Optimization of Real Time Quantitative PCR (Q-PCR) for *Fusarium pseudograminearum* and *F. culmorum* on wheat

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Fusarium crown rot of wheat is caused by a complex of *Fusarium* species, of which *F. pseudograminearum* and *F. culmorum* are the most important. Crown rot reduces wheat yields by an average of 9% in the Pacific Northwest. Traditional methods of species identification have included morphological characteristics of macroconidia. With the advent of Q-PCR techniques and the development of primers for *F. pseudograminearum* and *F. culmorum,* the potential exists for more accurate species identification and fungal DNA quantification from infected wheat stems. Primers developed in previous studies were evaluated for use in Q-PCR and DNA extraction kits were tested and optimized to accurately assess *Fusarium* species and DNA concentrations in wheat stem tissue. The 'OPT' primers (Shilling et al. 1996) for the amplification of *F. culmorum* and the 'FPG' primers (Williams et al. 2002) for the amplification of *F. pseudograminearum* yielded the most consistent results. The MO-BIO© Ultra Clean Soil Kit for DNA extraction produced the most consistent Q-PCR amplification from infected wheat tissue. The most optimal results were obtained by grinding with liquid nitrogen and soaking prior to bead beating (using a ceramic bead (MP Biomedicals© - FastDNA extraction kit)) and a Fast Prep speed of 5 for 45s. Addition of polyvinylpolypyrrolidone (PVPP) was necessary for adequate DNA extraction.