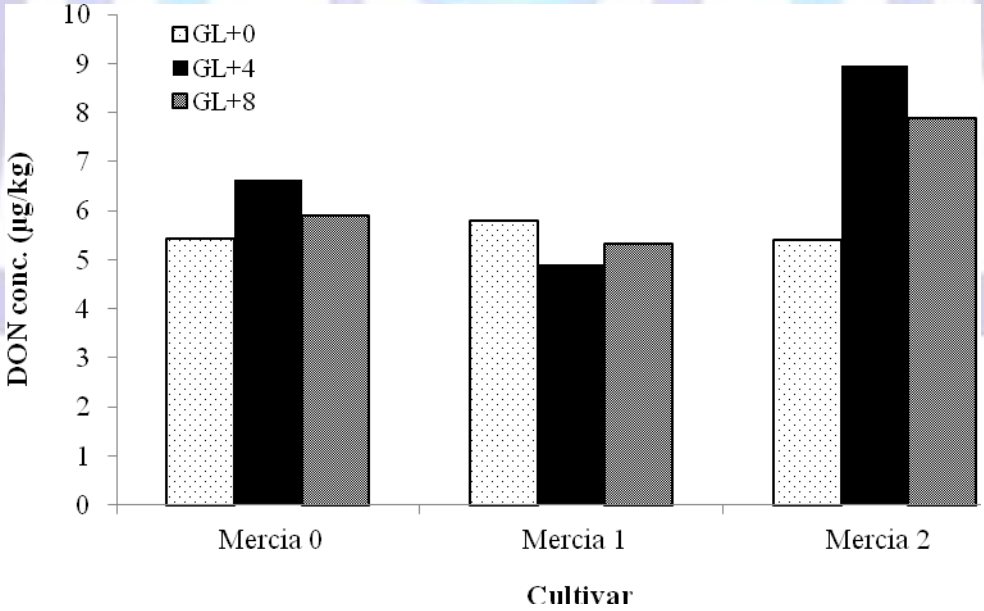

Figure 8: Effect of time of inoculation on DON concentration of wheat cultivars: (a) Mercia 0 (b) Mercia 1 (c) Mercia 2inoculated with *F. graminearum* and maintained at two temperatures. SED= 0.39.

DISCUSSION

Fusarium graminearum grows well at 20 - 30^{o}C (Brennan *et al*., 2003; Doohan *et al*., 2003; Brennan *et al*., 2005; Xu *et al*., 2007) with optimal temperature at 25 – 29^{o}C (Kang and Buchenauer, 2002; Xu, 2003). It would be expected that higher fungal activity would occur at the optimal temperatures which were super-optimal for the wheat. For some cultivars fungal development and/or visibility of infection was accelerated under higher temperatures with spikes becoming necrotic compared to low temperature due to either the aggressiveness of the fungus or the susceptibility of the host at high temperature. There may have also been a delay in the time taken for the symptoms to be expressed at the lower temperature (Brennan *et al*., 2005). Our results showed that in the Mercia background, Rht-D1b on average had higher FHB severity. This agrees with Gosman *et al*. (2007), Miedaner and Voss (2008) and Srinivasachary *et al*. (2009) that Rht-D1 is strongly linked with FHB susceptibility, although in this study, the level of susceptibility was strongly influenced by the time of inoculation. Nicholson *et al*. (2008) advocated same day inoculation of wheat NIL since the period of optimal susceptibility to FHB can significantly differ among cultivars. In the current study, in addition to the two temperature regimes, the Rht effects proved to be influenced by the inoculation timings. The sensitivity of the NIL of Mercia to the timing of inoculation was more in the wild type and Rht-B1b, both showing different sensitivity to temperature at the different inoculation timings unlike Mercia 2. The works of Del Ponte *et al*. (2007) which found different levels of FHB susceptibility on wheat spikes inoculated at different growth stages under controlled environment study further supports these results. In terms of DON accumulation as proposed by Bai and Shaner (2004), temperature might affect; (i) level of DON production by the fungus, (ii) the degradation of DON by plant enzymes during kernel development and (iii) the promotion or restriction of the movement of DON from other spike tissues into the kernels. Van der Fles-Klerx (2013) reported that DON concentration could be reduced with reduction in grain filling period possibly because of the shorter time for fungal growth and toxin formation. There was no definite pattern to DON accumulation by the different experimental cultivars under high temperature stress. DON was produced at any time during the flowering stages regardless of the level of severity of infection and this agrees with Cowger *et al*. (2009). This phenomenon has also been recognized by Siou *et al*., (2013) who inoculated wheat spikes before anthesis, at mid-anthesis and days after anthesis. The authors obtained the highest disease and toxin levels at inoculations around anthesis, although early and later infections led to detectable levels of toxin for the most aggressive isolates. Dill-Macky, (2003) and Del Ponte *et al*., (2007) reported that cultivars could develop visual symptoms with no evidence of grain damage at harvest. Although, there is lack of agreement on the exact size of time of vulnerability to DON accumulation, most authors have identified that infection occurs mainly at anthesis (Shaner, 2003, Del Ponte *et al*., 2007, Cowger and Arrellano, 2013; Wegulo, 2012). Responses of the main stem appear important for the sensitivity of genotypes to heat stress because of their final contribution to the grain yield. The different sensitivities shown by the cultivars to inoculation timings are an indication of how important infection timing could influence FHB susceptibility in wheat cultivars regardless of the linked semi-dwarfing alleles. GA-signalling in semi-dwarfs is known to be critical for the normal development and functioning of reproductive tissues (Mutasa-Gottgens and Hedden, 2009) and has also been linked to stress tolerance (Colebrook *et al*., 2014). Shaner (2003) and Wegulo (2012) also identified that infections during flowering and shortly after flowering are the most damaging. FHB disease from production of inoculum to infection of wheat heads could be influenced by temperature. Reports by Del Ponte *et al*. (2007); Yoshida *et al*. (2007) and Wegulo, (2012) that wheat growth stages are important in FHB susceptibility are consistent with the results obtained in this study. Our results clearly showed that Alchemy because of its particularity in anther maturity (results not shown) was more susceptible to FHB infection at GL+0 as reflected in both the severity of the disease and DON accumulation. In other words, flowering behaviour contributed a lot to influence the behavioural pattern of the cultivars to FHB infection. Nicholson *et al*., (2008) had advocated same day inoculation of wheat NIL since the period of optimal susceptibility to FHB can significantly differ among cultivars. Schroeder and Christensen (1963) earlier reported that wheat cultivars differ with growth stage at which they are most susceptible and this is in line with the findings of the current study. It could be possible that the longer duration of flower opening might have promoted the spread of the fungus and accounted for the high visual symptoms especially at GL+8 in some cultivars. Gilsinger *et al*., (2005) found that wheat genotypes with brief flower opening are at lower risk of infection due to reduced time and area for *Fusarium* spores to initiate infection. Although inoculations at GL+8 may have a lower impact on grain yield in the experiment, infected and DON contaminated healthy grains are likely to contribute to the final DON levels in mature grains (Del Ponte *et al*., 2003). For practical purposes, cultivar sensitivities to time of inoculation could be useful in estimating FHB infection and also a useful estimator of cultivar response to FHB disease.

CONCLUSION

The results of the current work therefore contribute to defining better inoculation times that make *F. graminearum* infection probable under plant stress conditions. An interesting aspect of this work shares the possible use of the results from the current findings in predicting FHB disease. The variation in the flowering habits in wheat cultivars influenced FHB susceptibility and cultivars were found to have varying levels of sensitivity to inoculation timing and temperature. Reduced DON at the most susceptible stage might not be associated with the linked Rht-B1b but cultivar specific. However, the use of glume loose in the current study gave a precise timing for fungal entry and thus a potential facilitator of disease development and inoculation at GL+4 was consistently the most vulnerable stage for FHB infection in most UK wheat cultivars used in the experiments.

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