O. A. Vogel Wheat Research Fund
Annual Progress Report

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Progress Report Year: 2008

Title: Resistance to and Seed Transmission of Cephalosporium Stripe in Wheat

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Results:

Seed transmission of *C. gramineum*. The goal of this project is to develop a PCR-based assay that can be used to detect *Cephalosporium gramineum* in wheat seed and to estimate the proportion of infected seed. Six oligonucleotide sequences from the ITS region of *C. gramineum* were previously identified by this laboratory for use as forward and reverse primer pairs in PCR amplification of *C. gramineum* DNA. The primer pair F3/R3 successfully amplified DNA purified from cultures of *C. gramineum* isolates at concentrations as low as 1 pg/µl, and was selected for further development. No primer pair reliably amplified DNA extracted from the root, crown or leaf tissue of wheat plants infected with *C. gramineum*. Dilution of *C. gramineum* DNA into DNA from healthy wheat leaf tissue resulted in weaker amplification compared to *C. gramineum* DNA diluted to the same concentration in water, suggesting that one or more compounds in the plant extractions were acting to inhibit the polymerase chain reaction. The addition of bovine serum albumin to the reaction mix at 300 ng/µl improved amplification of template diluted in wheat DNA and of total DNA extracted from infected wheat plants. DNA from isolates of fungal pathogens of wheat were evaluated as templates for PCR amplification (Fig. 1). The F3/R3 assay was largely specific to *C. gramineum;* however, very faint bands were detected with *Fusarium avenaceum* and healthy wheat leaf tissue suggesting a potential for false positive readings.

Evaluation of the F3/R3 primers for detection of *C. gramineum* in infected seed was performed using seed from the susceptible winter wheat cultivar 'Stephens' grown in a 2008 field trial at the Palouse Conservation Field Station near Pullman, WA. Estimates of the proportion of infected seed in the lot were made by evaluating total DNA extractions from 850 individual seeds for PCR amplification of the *C. gramineum* ITS region and by evaluating colony development on a second sample of 850 seeds after 3 weeks on a semi-selective agar medium at 15 ºC. Prior to both methods, seed was surface disinfected with 95% ethanol for 2 minutes and then with a 20% bleach solution for 2 minutes. Seed for the colony evaluation was frozen for 24 hours after a 24 hour germination period at room temperature then placed on growth media. Estimates of the proportion of infected seed were similar for both methods. Colony evaluation yielded an estimate of 0.4% infected seed and the PCR assay estimate was 0.6% infected based on a strong amplification signal at the expected band size. However, at least three additional seed extractions evidenced faint amplification at the correct band size. Further evaluation of those and other randomly selected seed extractions revealed a tendency of the F3/R3 PCR assay to produce faint false positive signals that were not consistent across multiple PCR runs.

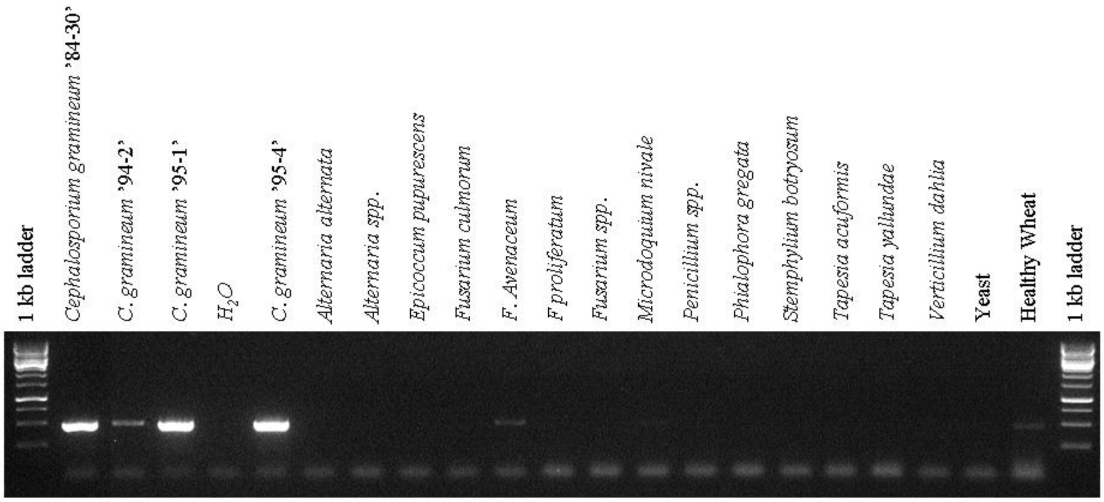

Figure 1. PCR amplification from *Cephalosporium gramineum* isolates and wheat mycoflora using the *C. gramineum*-specific primers F3/R3.

Genetic characterization of resistance genes / Mapping and tagging resistance genes. Research to confirm that a translocation from wheatgrass in line J99C0009 confers resistance to Cephalosporium stripe continues. However, ambiguous results from previous field tests has resulted in the need to confirm presence of the wheatgrass translocation in the resistant parent line and then to re-make crosses for genetic study. To rapidly and effectively identify plants containing the translocation, we identified wheatgrass genome-specific PCR primers and are testing potential parent plants to determine whether the translocated wheatgrass chromosome is present. Once confirmed, crosses will be made between these plants and susceptible genotypes for genetic study.

Accomplishments:

Outcomes: A single-seed evaluation method would be of little utility for large-scale seed testing. Therefore, we are developing a quantitative method based on real-time PCR (rtPCR) with the intent of correlating the template starting quantity to the proportion of infected seed in a bulk extraction. This assay, using the F3 primer and a non-*C. gramineum*-specific primer optimized for rtPCR, is specific for *C. gramineum* isolates without false positive detection in *F. avenaceum* or in healthy wheat extractions. We do not yet know if this assay will prove sensitive enough to detect a single infected seed within the total DNA extracted from samples of 50 to 100 bulked seeds. If this assay should prove successful, then we intend to evaluate the statistical correlation between the proportion of bulked seed DNA attributable to *C. graminium* as estimated by rtPCR and the proportion of infected seed as estimated by colony evaluation. From this correlation, it should be possible to obtain an estimate of the proportion of infected seed in a sample which is comparable to that obtained with the colony growth evaluation method.

Impacts: Little is known about seed transmission of *C. gramineum* in wheat, partly because a rapid and effective method of determining whether wheat seed is infected by this pathogen does not exist. Unfortunately, Brazil established a quarantine against regulating import of wheat seed containing *C. gramineum*; the lack of an effective detection tool makes it impossible to determine whether seed lots are infected and thus wheat from areas of the world where the disease occurs may be subject to this quarantine. Development of a specific and sensitive PCR assay for *C. gramineum* in seed could provide a useful tool for trade and for developing a greater understanding of this phenomenon in wheat.

Publications:

Li, H. J., R. L. Conner, and T. D. Murray. 2008. Resistance to soil-borne diseases of wheat: Contributions from the wheatgrasses *Thinopyrum intermedium* and *Th. ponticum*. Can. J. Plant Sci. 88:195-205.

Presentations and Reports:

Murray, T. 2008. Wheatgrass as a Source of Genes for Perennial Habit and Disease Resistance in Wheat. Tokachi Nogaku Danwakai, pages 1-12.