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Relationship Between Time of *Fusarium* Inoculation and Grain Positions Within Spike on Grain Set Among Wheat Cultivars

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Abstract
This study investigated the effect of time of *Fusarium* inoculation during anthesis on possible grain number within spikelets and florets in *Fusarium* infected wheat spike in controlled environment. Wheat cultivars; two each of commercial cultivars (Oakley and Soissons) and near isogenic lines (NILs) of Mercia background (Mercia 1 and Mercia 2) were grown in pots, inoculated with *Fusarium graminearum* spores at three different stages of anthesis; at anthesis (AA), 4 and 8 days after anthesis (4 DAA and 8 DAA), and incubated at either 23/15^{o}C or 28/20^{o}C for 14 days. After harvest, wheat spikes were assessed for the presence of healthy or shriveled grains across the spikelets and florets. Results of the study showed that cultivars responded differently to the time of inoculation at both the spikelet and floret and inoculation at 4 DAA which corresponded with mid-anthesis in most cultivars was the most severe stage. Soissons showed less sensitivity to the time of inoculation, while Mercia 2 linked with Rht-D1b was the most susceptible at inoculation done at 4 DAA when compared with the other cultivars. Grains in floret 'd' especially in the NILs had very low grains due to poor grain set relative to the control. This study therefore indicates that the relationship between time of inoculation and grain positions within wheat spike infected with *Fusarium* at different stages of anthesis could vary among cultivars with different genetic backgrounds.

1. Introduction

Fusarium head blight (FHB), commonly known as scab, is a fungal disease caused by *Fusarium graminearum* and other related species (Parry et al. 1995; Nicholson et al. 2007; Edward and Godley 2010) that affect small grain crops such as wheat, barley, and rye in many regions around the world. Wegulo (2012) identified plant growth stage as one of the factors that could influence *Fusarium* infection and the associated mycotoxins. Schroeder and Christensen (1963) also suggested in their work that wheat cultivars differ with the growth stage at which they are most susceptible to *Fusarium* infection. Using several *Fusarium* species, Lacey et al., (1999) inoculated winter wheat cultivars at different times from spike emergence to harvest. Little or no disease was observed for inoculation performed between spike emergence and the start of anthesis, but anthesis was the only infection time for which high mycotoxin concentrations was observed. Moreover, *Fusarium* infection sharply decreased for inoculations performed after mid-anthesis. Bai and Shaner (1996) comparing the susceptibility of wheat to FHB when inoculated at heading; flowering and late milk to early dough after inoculation by

injection of conidia into a central floret, reported that the mean disease incidence for the three growth stages was 98%, 100% and 92%, respectively.

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. 1998a; Knopf et al. 2008). The semi-dwarfing 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 and increase harvest index and thereby grain yield (Chapman et al. 2007). However, wheat cultivars carrying Rht-D1b have been linked to higher susceptibility to FHB possibly due to the shortened distance from the spike to infected crop debris (Mesterházy, 1995; Gosman et al. 2007).

For the development of highly effective strategies for controlling FHB, particularly for individual grain yield and toxin reduction in *Fusarium* infected wheat grains, it is important to understand the resultant effect when infection occurs at different stages during flowering using cultivars linked with the Rht alleles. Culler et al. (2007) advocated studies aimed at investigating the role of environment on *Fusarium* infection following infection of wheat spikes at different growth stages in a range of environments.

Consequently, the lack of knowledge on infection timing has also led to the problems associated with the variable efficacy of fungicide sprays (Parry *et al*., 1995; Yoshida et al. 2007). The numbers of spikelets and florets which are vulnerable during these predicted periods of either biotic or abiotic stress could have direct implications for the resultant level of grains per spike. Since flowering is recognised as the most favourable host stage for infection (Obst et al. 1997 and Lacey et al. 1999 and Yoshida et al. 2007), a better understanding of the infection timing within the flowering stages on grain set within spikes is necessary for accurate predictions of the possible interaction between *Fusarium* infection and the positions of the grain within spikes. Understandably progress has been made for years now, but *Fusarium* disease control is still challenging due to the complex disease nature. The disease still poses a major threat to yield and quality of grains (McMullen et al. 1997).

However, most cultivars do not possess desirable levels of resistance that could lead to good genetic control (Bai et al. 2001).

Little attempts have been made to compare the effect of *Fusarium* infection timing during anthesis on grain development and final grain yield of wheat 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 their sensitivity. This study was therefore aimed at determining the effect of different *Fusarium* infection timings during anthesis and evaluating also 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 sensitivity to within spike (spikelet and floret positions) grain set.

2. Materials and Methods

2.1. Growing Conditions and Experimental Design

A complete factorial pot experiment with three replicates at Plant Environment Laboratory, University of Reading, UK (51^o 27' N latitude, 00^o 56' W longitude) evaluated two commercial wheat cultivars (Oakley and Soissons) and near isogenic lines (NILs) of Mercia background (Mercia 1 and Mercia 2) (Table 1) which differed in the linked semi-dwarfing allele (Rht) at different *Fusarium* inoculation times. Rht-B1b is linked with FHB resistance while Rht-D1b is linked with FHB susceptibility. Soissons is also linked with Ppd-D1a which confers early flowering. The commercial 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' x Robigus) x Access	Rht-B1b
Soissons	Jena x 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 water drainage. Five seeds each of the four cultivars were sown in the pot 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.

2.2. Spike Inoculation and Assessment

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 x 10^5/ml spore suspension using a hand sprayer. Spike inoculation was carried out at the start of anthesis (AA), 4 days (4 DAA) and 8 days after anthesis (8 DAA) and the corresponding control plants sprayed with sterile distilled water. 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 either 23/15°C or 28/20°C at 88 – 93% relative humidity for 14 days. At the end of 14 day 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'. Spikelet 1-7, 9-15 and 17-23 represent the lower, middle and upper spikelets, respectively. 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 either healthy or shriveled grains on the odd numbered spikelets only.

2.3. Data Analysis

Data from individual cultivar and the average of the two temperatures 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.

3. Results

3.1. Spikelet Position

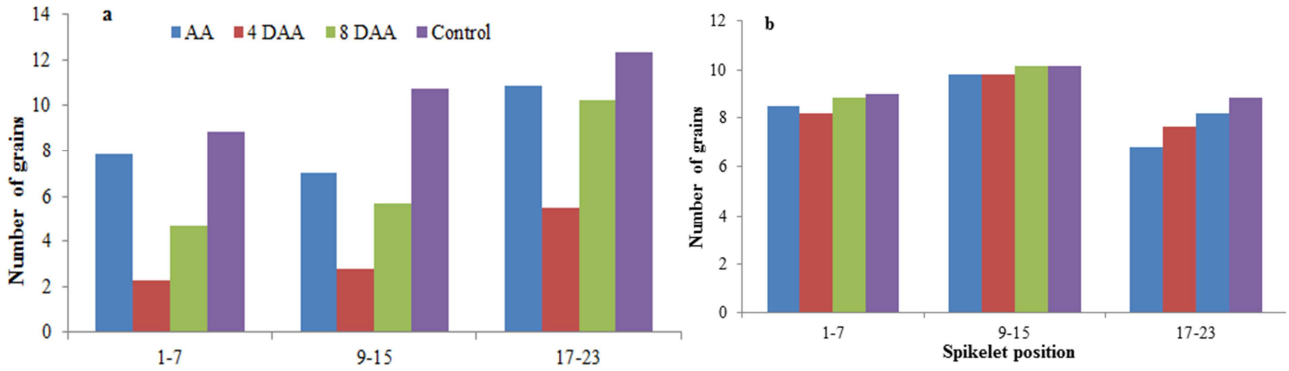

Figure 1. *Effect of time of inoculation on number of grains within spikelet in (a) Oakley and (b) Soissons in Fusarium graminearum infected wheat spikes. Data are means of two temperatures and were taken from one side (odd) of the wheat spike. LSD = 3.8 and 1.9 for Oakley and Soissons, respectively.*

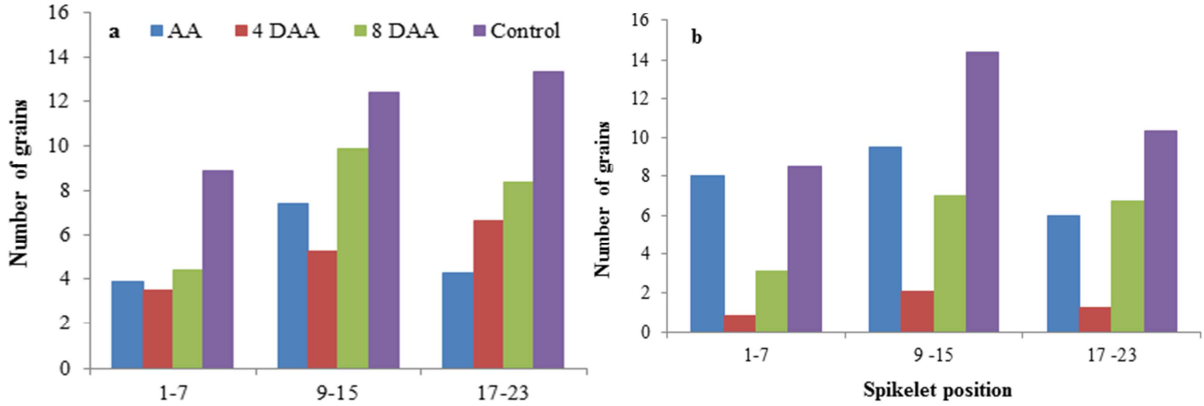

Figure 2. *Effect of time of inoculation on number of grains within spikelet in (a) Mercia 1 and (b) Mercia 2 in Fusarium graminearum infected wheat spikes. Data are means of two temperatures and were taken from one side (odd) of the wheat spike. LSD = 3.8 and 3.2 for Mercia 1 and Mercia 2, respectively.*

The effect of time of inoculation on grain reduction was compared between two commercial cultivars and NILs of Mercia background to ascertain the effect of *Fusarium* infection at different stages during flowering on grain distribution within spikelets and florets. The grain number per measurement showed considerable variation among cultivars and between the times of inoculation. For Oakley, grain number was severely reduced at inoculation at 4 DAA compared with the other inoculation times (Fig. 1a). The upper (17-23), middle (9-15) and lower (1-7) spikelets all have reduction of grains with the upper spikelets more sensitive to inoculation at 4 DAA when compared with the other inoculation times. Soissons showed no significant effect but on average middle spikelets was associated with marginally higher grains and very much less sensitive to the time of inoculation (Fig. 1b). In Mercia 1, lower spikelets (1-7) had the lowest grains at all the inoculation times and the reduction value followed this order; 8 DAA > AA > 4 DAA, for all spikelet positions except the lower spikelets where inoculation at AA had the least number of grains (Fig. 2a). For inoculations

at 8 DAA, the middle spikelets (9-15) were less sensitive and had the highest grains. Inoculation at 4 DAA reduced grains at all inoculation times for Mercia 2 (Fig. 2b) and the spikelet position sensitivity was greatest at middle spikelets for all inoculation times when compared with the control.

3.2. Floret Position

In Oakley, least sensitivity to the time of inoculation was observed at floret 'd' as a result of the developmental stage of the grains at this positions at the time of *Fusarium* inoculation (Fig. 3a). However, highest sensitivity was observed at florets 'a', 'b' and 'c' for inoculations done at 4 and 8 DAA, but with inoculation at AA showing no difference with the control at all florets. Soissons on the other hand, showed no significant interaction effect between time of inoculation and floret positions, although inoculation at 4 DAA consistently had the lowest grains across floret positions (Fig. 3b). Inoculation at 4 DAA consistently had the lowest grains in the NILs with the lowest sensitivity obtained also at floret 'd' (Fig. 4a&b). However, Mercia 2 showed substantial grain reduction at floret 'a', 'b' and 'c' for inoculation done at 4 DAA contrasting the effect of Mercia 1 at these floret positions (Fig. 4b). When compared with the control, the NILs showed varying sensitivity at the floret positions but with higher grain reduction obtained at florets 'a' and 'b'. No grains was recorded in Mercia 2 at floret 'd' for inoculation done at 4 DAA.

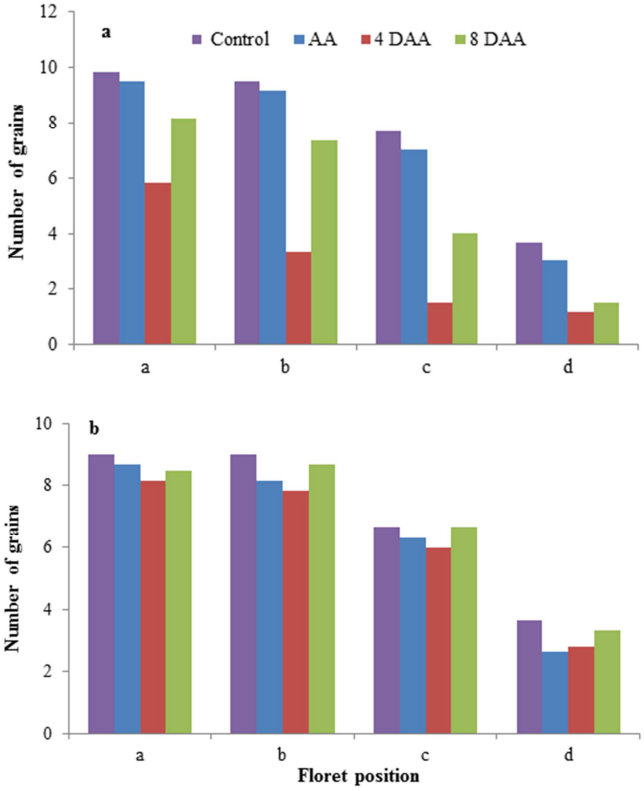

Figure 3. *Effect of time of inoculation on number of grains within spikelet in (a) Oakley) and (b) Soissons in Fusarium graminearum infected wheat spikes. Data are means of two temperatures and were taken from one side (odd) of the wheat spike. LSD = 1.8 and 1.4 for Oakley and Soissons, respectively.*

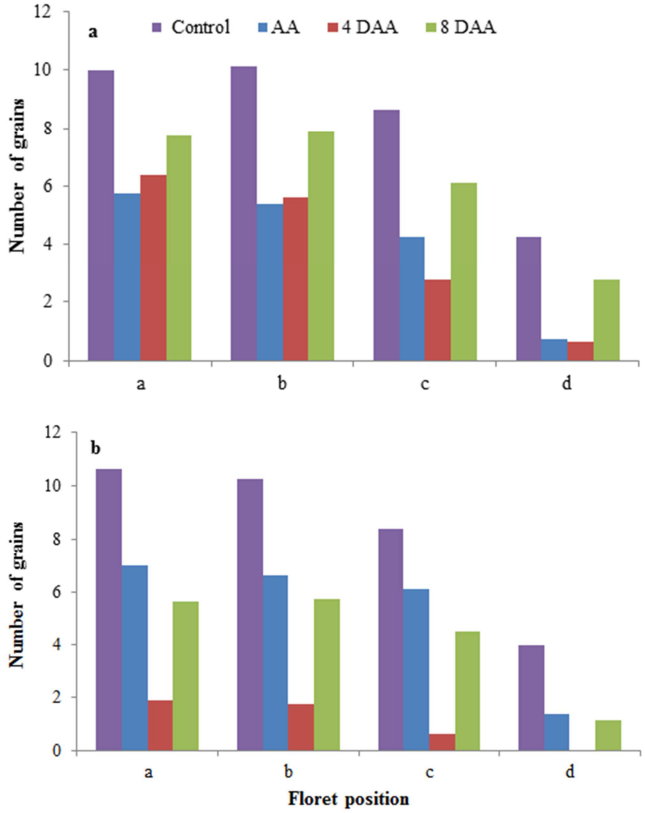

Figure 4. *Effect of time of inoculation on number of grains within floret in (a) Mercia 1 and (b) Mercia 2 in Fusarium graminearum infected wheat spikes. Data are means of two temperatures and were taken from one side (odd) of the wheat spike. LSD = 2.1 and 1.6 for Mercia 1 and Mercia 2, respectively.*

4. Discussion

Data analysis from the study revealed a possible relationship between the time of *Fusarium* inoculation and grain positions within spike on grain number reduction among wheat cultivars. The implication of the current result is that the number of grains in each spike varied with the stage at which the spikes were inoculated. This was unlike in the grain weight which was independent of the inoculation times (data not shown). It could be that in some cultivars grain weight was usually compensated for reduced grain number as revealed by Borner et al. (1993) in their findings. Apparently, the rate of grain growth might have increased under FHB epidemics regardless of whether the number of grains per spike was affected, although, a premature ripening at the upper spikelet instead of infection might sometimes lead to reduced grain number. Wegulo (2012) stated in his review that few or no grains could develop when heads were inoculated at flowering. The middle spikelets and florets 'a ' and 'b' which generally have the highest number of grains in wheat ears were affected more in some cultivars than the other, and this could have an implication on the overall grain yield and mycotoxin levels. However, this variation in the grain number reduction among cultivars under different stages of inoculation confirms the report of Nicholson et al. (2008) that some wheat cultivars have either narrow or longer period of optimal susceptibility to *Fusarium* infection.

Similarly, grain distribution within the spike and within floret also confirmed that mid-anthesis (inoculation at 4 DAA) was most critical for most cultivars and this is in line with the views of many authors investigating Fusarium head blight in wheat (Edwards and Godley, 2010; Cowger and Arellano, 2013; Angelo et al. 2014). This stage represents the peak of flowering in most UK wheat cultivars and could explain the reason for increased susceptibility to FHB.

Furthermore, the higher susceptibility of Mercia 2 at 4 DAA could be partly linked to the presence of the Rht-D1b which is widely associated with *Fusarium* susceptibility (Gosman et al. 2007), while the non-sensitivity of Soissons to time of inoculation at the spikelet positions could be due to early flowering conferred by the presence of Ppd-D1a which confers photoperiod insensitivity (Worland et al. 1998b; Korzun et al. 1998). This attribute clearly distinguished the cultivar from the other cultivars. Ppd1-D1a shortens the plant's life cycle, thus leading to higher grain set before the event of the pathogen. However, the fewer number of grains found in floret 'd' in all cultivars (although more severe in NILs) could be attributed to the ability of the pathogen to prevent grain development at this position. This somewhat agrees with Dias and Lidon (2009) who associated the poor grain set found in wheat cultivars under stress to both cultivar sensitivity to stress and the exact growth stage at which the stress was applied.

5. Conclusion

The time of *Fusarium* infection during anthesis is important in wheat grain set. However, this study demonstrated that the most critical time for *F. graminearum* infection on wheat grain set might differ and appears to be influenced by the position of either the floret or spikelet within the spike. In addition, cultivar sensitivity depended on the time of inoculation, and 4 days after the start of anthesis was confirmed to be the most vulnerable stage for most of the cultivars. The middle spikelets and florets 'a ' and 'b' which usually have higher grains in wheat spikes were affected more in some cultivars than the other, and this could have an adverse effect on the overall grain yield and also influence the final mycotoxin levels in the grains. Also full grain development at floret 'd' especially in the NILs was hindered by the pathogen resulting in poor grain set at this floret position. The study also showed that the linked semi-dwarfing allele and/or the cultivar background could also affect cultivar susceptibility to reduced grain set especially when *Fusarium* infection occurs at different stages during anthesis.

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