Genotype and time of inoculation effects on DON per grain and grain weight of winter wheat under two environmental conditions
Victor C. Okereke
Department of Crop and Soil Science, University of Port Harcourt, Choba, Port Harcourt, Nigeria.

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 x 10^7/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 Arellano, 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; Schaaafsma 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 (µg/g), grain DON content (µg) and mean grain weight (mg) at controlled environment.

| Treatment         | DON Conc. (µg/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.

REFERENCE

1. Börner, A., Worland, A. J., Plaschke, J., Erika Schumann., Law, C. N. 1993. Pleiotropic effects of genes for reduced height (Rht) and day-length insensitivity (Ppd) on yield and its components for wheat grown in Middle Europe. Plant Breeding 111: 204-216.
2. Cowger, C., Arellano, C. 2010. Plump kernels with high deoxynivalenol linked to late Gibberella zeae infection and marginal disease conditions in winter wheat. Phytopathology 100: 719-728.
3. Del Ponte, E.M., Fernandes, M.C., Bergstrom, G.C. 2007. Influence of growth stage on Fusarium head blight anddeoxynivalenol production in wheat. Phytopathology 155: 577-581.
4. Edwards, S.G., Pirgozliev, S.R., Hare, M.C., Jenkinson, P. 2001. Quantification of Trichothecene-producing Fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. Applied and Environmental Microbiology 67: 1575-1580.
5. Farooq, M., Bramley, H., Palta, J.A. Siddique, K.H.M. 2011. Heat stress in wheat during reproductive and grain-filling phases. Critical Reviews in Plant Sciences 30: 491-507.

6. Flintham, J. E., Borner, A., Worland, A.J., Gale, M. D. 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. Journal of Agricultural Science 128: 11-25.

7. Gosman, N., Bayles, R., Jennings, P., Kirby, J., Nicholson, P. 2007. Evaluation and characterization of resistance to Fusarium head blight caused by *Fusarium culmorum* in UK winter wheat cultivars. Plant Pathology 56: 264-276.

8. Lukac, M., Gooding, M.J., Griffiths, S., Jones, H. 2012. Asynchronous flowering and within-plant flowering diversity in wheat and implication for crop resilience to heat. Annals of Botany 109: 843-850.

9. Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding 114: 377-386.

10. Miedaner, T., Voss, H.H. 2008. Effect of dwarfing Rht genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Science 48: 2115-2122.

11. Schaaftsma, A.W., Savard, M.E., Clear, R., Dexter, J. 2004. Methods and issues regarding detection of deoxynivalenol, Fusarium-damaged kernels, and *Fusarium* spp. in commercial grain Canada. Canadian Journal of Plant Pathology 26: 443-452.

12. Semenov, M., Shewry, P.R. 2011. Modelling predicts that heat stress, not drought, will increase vulnerability of wheat in Europe. *Europe Science Reports-UK* 1: 66.

13. Siou, D., Gelisse, S., Laval, V., Repincay, C., Canales, R., Suffert, F., Lannou, C. 2013. Effect of wheat spike infection timing on Fusarium head blight development and mycotoxin accumulation. Plant Pathology 63: 264-276.

14. Stack, R.W. 2003. History of Fusarium head blight with emphasis on North America. In: K.J. Leonard and W.R. Bushnell, editors, Fusarium head blight and barley. APS Press, St. Paul, MN, USA, Pp 1-34.

15. Van der Fels-Klerx, H.J., van Asselt, E.D., Madsen, M.S., Olesen, J.E. 2013. Impact of climate change effects on contamination of cereal grains with deoxynivalenol. PLOS ONE, www.plosone.org.

16. Wegulo, S. 2012. Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins 4: 1157-1180.

17. West, J.S., Hodgate, S., Townsend, J.A., Edwards, S.G., Jennings, P., Fitt, B.D.L. 2012. Impacts of changing climate and agronomic factors on Fusarium ear blight of wheat in the UK. Fungal Ecology 5: 53-61.

18. Worland, A. J., E. J. Sayers, and V. Korzun, 2001. Allelic variation at the dwarfing gene Rht8 locus and its significance in international breeding programmes. Euphytica119: 155-159.

19. Youssefian, S., Kirby, E.J.M., Gale, M.D. 1992. Pleiotropic effects of the GA-insensitive Rht dwarfing genes in wheat. 2. Effects on leaf, stem, ear and floret growth. Field Crops Research 28: 191-210.

**ACKNOWLEDGEMENT**

The author is grateful to Commonwealth Scholarship Commission in the UK and University of Port Harcourt, Nigeria for sponsoring this work.

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**DOI**: 10.24297/jaa.v7i4.6358