

Original Research Article

Cultivar Response to within Spike Grain Number Reduction in *Fusarium* Infected Wheat under Climate Change Scenario

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Abstract	Keywords
<p>The effect of temperature immediately after anthesis on potential grain number within spikelets and florets of <i>Fusarium</i> infected wheat spike was investigated in controlled environment. Four wheat cultivars; two each of elite cultivars (Oakley and Soissons) and near isogenic lines (NIL) of Mercia background (Mercia 1 and Mercia 2) were grown in pots, inoculated with <i>Fusarium graminearum</i> spores during anthesis and incubated at two contrasting temperatures (23/15°C and 28/20°C) for 14 days. Wheat spikes were assessed for number of healthy or shrivelled grains in spikelet and floret positions after harvest. Data showed that elite cultivars differed in response to higher temperature at the spikelets positions where higher temperature reduced grains at the upper spikelets in Oakley and not in Soissons. Near isogenic lines showed similar pattern in grain reduction and with no significant temperature effect. Grains in floret positions were not affected by temperature in all cultivars. The result therefore reveals that reduction in the total number of grains as a result of reduced grains within spikelet positions of individual wheat spike may vary under heat stress condition depending on the choice of cultivar used.</p>	<p>Elite cultivars Florets <i>Fusarium graminearum</i> Near Isogenic Lines Spike Spikelets</p>

Introduction

Cereals are mostly susceptible to heat and drought stress during booting and anthesis (Barnabas et al., 2008). In wheat for instance, temperature above 30°C causes severe grain reduction (Wheeler et al., 1996; Ferris et al., 1998). The sensitivity of wheat to heat stress could be attributed to the prevalence of the semi-dwarfing alleles that supported the *Wheat Green Revolution*. By the 1990s, 80% of available

wheat cultivars contained a semi-dwarfing Rht allele and they are mostly either Rht-B1b or Rht-D1b (Worland et al., 1998). Although their widespread use, early researchers advocated that both Rht-B1b and Rht-D1b could confer increased sensitivity to drought and heat stress (Gale and Youssefian, 1985). Variation to high temperature stress tolerance during grain filling among wheat

cultivars has been reported (Wardlaw et al., 1989; Tahir and Nakata, 2005).

Temperatures of 25°C to 32°C are favourable for *Fusarium* head blight (Brennan et al., 2005; Xu et al., 2007) with optimal temperature at 25–29°C (Kang and Buchenauer, 2002; Xu, 2003). Presently, likely losses from diseases such as FHB in combination with a change in climate cannot be predicted due to a lack of understanding of the influence of climate change on plant disease (Chakraborty et al., 2002).

With high temperature stress predicted to be important in the world over, average maximum daily temperatures in major wheat-growing areas could reach between 28°C and 30°C (Lukac et al., 2002) and above 38°C (Langer and Olugbemi, 1970) in some areas during flowering. Exposure to such temperatures which are optimum for FHB pathogens could result in significant reductions in grain set, grain weight and grain yield loss (Snijders, 2004). The numbers of spikelets and florets which are vulnerable during these predicted periods of stress could have direct implications for the resultant level of mycotoxin in the grains.

With the predicted increase in summer temperature and the resultant effect on the flowering habits of most wheat cultivars, flowering was predicted to occur earlier in the season and FHB infections projected to be more severe (Van der Fels-Klerx et al., 2013).

Few attempts have been made to compare the effect of temperature during grain-filling on grain development on final grain yield of *Fusarium* infected wheat spikes but there is no detailed information on cultivar response and which stage is the most responsive, or whether floret or spikelet positions within spike differ in sensitivity. This study was therefore aimed at determining the effect of temperature (23/15°C and 28/20°C) applied after anthesis on grain set, to evaluate the cultivar {elite cultivars and near isogenic lines (pairs of lines that only differ in the genomic region of interest with other genes affecting the traits of interest remaining same in both lines)} response and differences in sensitivity to high temperature between spikelet and floret positions.

Materials and methods

Crop husbandry

A pot experiment was carried out at Plant Environment Laboratory (PEL), University of Reading, UK. Two elite cultivars (Oakley and Soissons) and near isogenic lines of Mercia background (Mercia 1 and Mercia 2) (Table 1) which differed in the linked semi-dwarfing allele (Rht) were used in a complete factorial experiment. Rht-B1b is linked with FHB resistance while Rht-D1b is linked with FHB susceptibility. The elite cultivars which are in Home-Grown Cereals Authority (HGCA) recommended list for 2012-2013 are high-yielding.

Table 1. Pedigree and the associated semi-dwarfing allele of the cultivars used in the experiment.

Cultivar	Pedigree	Semi-dwarfing allele
Oakley	(Aardvark ‘Sib’ × Robigus) × Access	Rht-B1b
Soissons	Jena × HN 35	Rht-B1b
Mercia 1	Near isogenic line	Rht-B1b
Mercia 2	Near isogenic line	Rht-D1b
Source: HGCA recommended list for 2012/2013.		

The plants 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 laid outside to a fenced off area and raised to a height of approximately

10cm on bricks to allow free drainage. Five seeds each of the four cultivars were sown on the pot on at a depth of 2-2.5cm and then thinned to three per pot at the three leaf stage. Pots were irrigated automatically through a drip irrigation system twice daily. Plants were treated with Flexity [300g/L (25.2% w/w) metrafenone (BASF Plc, UK) at 0.5L ha⁻¹] against powdery mildew at Growth Stage 39 (GS 39). At Growth Stage 40 (GS 40), the first

tillers were tagged so that they could be identified for spore inoculation and subsequent disease evaluation.

Spike inoculation

Flowers on the main stem were monitored for progress in growth, from GS 40 onward. Each spike of the main stem was spray inoculated with 1ml of 1×10^5 /ml spore suspension using a hand sprayer at the start of flowering, 4 and 8 days after. After inoculation, the plants were enclosed for 24 hours using clear polythene bags to increase humidity and promote disease development and transferred to a growth cabinet set at two temperatures, 23/15°C (cool) and 28/20°C (hot) at 88 – 93% relative humidity for 14 days.

Spike assessment

After 14 days of incubation, the plants were taken out from the cabinets and then taken back outside to mature. Harvesting was done when the plants were fully senesced and the grain below 15% moisture content and carefully threshed. The spikelets of an ear were numbered from the collar upwards, the lowest being '1' and the subsequent numbers alternating between sides such that one side of the ear was 'odd' and the other 'even'. Floret labelling followed the scheme of Kirby and Appleyard (1984), with the first floret from the lower glume labelled as 'A', subsequent florets up the spikelet alternated between sides such that floret 'B' and 'D' were on the same side. The spikes were scored for presence of grains (healthy and shrivelled) on the odd numbered spikelets only.

Data analysis

Data from individual cultivar were subjected to ANOVA using GenStat (GenStat® 13th Edition, (VSN International Ltd., UK) and means separated using the Least significant difference (LSD) at 5% probability level.

Results

Spikelets

The effect of temperature stress during grain filling was compared between elite wheat cultivars (Oakley and Soissons) and near isogenic lines of

Mercia background (Mercia 1 and Mercia 2) to ascertain the effect *Fusarium* infection will have on grain set within spikelets and florets. For elite wheat cultivars, cultivar difference was observed between Oakley and Soissons. Significant ($P=0.03$) temperature effect was observed in Oakley at the upper spikelets (17-23) where higher temperature reduces grain numbers (Fig. 1a), however, this effect was not observed at both lower (1-7) and middle spikelets (9-15). Soissons showed no temperature effect at all the spikelet positions, although higher temperature marginally reduced grains at the upper spikelets (Fig. 1b). The *Fusarium* infected wheat spikes of the near isogenic lines of Mercia background showed similar pattern in grain distribution across spikelets at both temperatures. Low temperature maintained higher grains in both cultivars, although the difference was not statistically significant ($P=0.05$) (Fig. 1c and d).

Florets

The effect of temperature across florets was similar for all the cultivars. In most cases, low temperature maintained relatively higher grains across florets, although these differences were not statistically significant ($p=0.05$) (Fig. 2) from the higher temperature. For NIL, the sensitivity of temperature could be most severe at florets 'a' and 'b' and could be assumed as the most critical stage for these cultivars (Fig. 2c and d). Soissons showed 8% grain reduction at lower temperature at the floret 'a' which contrasted with the other cultivars (Fig. 2b).

Discussion

A change in the climate is expected to have profound effect on our food production worldwide (Semenov, 2009 and Lobell et al., 2011). Gradual changes in mean environmental parameters, in addition to sudden extreme temperature events can potentially alter both the crop performance and interaction with plant pathogens (Brennan et al., 2005). Temperature has been showed to play important role in FHB disease from production of inoculum to infection of wheat spikes (Campbell and Lipps, 1998; Brennan et al., 2003); small temperature changes, consequently, may affect the severity of FHB disease. Semenov and Shewry (2011) also established that higher temperatures during anthesis could limit the grain number and grain size in wheat.

Farooq et al. (2011) in their work observed that rate of grain filling is accelerated during heat stress leading to reduction in the duration of grain filling. The number of grains per spike was reduced by the number of spikelets and also the viable florets. On average the spikelet number of the cultivars used in the experiment was about 20 and the floret numbers varied from two in the lower spikelets to four at the middle spikelets. The florets at the middle spikelets were the first to reach anthesis and the lower

spikelets the last and individual florets within a spike differed in the timing of anthesis and also on the rate of grain growth. Moreover, the temperature regimes employed in this study encouraged FHB infection in all cultivars as FHB rating at both temperatures was similar (data not shown). Generally, elite cultivars responded differently to temperature change in relation to the number of grains within spike when compared with the NIL which had a definite pattern.

Fig.1: Effect of temperature on number of grains within spikelet in (a) Oakley, (b) Soissons, (c) Mercia 1 and (d) Mercia 2 in *Fusarium graminearum* infected wheat spikes. Data are means of three inoculation times and scores taken from one side (odd) of the wheat spike. Errors bar indicate the LSD.

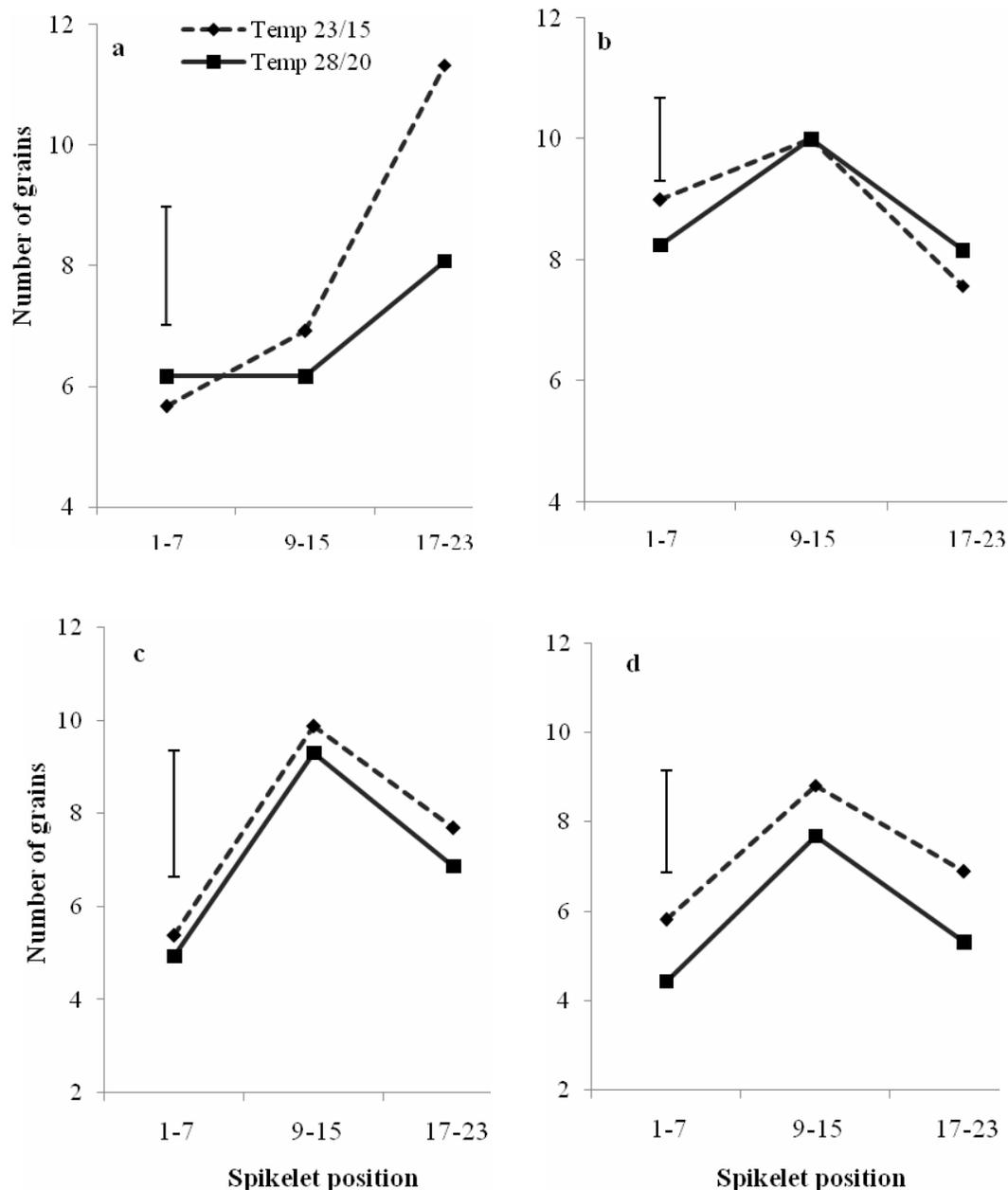
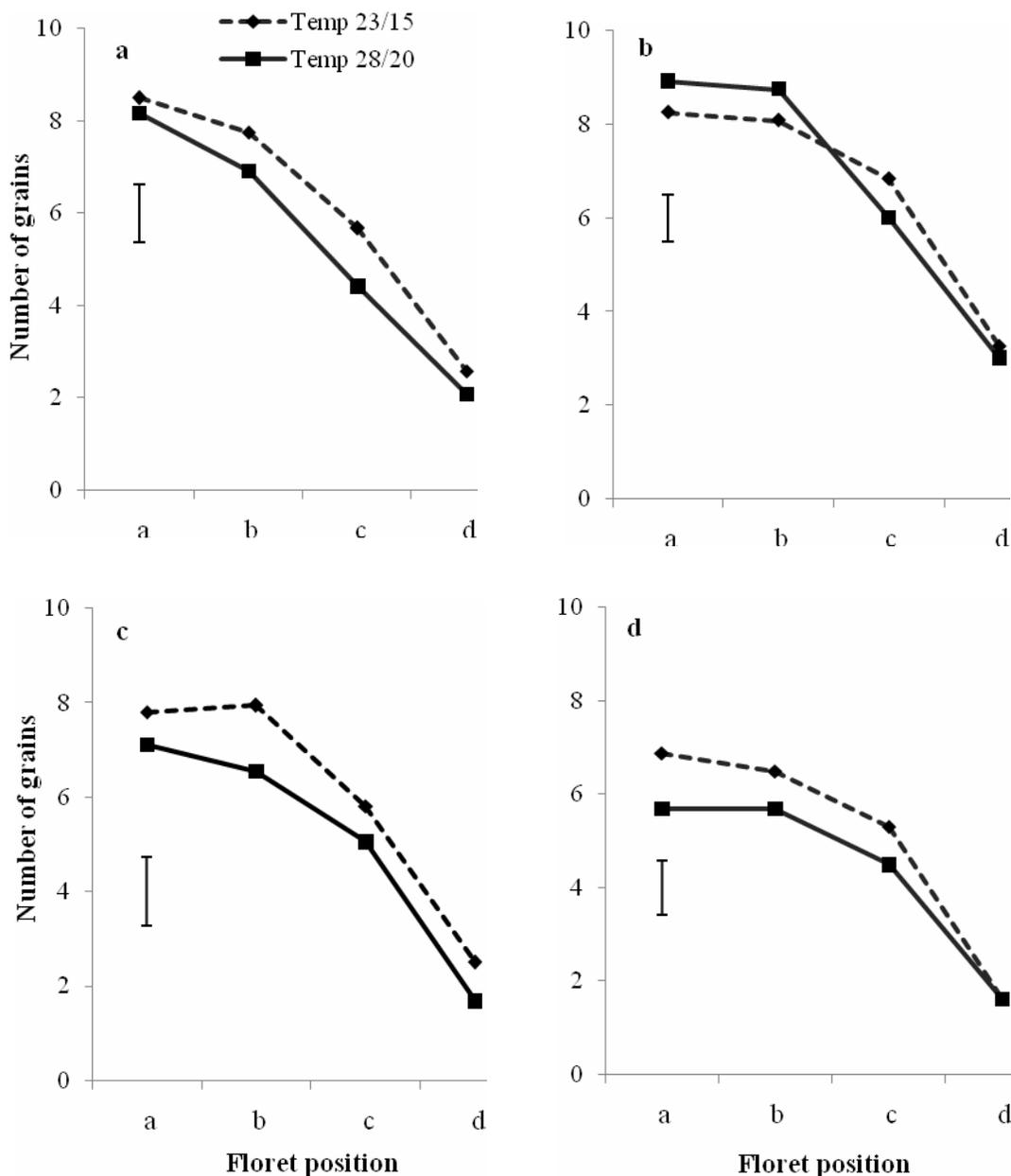


Fig. 2: Effect of temperature on number of grains within floret in (a) Oakley, (b) Soissons, (c) Mercia 1 and (d) Mercia 2 in *Fusarium graminearum* infected wheat spike. Data are means of three inoculation times and scores taken from one side (odd) of the wheat spike. Errors bar indicate the LSD.



Oakley showed more sensitivity to the high temperature treatment especially at upper spikelets, while the non-sensitivity of Soissons to temperature stress may be due to early flowering conferred by the presence of Ppd-D1a and this distinguished the cultivar as a potential candidate to be used under heat stress conditions.

The overall reduction in grain number may be associated with the ability of the fungus to prevent assimilate supply to the affected spikelets position

for grain development which seemed to have differed among elite cultivars. However, the outer floret 'c' and 'd' with generally lower grains could also be attributed to the adverse effects of the pathogen on grain development. Although, factors like premature ripening at the apical area instead of infection might lead to reduced grain number in some wheat cultivars, Tashiro and Wardlaw (1990) illustrated up to 11% grain reduction under higher temperature when spikes were inoculated immediately following anthesis.

The middle spikelets and floret 'a' and 'b' which generally have the highest number of grains in wheat ears were affected more in the NIL and this could have a consequence effect on the final mycotoxin levels in the grains at these positions. However, cultivar response to grain reduction within spikelets and within florets in *Fusarium* infected wheat spike under heat stress condition could determine the final grain yield and estimation of grain yield on spikelet and floret basis under the climate change situation would be most achieved using the near isogenic lines of wheat.

Conclusion

The implication of the results of this study is the need to evaluate, in contrasting cultivars, the sensitivity to *Fusarium* infection and temperature stress to stages of grain development in order to provide an effective base for disease management and grain yield prediction in the field. These results thus illustrated that the interaction between *Fusarium* infection and heat stress during grain filling and plant growth stage could contributed in a different way to shifts in mycotoxin produced by this fungus in wheat cultivars. There could be great fears related with prediction of climate change effects on grain yield, which are affected by slight increase in temperature events when working with cultivars differing in their background.

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