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Project Title: Quantifying Fusarium in Seeds and Grain Products for Laboratory and Industrial Use.

PROJECT 1 ABSTRACT
(1 Page Limit)

Specific and rapid analytical methods for determining *Fusarium graminearum* are important to understanding Fusarium Head Blight (FHB). Deoxynivalenol (DON) is used to test for toxicity potential of seed, but DON content on seed is not always indicative of DON in malt because of environmental conditions of the maturing grain, physiological development of the fungus, and storage conditions of the grain. Thus, if we can assess *Fusarium* contamination of incoming grain and correlate it to disease symptoms and presence of secondary metabolites, we should be able to predict grain quality. Visual estimations of FHB are not accurate assessments of *F. graminearum* and ergosterol, a non-specific fungal marker, is not quantitative for FHB. Thus, a need exists to develop rapid analytical methods for *F. graminearum*. We have developed monoclonal antibodies to *F. graminearum* and have been able to quantify *F. graminearum* in wheat and barley using ELISA and immunoblot systems, but need to test their utility for analyzing grain products and develop technologies for on-farm and laboratory use. Accordingly, our objectives are to: 1) examine the relationship between fungal mass, DON, and antigen mass in *Fusarium graminearum* and *Fusarium* spp. isolates grown under different environmental stresses, 2) determine the relationship between quantitative values of *F. graminearum* and DON in single kernels of barley collected from seed heads with varying FHB severity ratings, and 3) compare immunoquantification with real-time PCR, FHB severity scores, and ergosterol quantifications of *Fusarium graminearum*.

Three isolastes of *F. graminearum* will be grown in Petri dishes containing potato dextrose agar (PDA), Shenk-Hildebrandt media, ground barley extract agar, or ground wheat extract agar to determine best media for DON production. Isolates will then be grown on the best media containing 0, 0.5, or 1.0 M KCl and grown at 15, 25, and 35^{o}C. Mycelia will be quantified by weight and immunoligically, and DON analyzed to compare conditions necessary for each (Objective 1). Kernels from 10 seedheads ranging in FHB scores will be harvested from field plots. Each kernel location will be noted and analyzed for DON and *Fusarium*. Regression analysis will be used to establish whether a minimum *F. graminearum* seed colonization is necessary for DON production, and analysis of variance conducted to determine the effect of location within the spike on DON (Objective 2). Finally, *Fusarium graminearum* of will be quantified in 70 barley samples using real-time PCR, visual damage scores (FHB), DON, ergosterol, and immunoquantification using monoclonal antibodies. Regression analyses will be conducted comparing each method with the others. Agreement of the two methods will be based upon slope, intercept, and coefficients of determination.