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PROJECT 1 ABSTRACT
(1 Page Limit)

FHB resistance breeding programs for hard red winter wheat varieties adapted to Kentucky and the southeastern U.S. are underway at the University of Kentucky. Population screening tools that have been used in Kentucky include (i) visual assessment of disease severity, (ii) counts of infected ears or spikelets postanthesis, and (iii) counts of *Fusarium*-damaged or -infected kernels at harvest. Reduction of mycotoxins is a primary goal of our breeding program, but we have found that the correlation between our assessments of FHB disease severity and DON contamination of the wheat at harvest is relatively poor. The literature suggests that DON contamination depends on fungal biomass and fungal genotype, host genotype, moisture levels, and host physiological status (e.g. nitrogen levels). Although DON levels in the grain can be measured directly, different methods often result in different estimates of contamination, and the most accurate techniques are too costly, inconvenient, or time-consuming for routine use in a breeding program. Two previous studies have reported that a direct, quantitative measurement of *F. graminearum* biomass in the seed is highly correlated with levels of mycotoxin contamination, and thus evaluation of fungal biomass may provide a useful new tool for screening of breeding populations. Quantitative polymerase chain reaction (PCR) protocols for fungal pathogens, including *Fusarium spp.*, offer a convenient and cost-effective means for rapid analyses of fungal biomass. For example, a quantitative assay for trichothecene-producing *Fusarium spp.* was developed by European researchers based on real time (RT) PCR using the LightCycler® system and primers specific for the *tri5* gene sequence. **Our objectives** in this study include: 1) Develop and optimize a protocol for quantification of *F. graminearum* fungal biomass in developing and mature wheat kernels based on the *tri5* primers and SYBR-Green quantification using RT PCR in the highly automated and affordable SmartCycler® system. 2) Correlate fungal biomass with DON levels in kernels of three different breeding lines from the Kentucky breeding program that are highly resistant, moderately resistant, and susceptible to FHB 3) Use cytological methods to relate fungal development and localization over time to levels of DON and symptom development in infected seeds from resistant, moderately resistant, and susceptible wheat varieties. **Our goals** are 1) to develop useful and practical tools for screening of germplasm for the Kentucky FHB breeding program, and 2) to improve our basic understanding of the mechanism of seed infection by *F. graminearum* and its relation to FHB symptoms and DON production. Although two recent European studies have already demonstrated high correlations between *F. graminearum* biomass in wheat seeds, measured by quantitative PCR, and DON contamination, we think it will be useful to validate these results and develop a PCR quantification protocol for routine use in the breeding program in Kentucky. It is likely that environmental and other variables have different effects on the relationship between fungal biomass and DON production in different locations, there is considerable value in optimizing screening tools for each site and for each set of adapted genotypes. Comparisons of the results of parallel investigations in divergent systems will allow recognition of common themes. This in turn will aid in transfer of this technology to new sites and additional genotypes.